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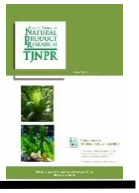

Antibiotic Susceptibility of *Escherichia coli* O157:H7 and *Salmonella* sp. in Water, Sediment and Irrigated Vegetables from Rivers in Ilorin Metropolis, Nigeria

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ABSTRACT

The assessment of the incidence of *Escherichia coli* and *Salmonella* sp. in irrigated fresh fruits and vegetables, and its nexus with the irrigation water is essential to prevent transfer of pathogens to humans. This study aimed at the detection and antibiogram profiling of *E. coli* O157:H7 and *Salmonella* sp. in the three prominent rivers (Asa, Oyun and Afon) in Ilorin metropolis. Ninety samples of water, sediment, and irrigated vegetables were collected over a period of 12 weeks and analyzed for total heterotrophic bacteria, *E. coli* and *Salmonella/Shigella* populations. Antibiotic susceptibility profiling of *E. coli* O157:H7 and *Salmonella* sp. was carried out using the disc diffusion method. The heterotrophic bacteria populations were mostly higher in sediments ($3.90 \times 10^5 \pm 1.15 \times 10^5 - 2.35 \times 10^6 \pm 8.75 \times 10^5$ cfu/g) than in waters ($5.60 \times 10^4 \pm 7.00 \times 10^3 - 2.16 \times 10^5 \pm 2.00 \times 10^4$ cfu/ml) obtained from the same point. The high counts of *E. coli* ($0 - 1.53 \times 10^3 \pm 5.65 \times 10^2$ cfu/g) and *Salmonella/Shigella* sp. ($0 - 1.59 \times 10^3 \pm 6.95 \times 10^2$ cfu/g) on the irrigated vegetables may be due to the observed direct contamination from polluted water. All *E. coli* O157:H7 isolates showed extensive drug-resistance while *Salmonella* sp. exhibited a combination of extensive and pan-drug resistance to standard antibiotics belonging to penicillins, aminoglycosides, macrolides, and fluoroquinolones. The occurrence of extensively drug-resistant strains of these pathogens in the environment portend a great risk to public health and can increase the chances of an outbreak of fatal infections among the human population.

Keywords: *Escherichia coli*, *Salmonella* sp., Irrigation, Antibiotic susceptibility, Drug-resistance

Introduction

Sub-Sahara Africa has very few population of people with access to drinking water through the household connection.[1] The issue of access to clean water and sanitation in rural Africa is a major challenge. Young children and some adults die from diarrheal illnesses that could be prevented by clean water and good hygiene.[2]

Uncontaminated freshwater and safe drinking water are imperative for human development and public health. Water sources (especially drinking water) free from enteric pathogens are crucial since most primary diseases in developing countries are related to water and sanitation.[3] World population explosion has led to the deficiency in water supply and an influx of human waste that has outpaced the development of wastewater management systems. Consequently, there are pollution of natural water bodies, unintentional use of wastewater in irrigated agriculture, irregular water supply, and environmental concerns for aquatic life due to the high concentration of pollutants flowing into water bodies.[4]

Irrigation is a very important input in farming, especially in areas with water deficiency. In Nigeria, many farmers are involved in irrigated vegetable cultivation because of water deficiency and they usually grow highly valued and easily perishable exotic vegetables.[5] These include lettuce, carrot, cabbage, spring onions, green pepper and green beans.

Enteric pathogens can be transmitted to humans through consumption of this irrigated produce, especially crops eaten raw.[6] Several studies have demonstrated very close relationship between the consumption of fruits and vegetables irrigated with raw wastewater and many food-borne diseases like gastroenteritis and cholera.[7]

Most *E. coli* strains are harmless, but *E. coli* O157:H7 strain produces a powerful toxin that causes severe illnesses. The *E. coli* O157:H7, a Gram-negative rod-shaped bacteria is a known leading cause of food borne illnesses such as acute diarrhea, hemorrhagic colitis and hemolytic uremic syndrome.[8,9] *Salmonella* species are widely dispersed in nature and often found in the intestinal tract of animals and humans; pathogenic *Salmonella* species are leading causes of food-borne bacterial illnesses in humans.[10] These pathogens primarily disseminate through the feces of wildlife, domestic animals and humans, contaminated water, and contaminated irrigation water used for agricultural practices.[8,9] There are several reports of outbreaks of Salmonellosis traced to consumption of raw fruits and vegetables, generally contaminated from manure on the outer surface of the fruit or vegetable.[11] Studies on environmental sources of *Salmonella* contamination implicated water as an important source, particularly irrigation water containing manure, wildlife feces or sewage effluents.[10,12] Therefore, sources of fecal pollution in waters devoted to human activity must be strictly controlled. Antibiotic resistance is a growing problem due to its overuse by humans especially without appropriate prescription in Nigeria as well as, use as growth promoters in food animals.[13,14] Resistance to beta–lactam antibiotics has become more serious in recent decades as strains producing extended–spectrum beta–lactamases render many, if not all, of the penicillins

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and cephalosporins ineffective in therapy.[14,15] Hence, this study aimed at assessing the antibiotic susceptibility of the pathogens *E. coli* O157:H7 and *Salmonella* sp. in fresh fruits and vegetables in irrigated farmlands along the major river courses in Ilorin metropolis. The need to assess the microbial quality of irrigation water sources and irrigated vegetables and its public health implications are highlighted.

