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Molecular Characterization of Pathogenic Bacteria in Irrigation Water from Zamfarawa River Jega, Kebbi State, Nigeria

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Abstract

This study aimed to evaluate the bacterial load and pathogenic bacterial contamination of irrigation water used in Zamfarawa Farms, Jega Town. A total of nine irrigation water samples were collected from three different farms. Bacterial enumeration was conducted using the standard plate count method, while coliform levels were estimated using the Most Probable Number (MPN) technique. Biochemical tests were performed for bacterial identification, and conventional polymerase chain reaction (PCR) followed by agarose gel electrophoresis was used to confirm selected bacterial isolates. The overall mean bacterial count in the irrigation water samples was 5.7×10^8 CFU/mL. Farm A recorded the lowest bacterial load, followed by Farm B, while Farm C showed the highest bacterial load. The occurrence data indicated that *Salmonella typhi* was the most frequently isolated bacterium from Zamfarawa River irrigation water, appearing in 78% of the samples, followed by *Bacillus cereus* at 22%. Coliform contamination was also detected, with MPN indices of 17 (95%

CI: 5–46) in Farms A and C and 14 (95% CI: 4–34) in Farm B, exceeding the reported international standard of 1 $\log_{10}$ CFU/mL. PCR results confirmed the presence of bacterial DNA fragments corresponding to *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella typhi*, and *Bacillus subtilis*. These findings indicate that irrigation water in the study area was contaminated with pathogenic bacteria, likely associated with untreated wastewater, sewage-contaminated water supplies, untreated organic manure, and poor hygiene practices during farming activities. The study concludes that the use of contaminated irrigation water may represent an important route for the transmission of pathogenic microorganisms to agricultural produce and consumers. This study contributes to agricultural microbiology and public health by highlighting the need for routine microbial monitoring, improved wastewater management, and safer irrigation practices in vegetable production systems.

Keywords: Irrigation Water; Pathogenic Bacteria; Coliforms; Microbial Contamination; Public Health Risk

INTRODUCTION

According to the World Health Organization (World Health Organization, 2018), 2.6 billion people are without access to clean water, and water-related diseases result in 3.4 million deaths annually, primarily among children. The United Nations Children's Fund (UNICEF) reports that contaminated water is responsible for 70,000 deaths of children under five in Nigeria each year (United Nations Children's Fund [UNICEF], 2014). Improving water quality could potentially reduce the global disease burden by around 4% (Allende et al., 2006). The microbiological quality of surface waters is influenced by factors such as sewage discharges, runoff from informal settlements, and contamination by human and animal fecal waste (WHO, 2020). Water is considered contaminated when it contains infectious and parasitic agents. Faecal coliforms (FC) are commonly used as bacterial indicators of fecal pollution and are therefore employed to assess the microbiological quality of drinking water (Arora et al., 2016). According to WHO standards, drinking water should be free from any pathogenic microorganisms or bacteria indicative of fecal contamination (World Health Organization, 2018).

MATERIALS AND METHODS

Study Area

The study was conducted in the Jega local government area of Kebbi State, situated in the extreme northwestern region of Nigeria, between latitudes 10° to 15° N and longitudes 33° to 60° E. The state is bordered by the Republic of Benin to the west, the Republic of Niger to the north, Sokoto State to the east, and Niger State to the south. The local government spans a land area of 891 square kilometers and is bordered to the east by Aliero local government (Uzondu et al., 2015). The warmest months are April and May, with average midday temperatures of 40.3℃. December is the coolest month, with nighttime temperatures averaging 16.2℃. The Zamfarawa River flows through Jega town, where farms are located. The residents of Jega utilize this river for a variety of activities, including swimming, bathing, washing vehicles, fishing, farming, and animal husbandry, which are the primary occupations in the area (Uzondu et al., 2015).

Sampling Design

The sampling method used was systematic sample collection. Three samples were taken from farms within the study area, designated as farm A, farm B, and farm C. In each farm, three samples of irrigation water were collected and transported to the Microbiology laboratory at Kebbi State University of Science and Technology, Aliero, for analysis.

Irrigation Water Sample Collection

Irrigation water samples were gathered from the Zamfarawa River in Jega. Three different sections of the river were used for sampling: upstream, midstream, and downstream. A total of nine samples three from each farm were collected and labeled as A, B, and C, respectively. These samples were placed in sterile amber plastic containers and transported to the Microbiology Laboratory at Kebbi State University of Science and Technology, Aliero, for analysis (Cheesbrough, 2006).

