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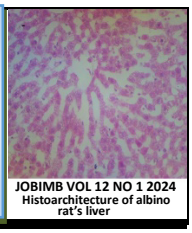


# Survey for Enterobacteria and *Vibrio* spp. Associated with Irrigated Vegetables in Irrigation Areas of Northern Bauchi State, Nigeria

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**ABSTRACT**

This study was prompted in a bid to determine the level of public health hazards associated with the presence of enterobacteria and *Vibrio* spp. in irrigated vegetables in irrigation areas of Northern Bauchi State, Nigeria. This was to address the question of what the recent circumstances behind the report of outbreak of gastroenteritis in the region were, thus the impetus for the current investigation. Irrigation Water and irrigated vegetable samples (72) from across the four local government areas were collected from different irrigation sites and analysed for bacteriological contamination indices. The River Zaki, River Jama'are, and River Zigau were observed to be the primary sources of irrigation water. The overall mean aerobic mesophilic count and the Most Probable Number values for coliforms observed in this study ranged from $1.6x10^4$ to $8.x10^8$ Cfu/mL and 14 to 33, respectively. Up to 62 isolates comprising 42 (81.82%) enterobacterial strains, eight *Pseudomonas* spp. and ten representing *vibrio* spp. were isolated from the analysis of vegetables and irrigation water samples. In descending order of dominance, the organisms isolated were *E. coli* 14 (22.6%), *Vibrio* spp. 10 (16.1%), *Citrobacter* spp. 9 (14.5%), *Pseudomonas aeruginosa* 8 (12.9), *Salmonella enterica* 8 (12.9%), *Klebsiella oxytoca* 8 (12.9%) and *Klebsiella pneumoniae* 5 (8.1%). All the isolates were sensitive to most of the antibiotics used except ampicillin (10 µg) and amox-clav (30 µg). No resistance was recorded against ciprofloxacin (5 µg). The findings revealed that the river water used for irrigation in this study was a possible pre-harvest source of contamination of fresh vegetables, potentially constituting a health risk to consumers.

## INTRODUCTION

Fresh and minimally processed vegetables and fruits provide the most important human diet that contains carbohydrates, proteins, vitamins, minerals, and fibre. The nutritional and other benefits of a regular intake of vegetables, including the ability to reduce the associated risk of illnesses such as heart disease, diabetes, and cancer, has been reported and documented internationally. This has resulted in a further increase in desirability and consumption [1]. High-quality irrigation water is becoming scarce; the risk of outbreaks of food-borne illnesses due to consumption of vegetables irrigated with contaminated water is increasing [2]. A wide range of microbial pathogens have been found in water and can be transferred to crops during irrigation, including coliform bacteria. These bacteria include many bacteria found in human or animal intestinal tracts, as well as plants and the environment so also allochthonous to water and vegetation under natural conditions [3]. Cholera and Enteritis caused by *Vibrio cholera* and Enterobacteria, respectively, continue to be a global threat to public health and a key indicator of lack of social development. At the nationwide level, Bauchi has been the hardest-hit state due to cholera activity in Nigeria 2021.

So far, the state has the highest number of cases among the top ten states which reported cases in the country [4]. However, the typical genera of this large heterogeneous family include *Citrobacter* species., *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella* species, *Proteus* species, *Salmonellae* species, *Serratia* species, *Shigellae* species, *Vibrio* species and *Yersinia enterocolitica*. *Shigellae*, *Salmonellae,* and *Vibrio cholarae* as well as *Vibrio parahaemolyticus* [5]

## METHODOLOGY

### Study area

The present study was conducted in four local government areas of the northern part of Bauchi state ($9^0$ 0'00''E-$11^0$0'0''E; $10^0$0'0'0N-$12^0$'0'0N) which include; Itas/Gadau, Jama'are, Zaki and Shira Local Government Areas of Bauchi State. These areas have a tropical climate, marked with two distinct seasons which

can be wet-season (April-September) and dry-season (October to March) [6] .

