

Full Length Research Paper

Assessment of microbiological quality and drug resistance patterns of raw vegetables irrigated with Hasassa River, West Arsi Zone, Oromia Region, Ethiopia

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Irrigation scheme is among the major transmission modes of enteric human pathogens. Irrigation of vegetables with polluted water and untreated wastewater is a common practice worldwide. This practice is common in urban areas of low-income countries including Ethiopia, where there is increasing demands for fresh vegetables with poor wastewater treatment. The aim of the present study was to assess the microbial quality and prevalence of antimicrobial resistance of bacteria isolated from vegetables irrigated with Hasassa River, Southeastern Ethiopia. Irrigation water and vegetable samples (carrot, lettuce, and garlic) were collected from irrigation sites and analyzed for their bacteriological load and presence of any pathogenic microbes. The resistance patterns were detected following standard procedures. Appropriate serial dilutions of the suspension from 10^{-4} , 10^{-5} and 10^{-6} were spread-plated on a suitable solid media for counts of aerobic Mesophilic bacteria, Gram-negative Enterobacteriaceae and Staphylococci, and homogenized samples were heated at 80°C for 10 min in a water bath to count aerobic spore formers, respectively. The maximum overall mean counts of aerobic Mesophilic bacteria, Enterobacteriaceae, aerobic spore formers, Staphylococci, and total coliform counts were 8.21, 6.58, 6.88, 6.88 log CFU ml^{-1} and >1100 MPN 100 ml^{-1} , respectively. The microflora of vegetable samples were dominated by *Bacillus* species (21.9%) followed by *Corynebacterium* species (12.5%), *Lactobacillus* species (12.5%). *Staphylococcus aureus* and *Salmonella* species were detected in 21.9 and 15.6% of the samples, respectively. The result of antimicrobial test showed that all the isolates 32 (100%) were resistant to Penicillin, 26 (81.3%) to Vancomycin, 23 (71.9%) to Ampicillin, 15 (46.9%) to Chloramphenicol, 15 (46.9%) to Erythromycin, and the least 3 (9.4%) to Perfloxacin. The present finding revealed that vegetables irrigated with Hasassa River appears to pose microbial contamination which may be transferred directly or indirectly during pre-harvest and post-harvest handling to fresh vegetables which potentially constitutes a health risk to consumers.

Key words: Antibiotic resistance, Hasassa River, irrigation water, vegetables.

INTRODUCTION

Despite the fact that fresh and minimally processed vegetables and fruits provide the most important human

diet, consumption of fresh vegetables and fruits has been associated health risk for consumers. It has been

reported that disease outbreaks in recent years have been linked to lettuce, tomatoes, spinach, and seed sprouts particularly when freshly contaminated by foodborne pathogens (Jung et al., 2014). Surveillance of vegetables has indicated that these foods can be contaminated with various bacterial pathogens, including Shiga toxin-producing *Escherichia coli* (STEC), *Staphylococcus aureus*, *Salmonella* species, *Shigella* species, *Listeria monocytogenes*, and *Campylobacter* species (Adane and Tsehayneh, 2017).

The sources of those pathogens and other microbes traverse the continuum from farm to plant and include contaminated agricultural water, soil amendments, contaminated harvesting equipment, field workers, processing plants and retail handling (Jung et al., 2014). As major component of agriculture, irrigation is considered as one of the most important transmission modes of enteric human pathogens to human through consumption of vegetables (Adane and Tsehayneh, 2017). It has been reported that irrigation of vegetables with polluted water and untreated wastewater is practiced worldwide. This practice is most common in the urban areas of low-income countries, which have no capacity to effectively treat wastewater and face increasing demands for fresh vegetables (Keraita et al., 2007). Keraita et al. (2007) also pinpointed that wastewater provides water and nutrients as important resources for irrigation, but has high levels of pathogens. The major pathogens associated with the use of highly polluted water are the fecal coliforms, *E. coli* and eggs of some helminthes such as *Ascaris lumbricoides*, *Trichuris trichiura* and others (Samuel et al., 2013).

Several studies reported the association of health risk and microbiological quality of irrigated vegetables using ponds or rivers. Studies in Jimma town, Western Ethiopia reported that the microflora of vegetables and irrigation river samples was dominated by *Bacillus* species (32.7%) followed by Enterobacteriaceae (25%) and Micrococcus (16%). Other pathogens such as *S. aureus* and *Salmonella* spp. were detected in 24.0 and 20.7% of the samples, respectively (Desta and Diriba, 2016). The study also suggested that 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.

