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Research Article

Bacteriological Contaminants of Some Fresh Vegetables Irrigated with Awetu River in Jimma Town, Southwestern Ethiopia

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The main purposes of this study were to determine the bacteriological load and safety of some fresh vegetables irrigated with Awetu River in Jimma town, southwestern Ethiopia. Water and vegetable samples were collected from three different irrigation sites and analyzed for their bacteriological contaminants following standard procedures. The maximum overall means of aerobic mesophilic bacteria, Enterobacteriaceae, aerobic spore formers, staphylococci, and total and fecal coliform counts were 8.06, 7.10, 6.54, and 2.97 log CFU g^{-1} and 1036 and 716 MPN 100 mL^{-1}, respectively. The microflora of vegetable samples was dominated by *Bacillus* species (32.7%) followed by Enterobacteriaceae (25%) and *Micrococcus* (16%). *Staphylococcus aureus* and *Salmonella* spp. were detected in 24.0% and 20.7% of the samples, respectively. All the *Staphylococcus aureus* isolates were resistant to ampicillin, cefuroxime sodium, and penicillin G (100.0% each). All the *Salmonella* isolates were also resistant to tetracycline, erythromycin, cefuroxime sodium, and penicillin G (100.0% each). The findings reveal that the river water used for irrigation in this study is a possible preharvest source of contamination to fresh vegetables which potentially constitutes a health risk to consumers.

1. Introduction

Fresh and minimally processed vegetables and fruits provide the most important human diet that contains carbohydrates, proteins, vitamins, minerals, and fiber. Lerici et al. [1] reported that nutritional and other benefits of a regular intake of fruits and vegetables are well documented internationally. 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 [2]. 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 because correct fresh produce intake alone could save 2.7 million lives a year [3].

In contrast to their health benefits, the consumption of fresh fruits and vegetables has also been associated to risk for consumers [4]. Outbreaks of food infections associated with consumption of ready-to-eat vegetables have been increasing [5]. Garg et al. [6] show 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,* and *Campylobacter* spp. [7].

Ijabadeniyi [2] reported that prepackaged fresh spinach was recalled by the Food and Drug Administration (FDA) as a result of *E. coli* outbreak in California, USA. The author also noted that fresh tomatoes consumed at restaurants in the USA were also blamed for an outbreak of *S. typhimurium.* In addition, there was an *E. coli* O157:H7 outbreak linked to lettuce from Taco Bell restaurants in northern USA [8].

The increase in outbreaks of foodborne illnesses due to fresh produce is a result of changes in dietary habits, including a higher per capital consumption of fresh or minimally processed fruits and vegetables and the increased use of

salad bars and meals eaten outside the home, Ijabadeniyi [2]. Changes in production and processing methods of distribution, consumption patterns, and practices are other factors that have also contributed to increasing foodborne diseases due to raw consumed vegetables [9].

Ijabadeniyi [2] reported that other reasons given by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) [10] for the increasing of foodborne infection/poisoning outbreaks are as follows: microbial adaptation, increase in international trade and in susceptible population, and increase in worldwide travelling. Furthermore, changes of a lifestyle of convenience, consumer demands regarding healthy food with no chemical preservatives and with an extended shelf life, and changes in human demographics and behavior have also contributed to increase of foodborne infections.

According to Suslow et al. [11], the microbial quality of irrigation water is critical because water contaminated with animal or human wastes can introduce pathogens into vegetable products during preharvest and postharvest activities via direct or indirect contamination. Therefore, microbiological quality of irrigation water has a paramount importance to the safety of fresh and minimally processed vegetables [12]. Moreover, Ibenyassine et al. [13] reported that contaminated irrigation water and surface runoff water may be the major sources of pathogenic microorganisms that contaminate fruits and vegetables in fields. Water from the river that received both human and animal waste disposal poses a health risk due to contamination with all microorganisms of human and animal intestinal habitat such as *Salmonella* and *Listeria* spp. [14, 15]. Consequently, the microbiological quality and safety of fresh vegetables is a significant concern to all stakeholders all over the world.

Tambekar and Mundhada [16] reported that foodborne bacterial pathogens commonly detected in fresh vegetables were *E. coli*, *S. aureus,* and *Salmonella* spp. Ayçiçek et al. [17] have also stated that plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality in addition to the common indicators such as fecal coliform. Information on the microbial load on fresh vegetables in Ethiopia has been studied by several investigators [18–20].

According to Hailu [21], the use of rivers and hand-dug wells for various purposes is common in Jimma town. The author further noted that Awetu River is the primary source of water for a range of activities such as recreation, bathing, washing clothes, and household utensils, small scale agricultural irrigation, car washing, and other uses. The deterioration of the quality of Awetu River as a result of discharge of municipal wastes and urban runoff has been well studied, Hailu [21]. For instance, Deneke [22] investigated about the pollution profiles along Awetu River and found high load of fecal coliforms. According to Sofonias and Tsegaye [23] some of the water sources in Jimma town such as unprotected springs, wells, and Awetu River have greater chance to be contaminated via human and animal fecal matters and wastes disposed from households, hotels, and small scale industries. Thus, these water sources could transmit diseases as a result of contamination. However, a comprehensive investigation of the quality and safety of vegetables irrigated with Awetu River including bacteriological quality and safety analyses is lacking. Therefore, this study was aimed at investigating the bacteriological contaminants of some fresh vegetables irrigated with Awetu River, in Jimma town, southwestern Ethiopia.