**Field Survey of the sampling sites**
This aspect was conducted to achieve the objective of field observation. The vegetables and irrigation water were sampled from three irrigation farms that are situated away from one another. In Zaki Local Government, the collection sites are fadaman Arewa, Kore ta Kudu and Kore ta Arewa. The corresponding sites for the other three (3) Local Government collections in Shira, Jamaare, and Gadau are; Zigau hayin Gada, Zigau Mazin and Zigau Kurmi; Honare, Digiza, and Agayayi; and Rafin Kabu, Rafin Gulewa and Rafin Banja respectively. A preliminary survey was carried out on the distribution and location of irrigated vegetable growers in the study areas before resuming the actual sample collection.

**Samples Collection**
A total of 72 samples were collected from four Local Governments involved in this study. Items comprising of twenty four (24) lettuces in four Local Governments, six (6) each per Local Governments and two (2) each per site, the same quantity was collected for tomatoes and irrigation water.

**Microbiological analysis of irrigation water and irrigated vegetables samples**

**Sample preparation**.
Mixed vegetable samples (unprocessed and large sized) were aseptically chopped into smaller pieces using a sterile stainless-steel knife before weighing. A 25 g of sub-sample of each vegetable was aseptically weighed and vigorously shaken in a conical flask containing 225 mL of sterile 0.1% buffered peptone water for 3min separately to homogenize the samples. In addition, a tenfold serial dilution was made. A 10 mL of irrigation water sample was also mixed with 90 mL of peptone water using vortex mixer. Finally, appropriate serial dilutions of the suspension were made as demonstrated by [7].

**Enumeration of total heterotrophic bacteria in the water and vegetable items**
This aspect was undertaken to enumerate the mesophilic, enterobacteria and coliforms in the irrigation water and vegetable samples. Total heterotrophic bacteria (THB) count in fresh irrigated vegetables, irrigation water and public water samples was carried out according to standard methods as demonstrated by [7], using nutrient agar as a growth medium. The pour plate method was used and 1 mL aliquots of each the $10^3$- $10^6$ dilution factor was dispensed aseptically into sterile Petri-dish in duplicate and then followed by 15ml of nutrient agar. The content was allowed to gel and the plates were incubated at 37ºC for 24 hours under aerobic conditions. Plates containing 30 to 300 colonies were selected and counted, and the number of colony forming units per mL (CFU/mL) was calculated by multiplying the number of colonies by the dilution factor.

**Enumeration of coliform bacteria**
Total coliform bacterial counts in fresh irrigated vegetable samples were carried out according to standard methods as demonstrated by [7], using MacConkey agar as a growth medium. The pour plate method was used and 1 mL aliquots of each the $10^3$ to $10^6$ dilution factor was dispensed aseptically into sterile Petri-dish in duplicate and then followed by 20 mL of MacConkey agar. The content was allowed to gel and the plates were incubated at 37ºC for 24 hours. Plates containing 30 to 300 colonies were selected and counted, and the number of coliform forming units per mL (cfu/mL) was calculated by multiplying the number of colonies by the dilution factor.

**Enumeration of coliform using MPN fermentation technique**
Most probable number (MPN) procedure was carried out for enumeration of the most probable number of coliform in irrigation water according to standard methods as demonstrated by [7], using lactose broth as the growth medium. Each test tube is contained in it an inverted Durham tube before the broth is sterilized. After sterilization of the test tubes containing Durham tubes and ensuring no air bubbles is trapped. 10 mL of water sample was added in to each of the 5 test tubes containing 10 mL of double-strength broth medium. Similarly, 1 mL of water sample was added to each of the 5 test tubes containing 10 mL of double strength broth medium likewise, 0.1 mL of water sample was added to the remaining 5 test tubes containing 10 mL double strength medium. Formation of gas at 37°C within 48 hours constitutes a positive presumptive test and number determined with reference to MPN table.

**Confirmatory test for coliform bacteria**
A loopful from each gas-producing tube was inoculated into separate tubes containing lactose broth (LB) broth, after which the tubes were incubated at 37ºc for 48 h. The formation of gas confirmed the presence of coliform bacteria, and the numbers of the positive tubes was recorded and the most probable number of the coliform was determined from the MPN table as demonstrated by [7].

**Isolation and differentiation of members of *Enterobacteriaceae* (coliforms) detection and isolation of *E. coli***
This achieved by inoculating a loopful from gas-positive tubes of (LB) tubes on to Eosin-methylene blue (EMB) agar. The detection of dark - purple, with greenish metallic sheen was suggestive of *E.coli* as demonstrated by [7].