It has been observed that Hasassa 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 Hasassa River because of discharge of municipal wastes and urban runoff has been well observed. However, in Hasassa River, no study was reported on microbial

quality of irrigated vegetables and irrigation water. Therefore, this study was initiated to assess the microbial quality of raw vegetables and their composition and prevalence of antimicrobial resistance patterns including multiple drug resistance (MDR) of the isolate bacteria identified from fresh vegetable irrigated with Hasassa River in west Arsi zone, Southeastern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Hasassa, West Arsi zone in Oromia Regional State of Southeastern Ethiopia. It is located at 7°10'N 39°10'E / 7.167°N 39.167°E (Figure 1). Hasassa River starts from the center of the town flowing to Melka Wakena Dam, which was built on Wabe Shebelle River. In Hasassa town and the surrounding areas, the river has been used for various domestic purposes including small-scale agricultural irrigation of vegetables and fruits.

Sample collection

Laboratory based cross sectional study design was used. A total number of 12 samples including three each vegetable (lettuce, garlic, and carrot) were randomly picked from both leaf and root aseptically cut into pieces by using 90% ethanol sterilized scissors. All vegetable samples were collected aseptically in sterilized plastic bags and transported to Adama Science and Technology University. The samples were processed for bacteriological analysis within 1 to 8 h.

Samples from irrigation water

Three irrigation water samples were collected from Hasassa River at different sites. A total of 0.75 L water samples were collected at the open surface flowing along the river course at the same time of the irrigation period. Samples were aseptically collected in sterile glass bottles maintained at 4°C in a cooler box and taken to the Laboratory of Microbiology, Department of Applied Biology, Adama Science and Technology University.

Sample processing

Each vegetable samples (unprocessed and large sized) were aseptically chopped into smaller pieces. A 25 g of subsample of each vegetable was aseptically weighed and vigorously shaken in 225 ml of sterile 0.1% (w/v) peptone water for 3 min to homogenize the samples. They were prepared to appropriate serial dilutions from 10^{-4} , 10^{-5} and 10^{-6} . They were spread-plated on a suitable solid media and incubated aerobically at 37°C for 24 to 48 h. Similar procedures were followed for water samples.

Bacterial counts

A volume of 0.1 ml aliquot of appropriate dilution was spread-plated in duplicates on pre-solidified Plate Count Agar, MacConkey Agar and Mannitol Salt Agar for counts of aerobic Mesophilic bacteria,

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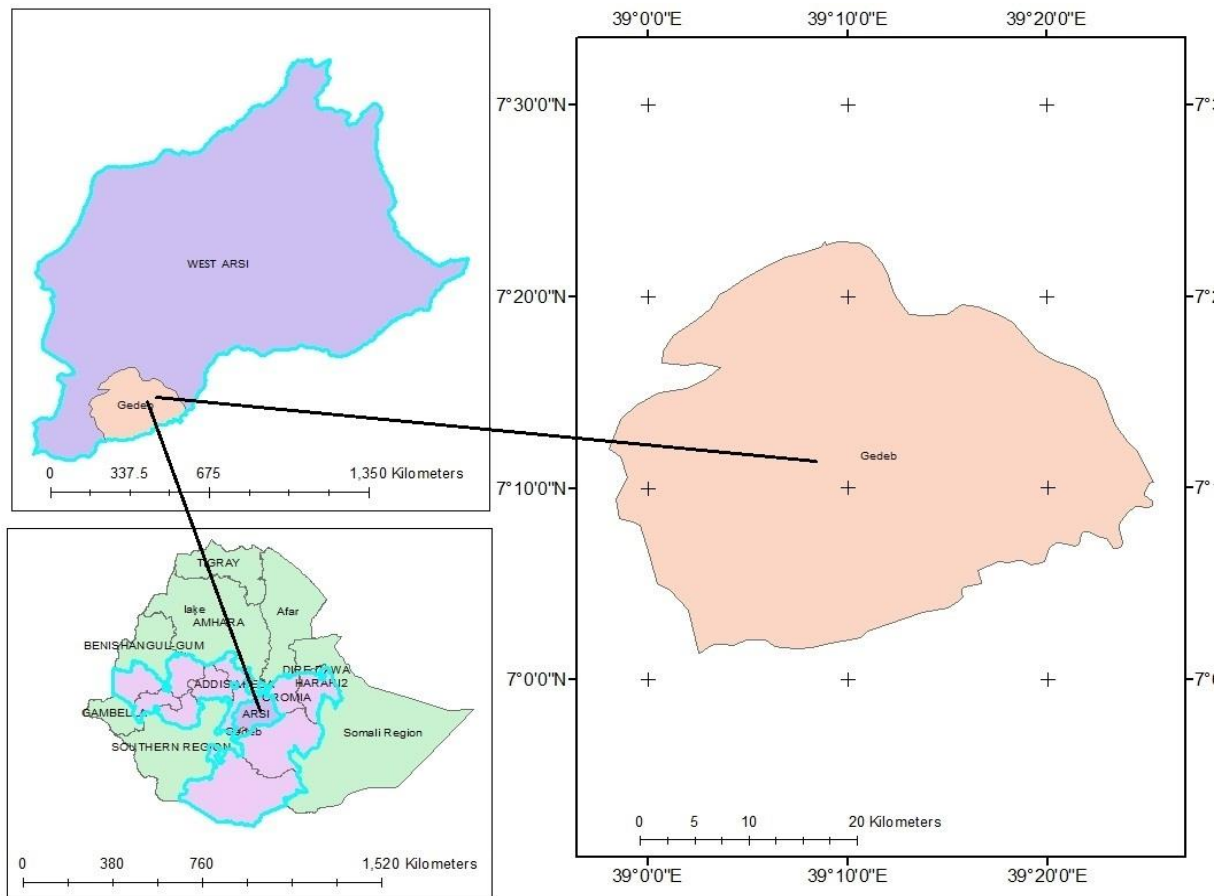