2. Materials and Methods

2.1. Description of the Study Area. The study was conducted at Jimma town, located 350 km southwest of Addis Ababa. The town's geographical coordinates are 7°41′N latitude and 36°50′E longitude. The town is found with abundant mean annual rainfall between 1800 and 2300 mm which makes this region one of the best watered Ethiopian highland areas, conducive for agricultural production [24].

2.2. Study Design and Study Population. Laboratory based cross sectional study design was used. The sampling sites were three irrigation farms around Awetu River designated as site A, B, and C along the river course at the same time of the irrigation period. The size of the farms is about 1.5 ha and the average production of vegetables from these farms is 30 kg/ha. On the average the frequency of irrigation for the target vegetables is 3–5 days/week depending on the humidity of the air at the farms.

There are many farmers who are members of Microenterprise Association around Awetu River. They are actively involved in irrigating their farms.

Preliminary survey was made on the distribution and location of vegetable growers in the study area prior to resuming the actual sample collection. The survey included individual residents and members of Microenterprise Association of vegetable growers who regularly use Awetu River to irrigate their farms in Jimma town. Purposive sampling was made to gather information on Awetu River from a total of 60 vegetable growers using structured questionnaire. This survey was performed in order to get general information about Awetu River including its pollution status and its effect on human health.

2.3. Collection of Samples. A total of 120 vegetable samples, 40 each from the three sites and 10 samples each of lettuce, cabbage, tomato, and carrot, were randomly picked (1.5 kg) aseptically using sterilized scissors and cut into pieces. In addition, a total of 30 irrigation water samples (200 mL each) were collected from three sites (10 samples each). The water samples were collected from the depth of below 5 cm surface water using sterilized stopper glass bottles. The samples were transported to Postgraduate and Research Laboratory of Biology Department, College of Natural Sciences, Jimma University using ice box. The samples were processed for bacteriological analysis within 1–4 hrs of collection.

2.4. Sample Preparation. Mixed vegetable samples (unprocessed and large sized) were aseptically chopped into smaller pieces using a sterile stainless steel knife prior to weighing. A 25 g of subsample of each vegetable was aseptically weighed and vigorously shaken in 225 mL of sterile 0.1% (w/v) buffered

peptone water (Oxoid) for 3 min separately to homogenize the samples [25]. In addition, a tenfold serial dilution was made. A 10 mL of water sample was also mixed with 90 mL of peptone water using vortex mixer. Finally, appropriate serial dilutions of the suspension were spread-plated on a suitable solid media.

2.5. Bacterial Counts. A volume of 0.1 mL aliquot of appropriate dilution was spread-plated in duplicate on presolidified plates of Plate Count Agar (Oxoid), Violet Red Bile Glucose Agar (Oxoid) and Mannitol Salt Agar (Oxoid) that then were incubated at optimum temperature and time for counts of aerobic mesophilic bacteria, Enterobacteriaceae, and staphylococci, respectively. Homogenized samples were heated at 80°C for 10 minutes in a water bath to count aerobic spore formers. Thereafter, a 0.1 mL appropriate dilution was spread-plated in duplicate on predried surfaces of Plate Count Agar (Oxoid) plates. Inoculated plates were incubated at 30°C–32°C for 24–48 hrs. For microbial counts, plates with colonies between 30 and 300 were considered. Total coliforms and fecal coliforms were enumerated by multiple tube fermentation tests as described by APHA [26]. The results were expressed as MPN 100 mL^{-1}.

2.6. Isolation and Characterization of Dominant Microflora. After enumeration of aerobic mesophilic bacteria, 10–20 colonies with distinct morphological difference such as color, size, and shape were randomly picked from countable plates and aseptically transferred into a tube containing 5 mL nutrient broth (Oxoid) and incubated at 30°C for 24–48 hrs. The cultures were purified by repeated plating and pure cultures were temporarily preserved on nutrient agar slants at 4°C for a month for further work. An overnight activated culture was further characterized using the following standard tests such as cell morphology, gram staining, motility, bacterial endospore staining, catalase, cytochrome oxidase, and Oxidation Fermentation (O/F) to differentiate into various bacterial groups such as genus and family levels [27].

2.7. Isolation and Biochemical Identification of Some Bacterial Pathogens. After counting staphylococci, yellow colonies on Mannitol Salt Agar plates were aseptically picked and transferred into 5 mL nutrient broth and incubated at 37°C for 24–36 hrs for further purification. Then, a loop of culture from the nutrient broth was streaked on presolidified surface of nutrient agar supplemented with 0.75% sodium chloride and again incubated at 37°C for 24–36 hrs so as to obtain distinct colonies. Finally, the distinct colonies were characterized using the established microbiological methods such as gram staining. Gram positive cocci with clustered arrangement under the microscope were subjected to preliminary biochemical tests (the catalase and coagulase tests) for conformation.

25 g or 25 mL of each sample was aseptically transferred into sterile flask containing 225 mL buffered peptone water (BPW), then was homogenized for 5 min, and then was incubated at 37°C for 24 hrs for recovery and proliferation of cells. Following the BPW enrichment, a 1 mL of culture was transferred into 10 mL of Rappaport Vassiliadis broth and was incubated at 43°C for 48 hrs. A loop full of culture from the Rappaport Vassiliadis broth was streaked onto Xylose Lysine Deoxycholate Agar (XLD) agar and incubated at 37°C for 24 hrs. Typical colonies that appeared red or colorless with no blackening were picked as presumptive *Shigella* and colonies which had slightly transparent zone of reddish color and a black center, a pink-red zone surrounding the colonies, were considered as presumptive *Salmonella*. The presumptive *Salmonella* and *Shigella* colonies were further confirmed following standard methods [28].