Irrigation Water Sample Preparation

Nutrient Agar medium was used for the isolation and enumeration of bacteria via the Standard Plate Count method. The medium was prepared and utilized according to the manufacturer's instructions. A 1.0 ml volume of serially diluted sample from three dilution factors (10^-5, 10^-6, and 10^-7) was aseptically poured onto the medium and incubated at

37ºC for 24 to 48 hours. The resulting colonies were counted and recorded as colony-forming units per milliliter of water sample.

Characterization of Bacterial Isolates

IndoleTest

Bacterial cultures were inoculated into 5 ml of peptone water and incubated at 37°C for 24 hours. After incubation, 3-8 drops of Kovac's reagent were added and gently shaken. The appearance of a red or pink color in the reagent layer above the broth within one minute indicated a positive reaction, while a yellow color indicated a negative reaction (Arora, 2016).

Citrate Utilization

This test involved inoculating the test organism into test tubes containing Simon's citrate medium, followed by incubation for 24 to 72 hours. A positive result was shown by the development of a deep blue color after incubation, whereas no color change indicated a negative result (Arora, 2016).

Methyl Red Voges-Proskauer Test

Five milliliters of Mr-Vp broth were inoculated with the test organism and incubated at 35°C for 48 to 72 hours. One milliliter of the broth was transferred to a small serological tube, and 2-3 drops of methyl red were added. A red color upon adding the indicator signified a positive methyl red test, while a yellow color indicated a negative test. For the Voges-Proskauer (Vp) test, 5 drops of 40% potassium hydroxide (KOH) were added to the remaining broth in the original tube, followed by 15 drops of 5% naphthol in ethanol. The tube was shaken, the cap loosened, and placed in a sloping position. A positive Vp test was indicated by the development of a red color starting from the liquid-air interface within 1 hour, while no color change signified a negative result (Holt et al., 1994).

Motility Test

The test organism was inoculated into nutrient agar medium by making a fine stab with a needle to a depth of 2 cm, stopping just short of the bottom of the tube. The tubes were then incubated at 37°C for 48 hours. After incubation, the line of inoculation would not be sharply defined, and the rest of the medium would appear somewhat cloudy if the organism was motile. If the organism was non-motile, growth would be restricted to the line of inoculation, which would remain sharply defined (Arora, 2016).

Urease Test

The test organisms were inoculated onto urea agar slopes and incubated at 37°C for 48 hours. A positive reaction was indicated by the development of a bright pink or red color (Holt et al., 1994).

Triple-Sugar Iron Agar Test

A sterile needle was used to obtain the test organism, which was then streaked across the surface of the slant and stabbed twice. The cap was loosely closed, and the tube was incubated at 35°C for 24 hours (Holt et al., 1994).

Identification of Coliforms from Irrigation Water Samples

Coliform counts were determined using the Most Probable Number (MPN) technique. Samples were incubated in Lactose broth tubes at 37°C for 48 hours. Double and single strength MacConkey broth (purple color) was sterilized in bottles containing inverted Durham tubes to detect gas production. The bottles were arranged in three sets containing MacConkey broth and inoculated with 10 ml, 1 ml, and 0.1 ml of water, then incubated at 37°C following standard methods (APHA). After 48 hours at 37°C, positive tubes producing acid and gas were used to estimate the count (MPN) and perform a presumptive test (Egberongbe et al., 2012). The confirmed test for fecal coliforms involved transferring a loopful of broth from a positive tube to E. coli broth, followed by incubation at 44.5°C for 24 hours, with the tubes observed for gas formation. The completed test for fecal coliforms was conducted by plating a loopful of broth from a positive EC tube onto an Eosin Methylene Blue (EMB) agar plate. The plates were incubated at 44.5°C for 48 hours and observed for a dark red color with a metallic green sheen, indicating coliforms. Pure cultures of total and fecal coliform colonies were prepared on nutrient agar slants and stored for further analysis.