Materials and Methods

Sampling sites

Three major rivers Asa, Oyun and Afon were selected for this investigation as shown in Fig. 1. The rivers do not originate from Ilorin metropolis, but they flow through parts of the city, as major freshwater resources to local residents, with both ecological and economic values. Water from Asa is dammed, treated by the Municipal Water Agency and supplied to the metropolis, some adjoining towns and communities with about a million inhabitants. A portion of Oyun River is dammed by the University of Ilorin and supplies treated water to over 50,000 people within the University and the surrounding communities. The bank of Afon River and its catchment landscape on the outskirt of the city is a major agricultural land used for all-year-round irrigation farming which supplies the city with varieties of fresh vegetables, fruits and crops.

Samples collection

Water, sediments and vegetable plant parts samples were collected from the rivers and adjacent farmlands. Water samples were collected into sterile 250-ml glass bottles, sediment and plant parts in clean sealable plastic bags. Sampling followed standard microbiological procedures for collection of water and sediment.[16] Sampling of water and sediment samples along the river banks were based on evidence of human and animal activities such as defecation and grazing. Plant parts were collected from the farms along river banks either directly by the researcher, where permission was given by the farmer, or supplied by the farmer to the researcher in clean sealable plastic bags. All plant parts were collected not more than 2–3 h after irrigation process. Two plants were chosen for this study, green leafy vegetable (*Telfairia occidentalis*) and red pepper (*Capsicum frutescens*), based on their availability at all sites and the willingness of farmers to allow access to the plots where they were cultivated.

From each of the rivers, six (6) samples (water, sediment and plant parts) were taken on every sampling day for five (5) visits at 2 weeks interval over a three-month period between April and June 2016. This gave a total of 30 samples from each river site. An aggregate total of 90 samples each of water, sediment and plant parts were analyzed from the three rivers in this study. The designated sample codes were: (1) RBW: water sample close to the river bank where irrigation water is drawn by the farmers; (2) RBS: sediment from the same portion where water samples were collected (3) IVP: irrigated leafy vegetable plant; (4) VPS: sediment around the plot where the vegetable was sampled; (5) IPP: irrigated pepper plant; and (6) PPS: sediment around the plot where the pepper was sampled. All samples were transported to the laboratory in an ice chest and processed immediately or within 2 h of collection in the laboratory.

Determination of total heterotrophic bacteria, Escherichia coli and Salmonella-Shigella population density

All samples of water, sediment and plant parts were analyzed for: (i) total heterotrophic bacterial count; (ii) *Escherichia coli* count; and (iii) *Salmonella/Shigella* density. A weighed amount [1 g of sediment or plant part (wet weight)] was placed in sterile peptone water (10 ml) and vortexed intermittently for 30 seconds to release the cell particles into suspension. In case of water samples, 1 ml was first introduced into 9 ml sterile peptone water and vortexed for 10 seconds. Using the standard pour plate method, 1 ml aliquot (after processing to appropriate serial dilutions) was introduced on specific culture agar plates. Duplicate plates were made for each sample. For heterotrophic bacteria and *Salmonella/Shigella* counts, nutrient agar (NA) and Salmonella-Shigella agar (SSA) were used for cultivation at 37 ºC respectively. MacConkey agar (MCA) was used to cultivate *E. coli* and incubation was at 45 ºC. The culture plates were enumerated after 18–24 h incubation period and the mean of the values presented.

Isolation of Escherichia coli O157:H7 and Salmonella sp.

To obtain isolates of *E. coli* O157:H7 and *Salmonella* sp. used in the antibiotic susceptibility test, distinct colonies on the MCA and SSA plates were screened by repeated sub-culturing at 45ºC and 37ºC respectively. Distinct *E. coli* colonies were streaked on Cefixime-Tellurite Sorbitol MacConkey (CT-SMAC) agar to differentiate and select for *E. coli* O157:H7 as sorbitol-non-fermenting whitish colonies. For *Salmonella* sp. distinct colonies with blackish pigmentation were picked and further sub-cultured on SSA for purity.

A number of tests, including morphological and biochemical were later used to further screen the isolates of *Salmonella* sp. The isolates were purified by further rounds of sub-culturing before stock cultures were made and stored in the refrigerator. A large number of isolates were obtained; however, 18 isolates each of *E. coli* O157 and *Salmonella* sp. were selected on the basis of site of sample collection.