2.8. Antimicrobial Susceptibility Testing for S. aureus and Salmonella spp. The antimicrobial susceptibility testing for *S. aureus* and *Salmonella* spp. was determined according to modified Kirby Bauer disc diffusion technique as described by Clinical Laboratory Standard Institute (CLSI; [29]). The following 9 drugs, namely, ampicillin (10 μg), gentamycin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg), erythromycin (15 μg), Ttrimethoprim-sulfamethoxazole (co-trimoxazole) (25 μg), cefuroxime sodium (5 μg), norfloxacin (10 μg), and penicillin G (10 μg), were used to determine the antibiogram of the isolates. The criteria used to select the antimicrobial agents tested in this study were based on availability and frequency of prescription of the drugs for the management of bacterial infection in Ethiopia. *Salmonella typhimurium* (ATCC13311) and *Staphylococcus aureus* (ATCC25923) were used as reference strains for quality control of the antibiotics used.

2.9. Data Analysis. Bacterial counts were calculated as colony forming units per gram (CFU g^{-1}) and colony forming units per milliliter (CFU mL^{-1}) and converted into $\log_{10}$ values. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by LSD's Post Hoc Multiple Comparison Test using statistical software (SPSS) package version 21. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Sociodemographic Characteristics of the Study Subjects. Males accounted for 71.7% of the respondents (data not shown). The ages of vegetable growers ranged from 18 to 67 years with average age of 35 years. A large number of the respondents (48.3%) were Muslims followed by Orthodox (33.3%). Regarding their marital status, 70% were married but 21.7% were unmarried. About 55% of the respondents were illiterate and the rest (45%) attended elementary school (data not shown).

3.2. Irrigation Conditions of Awetu River. All the respondents (100.0%) used Awetu River for different purposes (Table 1). From all the respondents, 98.3% used Awetu River for irrigation, 35% for washing their clothes, and the rest 8.3% for taking shower (Table 1). The majority of the respondents (96.7%) irrigated tomato followed by potato (75.0%) and carrot (51.7%) (Table 1).

Table 1: General information and irrigation conditions of Awetu River.

Characteristics	Number of respondents ($n = 60$)	
	Frequency	Percent (%)
Using of Awetu River for any means		
Yes	60.0	100.0
No	0.0	0.0
Application of Awetu River		
Irrigation	59.0	98.3
Washing clothes	21.0	35.0
Taking shower	5.0	8.3
Types of vegetables irrigated with Awetu River		
Tomato	58.0	96.7
Potato	45.0	75.0
Carrot	31.0	51.7
Cabbage	23.0	38.3
Lettuce	11.0	18.3
Green pepper	6.0	10.0
Onion	4.0	6.7
Use of irrigated vegetables		
Family consumption	53.0	88.3
Source of income	44.0	73.3
Suspected diseases due to consumption of the vegetables		
Typhoid	10.0	66.7
Diarrhea	7.0	46.7
Anemia	2.0	13.3
Suffering of Awetu River from any contamination		
Yes	60.0	100.0
No	0.0	0.0
Sources of contamination		
Wastes released from toilet	58.0	96.7
Domestic waste	55.0	91.7
Plastic and petrol washes	19.0	31.7
Fecal matter	8.0	13.3

A significant number of the growers (88.3%) used cultivated vegetables for family consumption, while 73.3% used them as their source of income (Table 1). The respondents complained of health problems due to consumption of the vegetables. Accordingly, some of the respondents suspected typhoid (66.7%), others diarrhea (46.7%), and the rest associated the issue with anemia (13.3%) (Table 1).

Generally, all the respondents (100.0%) believed that Awetu River suffers from wastes released from Jimma town that included toilet wastes (96.7%), domestic wastes (91.7%), plastic and petrol washes (31.67%), and fecal matter (13.3%; Table 1).

3.3. Bacterial Counts. The mean bacterial counts ($\log \text{CFU mL}^{-1}$ or g^{-1}) of aerobic mesophilic bacteria, Enterobacteriaceae, aerobic spore formers, and staphylococci at the three sites were 8.58, 7.42, 5.75, and 2.64 $\log \text{CFU mL}^{-1}$ for water; 6.94, 6.09, 5.24, and 2.97 for lettuce; 8.06, 7.10, 6.54, and 2.71 for carrot; 7.41, 6.24, 5.71, and 2.75 for tomato; 7.66, 6.70, 5.89, and 2.76 $\log \text{CFU g}^{-1}$ for cabbage, respectively (Table 2). The mean microbial counts of different samples of selected sites ranged from 2.55 to 9.42 $\log \text{CFU mL}^{-1}$.