Figure 1. Map of study area with its longitude and latitude during April-July, 2018

Enterobacteriaceae and Staphylococci, respectively. Homogenized samples were heated at 80°C for 10 min in a water bath to count aerobic spore formers. The inoculated plates were incubated at 32 to 37°C for 24 to 48 h. The mean colony counts on each given dilution were used to estimate the total viable count for the samples in colony forming units (CFU ml⁻¹). Liquid sample to be tested was diluted serially and inoculated in lactose broth. The number of total coliforms was determined by counting the number of tubes giving positive reaction (both color change and gas production) compared with standard statistical tables and the results were expressed as MPN 100 ml⁻¹.

Isolation and characterization of dominant microflora

After enumeration of aerobic Mesophilic bacteria, 21 colonies with distinct morphological characteristics of colonies such as color, size, and shape were randomly picked from countable plates and aseptically transferred into a tube containing 5 ml nutrient broth and incubated at 30°C for 24 to 48 h. The cultures were purified by repeated plating and pure cultures were temporarily preserved on nutrient agar slants at 4°C. An overnight activated culture of each isolates was further characterized by inoculating to the following standard tests such as cell morphology, endospore staining, Gram staining, motility, catalase, indole test, urease test, carbohydrate fermentation/utilization test, hydrogen sulfide production (H₂S), methyl red (MR) test, citrate utilization test, and oxidation fermentation (O/F) according to the protocol used by Oluyeye et al.

(2015) to differentiate into genus and family levels.

Isolation and identification of bacterial pathogens

For the identification of *S. aureus*, yellow colonies on Mannitol Salt Agar plates were aseptically picked and transferred into 5 ml nutrient broth and incubated at 37°C for 24 to 36 h for further purification. Then, a loop of culture from the nutrient broth was streaked on pre-solidified surface of nutrient agar supplemented with 0.75% sodium chloride and again incubated at 37°C for 24 to 36 h. Finally, the distinct colonies were characterized using the established microbiological methods such as Gram staining and preliminary biochemical tests (the catalase).

For isolation of *Salmonella* spp., 25 g or 25 ml of each sample was aseptically transferred into sterile flask, containing 225 ml buffered peptone water (BPW), and inoculated onto the *Salmonella-Shigella* (SS) agar and incubated at 35°C for 24 h. The presumptive *Salmonella* colonies were then sub-cultured by streaking onto the fresh SS agar and incubated for 24 h at 37°C. Since the selective media was used for the isolation, the presumptive *Salmonella* isolates were identified by two confirmatory biochemical tests, triple-sugar-iron (TSI) agar test. The presumptive *Salmonella* colonies were directly stabbed into the TSI agar slant and incubated with loosened caps for 24 h at 37°C. For the urease test, two loopful of pure and well-isolated *Salmonella* colonies were inoculated into the urea broth. The inoculated tubes were incubated with loosened caps for 48 h at 35°C in an incubator. The TSI agar was checked