Identification of Bacterial Isolates

The various pure isolates from the irrigation water samples underwent microscopic observation, gram staining, and standard biochemical tests, following the procedure outlined by Benson (Benson, 2002). The results were compared with known taxa using the scheme of Cheesbrough (2006) and Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Based on the Gram reaction, Gram-positive organisms were streaked onto Mannitol Salt Agar plates and incubated at 37°C for 24 hours. The presence of yellowish

pigments in Mannitol Salt Agar indicated Staphylococcus aureus (Scott et al., 2013). Tubes showing color change and gas production were shaken and streaked onto Levine's Eosin Methylene Blue (EMB) Agar and incubated at 37ºC for 24 hours. Small nucleated colonies with a greenish metallic sheen indicated E. coli (Egberongbe et al., 2012). The presence of black and pink colonies on Salmonella-Shigella agar suggested Salmonella and Shigella species, respectively (Odu & Adeniji, 2013). The percentage of occurrence distribution was calculated by determining the percentage of bacteria present in each farm and sample collected.

RESULTS

Table 1: Result of Total Plate Count for Irrigation water and Soil from Farm A, B, C

Sample	Dilution Factor	FA Colony Count /cfu/g	FBColony count /cfu/g	FC Colony Mean±SD Count /cfu/g	
Irrigation water	10^{-5}	7	7	7	
	10^{-6}	7	5	5	5.7 x 10^{8} CFU/ml.
	10^{-7}	2	3	2	

KEY
FA = Farm A
FB = Farm B
FC = Farm C

Table 2: Result of Most Probable Number Technique from Farm One

Volume of Water(ml)	Number of Tubes	Volume of Media	Positives A B C	MPN Index A and B	C
10	3	10ml SS	3 3 3		
1	3	10ml DS	2 2 2	17 (95% Cl: 5 - 46)	14 (95% Cl: 4 - 34)
0.1	3	10ml DS	1 0 1		

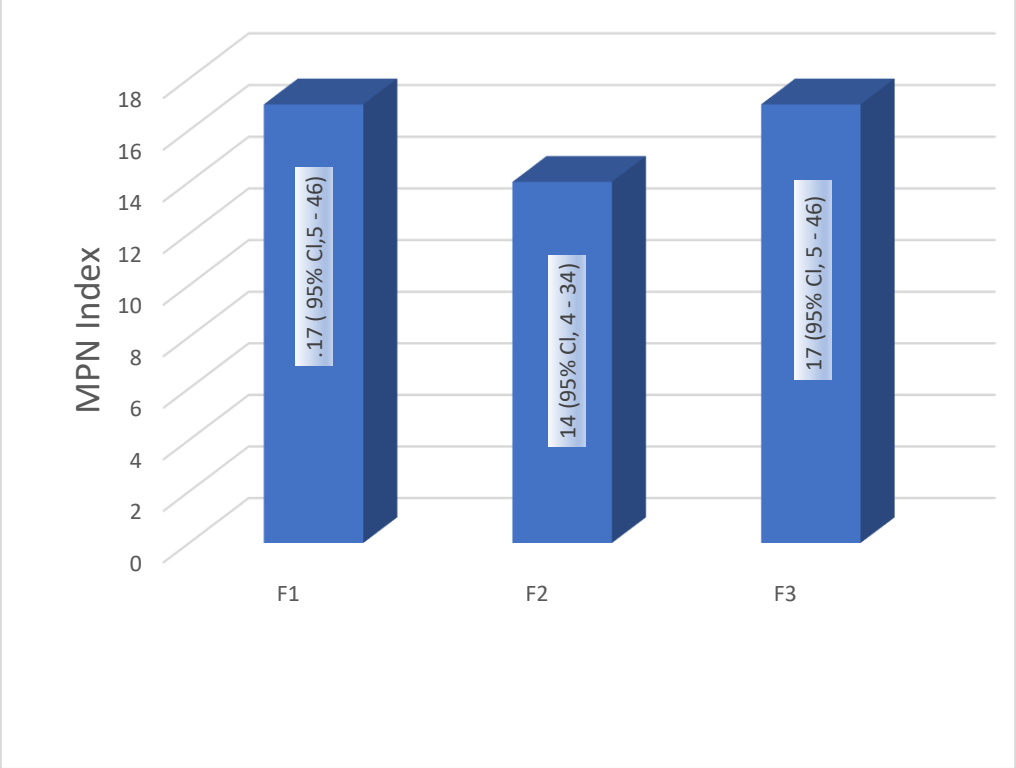

Figure 1: MPN Index Obtained from Combinations of Positive Tubes in a 5 Tube Dilution Inoculum Quantities of 10, 1, 0.1 for Water samples from three Zamfarawa Farms, Jega