**Isolation of bacterial species**
*Salmonella* species were isolated using the method demonstrated by [7], the samples were added to 9 mL of lactose broth and incubated for 18 hours at 37°C to resuscitate the organisms that might appear weak or even unable to grow when cultured directly on selective media. On the second day, the topmost surface of the enrichment was subcultured without shaking the broth, because most Salmonella species are more motile than E. coli and tend to accumulate at the top of the enrichment. This was achieved by plating onto Salmonella-Shigella agar (SSA). After overnight incubation at 37°C, the plates were observed. Salmonella colonies appeared as colorless and transparent, with black-centered colonies indicating the presence of lactose-fermenting Salmonella species.

The black centers are due to the production of hydrogen sulfide, which reacts with iron salts in the medium to form a black precipitate, distinguishing them from other organisms that do not produce hydrogen sulfide. The selective and differential properties of SSA facilitate the isolation and identification of Salmonella from mixed microbial populations. Isolation of *Klebsiella* spp. was carried out using the MCA and Eosin methylene blue agar medium was used as a growth medium [7]. Isolation of *Citrobacter* spp. was carried out using the MCA and Eosin methylene blue agar medium was used as a growth medium [7]. Isolation of *Vibrio* species. was carried out by taking 1 mL of homogenized sample, irrigation water samples, was added to 9 mL of Alkaline Peptone Water (APW) and incubated at 37°C for 4-6 hours.

Enriched APW was streaked onto Thiosulfate Citrate Bile salts Sucrose (TCBS) agar medium. Plates were further incubated at 37°C overnight. The colonies characteristically yellowish, 2-3 mm in diameter after 18-24 h at 37 ºC was considered *Vibrio cholera* as demonstrated by [7].

**Biochemical analysis**
All bacterial isolates were identified by microscopic, morphological, and biochemical characterization. The biochemical tests were interpreted to determine the presumptive nomenclature of the potential pathogenic bacteria isolates (mostly enteric) through Bergey's Manual of Determinative Bacteriology, API (Analytical Profile Index) 20-E and molecular technique using polymerase chain reaction.

**Statistical analyses of the data**
Results were presented using descriptive statistics (tables, bar chart, and pie chart), Statistical analysis was performed to determine whether there were significant differences between mean bacterial counts obtained from irrigation water, domestic drinking water sources, and fresh vegetable samples; two-sample t test was used with P value 0.05 as an indicator of statistical significance. SPSS 16.0 (a software package for statistical analysis) was used to perform the analyses [8].

**Antimicrobial sensitivity test against isolated organisms**
All bacterial isolates were tested in-vitro for sensitivity to antibiotics using disk diffusion techniques as described by Clinical and Laboratory Standards Institute. An overnight Mueller- Hinton broth culture was prepared by inoculating 2-3 loopfuls of the test organism in nutrient broth and incubating overnight (usually 18 h) at 37ºC. A standard inoculum test culture was then achieved by diluting 0.1 mL of the culture suspension to 20 mL of sterile distilled water. This is 1:200 dilutions which marched the turbidity of 0.5 McFarland standards. This was carried out according to [9]. A replicate number of nutrient agar plates (usually two) for each isolate were prepared, dried in a drier (for 10-15 min) to remove excess moisture. These were seeded as evenly as possible with standard swab of the inoculum and allowed to settle and dry for 5-10 min. Excess inoculum moisture that persists was removed with the aid of a sterile swab.

Standard single disc (06) discs of about 6mm diameter, far enough from one another, were firmly pressed with sterile forceps onto each test plate. The control plates were inoculated with the single disc alone without the test culture and all the plates were incubated aerobically at 37ºC for 18 to 24 h. A diameter of the zones of inhibition in millimeters was measured with the help of a millimeter ruler, and the mean for each replicate was recorded through a standard table that relates the inhibition zone diameter to the degree of bacteria resistance.

## RESULTS

**Field Observations on Sampling Sites**
Generally, across the 4 local governments, the Rivers (River Zaki, River Jama'are and River Zigau) were the main irrigation sources in each sampling site studied. The common anthropogenic activities around the irrigation areas such as washing clothes, wading, use of human and animal dung as manure, animal rearing, fishing, and fetching of drinking water were observed.