Table 1. Bacterial counts from irrigation water and vegetable samples irrigated with Hasassa River during April-July, 2018

Samples type	Average of total viable bacteria in log of CFU mL ⁻¹ ± S.D				
	AMB	Enterobacteriaceae	ASF	Staphylococci	Total coliform MPN100 ml ⁻¹
Carrot	7.62 ± 0.40	5.59 ± 0.11	6.58 ± 0.58	6.38 ± 0.7	>1100 ± 0
Lettuce	8.21 ± 0.12	6.58 ± 0.58	6.88 ± 0.49	6.88 ± 0.52	813 ± 496.5
Garlic	6.76 ± 0.55	5.95 ± 0.66	5.68 ± 0.45	6.12 ± 0.57	191 ± 84.9
Irrigation water	7.09 ± 0.16	6.05 ± 0.49	6.77 ± 0.53	6.40 ± 0.61	>1100 ± 0

AMB: Aerobic mesophilic bacteria; ASF: Aerobic spore formers.

for the production of hydrogen sulphide (H₂S) gas, while the urease test was checked for the degradation of urea.

Antimicrobial susceptibility testing

Once the bacteria were isolated and identified from each sample, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates. Bacterial inocula were prepared by suspending the freshly grown bacteria in 4 to 5 ml sterile nutrient broth and the turbidity was adjusted to that of a 0.5 McFarland standard prepared from adding 0.5 mL of 1.0% BaCl₂ to 99.5 mL of 1% H₂SO₄ solution. The antimicrobial susceptibility testing was performed using Mueller-Hinton agar medium against selected antibiotics, namely: Chloramphenicol 30 µg, Penicillin G 10 µg, Vancomycin 30 µg, Erythromycin 15 µg, Perfloracin 5 µg, and Ampicillin 10 µg were obtained from pharmacy. The bacterial suspension was aseptically streaked uniformly on the entire agar surface using sterile cotton swab in three different directions by rotating the plates at 60° angles after each streaking. The Petri plates were left to dry for 20 min. Afterwards, the antibiotics containing discs were placed on the agar surface with sterile forceps and pressed carefully down to ensure contact. The plates were incubated aerobically at 35°C for 18 to 24 h. The plates were examined after incubation for diameters of zone of inhibition around the discs, which was measured using ruler (mm). The data obtained was interpreted with reference to CLSI (2013). 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. *E. coli* (ATCC25922) and *S. aureus* (ATCC6538) standard strains from Ethiopian Institute of Biodiversity were used as reference strains for quality control of the antibiotics used.

Data analysis

Bacterial counts were calculated as colony forming units per milliliter (CFU ml⁻¹) and converted into log₁₀ values before computing means and standard deviations. Total coliform was expressed by MPN100 ml⁻¹. Then mean of bacterial counts and standard deviation was calculated using Microsoft Excel. The statistical analysis was performed by single factor (ANOVA) at $p < 0.05$.

RESULTS

Bacterial counts

The mean bacterial counts (log CFU ml⁻¹) of aerobic

Mesophilic bacteria, Enterobacteriaceae, aerobic spore formers, and Staphylococci were determined for the three vegetable and irrigation water samples. The average microbial counts of different vegetable samples of selected sites ranged from 5.59 to 8.21 log CFU ml⁻¹ and MPN of total coliforms count and their overall mean in vegetables ranged from 191 to >1100 and in irrigation water total coliform was >1100 (Table 1). Mean counts of all bacterial groups including coliforms identified from the samples showed statistical significance at $p < 0.05$.

Analysis of microflora and the pathogens

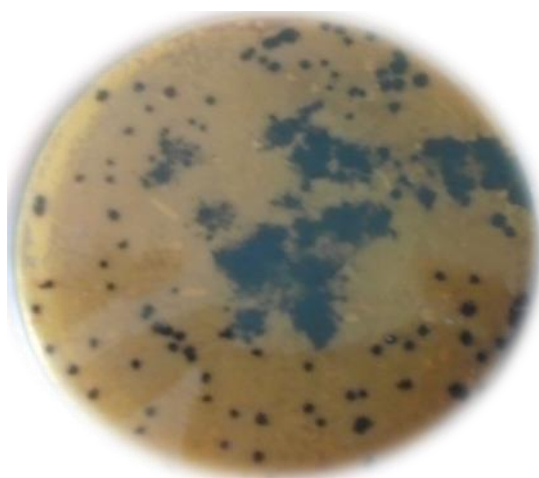
In the present study, eight genera of Gram positive and Gram-negative bacterial species were identified. Aerobic mesophilic bacterial flora identified from the water samples and different vegetables collected from the downstream of Hasassa River were dominated by *Bacillus* spp. (21.88%) followed by *Corynebacterium* species (12.5%) and *Lactobacillus* species (12.5%) and the least isolation was for *Neisseria* species (3.13%) (Table 2). Lettuce was the vegetable most contaminated, followed by the samples of carrot, irrigation water and garlic (Table 2).