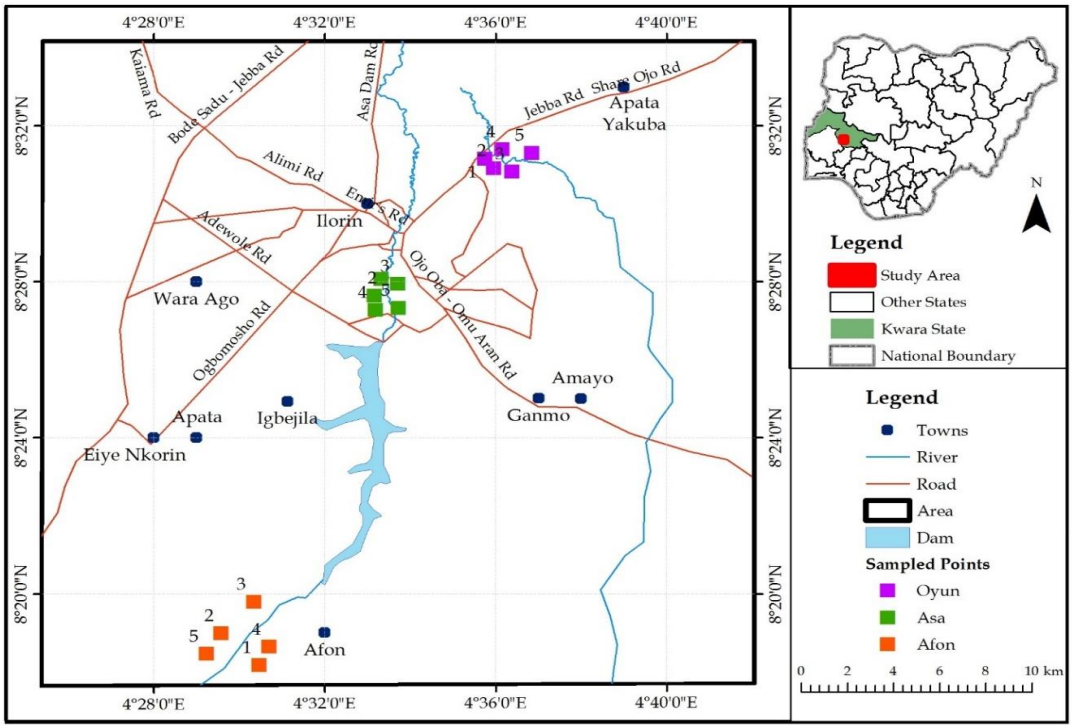

Figure 1: Study area showing sampling sites.

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Antibiotic susceptibility of isolates recovered from samples

A total of 36 probable isolates, comprising 18 *E. coli* O157:H7 and 18 *Salmonella* sp. were screened for antimicrobial susceptibility, using the agar disk diffusion method.[17] The following antibiotics (Oxoid Ltd., Basingstoke, UK) were used: ceftazidime (30 µg), gentamycin (10 µg), nitrofurantoin (30 µg), augmentin (30 µg), amoxicillin (30 µg), ciprofloxacin (30 µg), ofloxacin (30 µg), and cefuroxime (30 µg). Briefly, the cell cultures were grown for 24 h in nutrient broth and then harvested by centrifugation (3500 rpm for 20 mins) repeated until cell pellets were obtained. The inoculum size of the cell pellets in suspension was standardized to approximately 1.2×10^8 cfu/ml using the 0.5 McFarland's standard. The cultures (1 ml) were uniformly streaked on Muller-Hinton agar (Oxoid Ltd., Basingstoke, UK) plates and pre-incubated for at least 6 h before the antibiotic-impregnated discs were placed onto the inoculated plates using sterile forceps. The plates were then incubated at 37 ºC for a further 24 h, after which clear zones of inhibition around each antibiotic disk were measured. The results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria.[18]

Results and Discussion

Total bacterial population in the samples

This study assessed the total heterotrophic bacteria, *E. coli* and *Salmonella* sp. counts in water, sediments and vegetable plant samples collected from three rivers adjacent to irrigated farm lands in Ilorin metropolis. Figures 2, 3 and 4 present the results obtained from five rounds of sampling in the three different rivers. Generally, in Asa River, heterotrophic bacteria population was higher in RBS ($5.20 \times 10^5 \pm 4.50 \times 10^4 - 2.00 \times 10^6 \pm 4.10 \times 10^5$ cfu/g) than in RBW ($5.60 \times 10^4 \pm 7.00 \times 10^3 - 1.92 \times 10^5 \pm 4.32 \times 10^4$ cfu/ml). Also, heterotrophic bacteria population was higher in IVP ($4.20 \times 10^3 \pm 2.10 \times 10^2 - 1.44 \times 10^4 \pm 3.40 \times 10^3$ cfu/g) than in IPP ($1.40 \times 10^3 \pm 2.50 \times 10^2 - 4.80 \times 10^3 \pm 5.50 \times 10^2$ cfu/g). The bacterial loads in VPS ($3.00 \times 10^5 \pm 1.20 \times 10^4 - 1.12 \times 10^6 \pm 6.30 \times 10^3$ cfu/g) and PPS ($3.60 \times 10^5 \pm 5.34 \times 10^4 - 8.30 \times 10^5 \pm 9.00 \times 10^4$ cfu/g) were higher than those in RBW used to irrigate the plots (Figure 2). For Oyun River, similar trend was observed with the heterotrophic bacteria population in RBS ($5.90 \times 10^5 \pm 1.75 \times 10^5 - 2.35 \times 10^6 \pm 8.75 \times 10^5$ cfu/g) being higher than in RBW ($9.80 \times 10^4 \pm 3.40 \times 10^4 - 2.16 \times 10^5 \pm 2.00 \times 10^4$ cfu/ml). Populations of bacteria in IVP ($4.60 \times 10^3 \pm 1.30 \times 10^3 - 1.54 \times 10^4 \pm 5.70 \times 10^3$ cfu/g) and IPP ($5.20 \times 10^3 \pm 1.60 \times 10^3 - 2.16 \times 10^4 \pm 8.80 \times 10^3$ cfu/g) were similar (Figure 2).

Likewise, in Afon River, heterotrophic bacteria population was higher in RBS ($3.90 \times 10^5 \pm 1.15 \times 10^5 - 2.03 \times 10^6 \pm 6.65 \times 10^5$ cfu/g) than in RBW ($5.70 \times 10^4 \pm 1.25 \times 10^4 - 9.50 \times 10^4 \pm 2.35 \times 10^3$ cfu/ml). Bacteria populations in IVP ($2.40 \times 10^3 \pm 1.10 \times 10^3 - 5.80 \times 10^3 \pm 2.00 \times 10^3$ cfu/g) were not different from that in IPP ($2.60 \times 10^3 \pm 1.00 \times 10^3 - 5.70 \times 10^3 \pm 1.85 \times 10^3$ cfu/g) collected from farms close to Afon River (Figure 2). The similarity in the heterotrophic bacterial population of the vegetable plots sediments could be as a result of the deposition of contaminated irrigated surface water and sludge from the same sources to the plots.[19,20] Generally, the high level of bacterial populations observed in the sediments were similar to previous reports which showed sediments as major reservoirs for microorganisms.[20,21,22,23] The survival of microorganisms in sediments has been attributed to availability of nutrient, organic matter content, temperature, radiation, pH and competition with other flora.[24]