**Bacteriological contaminations of the samples**
The mean viable mesophilic bacterial count in Colony Forming Unit per 100 mL (CFU/100 mL) of irrigated vegetables and irrigation water samples (**Table 1**) showed that total viable count in IW ranged between $8.83x10^7$to $1.22x10^7$ CFU/100 mL, corresponding to an MPN of 27 MPN /100 mL. In LT Samples, $8.7x10^4$ to $9.32x10^7$ CFU/10 mL was the highest mean bacterial count, but the coliform count of 33 MPN/100 mL established their unfitness for public consumption. Tomatoes showed a minimum of $5.05x10^4$ to a maximum of $1.46x10^8$CFU/100 mL. Meanwhile, 33MPN/100 mL coliform counts.

**Cultural morphology and biochemical characteristics of bacteria isolated from four different local government areas of Northern Part of Bauchi State**
Based on the identification criteria employed; gram reaction, ability to ferment lactose on MaConkey agar medium, confirmation of *E.coli* and other coliform on Eosin methylene blue agar medium (EMB), Salmonella Shigella Agar for differentiation of *Salmonella* and *Shigella* spp. Base on hydrogen sulphide production and Thiosulphate citrate bile sucrose (TCBS) selective medium for differentiation of *Vibrio* spp. Subsequently, there is a set of biochemical reactions such as the Indole test, Methyl red, Voges Proskauer, Oxidase test, and Urease and Citrate utilization test. The organisms identified (**Table 2**) were found to be *E.coli, Klebsiella oxytoca, Salmonella enterica, Citrobacter freundii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Vibrio cholera, and Vibrio parahaemolyticus.*

**Confirmatory test of the isolates by Analytical Profile Index (API-20E)**
The organisms identified in **Table 2** were further confirmed using the Analytical profile index (API) (**Table 3**).

**Total number of isolates and genera obtained from each sampling area**
A total of 62 isolates comprising 42 (81.82%) enterobacterial strains and 8 representing *Pseudomonas* spp., and 10 representing *Vibrio* spp. were isolated from the 72 irrigated vegetables and irrigation water samples analysed in the four local government areas of northern Bauchi State. The most dominant genus isolated, was found to be *E. coli* 14(22.6%). Next in line in terms of dominance were *vibrio* spp. 10(16.1%), *Citrobacter* spp. 9(14.5%), *Pseudomonas aeruginosa* 8(12.9), *Salmonella enterica* 8(12.9), *Klebsiella oxytoca* 8(12.9%), and the least common being *Klebsiella pneumoniae* 5(8.1) (**Table 4**).

**Total number of isolates and the genera obtained from each sampling types**
Dominance was also found to vary with the source of samples. Therefore among the whole samples analysed the predominant in terms of number of organisms isolated in each sample type was found to be irrigation water 17(27.4%) then the next in the line was found to be lettuce 15(24.2%) followed by well water 12(19.4%) second to the last was found to be borehole water 10(16.1%) and the least in terms of dominance was found to be tomatoes 8(27.4%) as shown in **Table 6**.

**Percentage susceptibility pattern of the isolates to some commonly used antibiotics**
Amoxiclave, ampicillin, and chloramphenicol were the most resisted antibiotic agents by the isolated organisms. However, ciprofloxacin, doxycycline, and streptomycin are the drugs of choice to be recommended for the treatment of all the isolated organisms, as shown in **Table 6.**

**Table 1.** Mean Bacterial Counts (CFU/mL) of irrigated vegetables and water samples from irrigation areas of Northern Bauchi state Nigeria.

| Sample type | Site | Zaki | Jamaare | Gadau | Shira | WHO Ref Standard | MPN/100ml |
|---|---|---|---|---|---|---|---|
| IW | A | $1.8x10^8$ | $1.41x10^8$ | $1.03x10^8$ | $9.2x10^7$ | ≤500 | 26, 27, 27, 33 |
| | B | $1.99x10^8$ | $8.8x10^8$ | $1.09x10^8$ | $9.15x10^7$ | ≤500 | 17, 17, 26, 14 |
| | C | $1.98x10^8$ | $6.4x10^8$ | $9.75x10^7$ | $8.15x10^7$ | ≤500 | 22, 26, 17, 14 |
| LT | A | $1.49x10^8$ | $1.04x10^8$ | $9.0x10^7$ | $9.35x10^4$ | $1.0x10^2$ | NA |
| | B | $1.21x10^8$ | $1.01x10^8$ | $1.16x10^8$ | $8.25x10^4$ | $1.0x10^2$ | NA |
| | C | $9.5x10^8$ | $1.36x10^8$ | $7.35x10^7$ | $8.4x10^4$ | $1.0x10^2$ | NA |
| TM | A | $1.46x10^8$ | $1.12x10^8$ | $9.95x10^7$ | $6.0x10^4$ | $1.0x10^2$ | NA |
| | B | $1.33x10^8$ | $9.4x10^7$ | $6.7x10^7$ | $7.0x10^4$ | $1.0x10^2$ | NA |
| | C | $8.75x10^7$ | $5.9x10^7$ | $8.35x10^7$ | $5.05x10^4$ | $1.0x10^2$ | NA |