The minimal and maximal mean counts of aerobic mesophilic bacteria, Enterobacteriaceae, aerobic spore formers, and staphylococci obtained from different samples of the three sites ranged from 5.92 to 9.42, 5.0 to 8.36, 4.30 to 7.04, and 2.55 to 3.24 $\log \text{CFU g}^{-1}$ or mL^{-1}, respectively (Table 2). The mean count of AMB was the highest (9.42 $\log \text{CFU mL}^{-1}$) in water samples of site C. The count of Enterobacteriaceae was also relatively higher (8.36 $\log \text{CFU mL}^{-1}$) in water samples of site C but the range of mean count for staphylococci relatively in all samples was similar (Table 2). In addition, MPN of total and fecal coliforms and their overall mean in vegetables also ranged from 865.3 to 1036.0 and 524.0 to 716.0 MPN 100 mL^{-1}, respectively. However, both were >2400.0 MPN 100 mL^{-1} in water samples (Table 2).

The mean counts ($\log \text{CFU mL}^{-1}$ or g^{-1}) of AMB, Enterobacteriaceae, and aerobic spore formers revealed statistically significant ($p < 0.05$) difference between all samples of the three sites. However, there was no significant ($p > 0.05$) difference between mean counts of total coliform and fecal coliforms of all the samples collected from the three irrigation sites (Table 2). Likewise, the mean counts of staphylococci did not differ significantly ($p > 0.05$) between the different site of irrigation farms for carrot and cabbage (Table 2). Mean counts of all bacterial groups differed significantly ($p < 0.05$) between the different sample types with the highest mean count in carrot samples (data not shown).

3.4. Microflora Analysis. Aerobic mesophilic bacterial flora of water samples collected from the three sites of the downstream of Awetu River was dominated by *Bacillus* spp. (35.3%), Enterobacteriaceae (26.7%), *Micrococcus* spp. (12.0), *Pseudomonas* spp. (8.3%), *Staphylococcus* spp. (6.7%), *Aeromonas* spp. (6.0%), and *Streptococcus* spp. (5.0%). Similarly, the most predominant genera/families in lettuce, carrot, tomato, and cabbage samples were *Bacillus* spp. (30.0, 34.0, 31.7, and 32.3%), Enterobacteriaceae (23.3, 26.0, 24.0, and 25.0%), *Micrococcus* spp. (16.0, 16.3, 18.7, and 17.3%), *Pseudomonas* spp. (13.0, 9.0 10.3, and 9.3%), *Staphylococcus* spp. (9.3, 6.7, 8.0, and 8.7%), *Aeromonas* spp. (6.0, 5.0 5.7, and 5.0%), and *Streptococcus* spp. (2.3, 3.0, 1.7, and 2.3%), respectively (Figure 1). Generally, the same dominant genera were isolated in both water and vegetable samples even though their distributions were relatively varied.

3.5. Prevalence of S. aureus, Shigella, and Salmonella Isolates. The total prevalence of *S. aureus* and *Salmonella* was 24.0% and 20.7%, respectively (Table 3). The distribution of these pathogens varied depending on the nature of vegetables. With regard to sample types, prevalence of *S. aureus* was higher

Table 2: Bacterial counts from water and vegetable samples.

Sample type	Site	Average log CFU mL^{-1} or G^{-1} ± S.D				Average MPN 100 mL^{-1} ± S.D.	
		AMB	Entero.	ASFs	Staph.	Total coliform	Fecal coliform
Water	A	$7.65^{c} \pm 0.4$	$6.55^{c} \pm 0.3$	$4.88^{c} \pm 0.5$	$2.55^{b} \pm 0.1$	$>2400.00^{a} \pm 0$	$> 2400.00^{a} \pm 0$
	B	$8.69^{b} \pm 0.5$	$7.34^{b} \pm 0.3$	$5.70^{b} \pm 0.5$	$2.66^{ab} \pm 0.1$	$>2400.00^{a} \pm 0$	$>2400.00^{a} \pm 0$
	C	$9.42^{a} \pm 0.1$	$8.36^{a} \pm 0.1$	$6.65^{a} \pm 0.5$	$2.71^{a} \pm 0.1$	$>2400.00^{a} \pm 0$	$>2400.00^{a} \pm 0$
	Average	8.58 ± 0.3	7.42 ± 0.2	5.75 ± 0.5	2.64 ± 0.1	$> 2400.00 \pm 0$	$> 2400.00 \pm 0$
Lettuce	A	$5.92^{c} \pm 0.5$	$5.00^{c} \pm 0$	$4.30^{c} \pm 0.1$	$2.77^{b} \pm 0.2$	$844.00^{a} \pm 330.5$	$524.00^{a} \pm 202.4$
	B	$6.90^{b} \pm 0.5$	$6.10^{b} \pm 0.6$	$5.41^{b} \pm 0.3$	$2.89^{b} \pm 0.3$	$908.00^{a} \pm 309.1$	$588.00^{a} \pm 269.8$
	C	$7.99^{a} \pm 0.5$	$7.17^{a} \pm 0.4$	$6.02^{a} \pm 0.5$	$3.24^{a} \pm 0.4$	$972.00^{a} \pm 269.8$	$652.00^{a} \pm 309.1$
	Average	6.94 ± 0.5	6.09 ± 0.3	5.24 ± 0.3	2.97 ± 0.3	908 ± 303.2	588 ± 260.5
Carrot	A	$7.05^{c} \pm 0.5$	$6.43^{c} \pm 0.3$	$5.98^{c} \pm 0.4$	$2.63^{a} \pm 0.1$	$972.00^{a} \pm 269.8$	$652.00^{a} \pm 309.1$
	B	$7.99^{b} \pm 0.5$	$7.08^{b} \pm 0.4$	$6.59^{b} \pm 0.4$	$2.74^{a} \pm 0.1$	$1036.00^{a} \pm 202.4$	$716.00^{a} \pm 330.5$
	C	$9.15^{a} \pm 0.4$	$7.79^{a} \pm 0.5$	$7.04^{a} \pm 0.5$	$2.76^{a} \pm 0.2$	$1100.00^{a} \pm 0$	$780.00^{a} \pm 337.3$
	Average	8.06 ± 0.5	7.1 ± 0.4	6.54 ± 0.5	2.71 ± 0.2	1036 ± 157.4	716 ± 325.7
Tomato	A	$6.57^{c} \pm 0.4$	$5.38^{c} \pm 0.4$	$5.12^{c} \pm 0.4$	$2.62^{c} \pm 0.1$	$780.00^{a} \pm 460$	$460.00^{a} \pm 0$
	B	$7.53^{b} \pm 0.4$	$6.34^{b} \pm 0.3$	$5.72^{b} \pm 0.5$	$2.76^{b} \pm 0.1$	$844.00^{a} \pm 330.5$	$524.00^{a} \pm 202.4$
	C	$8.12^{a} \pm 0.4$	$6.99^{a} \pm 0.5$	$6.29^{a} \pm 0.1$	$2.88^{a} \pm 0.1$	$972.00^{a} \pm 269.8$	$588.00^{a} \pm 269.8$
	Average	7.41 ± 0.4	6.24 ± 0.4	5.71 ± 0.3	2.75 ± 0.1	865.33 ± 353.4	524 ± 157.4
Cabbage	A	$6.82^{c} \pm 0.5$	$5.89^{c} \pm 0.5$	$5.24^{c} \pm 0.3$	$2.71^{a} \pm 0.2$	$844.00^{a} \pm 330.5$	$588.00^{a} \pm 269.8$
	B	$7.58^{b} \pm 0.4$	$6.81^{b} \pm 0.5$	$5.81^{b} \pm 0.5$	$2.73^{a} \pm 0.2$	$908.00^{a} \pm 309.1$	$652.00^{a} \pm 309.1$
	C	$8.57^{a} \pm 0.4$	$7.40^{a} \pm 0.5$	$6.63^{a} \pm 0.5$	$2.85^{a} \pm 0.2$	$1036.00^{a} \pm 202.4$	$716.00^{a} \pm 330.5$
	Average	7.66 ± 0.4	6.7 ± 0.5	5.89 ± 0.4	2.76 ± 0.2	929.33 ± 280.7	652 ± 303.2