Escherichia coli count in the samples

At Asa River, *E. coli* number was higher in RBS ($6.00 \times 10^3 \pm 1.70 \times 10^3 - 1.95 \times 10^5 \pm 9.75 \times 10^3$ cfu/g) than in RBW ($3.00 \times 10^2 \pm 8.00 \times 10^1 - 1.81 \times 10^4 \pm 5.20 \times 10^3$ cfu/ml). *E. coli* populations in IVP ($0 - 3.40 \times 10^2 \pm 2.70 \times 10^2$ cfu/g) and IPP ($0 - 8.80 \times 10^2 \pm 2.40 \times 10^2$ cfu/g) were similar. However, *E. coli* load in PPS ($0 - 1.26 \times 10^5 \pm 9.30 \times 10^3$ cfu/g) was often higher than in VPS ($0 - 3.00 \times 10^4 \pm 8.30 \times 10^3$ cfu/g) (Figure 3). In Oyun River, *E. coli* population in RBW ($1.00 \times 10^3 \pm 3.00 \times 10^2 - 7.40 \times 10^3 \pm 3.70 \times 10^2$ cfu/ml) was lower than in RBS ($1.20 \times 10^4 \pm 4.60 \times 10^3 - 1.23 \times 10^5 \pm 1.55 \times 10^4$ cfu/g). *E. coli* load on IVP ($0 - 1.80 \times 10^2 \pm 4.00 \times 10^1$ cfu/g) was often lower than on IPP ($0 - 1.53 \times 10^3 \pm 5.65 \times 10^2$ cfu/g) collected from farms around Oyun River (Figure 3). A similar trend was also observed in Afon River, where *E. coli* number was higher in RBS ($3.00 \times 10^3 \pm 9.20 \times 10^2 - 5.30 \times 10^4 \pm 6.65 \times 10^3$ cfu/g) than in RBW ($1.10 \times 10^3 \pm 3.50 \times 10^2 - 4.90 \times 10^3 \pm 8.45 \times 10^2$ cfu/ml). *E. coli* population in IVP ($0 - 1.33 \times 10^3 \pm 4.65 \times 10^2$ cfu/g) was not different from that on IPP ($0 - 3.30 \times 10^2 \pm 1.25 \times 10^2$ cfu/g) (Figure 3).

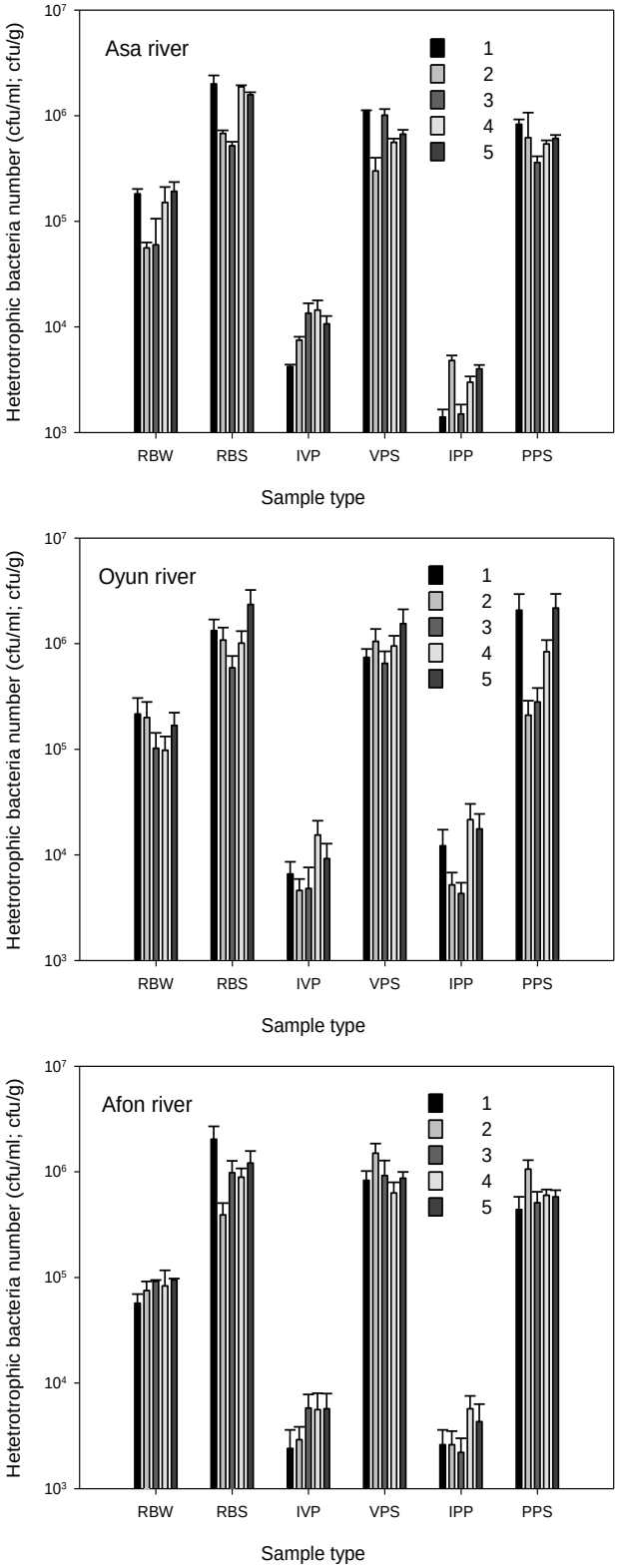

Figure 2: Population of total heterotrophic bacteria in the three rivers. RBW: water samples, RBS: sediment from water sampled area, IVP: irrigated leafy vegetable plant VPS: sediment where vegetable was sampled, IPP: irrigated pepper plant, PPS: sediment where pepper was sampled.