Key: IW = Irrigation water, LT = Lettuce, Tomatoes, A, B and C = Three irrigation Sites of Collection.

**Table 2.** Culture, morphology and biochemical characteristics of bacteria isolated from four different local government areas of Northern Part of Bauchi State, Nigeria.

| TEST | *E. coli* N=14 | *K. pneumonia* N=8 | *K. oxytoca* N=8 | *C. fraundii* N=9 | *S. enterica* N=8 | *V. cholarae* N=5 | *V.* species. N=5 | *P. aeruginosa* N=8 |
|---|---|---|---|---|---|---|---|---|
| MOR | Rod | Rod | Rod | Rod | Rod | Rod curved | Rod | Rod |
| GRM | - | - | - | - | - | - | - | - |
| MCA | Pink | Mucoid pink | Pink | colorless | colorless | NA | NA | colorless |
| SSA | NA | NA | NA | Clrless BC | Clrless BC | NA | NA | NA |
| EMB | Blue (MSH) | Pink | Blue | NA | NA | NA | NA | NA |
| TCBS | NA | NA | NA | NA | NA | Yellowish | Greenish | NA |
| CAT | + | + | + | + | + | + | + | + |
| OXI | - | - | - | - | - | + | + | + |
| CIT | - | + | + | + | + | + | + | + |
| IND | + | - | + | - | - | + | + | - |
| MR | + | - | + | + | + | - | - | - |
| VP | - | + | - | - | - | - | - | - |
| URE | - | + | - | - | - | - | - | - |

Key: MOR = morphology, GRM = Gram reaction, MCA = Macconkey Agar, SSA= Salmonella Shigella Agar, EMB= Eosin metalin blue agar, TCBS= Thiosulphate Citrate Bile Succrose, CAT= Catalase, OXI= Oxidase, CIT= Citrate, IND= Indole, MR= Methyl red, VP= Vogesproscauer, URE= Urease, *E.coli= Escherichia Coli, K.pneumoniae= Klebsiella pneumonia, K.oxy= Klebsiella Oxytoca, C.fraundii= Citrobacter Fraundii, S.enterica= Salmonella Enterica, V.cholarae= Vibrio cholarae, V.* species= *Vibrio Parahaemolyticus,.aeruginosa = Pseudomonas aeruginosa,* clrless(BC)= colorless with black centered, Rod (cvd)= Rod curved.

**Table 3.** API for biochemical characteristics of bacteria isolated from four different local government areas of Northern Part Bauchi State.

| TEST | *E. coli* N=14 | *K. pneumoniae* N=8 | *K. oxytoca* N=8 | *C. fraundii* N=9 | *S. enterica* N=8 | *V. cholarae* N=5 | *V.* species N=5 | *P. aeruginosa* N=8 |
|---|---|---|---|---|---|---|---|---|
| ONPG | + | + | + | + | + | + | - | - |
| ADH | + | - | + | + | + | - | - | + |
| LDC | + | + | + | + | + | + | + | - |
| ODC | + | - | + | - | + | + | + | - |
| CIT | - | - | + | + | + | - | + | + |
| $H_2S$ | - | - | - | + | + | - | - | - |
| URE | - | + | - | - | - | - | - | - |
| TDA | - | - | + | - | - | - | - | - |
| IND | + | - | + | - | - | + | + | - |
| VP | - | + | + | - | - | + | - | - |
| GEL | - | - | + | - | - | + | - | - |
| GLU | + | + | + | + | + | - | + | + |
| MAN | + | + | + | + | + | - | + | - |
| INO | - | + | + | + | - | - | - | - |
| SOR | + | + | + | + | + | - | - | - |
| RHA | + | + | + | + | + | - | - | - |
| SAC | - | + | + | + | - | + | - | - |
| MEL | + | + | + | + | + | - | - | + |
| AMY | + | + | + | + | + | - | + | + |
| ARA | + | + | + | + | + | - | - | - |
| OXI | - | - | - | - | - | + | + | + |