Mean values followed by different alphabets within a column of each sample types are significantly different using *post hoc* multiple comparisons test ($p < 0.05$), where AMB stands for aerobic mesophilic bacteria, ASFs, aerobic spore formers, Entero., Enteriobacteriaceae, and Staph., staphylococci.

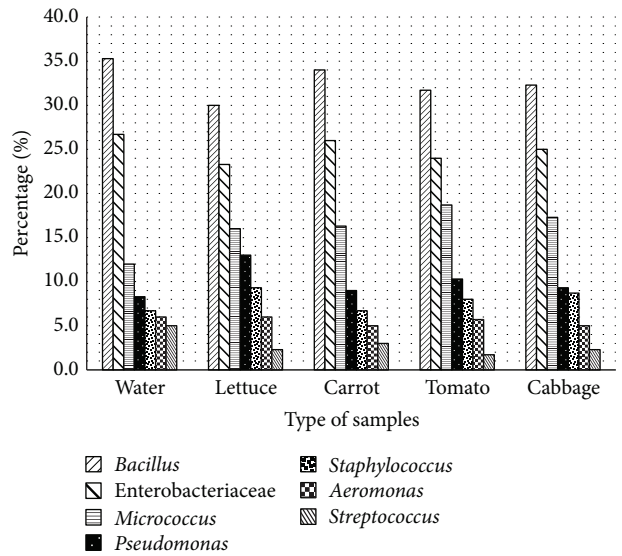

Figure 1: Distribution of dominant bacterial isolates from water and vegetable samples from irrigated farm land along the Awetu River.

in lettuce (33.3%), cabbage (26.7%), and tomato (23.3%), but relatively lower in water (20.0%) and carrot samples (16.7%). Higher frequency of *Samonella* spp. was encountered in cabbage (30.0%) but it was lower in lettuce (13.3%, Table 3). *Shigella* was not encountered in any of the samples analyzed. The prevalence of these all bacterial pathogens was not significantly ($p > 0.05$) different with respect to sample types (data not shown). However, in terms of sample sites, there was significant ($p < 0.05$) difference for *S. aureus* but not for *Salmonella* spp. (data not shown).

3.6. Antimicrobial Susceptibility Patterns of S. aureus and Salmonella Isolates. *Staphylococcus aureus* isolates were most susceptible to chloramphenicol (100.0%), norfloxacin (94.4.0%), gentamycin (86.1%), and erythromycin (80.6%; Table 4). Out of the tested drugs, the highest resistance was observed against ampicillin, cefuroxime sodium, and penicillin G (100.0% each).

All *Salmonella* isolates were also susceptible to chloramphenicol, norfloxacin, gentamycin (100.0% each), and cotrimoxazole (93.5%). Out of the tested drugs, the highest resistance was observed against tetracycline, erythromycin, cefuroxime sodium, penicillin G (100.0% each), and ampicillin (90.3%) (Table 4).