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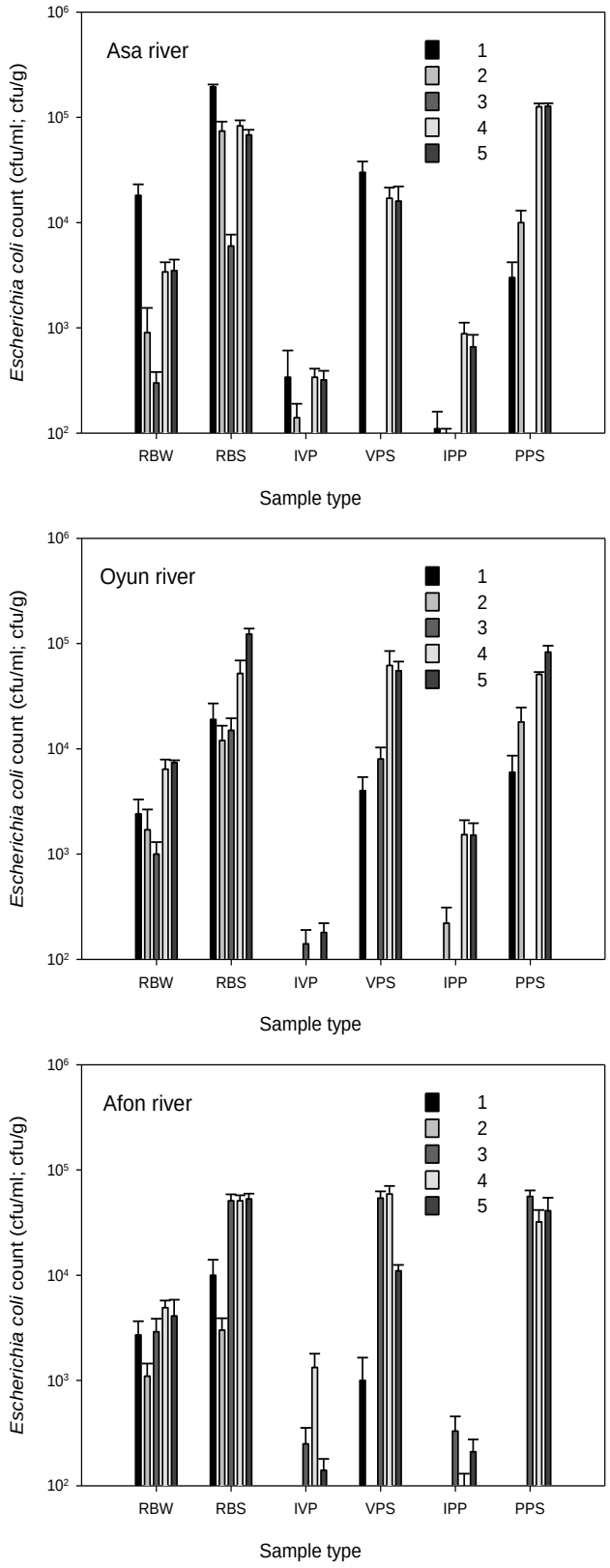

Figure 3: Population of *E. coli* in the three rivers. RBW: water samples, RBS: sediment from water sampled area, IVP: irrigated leafy vegetable plant VPS: sediment where vegetable was sampled, IPP: irrigated pepper plant, PPS: sediment where pepper was sampled.