**Keys:** ONPG= O-nitrophenyl-b-D-galactopyranoside, ADH= arginine dihydrolase, LDC= lysine decarboxylase, ODC= ornithine decarboxylase, CIT= citrate, $H_2S$= hydrogen sulfide, URE= urea, TDA= Tryptophan deaminase, IND= Indole, VP= Voges-Proskauer, GEL= gelatin, GLU= glucose, MAN=Mannitol, INO= inositol, SOR= sorbitol, RHA= rhamnose, SAC= MEL= melibiose, AMY= amygdalin, ARA= Arabinose, OXI=Oxidase, *E.coli= Escherichia Coli, K.pneumoniae= Klebsiella pneumonia, K.oxy= Klebsiella Oxytoca, C.fraundii= Citrobacter Fraundii, S.enterica= Salmonella Enterica, V.cholarae= Vibrio cholarae, V.* species= *Vibrio Parahaemolyticus,.aeruginosa = Pseudomonas aeruginosa*

**Table 4.** Percentage frequency of occurrence of different isolates from each sampling area.

| Organism | Sampling area | | | | Total n(%) |
|---|---|---|---|---|---|
| | ZK | JMR | GDU | SHR | |
| | n(%) | n(%) | n(%) | n(%) | |
| *E. coli* | 4(28.6) | 4(28.6) | 3(21.4) | 3(21.4) | 14(22.6) |
| *S. enterica* | 3(37.5) | 0(0) | 3(37.5 | 2(25) | 8(12.9) |
| *C. fraudii* | 3(33.3) | 3(33.3) | 0(0) | 3(33.3) | 9(14.5) |
| *K. pneumoniae* | 0(0) | 2(40) | 3(60) | 0(0) | 5(8.1) |
| *P. aeruginosa* | 2(25) | 2(25) | 2(25) | 2(25) | 8(12.9) |
| *K. oxytoca* | 3(37.5) | 3(37.5) | 0(0) | 2(25) | 8(12.9) |
| *V. cholarae* | 3(60) | 0(0) | 2(40) | 0(0) | 5(8.1) |
| *V. species* | 1(20) | 2(40) | 2(40) | 0(0) | 5(8.1) |
| Total | 19(30.6) | 16(25.8) | 15(24.2) | 12(19.4) | 62(100) |

Key: Org = Organisms, *E.coli= Escherichia Coli, K.pneumoniae= Klebsiella pneumonia, K.oxy= Klebsiella Oxytoca, C.fraundii= Citrobacter Fraundii, S.enterica= Salmonella Enterica, V.cholarae= Vibrio cholarae, V.* species= *Vibrio Parahaemolyticus,.aeruginosa = Pseudomonas aeruginosa,* ZK= Zaki, JMR= Jamaare, GDU= Gadau, SHR= Shira, n= number of Isolates, %= Percentage

**Table 5.** Total number of isolates and the genera obtained from each sample type.

| Organism | | | Sample Type | Total n (%) |
|---|---|---|---|---|
| | IW (n%) | LT n(%) | TM n(%) | |
| *E. coli* | 6(42.9) | 4(28.6) | 4(28.6) | 14(22.6) |
| *S. enterica* | 4(50) | 2(25) | 2(25) | 8(12.9) |
| *C. fraundii* | 3(33.3) | 4(44.4) | 2(22.2) | 9(14.5) |
| *P. aeruginosa* | 2(25) | 4(50) | 2(25) | 8(12.9) |
| *K. oxytoca* | 4(50) | 2(25) | 2(25) | 8(12.9) |
| *K. pneumoniae* | 2(40) | 2(40) | 1(20) | 5(8.1) |
| *V. cholarae* | 2(40) | 2(40) | 1(20) | 5(8.1) |
| *V. species*. | 3(60) | 2(40) | 0(0) | 5(8.1) |
| Total | 26(41.9) | 22(35.5) | 14(22.6) | 62(100) |