3.7. Multiple Drug Resistance of S. aureus and Salmonella Isolates. Pattern of multiple drug resistance (MDR) among *S. aureus* isolates varied from three to seven antibiotics (Table 5). The highest MDR noted was Amp/Pen/Cef/Te/Cot (11/36, 30.6%), followed by Amp/Pen/Cef and Amp/Pen/Cef/Te (7/36, 19.4% each). The maximum MDR registered was resistance to 7 antibiotics with the combination Amp/Pen/Cef/Te/Cot/Ery/Nor. MDR to five antibiotics dominated the resistance patterns (13/36, 36.1%; Table 5).

Table 3: Prevalence of *S. aureus* and *Salmonella* isolates from water and vegetable samples.

Sample type	Sites	Sample size	Number of *S. aureus* positive samples (%)	Number of *Salmonella* positive samples (%)
Water	A	10	0	0
	B	10	2 (20)	2 (20)
	C	10	4 (40)	5 (50)
	Subtotal	30	6 (20)	7 (23.3)
Lettuce	A	10	3 (30)	1 (10)
	B	10	2 (20)	1 (10)
	C	10	5 (50)	2 (20)
	Subtotal	30	10 (33.3)	4 (13.3)
Carrot	A	10	0	1 (10)
	B	10	1 (10)	2 (20)
	C	10	4 (40)	2 (20)
	Subtotal	30	5 (16.7)	5 (16.7)
Tomato	A	10	1 (10)	1 (10)
	B	10	3 (30)	2 (20)
	C	10	3 (30)	3 (30)
	Subtotal	30	7 (23.3)	6 (20)
Cabbage	A	10	1 (10)	2 (20)
	B	10	3 (30)	3 (30)
	C	10	4 (40)	4 (40)
	Subtotal	30	8 (26.7)	9 (30)
	Total	*150*	*36 (24)*	*31 (20)*

Table 4: Antibiotic susceptibility of *S. aureus* and *Salmonella* spp. isolated from water and vegetable samples.

Antimicrobial agent	Disc content (μg)	*S. aureus* ($n = 36$)				*Salmonella* spp. ($n = 31$)			
		S	%	R	%	S	%	R	%
Ampicillin	10	0	0	36	100	3	9.7	28	90.3
Gentamycin	10	31	86.1	5	13.9	31	100	0	0
Chloramphenicol	30	36	100	0	0	31	100	0	0
Tetracycline	30	7	19.4	29	80.6	0	0	31	100
Erythromycin	15	29	80.6	7	19.4	0	0	31	100
Co-trimoxazole	25	18	50	18	50	29	93.5	2	6.5
Cefuroxime sodium	5	0	0	36	100	0	0	31	100
Penicillin	10	0	0	36	100	0	0	31	100
Norfloxacin	10	34	94.4	2	5.6	31	100	0	0

Where S stands for sensitive and R, resistance.

Pattern of multiple drug resistance (MDR) in *Salmonella* isolates also varied from four to six antibiotics (Table 5). The highest MDR noted was Amp/Te/Ery/Pen/Cef (26/31, 83.9%), followed by Te/Ery/Pen/Cef (3/31, 9.7%). The maximum MDR registered was resistance to 6 antibiotics with the combination of Amp/Te/Ery/Pen/Cef/Cot (Table 5).

4. Discussion

In the current study, all the respondents used Awetu River without any treatment for different purposes, mainly for irrigation of vegetables that may be eaten raw. Similarly, Prabu [30] reported that many people used Akaki River in Addis Ababa, Ethiopia, for different activities including irrigation of vegetables that are served in the households of Addis Ababa city without any treatment.

Some of the respondents complained of health problems such as typhoid due to consumption of vegetables irrigated with Awetu River. Faruqui et al. [31] demonstrated the prevalence of typhoid epidemics in Santiago and Dakar, Senegal, which could be traced to fecal contamination of water or fresh vegetables as noted in this study. Moreover, in agreement with the current study, Gerardi and Zimmerman [32] reported *Clostridium perfringes*, *Staphylococcus aureus*, and some other bacteria from wastewater.

Table 5: Multiple drug resistance (MDR) of *S. aureus* and *Samonella* spp. isolated from water and vegetable samples.

Isolates	Number of drugs resisted	Drugs resisted	Resistant isolates	
			Subtotal number (%)	Total Number (%)
S. aureus (36 isolates)	Three	Amp/Cef/Pen	7 (19.4)	7 (19.4)
	Four	Amp/Te/Cef/Pen	7 (19.4)	7 (19.4)
	Five	Amp/Te/Cot/Cef/Pen	11 (30.6)	13 (36.1)
		Amp/Gen/Te/Cef/Pen	2 (5.6)	
	Six	Amp/Te/Ery/Cot/Cef/Pen	4 (11.1)	7 (19.4)
		Amp/Gen/Te/Ery/Cef/Pen	1 (2.8)	
		Amp/Gen/Te/Cot/Cef/Pen	2 (5.6)	
	Seven	Amp/Te/Ery/Cot/Cef/Pen/Nor	2 (5.6)	2 (5.6)
Salmonella spp. (31 isolates)	Four	Te/Ery/Pen/Cef	3 (9.7)	3 (9.7)
	Five	Te/Ery/Pen/Cef/Amp	26 (83.9)	26 (83.9)
	Six	Te/Ery/Pen/Cef/Amp/Cot	2 (6.5)	2 (6.5)

Where Amp stands for ampicillin, Pen, penicillin, Cef, cefuroxime sodium, Te, tetracycline, Cot, co-trimoxazole, Gen, gentamycin, Ery, erythromycin, and Nor, norfloxacin.