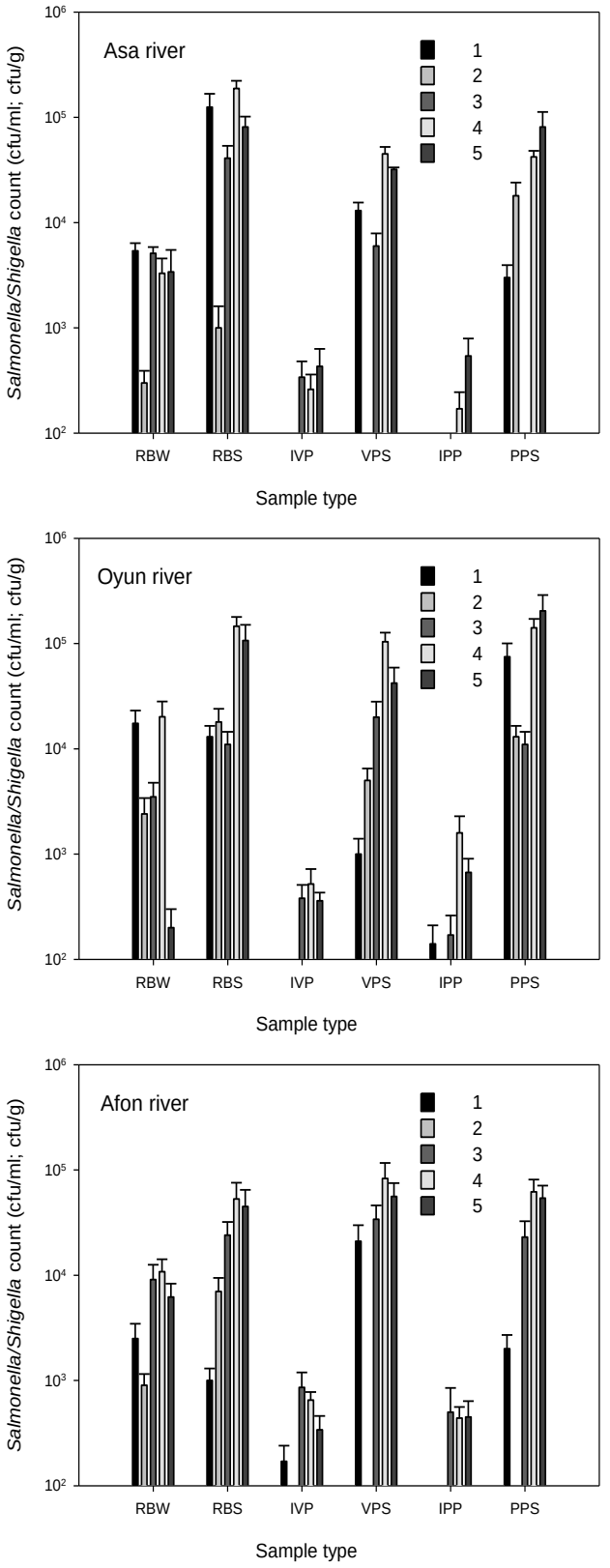

Figure 4: Population of *Salmonella/Shigella* in the three rivers. RBW: water samples, RBS: sediment from water sampled area, IVP: irrigated leafy vegetable plant VPS: sediment where vegetable was sampled, IPP: irrigated pepper plant, PPS: sediment where pepper was sampled.

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Table 1: Antibiotic susceptibility patterns of *E. coli* O157:H7 isolates from the three rivers.

Tag	Sample sources	Zone of inhibition (mm)							
	Asa River	AMP	CAZ	GEN	NIT	AUG	CPR	CRX	OFL
RBW	River bank water	0	0	10.0 ± 0.8	0	0	0	0	18.2 ± 2.1
RBS	River bank sediment	0	0	23.3 ± 4.2	0	0	0	0	20.3 ± 1.2
IVP	Irrigated vegetable plant	0	0	10.0 ± 4.5	0	0	0	0	15.7 ± 1.6
VPS	Vegetable plot sediment	0	0	12.7 ± 2.5	0	9.3 ± 1.7	0	25.4 ± 4.8	21.3 ± 2.2
IPP	Irrigated pepper plant	0	0	0	0	0	0	0	13.3 ± 1.7
PPS	Pepper plot sediment	0	0	6.0 ± 0.8	0	9.0 ± 0.8	0	0	19.7 ± 2.1
	Oyun River								
RBW	River bank water	0	0	13.0 ± 0.9	0	0	0	0	21.7 ± 1.6
RBS	River bank sediment	0	0	13.0 ± 0.9	0	0	0	0	14.3 ± 0.8
IVP	Irrigated vegetable plant	0	0	12.7 ± 0.9	0	0	0	0	19.7 ± 0.9
VPS	Vegetable plot sediment	0	0	3.7 ± 1.2	0	0	0	0	16.7 ± 1.5
IPP	Irrigated pepper plant	0	0	10.7 ± 0.9	0	0	0	21.0 ± 1.2	20.3 ± 0.8
PPS	Pepper plot sediment	0	0	7.7 ± 0.9	0	0	0	0	19.7 ± 0.8
	Afon River								
RBW	River bank water	0	0	18.0 ± 0.8	0	0	21.6 ± 2.1	0	16.0 ± 0.7
RBS	River bank sediment	0	0	15.7 ± 0.8	0	0	11.0 ± 0.7	0	14.7 ± 0.7
IVP	Irrigated vegetable plant	0	0	9.0 ± 0.7	0	0	0	0	20.6 ± 0.7
VPS	Vegetable plot sediment	0	0	0	0	0	17.3 ± 0.7	0	19.7 ± 0.7
IPP	Irrigated pepper plant	0	0	0	0	0	10.7 ± 0.7	0	12.7 ± 0.7
PPS	Pepper plot sediment	0	0	0	0	0	0	0	17.7 ± 0.7
	Resistant	≤13	≤17	≤12	≤14	≤13	≤15	≤14	≤12
	Intermediate	14-16	18-20	13-14	15-16	14-17	16-20	15-22	13-15
	Sensitive	≥17	≥21	≥15	≥17	≥18	≥21	≥23	≥16

CAZ: ceftazidime (30 µg); GEN: gentamycin (10 µg); NIT: nitrofurantoin (30 µg); AUG: augmentin (30 µg); AMP: amoxicillin (30 µg); CPR: ciprofloxacin (30 µg); OFL: ofloxacin (30 µg) and CRX: cefuroxime (30 µg)

Table 2: Antibiotic susceptibility patterns of *Salmonella* sp. isolate from the three rivers.

Tag	Sample sources	Zone of inhibition (mm)							
	Asa River	AMP	CAZ	GEN	NIT	AUG	CPR	CRX	OFL
RBW	River bank water	25.0 ± 4.9	0	0	0	0	0	0	0
RBS	River bank sediment	0	0	0	0	0	0	0	0
IVP	Irrigated vegetable	0	0	0	0	0	0	0	0
VPS	Vegetable plot sediment	0	0	0	12 ± 1.1	0	0	3.2 ± 1.1	20.0 ± 1.0
IPP	Irrigated pepper	0	0	15.7 ± 1.2	0	0	0	23.3 ± 1.1	16.7 ± 1.0
PPS	Pepper plot sediment	19.3 ± 2.4	0	18.0 ± 4.3	0	0	0	*22.0 ± 1.1*	20.3 ± 2.4
	Oyun River	Zone of inhibition (mm)							
RBW	River bank water	0	0	0	0	0	0	0	0
RBS	River bank sediment	0	9.7 ± 0.7	0	0	0	0	16.3 ± 0.8	11.7 ± 0.8
IVP	Irrigated vegetable	0	0	0	0	0	0	0	0
VPS	Vegetable plot sediment	0	0	0	0	0	0	0	0
IPP	Irrigated pepper	0	0	0	0	0	0	0	0
PPS	Pepper plot sediment	16 ± 0.8	0	0	0	0	0	0	0
	Afon River	Zone of inhibition (mm)							
RBW	River bank water	0	0	0	0	0	0	0	0
RBS	River bank sediment	0	0	0	0	0	0	0	0
IVP	Irrigated vegetable	0	0	0	0	0	0	0	0
VPS	Vegetable plot sediment	0	0	0	0	0	3.7 ± 0.6	0	0
IPP	Irrigated pepper	0	0	0	0	0	15.7 ± 0.6	0	0
PPS	Pepper plot sediment	0	0	0	10.3 ± 0.7	0	0	10 ± 0.6	0
	Resistant	≤13	≤17	≤12	≤14	≤13	≤20	≤14	NA
	Intermediate	14-16	18-20	13-14	15-16	14-17	21-30	15-22	NA
	Sensitive	≥17	≥21	≥15	≥17	≥18	≥31	≥23	NA