Key: IW = Irrigation Water, LT = Lettuce, TM =Tomatoes, *E.coli= Escherichia Coli, K.pneumoniae= Klebsiella pneumonia, K.oxy= Klebsiella Oxytoca, C.fraundii= Citrobacter Fraundii, S.enterica= Salmonella Enterica, V.cholarae= Vibrio cholarae, V.* species= *Vibrio Parahaemolyticus,.aeruginosa = Pseudomonas aeruginos*

**Table 6.** Percentage (%) sensitivity of isolates sourced from the samples used in the study.

| Antibiotics | Disc content (µg) | *E.coli* (n=14) | | *K. pneumoniea* (n=8) | | *S. enterica* (n=8) | | *K. oxytoca* (n= 8) | | *V.* species (n=10) | | *C. fraundii* (n=9) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | R | S | R | S | R | S | R | S | R | S | R |
| Ciprofloxacin | 5 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 |
| Ampicilin | 10 | 43.9 | 57.1 | 80 | 20 | 62.5 | 38 | 75 | 25 | 40 | 60 | 44.4 | 56.6 |
| Amoxyclave | 30 | 0 | 100 | 20 | 80 | 0 | 100 | 0 | 100 | 20 | 80 | 0 | 100 |
| Doxycycline | 30 | 57.1 | 53.9 | 100 | 0 | 87.5 | 13.5 | 87.5 | 13.5 | 80 | 20 | 77.8 | 23.2 |
| Imipenem | 10 | 78.6 | 22.4 | 100 | 0 | 87.5 | 13.5 | 75 | 25 | 60 | 40 | 66.7 | 34.3 |
| Streptomycin | 300 | 85.7 | 15.3 | 100 | 0 | 100 | 0 | 75 | 25 | 80 | 20 | 100 | 0 |
| Chloramphenicol | 30 | 57.1 | 43.9 | 80 | 20 | 5 | 95 | 62.5 | 8.5 | 40 | 60 | 33.3 | 67.7 |

Key: AMP = Ampicillin, AMC = Amoxiclave, DOXY = Doxycycline, IMP = Imipenem, CPR = Ciprofloxacin, STREP = Streptomycin, CHL = Chloramphenicol, *E.coli= Escherichia Coli, K.pneumoniae= Klebsiella pneumonia, K.oxy= Klebsiella Oxytoca, C.fraundii= Citrobacter Fraundii, S.enterica= Salmonella Enterica, V.cholarae= Vibrio cholarae, V.* species= *Vibrio Parahaemolyticus,.aeruginosa = Pseudomonas aeruginosa,*R= resistance, S= sensitive

## DISCUSSION

In the current study, River Zaki, River Jama'are and River Zigau serves as irrigation sources. All the irrigation sites used water from the rivers for irrigation without any treatment, which may affect the quality of the crops they produced in terms of chemical contamination and microbiological contaminants. Similarly, the various human activities being carried out along the river sides such as washing and bathing may increase the water's pollution level as people urinate or defecate in water while swimming. Similarly, [5] reported that many people used Jakara River in Kano, Nigeria, for different activities including irrigation of vegetables served in Jakara Area households without any treatment.

The overall mean aerobic mesophilic count and the Most Probable Number values for coliforms observed in this study ranged from $1.6x10^4$ to $8.0x10^8$cfu/mL and 17 to 33, relatively higher than previous reports from Kano, [10] but other studies reported a lower count that ranged from 2 to 6 logCFUg−1 [11]. The overall mean count of Enterobacteriaceae in the present study ranged from $1.6 x10^4$ to $8.0x10^8$ CFU/mL. This is higher than other studies conducted on lettuce and green pepper 4.08 and 5.84 log CFU g−1, respectively, by [5] and [3]. in Ethiopia and Morocco, respectively. The total viable bacterial count and the Most Probable Number values for coliform, which in most cases were greater than the standard acceptable range of 2 to 4 per 100 mL set by [5], it was observed that the inhabitants had available to them for all purposes, water from borehole and wells that were seldom closed and/or protected. These were found to be bacteriologically unfit for consumption. The isolates recovered from the irrigation water, vegetable and public water samples in this study are, in order of prevalence, *K pneumoniae* (27.9%), *P aeruginosa* (26.2%), and *E coli* (8.2%). Others include *Salmonella enteritidis, K oxytoca, V. cholera, V. parahaemolyticus* and *Citrobacter freundii*. The preponderance species were *E. coli, K. Oxytoca* and *P aeruginosa* in all the samples analysed is of great importance epidemiologically because the organisms have been known to cause nosocomial and opportunistic infections in debilitated individuals.