KEY
F1=Farm A
F2=Farm B
F3=Farm C

Table 3: Identified Bacteria Isolated from Irrigation Water from Farm A

Isolate Code	Gram/shape	Catalase	Coagulase	Oxidase	Urase	Citrate	Nitrate	Mr	Vp	Indole	H_2S	Gas	Motility	Glucose	Lactose	Sucrose	Mannitol	TSI	Spore	Stach	Species Identified
F1W2, F1W3	G- rod/ pairs	+	NA	-	-	+	+	+	-	-	+	-	+	+	-	-	-	K/A	-	+	*S. typhi*
F1W4	G- rod/ single	+	-	-	-	-	-	+	-	+	-	+	+	+	+	D	+	A/A	-	-	*E. coli*

Table 4 :Identified Bacteria Isolated from Irrigation Water from Farm B

Isolate Code	Gram/shape	Catalase	Coagulase	Oxidase	Urase	Citrate	Nitrate	Mr	Vp	Indole	H_2S	Gas	Motility	Glucose	Lactose	Sucrose	Mannitol	TSI	Spore	Stach	Species Identified
F2W2, F2W3	G- rod/ pairs	+	NA	-	-	+	+	+	-	-	+	-	+	+	-	-	-	K/A	-	+	*S. typhi*
F2W1	G+ rod	+	NA	+	-	+	D	-	+	-	-	-	+	+	-	D	-	K/A	+	+	*Bacillus cereus*
F2W4	G- rod/ single	+	-	-	-	-	-	+	-	+	-	+	+	+	+	D	+	A/A	-	-	*E. coli*

Table 5: Identified Bacteria Isolated from Irrigation Water, Soil, and Vegetable from Farm Three

Isolate Code	Gram/shape	Catalase	Coagulase	Oxidase	Urase	Citrate	Nitrate	Mr	Vp	Indole	H_2S	Gas	Motility	Glucose	Lactose	Sucrose	Mannitol	TSI	Spore	Stach	Species Identified
F3W1, F3W2, F3W3	G- rod/ pairs	+	NA	-	-	+	+	+	-	-	+	-	+	+	-	-	-	K/A	-	+	*S. typhi*

Table 6: Occurrences of Bacteria Isolated in Irrigation Water Samples

M/O	F1/W1	F1/W2	F1/W3	F2/W1	F2W2	F2W3	F3/W1	F3W2	F3W3	(%)
S. aures										0%
S. typhi		+	+		+	+	+	+	+	78%
E. coli										0%
Bacillus cereus	+			+						22%
L. monocytogenes										0%
Bacillus subtilis										0%

Fig. 2 shows agarose gel electrophoresis result of identified bacterial isolates after conventional PCR technique. Lane 1 shows fragment of *S. aureus* DNA at around 150bp, lane 2 shows that of *L. momocytogenes* at 200bp, *B. cereus* at 300bp *S. typhi* at around 350bp, *B. subtilis* at 400bp and *salmonella typhi* at 450bp.