S. aureus was observed fermenting Mannitol and producing a yellow zone surrounding colony on MSA plates from which isolated pure colonies were confirmed by salt tolerance and catalase test (Figure 2). Other colonies displayed typical *Salmonella* spp. morphological characteristics on *Salmonella-Shigella* agar, which were clearly with a black spot in the center due to H₂S gas production on *Salmonella-Shigella* agar (Figure 2). The presumptive colonies were scored to be *Salmonella* spp. after the colonies showed positive reaction to triple sugar iron test and negative to urease test.

S. aureus and *Salmonella* spp. isolated by special selective media accounted for a percentage of 21.88 and 15.6%, respectively, of the total isolates (Table 2). The distribution of these pathogens varied depending on the nature of vegetables. With regard to sample types, higher proportion (40.0%) of *Salmonella* spp. was encountered in lettuce while same proportion (28.6%) of *S. aureus* was identified from lettuce, garlic, and irrigation water, but relatively less proportion (14.2%) from carrot (Table 2).

Table 2. Prevalence of bacteria isolated from irrigation water and vegetables in Hasassa during April-July 2018.

Isolate	Sample				Total isolates	Prevalence (%)
	Irrigation water	Carrot	Garlic	Lettuce		
<i>E. coli</i>	1 (50)	1 (50)	-	-	2 (100)	6.25
<i>Bacillus</i> spp.	-	2 (31.7)	1 (14.3)	4 (57.1)	7 (100)	21.88
<i>Corynebacterium</i> spp.	2 (50)	1 (25)	1 (25)	-	4 (100)	12.5
<i>Streptococcus</i> spp.	1 (50)	1 (50)	-	-	2 (100)	6.25
<i>Staphylococcus aureus</i>	2 (28.6)	1 (14.2)	2 (28.6)	2 (28.6)	7 (100)	21.88
<i>Salmonella</i> spp.	1 (20)	1 (20)	1 (20)	2 (40)	5 (100)	15.6
<i>Neisseria</i> spp.	-	-	-	1 (100)	1 (100)	3.13
<i>Lactobacillus</i> spp.	-	1 (25)	1 (25)	2 (50)	4 (100)	12.5
Total	7 (21.88)	8 (25)	6 (18.75)	11 (34.37)	32 (100)	100

Black colonies on *Salmonella-Shigella* agar

Yellow colonies on MSA agar

Figure 2. Presumptive *Salmonella* spp. on SS agar and *Staphylococcus aureus* on mannitol salt Agar from Hasassa River and irrigated vegetables during April to July, 2018.

However, the prevalence of all bacterial microflora and pathogens was not significantly different at $p < 0.05$ with respect to sample types.

Antimicrobial resistance patterns of bacterial isolates

The proportion of antibiotic resistance was observed for 32 isolates against six different antibiotics of which the highest 32 (100%) isolates were resistant to penicillin g 10 µg followed by Vancomycin 30 µg with 26 (81.25%) and the least 3 (9.4%) to Perfloracin 5 µg (Table 3).

Multiple drug resistance patterns of bacterial isolates

Patterns of multiple drug resistance (MDR) among bacterial isolates of the present study varied from two to six antibiotics. The maximum MDR was recorded to all

the six antibiotics under experiment, namely Vancomycin (Van), Chloramphenicol (Chlora), Penicillin (Penic), Erythromycin (Eryth) Ampicillin (Ampic) and Perfloracin (Perf), followed by all of the antibiotics except Perf (Table 4). The highest MDR patterns were observed in *Bacillus* spp., *S. aureus* and *Salmonella* spp. respectively, with combination of (Van, Ampic), (Penic, Ampic), and (Van, Peni) antibiotics (Table 4).