Pathogenic bacteria *Salmonella* spp., *K. oxytoca* and *Vibrio* spp. isolated from the water used for irrigation and harvested vegetable samples have been implicated in various human health conditions, including salmonellosis, vibriosis, nosocomial, and opportunistic infections [12]. The probability is high that the bacteria from the contaminated water used for irrigation and postharvest washing of vegetables in the study site may survive on the vegetables in the field and when the produce is delivered to retail outlets or other local markets. In a study by [13], bacterial pathogens isolated from the vegetables irrigated with water from polluted stream included *E. coli*, *Salmonella enteritidis, K.*

*pneumoniae*, among others; all these Organisms are of public health significance, and their presence in any produce is an indication that contamination has occurred, thereby making it unsafe for human consumption in the raw, unprocessed form. Although the frequency of *K. pneumoniae* was low in this study, its presence is important because it has been implicated in food poisoning and food-borne infections. [14] reported high frequencies of *Salmonella* species. and *K. pneumoniae* on vegetables irrigated with contaminated river water and stream water. It is well documented that fecal contamination of irrigation water can cause numerous disease outbreaks [15]. In this study, *E. coli* was enumerated from each drinking water sample, indicating that the water was contaminated with feces. *Salmonella* spp., *Citroacter freundii*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa* bacteria, provides information on organisms that have been suggested as possible causes of waterborne diseases [15].

The antibiotic susceptibility testing to all 62 isolates revealed that 58% of the isolates were susceptible isolates. It is interesting to note that a high number of the isolates were susceptible to ciprofloxacin as well as streptomycin and doxycycline. None of the isolates were resistant to ciprofloxacin, while only 4.9% were resistant to streptomycin. It may be attributed to the high cost of ciprofloxacin. Ciprofloxacin is a relatively expensive drug when compared with most antibiotics frequently used in Nigeria, including the β-lactams and tetracycline; this is partly similar to a previous report by [16]. However, almost all the isolates showed a high resistance to amoxiclave, which was seen to be resisted by virtually all the organisms at a greater frequency. Throughout the study, only 42.9% *E. coli* were observed to show high sensitivity to ampicillin compared to all other genera studied. A similar study [17] reported that amoxicillin, clavulanate, chloramphenicol, streptomycin, and clindamycin appear less active than the doxycycline (30 ug/mL).

## CONCLUSIONS

Surface waters, such as rivers and groundwater, are common sources of agricultural irrigation water in northern Bauchi state Nigeria. However, most of these water resources are affected by water-based anthropogenic activities that result in various degrees of contamination or pollution with pathogenic micro-organisms, chemical contaminants, and other hazardous substances and, as such, have the potential to increase dissemination of micro-organisms onto growing vegetables and crops that are of public health importance. The hygienic quality of both water and vegetable samples was poor since higher mean bacterial counts were recorded beyond the standard safe limits. Amoxiclave, ampicillin, and chloramphenicol were the most resisted antimicrobial agents by the isolated organisms. However, ciprofloxacin, doxycycline, and streptomycin are the drugs of choice recommended for treating all isolated organisms. Based on the findings of this research and to prevent public health menace, the following recommendations should be observed: The use of untreated human and animal excrement as fertilizers should be discouraged, and if must be used, it should be properly fermented. A law restricting the use of untreated water for irrigation should be imposed to reduce the risk of disseminating agents of waterborne diseases through consumption of raw vegetables. Consumers should thoroughly wash all fruits and vegetables in a saline solution and rinse them, especially where the risk of contamination is high, before use. Government should increase its efforts to supply potable water to the public in rural and urban communities. Public health officials should devise more measures to halt rampant supplies of unhygienic irrigated vegetables from reaching public domains. The public should observe personal hygiene and environmental sanitation, and above all, they should adopt the ‘’maintenance culture’’, a quality which was significantly observed to be poor at the time of this study.

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