The overall mean aerobic mesophilic count observed in this study ranged from 6.94 to 8.06 log CFU g^{-1}, relatively higher than previous reports from Morocco, Ibenyassine et al. [33], but other studies reported a lower count that ranged from 2 to 6 log CFU g^{-1} [17, 34, 35]. 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 [36].

According to this guideline specifically the mean counts of AMB in all food samples (this study) were 6.94 log CFU g^{-1} and above. Hence, they belong to the category of poor and spoiled food. Aerobic organisms reflect the exposure of the sample to contamination and the existence of favorable conditions for multiplication of microorganisms [37]. Ayçiçek et al. [17] also stated that plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality, and most foods are regarded as harmful when they have large populations of aerobic mesophilic microorganisms, even if the organisms are not known to be pathogens [38].

The overall mean count of Enterobacteriaceae in the present study ranged from 6.09 to 7.10 log CFU g^{-1}. This is higher than other studies conducted on lettuce and green pepper 5.08 and 4.84 log CFU g^{-1}, respectively, by Guchi and Ashenafi [20] and Ibenyassine et al. [33], in Ethiopia and Morocco, respectively. According to Gilbert et al. [39] and guideline recommended for fresh fruit and vegetables in London, overall mean counts (log CFU g^{-1}) of Enterobacteriaceae in carrot (7.10), cabbage (6.70), tomato (6.24), and lettuce (6.09) revealed unsatisfactory level (≥4 log CFU g^{-1}). Guchi and Ashenafi [20] suggested that the high level of Enterobactericeae in vegetables might indicate that the water used for irrigation could be heavily contaminated with fecal matter from sewerage effluent. Although most of Enterobacteriaceae are normal flora of vegetables, Motarjemi et al. [40] stated that high number clearly proves that poor hygiene could be a source of foodborne pathogens.

In case of aerobic spore formers, the overall mean counts ranged from 5.24 to 6.54 log CFU g^{-1}. In all vegetables the counts were higher compared to reports by Guchi and Ashenafi [20] where the counts ranged between 3.47 and 3.50 log CFU g^{-1} in green pepper and lettuce, respectively, from Addis Ababa, Ethiopia.

In the present study, the overall mean count of staphylococci from vegetable samples ranged from 2.71 to 2.97 log CFU g^{-1}. This is lower than the microbiological studies made on lettuce and green pepper from super market in Addis Ababa, Ethiopia, Guchi and Ashenafi [20], who reported 4.55 and 4.97 log CFU g^{-1}, respectively. Higher counts of staphylococci from super market may be due to skin contact and environmental contamination. In the current study, although the counts of staphylococci were low, health risk cannot be avoided, since Erkan et al. [41] reported that contamination of food stuffs during distribution and handling may allow bacterial growth and subsequent production of toxins.

The overall mean counts of total and fecal coliforms from vegetable samples in the present study are relatively lower than Nipa et al. [42], who reported >1100 MPN 100 mL^{-1} from salad vegetables. In addition, the current finding is lower than the results obtained by Ashenafi [18], 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} [43]. 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.

The total and fecal coliform counts from water samples in the present study were >2400 MPN 100 mL^{-1} which is higher than the WHO recommended standard [10]. According to the standard, the fecal coliform level must not exceed 1000 counts 100 mL^{-1} for the safe use of wastewater for irrigation of vegetables. A relationship has been established between coliform levels and the incidence of pathogenic bacteria like *Salmonella* [44].

Microbial groups that belong to seven genera were isolated from the examined samples at varying percentages with gram positive and gram negative flora accounting for 59.5% and 40.5%, respectively. The gram positive cells were represented by bacteria from the genera: *Bacillus*, *Micrococcus*, *Staphylococcus,* and *Streptococcus* spp. while the gram negative microflora constituted members of Enterobacteriaceae, *Pseudomonas* spp., and *Aromonas* spp. This is partly similar to the previous reports by Guchi and Ashenafi [20] in lettuce and green pepper from Addis Ababa, Ethiopia. The predominant microflora of fresh vegetables in the present study was generally *Bacillus* spp. followed by members of Enterobacteriaceae and *Micrococcus* spp.

The predominance of *Bacillus* isolates (this study) among the gram positive bacteria is in agreement with Guchi and Ashenafi [20]. Enterobacteriaceae isolates were the dominant microflora among gram negative isolates unlike the previous reports on lettuce and green pepper from Ethiopia which showed the predominance of *Pseudomonas* isolates, Guchi and Ashenafi [20]. From water samples, Ikpeme et al. [45] also reported the predominant genera that include *Bacillus* spp. (86.51%) followed by *Pseudomonas* spp. (71.23%) and *Aeromonas* spp. (52.58%) from Nigeria similar to the present study. Kwashie [34] also reported dominant bacteria such as Enterobacteriaceae*, Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp., and *Clostridium* spp. from soil samples irrigated with wastewater. This could be due to direct transfer of bacterial cells from wastewater to soil which may eventually be internalized into the vegetables indicating serious consumer's health risk when consumed raw.