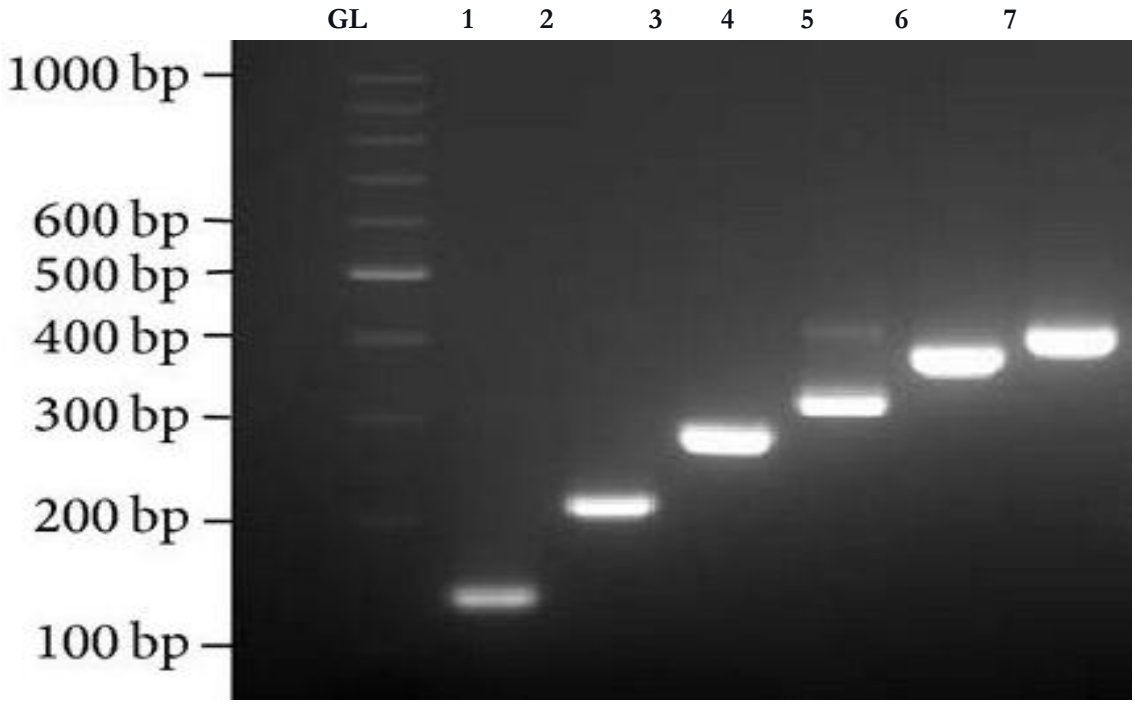

Figure 2: Agarose Gel Electrophoresis Result of Identified Bacterial Isolates After Conventional PCR Technique

Key
***GL*= gene ladder**
1= *S. aureus*
2= *L. monocytogenes*
3= *B. cereus*
4= *S. typhi*
5= *B. subtilis*
6= *E. coli*

DISCUSSION

The findings of this study indicate that the irrigation water used was contaminated with various species of pathogenic bacteria, including coliforms, which signal fecal contamination. The presence of these coliforms in all three selected farms is likely due to surface runoff, organic manure, and waste being washed into the river.

The study highlights that pathogenic bacteria are a significant cause of various foodborne infections within communities. Several bacterial species were identified in the collected samples, including S. aureus, S. typhi, E. coli, B. cereus, B. subtilis, and L. monocytogenes. These findings align with the study conducted by Onajite (Onajite & Ovie, 2022), who carried out a comparative analysis of water and reported a higher number of

coliforms in river and well water, with some well water and borehole water showing little to no coliforms, deeming them safe for drinking.

The primary sources of these bacteria in the water are attributed to both human and animal activities (Scott et al., 2013). Sources of bacterial contamination include surface runoff, animal waste deposition, and pasture. Additionally, human activities such as swimming, waste disposal, domestic activities, and fecal discharge (Egberongbe et al., 2012) are potential contributors to the introduction of foreign microorganisms into the water. These activities increase the availability of nutrients for microorganisms, thus promoting their growth in various water sources.

Farm A and C had a higher number of coliforms, 17 (95% CI: 5 - 46), compared to Farm B, which had 14 (95% CI: 4 - 34). This indicates that the water from these farms is not safe for drinking or irrigation purposes. Among the samples collected, Salmonella typhi had the highest occurrence, being isolated in nearly every irrigation water sample at a rate of 78%. In other studies, similar but lower percentages were reported; for example, in India, Salmonella spp. was found in 5.8% of different water and vegetable salad samples, and in Nigeria, it was reported in 6.7% of vegetables (Liwanag & Soriano, 2018).

Furthermore, a study from Brazil detected L. monocytogenes in only one out of 181 samples (0.6%) (Oliveira et al., 2019). The high occurrence of S. typhi and the presence of E. coli in the irrigation water confirm significant contamination, with the latter specifically indicating fecal contamination in the water samples.

CONCLUSION

This study revealed the presence of various levels of pathogenic bacteria in the collected samples, including *Salmonella typhi*, *Bacillus* spp., and *Listeria monocytogenes*. These findings suggest that irrigation water is a significant source of contamination for vegetables currently being sold, although other factors, such as post-harvest contamination, also contribute to the contamination of vegetables. The coliform levels observed in the irrigation water samples exceed international standards (1 log10 cfu/ml). The use of untreated wastewater and sewage-contaminated water for irrigation is a major source of pathogenic microorganisms, as demonstrated by the presence of different bacterial species in the irrigation water

Recommendations

Based on the results and conclusions of this study, the following recommendations are suggested to help reduce contamination in irrigation water:

i. Alternative irrigation methods should be considered to minimize the use of stream water, which is prone to surface runoff and is a primary source of contamination.

ii. Agricultural practices should be reviewed, and strict regulations should be enforced, particularly regarding the use of safe irrigation water and fertilizers.

iii. Further research on this topic is recommended, including the exploration of other methods for irrigating vegetables in different regions.

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