DISCUSSION

Microbial groups that belong to eight genera were isolated from the examined samples at varying percentages with Gram-negative flora accounting for 75 and 25%, respectively. The Gram-positive cells were represented by bacteria from the genera: *Bacillus* spp., *Lactobacillus* spp., *Corynebacterium* spp., *S. aureus*, and *Streptococcus* species, while the Gram-negative microflora constituted

Table 3. Proportion of antibiotic resistance among bacterial isolates identified from vegetable samples and irrigation water in Hasassa during April-July, 2018.

Identified isolate	Total (%)	Vancomycin 30 µg	Chloramphenicol 30 µg	Perfloxacin 5 µg	Penicillin g 10 µg	Ampicillin 10 µg	Erythromycin 15 µg
<i>Bacillus</i> spp.	7 (100)	5 (71.4)	2 (28.6)	-	7 (100)	4 (57.1)	1 (14.3)
<i>E. coli</i>	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)
<i>Corynebacterium</i> spp.	4 (100)	3 (75)	1 (25)	1 (25)	4 (100)	3 (75)	-
<i>Streptococcus</i> spp.	2 (100)	1 (50)	2 (100)	-	2 (100)	2 (100)	-
<i>Lactobacillus</i> spp.	4 (100)	3 (75)	3 (75)	1 (25)	4 (100)	1 (25)	3 (75)
<i>Neisseria</i> spp.	1 (100)	1 (100)	1 (100)	-	1 (100)	1 (100)	1 (100)
<i>Staphylococcus aureus</i>	7 (100)	6 (85.71)	3 (42.86)	-	7 (100)	7 (100)	6 (85.71)
<i>Salmonella</i> spp.	5 (100)	5 (100)	1 (20)	-	5 (100)	3 (60)	3 (60)
Total	32 (100)	26 (81.25)	15 (46.9)	3 (9.4)	32 (100)	23 (71.9)	15 (46.9)

Table 4. Multiple drug resistance patterns in isolates identified from irrigation water and vegetables in Hasassa during April-July, 2018.

Isolate	No. of antibiotics	Antibiotic resistance pattern observed	No. of isolates that demonstrated resistance
<i>Bacillus</i> spp.	5	Van, Chlora, Peni, Eryth, Ampic	1 (7)
	4	Van, Chloram, Penic, Ampic	2 (7)
	3	Van, Peni, Ampic	4 (7)
	2	Van, Ampic	5 (7)
<i>E. coli</i>	6	Van, Chlora, Peni, Eryth, Ampic, Perf	1 (2)
	4	Van, Chlora, Penic, Ampic	2 (2)
	2	Van, Penic	2 (2)
<i>Corynebacterium</i> spp.	5	Van, Perf, Peni, Chlora, Ampic	1 (4)
	3	Ampi, Peni, Van	3 (4)
<i>Streptococcus</i> spp.	3	Van, Peni, Ampic	1 (3)
	2	Penic, Ampic	2 (3)
<i>Lactobacillus</i> spp.	6	Van, Chlora, Peni, Eryth, Ampic, Perf	1 (4)
	4	Van, Chlora, Peni, Eryth	3 (4)
<i>Neisseria</i> spp.	5	Van, Chlora, Peni, Eryth, Ampic	1 (1)
<i>S. aureus</i>	5	Van, Chlora, Peni, Eryth, Ampic	2 (7)
	4	Eryth, Peni, Van, Ampic	4 (7)
	2	Penic, Ampic	5 (7)
<i>Salmonella</i> spp.	5	Van, Chlora, Peni, Eryth, Ampic	1 (5)
	4	Eryth, Ampic, Van, Peni	3 (5)
	2	Van, Peni	5 (5)

Vancomycin =Van, Penicillin =Peni, Ampicillin =Ampic, Chloramphenicol = Chlora, Perfloxacin = Perf, Erythromycin =Eryth.

members of *E. coli*, *Neisseria* spp., and *Salmonella* spp. This finding is partly similar to the previous reports by

Ankita et al. (2014) in raw fruits and vegetables from India.

The predominant microflora of fresh vegetables in the present study was *Bacillus* spp. that occurred in all samples except in irrigation water, followed by *Corynebacterium* spp. and *Lactobacillus* spp. The predominance of *Bacillus* isolates in this study among the Gram-positive bacteria was in agreement with Desta and Diriba (2016). *Salmonella* spp. isolates were the dominant bacteria among Gram-negative isolates contrary to the previous reports by Biniam and Mogessie (2010) on lettuce and green pepper from Ethiopia which showed the dominance of *Pseudomonas* isolates.