The predominance of *Bacillus* spp. was possibly due to the presence of spores in the water and soil as well as other environmental factors. The survival of *Bacillus* depends on several factors such as nature of the organism, resistance to a new physical environment, and ability to form spores [46]. High number of *Bacillus* spp. could cause food poisoning. In addition, endospores of *Bacillus* are more resistant than the vegetative cells to harsh weather conditions and even to antimicrobial treatments [47]. Therefore, the presence of high percentage of *Bacillus* spp. in fresh vegetables could have consumer's health risk.

The predominant Enterobacteriaceae in the present study indicates that the water used for irrigation was heavily contaminated with fecal matter and received sewerage from diverse sources. In addition, fresh fruit and vegetables often carry high levels of Enterobacteriaceae as part of their normal flora, Gilbert et al. [39]. In the present study, *Micrococcus* spp. was among the dominant isolates due to its occurrence in the wastewater and soil [48]. This is in agreement with Guchi and Ashenafi [20] who reported that *Micrococcus* spp. are the second dominant microflora isolated from lettuce and green pepper in Addis Ababa, Ethiopia. *Micrococcus* spp. are common environmental bacteria that could be introduced into the fresh vegetables through cross-contamination, for instance, from wastewater used by the growers during irrigation. *Micrococcus* is generally thought to be a saprotrophic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune system, such as HIV patients [49].

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. [50] who reported higher prevalence of *S. aureus* (51.5%) from Lebanon. In addition, Ijabadeniyi [2] 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. [45] (25–33%) from two rivers that are used for irrigation of vegetables in South Africa.

Shigella was not isolated from any of the samples tested in the current study. This is in line with Soriano et al. [51], who reported (0.0%) *Shigella* in all of the lettuces served in Spain University restaurants. However, *Shigella* was isolated from 8 (12.5%) samples of lettuces and 16 (25.0%) samples of green peppers by Guchi and Ashenafi [20] 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 [20] and Ijabadeniyi [2] who reported 10% in lettuce and green peppers as well as 11% in broccoli and cauliflower, respectively.

In the present study, all the bacterial pathogens isolated from the surface water were also isolated from all vegetables even if their prevalence was varied similar to the reports by Ijabadeniyi [2] in South Africa. Diverse prevalence of pathogens in different samples shows the possibility of variations in surface characteristics of the produce affecting pathogen attachment and survival [52].

The antibiotic resistance patterns of *S. aureus* isolates in the current study showed low percentage of resistance to norfloxacin (5.6%), gentamycin (13.9%), and erythromycin (19.4%). This is partly similar to previous report by Donokor et al. [53] from Ghana. In the current study, all *S. aureus* isolates were resistant to penicillin G, ampicillin, and cefuroxime sodium (100.0% each). This finding is partly in agreement with Sina et al. [54].

In the present study, high number of *Salmonella* spp. was susceptible to norfloxacin, chloramphenicol, and gentamycin (100% each) followed by co-trimoxazole (93.5%). This result is fairly in line with Akbarmehr [55] who reported that *Salmonella* spp. were highly susceptible to chloramphenicol (100%) followed by gentamycin (91.89%). However, isolates of *Salmonella* spp. exhibited resistance to tetracycline, erythromycin, penicillin G, and cefuroxime sodium (100% each) followed by ampicillin (90.3%). Cardoso et al. [56] have also reported 100% resistance of *Salmonella enteritidis* to both tetracycline and erythromycin from Brazil. The marked resistance of strains of *Salmonella* spp. to ampicillin as shown

in the present study agrees with the findings of Ash et al. [57] and Ikpeme et al. [45], working on rivers in the United States and Nigeria, respectively.

In this study, the two bacterial pathogens investigated (*S. aureus* and *Salmonella* spp.) showed high levels of multiple drug resistance. This trend has also been reported especially in the developing world [58, 59].

Generally, the surface water pollution in this study may have originated from both human and animal sewage disposal by the informal settlement that lacks proper sanitation. According to van Vuuren [60], lack of proper sanitation usually leads to disposal of both human and animal wastes in the wrong places including surface water. Almost any ready-to-eat vegetables that have been contaminated with pathogens either from the environment or from human or animal faeces or through storage, processing, and handling could potentially cause disease [4]. As a result consumption of these vegetables with elevated levels of bacterial pathogens may lead to health disorders. Thus regular monitoring of microbial contamination of vegetables grown using wastewater is necessary and consumption of contaminated vegetables should be avoided in order to reduce the health risk.

5. Conclusion

The present study showed the potential hazard of fresh vegetables collected from Jimma town which were irrigated with Awetu River. The hygienic quality of both water and vegetable samples was poor since higher mean bacterial counts were recorded beyond the standard safe limits. Microflora of vegetable and water samples was dominated by *Bacillus* and Enterobacteriaceae. The presence of high number of pathogenic bacteria such as *S. aureus* and *Salmonella* spp. could cause foodborne diseases. Ampicillin, penicillin G, and cefuroxime sodium were the most resistant antimicrobial agents by *S. aureus* and *Salmonella* spp. However, chloramphenicol, norfloxacin, and gentamycin are the drug of choice to be recommended for the treatment of both *S. aureus* and *Salmonella* spp. according to the *in vitro* assay of the present study. Wastewater should be properly treated when used for produce that may be eaten raw. This safety measure should be combined with Good Agricultural Practices during production of fresh vegetables. It is important to thoroughly wash vegetables and dip them in food grade antibacterial chemicals for a good time to eliminate pathogens and significantly reduce the microbial load.

Competing Interests

The authors declare that they have no competing interests.

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