CAZ: ceftazidime (30 µg); GEN: gentamycin (10 µg); NIT: nitrofurantoin (30 µg); AUG: augmentin (30 µg); AMP: amoxicillin (30 µg); CPR: ciprofloxacin (30 µg); OFL: ofloxacin (30 µg) and CRX: cefuroxime (30 µg); NA: Not applicable.

Similar to the population pattern observed in the heterotrophic bacteria counts, the sediments presented higher numbers of *E. coli* in all the three rivers studied (Figure 3).The densities of *E. coli* in the vegetables exceeded 1,000 cells in some of the samples while others were between 100 and 1000 cells. Regardless of the low numbers in the vegetables, consumption of these vegetables will still pose a health risk to humans as the infective dose of *E. coli* is less than 1000 cells.[19,22,23] Previous studies have shown that *E. coli* O157:H7 can persist on fruits and vegetable (parsley) for 177 days,[12] on lettuce for 25–77 days,[12] and about 21 days on salad vegetables, watermelons and iceberg lettuce.[19,20,25-27]

Salmonella/Shigella density in the samples

At Asa river, *Salmonella/Shigella* number was significantly higher in RBS ($1.00 \times 10^3 \pm 6.00 \times 10^2 – 1.25 \times 10^5 \pm 4.25 \times 10^4$ cfu/g) than in RBW ($3.00 \times 10^2 \pm 9.00 \times 10^1 – 5.40 \times 10^3 \pm 9.70 \times 10^2$ cfu/ml). *Salmonella/Shigella* populations in IVP ($0 – 3.40 \times 10^2 \pm 1.40 \times 10^2$ cfu/g) and IPP ($0 – 5.40 \times 10^2 \pm 2.50 \times 10^2$ cfu/g) were similar. *Salmonella/Shigella* load in VPS ($0 – 4.50 \times 10^4 \pm 7.25 \times 10^3$ cfu/g) was similar to that in PPS ($0 – 8.10 \times 10^4 \pm 3.15 \times 10^2$ cfu/g) (Figure 4). In Oyun River, *Salmonella/Shigella* population in RBW ($2.00 \times 10^2 \pm 1.00 \times 10^2 – 2.01 \times 10^4 \pm 8.05 \times 10^3$ cfu/ml) was lower than in RBS ($1.10 \times 10^4 \pm 3.50 \times 10^3 – 1.46 \times 10^5 \pm 3.30 \times 10^4$ cfu/g). *Salmonella/Shigella* loads in IVP ($0 – 5.20 \times 10^2 \pm 2.50 \times 10^2$ cfu/g) was not different from that in IPP ($0 – 1.59 \times 10^3 \pm 6.95 \times 10^2$ cfu/g) collected from farms around Oyun River (Figure 4). Unlike the trend seen in the other rivers, *Salmonella/Shigella* numbers in RBW ($9.00 \times 10^2 – 1.08 \times 10^4$ cfu/ml) and RBS ($1.00 \times 10^3 – 5.30 \times 10^4$ cfu/g) were similar in Afon River. *Salmonella/Shigella* population in IVP ($0 – 8.60 \times 10^2$ cfu/g) was similar to that in IPP ($0 – 5.00 \times 10^2$ cfu/g) (Figure 4). Though population of *Salmonella/Shigella* in sediment samples (RBS, VPS and PPS) were high and exceeded 1000 cells those of the vegetable samples were mostly below 100 cells (Figure 4). *Salmonella* sp. isolation from vegetables (spinach, kale and bok choy) in an irrigated farm in California was also similarly reported.[28] Persistent population of *Salmonella* in ponds was reported to be affected by season and environmental conditions.[29]

The density of pathogenic bacteria present in the irrigated vegetables is a reflection of the microbiota of the irrigation water. Surface waters are usually potential sources of pathogens when used for irrigation due to their pollution with agricultural and industrial wastewater effluents which usually contain high numbers of pathogenic microorganisms.[30] It has been reported that plants take up pathogens from sediments and irrigation water through their roots, stoma, tissues and damaged sections.[8,19,23,31,32] Pathogens can survive for several days both internally and externally on plants.[12] In plant tissues, it has been affirmed that pathogens are usually immune from various disinfection methods including use of UV light.[33] A couple of pathogenic infections have resulted from the consumption of raw or improperly cooked vegetables irrigated with contaminated water.[11] Ability of these pathogens to internalize and proliferate in the vegetables is also a major virulence factor.[24]

Antibiotic susceptibility of isolates recovered from samples

Antibiotic susceptibility profiling of *E. coli* O157:H7 and *Salmonella* sp. isolates recovered in each of the six samples collected from the three rivers were assessed and results presented in Tables 1 and 2. A total of thirty-six (36) isolates, comprising eighteen (18) *E. coli* O157:H7 and eighteen (18) *Salmonella* sp. were profiled. All 6 isolates of *E. coli* O157:H7 from Asa River showed complete resistance to ampicillin, ceftazidime, nitrofurantoin, augmentin and ciprofloxacin. One isolate was sensitive to both gentamicin (23.3 mm) and cefuroxime (25.4 mm), while most isolates were sensitive to ofloxacin (18.2–21.3 mm) (Table 1). Similar susceptibility profiles were recorded for *E. coli* O157:H7 isolates recovered from both Asa and Oyun Rivers (Table 1).