Gram-positive bacteria could be higher effect in the spoilage of vegetables than Gram-negative bacteria. From wastewater-irrigated and manure-treated farmland of Nigeria samples, Oluyeye et al. (2015) reported the predominant genera that include *Bacillus* spp. (33%), *S. aureus* and *Pseudomonas* species (15%). The presence of endospores that could make more resistant than the vegetative cells to harsh weather conditions and even to antimicrobial treatments may result in high percentage of *Bacillus* spp.

Corynebacterium spp. is inhabitants of the soil. Predominance of *Corynebacterium* spp. in the present study may come from the soil or from fecal-contaminated water used for irrigation and received sewage from diverse sources. Ankita et al. (2014) also reported dominant bacteria such as *Corynebacterium* spp., *Staphylococcus* spp., *Bacillus* spp., *Streptococcus* spp., *Lactobacillus* spp. and *Pseudomonas* spp. respectively, from raw fruits and vegetables. Oladele and Olakunle (2011) reported that the presence of *Bacillus subtilis*, *Serratia marcescens*, *Lactobacillus* spp. and *Proteus mirabilis* on the deteriorated spinach samples may be linked to the fact that these microbes are widely distributed in air, dusts and soils.

The prevalence of *S. aureus* in the current study was 21.9% lower as compared to 51.5% of the report of Halablab et al. (2011) from Lebanon. The presence of *S. aureus* (28.6%) in the irrigation water in this study was lower than those obtained by Ikpeme et al. (2011) (25 to 33%) from two rivers used for irrigation of vegetables in South Africa. On the other hand, the overall mean count of staphylococci from vegetable samples ranged from 6.12 to 6.88 log CFU ml⁻¹. This is higher than the bacterial count of 4.55 and 4.97 log CFU ml⁻¹, reports on lettuce and green pepper, respectively from super market in Addis Ababa, Ethiopia (Biniam and Mogessie, 2010). The relatively higher counts of staphylococci from the present study may be due to skin contact and environmental contamination. It has been reported that *S. aureus* is a dangerous pathogen with which surface of vegetables may be contaminated through human handling and other environmental factors and which is also able to survive for several weeks (Halablab et al., 2011). Human skin and nasal cavity are the main reservoir of staphylococci.

In the present study, the prevalence of *Salmonella* spp.

in all vegetable samples was higher (15.6%) as compared to previous reports by Biniam and Mogessie (2010) which reported 10% in lettuce and green peppers. This difference might be attributed to the variation of the test techniques employed (pre-enrichment steps), the origin of sample or geographical differences and difference in management practice. The presence of *E. coli* can represent the existence of fecal pathogens like *Salmonella* and *Shigella*. Thus, it can be good indicator of poor sanitary conditions of sources of water used and the use of water with fecal contamination.

In the present study, another important point was antimicrobial resistance rate was high and this may cause a serious challenge to the management of common infections. The overall resistance rates among the bacteria isolates remarkably, demonstrated 100% resistance to Penicillin g, 81.25% to Vancomycin, 71.9% to Ampicillin, and 46.9% were resistant to Chloramphenicol and Erythromycin, while least resistance (9.4%) was observed for Perfloraxilin. Similar study reports from two Spanish Lakes showed 71% resistance of isolates to at least one antibiotic including Penicillin (68.9%), Erythromycin (31.1%) and Chloramphenicol (22.2%) (Maria, 2013). Similarly, Golly et al. (2016) reported that all the Gram-positive bacteria isolates showed 100% overall total resistance to Penicillin, Ampicillin and Perfloraxilin. The antibiotic resistance pattern obtained in this study is a serious challenge to public health because of the higher demanding for raw vegetables in different homes, societies and functions.

Twenty different multiple resistance patterns were observed when analyzed with the number of antibiotics (Table 4). Only two isolates (*E. coli* and *Lactobacillus* spp.) were resistant to all six antibiotics tested and six isolates were resistant to only five antibiotics. Relatively higher proportion 19 (55.9%) of isolates showed resistance to any of the two antibiotics and 14 (41.1%) of them were resistant to four antibiotics, 8 (23.53%) of them were resistant to Vancomycin, Penicillin and Ampicillin, respectively. Multi antibiotic resistance (MAR) bacteria which were isolated also showed some level of resistance to almost all antibiotics tested. Such multi antimicrobial resistance patterns clearly indicate vegetables were potential vehicle for microbial food poisoning as well as a source of infectious diseases that cannot be treated with commonly used antibiotics.

There have been reports that bacteria are able to gain antibiotic resistance via mechanisms like mutational changes or acquisition of resistance genes through horizontal gene transfer from other bacteria or phages in different environments (Vaz-Moreira et al., 2014; Munita and Arias, 2016). The resistance ability of the isolates can be transferred from to another, through the antibiotic resistance plasmids (Olsen et al., 2004). Long ago, bacteria resistant to multiple drugs were found mostly in hospitals, where antimicrobial agents were used most extensively, however, resistance is currently found

anywhere almost as frequently in the community. This is therefore to emphasize the need to be given to good hygienic practices, proper handling, storage and retail of fresh vegetables in a sanitized environment.

Concerning the level of contamination among the target vegetables, the highest bacterial load in lettuce samples probably due to the larger surface area exposed to irrigation water. This is in line with the work of Halablab et al. (2011) which reported that lettuce carried higher incidence of *E. coli* and *S. aureus* organisms (42.30 and 50%) than parsley samples (13.80 and 37.93%), and the higher microbial loads on lettuce samples than parsley counterpart may be due to the large surface area of the former leaves. Other study also showed that high bacteria counts could likely be associated with the morphology of leaves which have a broad and rather rough surface, indeed, in both vegetables their large surface made easily coming in contact with the ground and the irrigation water (Cinzia et al., 2015).

The overall mean aerobic mesophilic count observed in the present study ranged from 6.76 to 8.21 log CFU ml⁻¹, relatively higher than previous reports (6.94 to 8.06 log CFU ml⁻¹) from Ethiopia, by Desta and Diriba (2016). However, lower bacterial counts that ranged from 6 to 7 log CFU ml⁻¹ were reported by Daniele et al. (2013).

The overall mean count of Enterobacteriaceae in the present study ranged from 5.56 to 6.88 log CFU ml⁻¹. This was lower than previous study conducted in Ethiopia by Desta and Diriba (2016) on lettuce, carrot and tomato which ranged from 6.09 to 7.10 log CFU g⁻¹, but higher than the microbial load of lettuce and green pepper 5.08 and 4.84 log CFU g⁻¹, respectively as reported by Biniam and Mogessie (2010) in Ethiopia. According to Gilbert et al. (2000) 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). Biniam and Mogessie (2010) suggested that the high level of Enterobacteriaceae in vegetables might indicate that the water used for irrigation could be heavily contaminated with fecal matter from sewerage effluent.

In case of aerobic spore formers, the overall mean counts ranged from 5.68 to 6.88 log CFU ml⁻¹. In all vegetables, the counts were higher compared to reports by Biniam and Mogessie (2010) where the counts ranged between 3.47 and 3.50 log CFU ml⁻¹ in green pepper and lettuce, respectively, from Addis Ababa (Ethiopia).

The overall mean counts of total coliforms from vegetable samples in the present study were relatively lower except for carrot >1100 MPN 100 ml⁻¹ than the report of Nipa et al. (2011), who observed >1100 MPN 100 ml⁻¹ from salad vegetables. The observed difference in the counts could be attributed in part to the degree of original contamination, storage conditions, and the hygienic conditions of utensils and vegetables handlers. The total coliform counts from water samples in the

present study were >1100 MPN 100 ml⁻¹ which was higher than the WHO recommended standard. 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. The presence of coliforms might be attributed to cattle faeces and excretion by farmers and others who use the farm environment as toilet.

Conclusion

The present study revealed the potential hazard of raw vegetables (lettuce, carrot and garlic) collected from Gedeb Asassa areas, which were irrigated by Hasassa River. This study was the first to evaluate the microbiological quality of vegetables grown by Hasassa River, where these vegetables harbored high microbial loads including aerobic mesophilic bacteria, coliforms, Enterobacteriaceae, aerobic spore formers, and staphylococci might be due to irrigation by untreated contaminated water in that area. The large number of aerobic mesophilic bacteria, indicator organisms (coliforms and *E. coli*) and pathogens (*S. aureus* and *Salmonella* spp.) detected in the vegetable samples revealed that the contamination of these foods by pathogenic microorganism might present a potential health hazard to consumers in the area. The *in vitro* assay result of the present study showed that irrigation water and samples (lettuce, carrot and garlic) contained bacteria with multiple antibiotic resistance patterns. This may be caused by high concentrations of microorganisms, nutrients, and antibiotics found in contaminated water makes it a favorable environment for bacterial growth and horizontal gene transfer of resistant genes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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