All isolates from Oyun River also demonstrated resistance to ampicillin, ceftazidime, nitrofurantoin, augmentin ciprofloxacin and cefuroxime; except one isolate (21.0 mm) that was intermediate to cefuroxime. Two isolates were also intermediate to gentamicin (13.0 mm) but most were sensitive to ofloxacin (16.7–21.7 mm) (Table 1). Likewise, in Afon River, all *E. coli* O157:H7 isolates recovered were resistant to ampicillin, ceftazidime, nitrofurantoin, augmentin and cefuroxime; whereas three isolates were resistant to gentamicin (1) and ciprofloxacin (2). All isolates except two were highly sensitive to ofloxacin (14.7–20.6 mm) (Table 1).

Interestingly, *Salmonella* sp. isolates had quite distinct antibiotic susceptibility profiles as compared to *E. coli* O157:H7 isolates which were similar. All 6 isolates of *Salmonella* sp. from Asa River demonstrated 100% resistance to ceftazidime, augmentin, nitrofurantoin and ciprofloxacin. Notably, two isolates each from RBS and IVP showed complete resistance to all eight antibiotics used. However, two isolates were sensitive to ampicillin (19.3; 25.0. mm) and gentamicin (15.7; 18.0 mm) while only one isolate was sensitive to cefuroxime (23.3 mm) (Table 2). Unlike what was observed for *E. coli* O157:H7 isolates in all rivers investigated, susceptibility profiles of *Salmonella* sp. isolates from Oyun River were different from those of the isolates from Asa River (Table 2). All 6 isolates demonstrated resistance to ceftazidime, gentamycin, nitrofurantoin, augmentin and ciprofloxacin (Table 2). Four of the *Salmonella* sp. isolates demonstrated total resistance to all eight antibiotics used. Only one isolate each was intermediate to ampicillin (16.0 mm) and cefuroxime (16.3 mm) (Table 2). All *Salmonella* sp. isolates recovered from Afon River however, exhibited resistance to all the eight antibiotics used in the study (Table 2). Findings from this study showed that *E. coli* O157:H7 and *Salmonella* sp. isolates from studied samples were highly drug-resistant. For *E. coli* O157:H7, 94.4% (17 of 18) of the isolates showed extensive drug resistance (XDR) with resistance to at least five of eight antibiotics, while 5.6% (1 of 18) of the isolates was pan-drug resistant, showing resistance to all eight antibiotics used in the study. The RBW isolates of the three rivers had Multi-antibiotic resistance index (MARI) range of 0.63 – 0.88; the RBS isolates were at a constant index of 0.75 while IVP and IPP isolates had MARI of 0.88 and 0.75 – 1.0, respectively. PPS and VPS samples had MARI of 0.88 and 0. 63 – 0.88, respectively.

For the Salmonella sp. 5.6% (1 of 18), 27.8% (5 of 18) and 66.7% (12 of 18) of the isolates exhibited multi-drug resistance (MDR), extensive drug resistance (XDR) and pan-drug resistance (PDR) respectively. The overall MARI of the isolates were 0.5 – 1.0. All the RBW isolates except from Asa River (sensitive to ampicillin) were completely resistant to the antibiotics used. RBS isolates in Asa and Afon Rivers exhibited complete resistance to all antibiotics but isolates from Oyun showed moderate sensitivity to cefuroxime. All the IVP isolates were resistant to the eight antibiotics while the IPP isolates from Asa River were sensitive to gentamicin and cefuroxime. VPS isolates were resistant to all antibiotics used while PPS isolate from Asa River was sensitive to ampicillin and gentamicin. Overall, 5.6% of the isolates had MARI of 0.5 and 0.63; 22.2% had MARI of 0.88 while 66.7% had MARI of 1.0. Notably, some isolates exhibited strict resistance to antibiotics belonging to six different classes; penicillins, aminoglycosides, macrolides, fluoroquinolones, second and third-generation cephalosporins. Ofloxacin was the most effective antibiotics as observed in the study (Table 1). The rate of resistance observed among these isolates in the environment and especially in the vegetables portends possible high risk of diseases outbreak. The results are in agreement with the findings by other researchers, who reported multidrug resistance among *E. coli* O157:H7 isolates,[34,35] and *Salmonella* isolates.[29] Sources of effluents or wastewater deposits into the rivers used for irrigation may be a determining factor for resistance. Run-off from farmlands, livestock production as well as domestic wastes with antibiotics deposits may be the sources of resistance genes.

Conclusion

Our findings affirmed the risk associated with using water with poor microbiological quality and resistant pathogenic organisms for irrigation during cultivation of vegetables. Consumption of vegetables containing XDR pathogenic bacteria constitute a major health risk, hence the need for the treatment of water used for irrigation. Contaminated irrigation water increases chances of producing and disseminating contaminated fruits and vegetables. Decontamination strategies and policies are required to remedy contaminated water sources in order to make them safe for irrigation of plants and vegetables of farmlands of the studied areas.

Conflict of interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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