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# Detections of antimicrobial resistance phenotypes and extended-spectrum beta-lactamase (ESBL)- producing *Salmonella* spps and *Escherichia coli* O157:H7 in raw vegetables and fruits from open markets in Jimma town, Ethiopia and evaluation of hygiene and handling practices of vendors

Ahmed Zeynudin[1], Teshome Degefa[1], Tariku Belay[1], Jiru Batu Mumicha[2], Abdusemed Husen[3], Jafer Yasin[4], Abdulhakim Abamecha[1], Andreas Wieser[5,6,7] and Mengistu Abayneh[1*]

## Abstract

**Objectives** Despite of the health benefits of consumption of fresh vegetables and fruits, this product could be associated with food-borne bacterial pathogens, including infections with antibiotic-resistant strains especially in developing countries due to limited in knowledge, and hygienic practices. This study was conducted to provide evidence data on the rates of *Salmonella* spp. and *E. coli* O157:H7 contamination, the antimicrobial resistance profile, and extended-spectrum β-lactamase (ESBL)-producing strains in fresh vegetables and fruits sold in open-air markets at Jimma town, southwest Ethiopia. In addition, this study provided data on the hygiene and handling practices of vendors, which can help as impute to improve food safety and safeguard public health. A total of 242 salad samples were collected from three different kebeles and examined for the presence of *Salmonella* spp. and *E. coli* O157:H7 in the microbiology laboratory of Jimma University by using conventional microbiological techniques.

**Results** Out of 242 samples tested, 12.8% (31/242) were contaminated with *Salmonella* spp. and *E. coli* O157. Of these, *Salmonella* spp. was detected in 10.7% (26/242) of the tested samples, whereas *Escherichia coli* O157:H7 was found in 2.1% (5/242) of samples. Fifty-three-point-8% of *Salmonella* spp. were resistant to ampicillin, 42.3% to co-trimoxazole, 46.2% to tetracycline, and 26.9% resistance was observed against each of ceftriaxone and cefotaxime. 40% of E. coli O157:H7 isolates were resistant against ampicillin, amoxicillin-clavulanic acid, and co-trimoxazole. Only one isolate was resistant to ceftriaxone and cefotaxime, and no resistance was observed against ceftazidime,

*Correspondence:
Mengistu Abayneh
menge.abay@gmail.com

Full list of author information is available at the end of the article

gentamicin, ciprofloxacin, chloramphenicol, and meropenem. Four *Salmonella* spp. and one *E. coli* O157:H7 isolate with a total of 5/31 (16.1%) isolates were confirmed as the ESBL producers. Multidrug resistance (MDR) was detected in 23.1% of *Salmonella* and 20.0% of *E. coli* O157:H7. Hygienic and handling practices of vendors were poor, which could contribute to contamination of vegetables and fruits in the area.

**Conclusions** Contamination of fresh salad vegetables with pathogenic bacteria could be a food safety concern in the study area. Hence, this finding suggests the need for attention by the concerned bodies to prevent the emergence and transmission of food-borne pathogens and antimicrobial-resistant strains through these food items in the study area.

**Keywords** Vegetables and fruits, Pathogenic bacteria, ESBL producers, Antibiotic resistance, Street vendors

## Background

The World Health Organization (WHO) and Food and Agriculture Organization (FAO) promote the daily consumption of fruits and vegetables as part of a healthy diet due to their high nutritional value [1]. On the other hand, the increased consumption of fruits and vegetables in recent years has been found to be accompanied by an increase in the number of human infections and outbreaks, as these can serve as reservoirs for pathogens or opportunistic pathogens. Antimicrobial resistance (AMR) can be transferred to humans through food, and fresh produce can be an ideal vector as it is often consumed raw or minimally processed [2, 3].

The microbiological contamination of foods of plant origin (e.g., fruits, vegetables, lettuce) that are consumed raw or undercooked can be contaminated with spoilage or pathogenic bacteria at any stage of the food chain, from primary production to consumption, and is responsible for foodborne illnesses, especially in developing countries, including outbreaks of disease caused by antimicrobial-resistant bacteria [4, 5]. For instance, the WHO says unsafe food causes 600 million cases of foodborne diseases worldwide and 420, 000 deaths, and some 30% of foodborne deaths occur among children under five years of age [6]. The prevalence of *Salmonella* on lettuce in developing countries was 6.4%, and in developed countries it was 2.8%, and the prevalence of *Escherichia coli* O157:H7 on lettuce was 4.1% [7]. Microbial contamination of fruits and vegetables regularly occurs in plantation fields through contact with soil, dust, contaminated irrigation water, and the use of raw sewage or manure fertilizers [8, 9]. The process of transporting these food products from the farm to households and to vending sites also contributes to the contamination of these fruits and vegetables, thus posing a serious problem in food safety [8, 9].

In Ethiopia, as it is a low- income country, facing multiple food safety challenges due to a lack of infrastructure and basic pre-requisites for food safety, such as clean water and environment, washing facilities, which compounded by limited implementation of food safety regulations [10]. Among some of clinically important foodborne pathogenic bacteria such as *Salmonella* spp. and *E. coli* O157:H7, showing high levels of resistance to most of the antibiotics prescribed in the country [10, 11]. For instance, the prevalence of *Salmonella* spp. detected in vegetables ranged from 0 to 10% [10], and the average prevalence of *E. coli* O157:H7 and *Salmonella*-related diarrhea in children and adults was 15.3% and 7.2%, respectively, and some of the factors associated with the infection were consumption of undercooked meat, raw vegetables, and/or fruit juice [12, 13].

To control infectious diseases, chemotherapy alone will not be enough; a concerted effort to limit and eliminate possible sources of infection is needed [14]. To address this, the periodic detection of infectious pathogens in food and related sources is a priority goal. Generating microbiological data of microbial contamination of vegetables and fruits is imperative to understand the burdens, guide the actions to prevent human health risks and other adverse consequences, and ensure produce safety on a sustainable scale. Therefore, this study was carried out to investigate the level of *Salmonella* spp. and *E. coli* O157:H7 contamination, antimicrobial resistance profiles, and extended-spectrum beta-lactamase (ESBL)-producing strains in fresh vegetables and fruits sold in open-air markets at Jimma town, southwest Ethiopia. Additionally, this study was conducted to provide evidences on the hygienic conditions and handling practices of vegetable and fruit vendors in a town. This preliminary data might help local authorities to achieve a better understanding of what may happen during the vegetables and fruits distribution from a farm grower to the market and to take further decisions to help both farmers and sellers for further consumer protection from developing diseases associated with the consumption of contaminated products with drug-resistant pathogens.

## Materials and methods

### Study design, area, and period

A descriptive cross-sectional study was carried out in three peri-urban areas (Hora Gibe, Bore, and Jiren markets) of Jimma town from first July to September 30, 2021. Jimma town is about 345 kilometers away from

the capital city, Addis Ababa, in the southwest direction. The town is located at 7° 40' North latitude and 36 ° 5' East longitudes, and the climate condition is relatively cool tropical monsoon climate with an average altitude of about 1780 m above sea level, a mean annual maximum temperature of 30 °C, and a mean annual minimum temperature of 14 °C. The annual rainfall ranges from 1138 mm to 1,690 mm. The fresh vegetables and fruits sold in these markets were brought from different agricultural areas found in the town and surrounding rural areas in a zone. As a result, diverse types of fruits and vegetables are frequently utilized as sources of food in the town.

### Data collection and processing

#### *Data on socio-demographic, hygienic and handling practices of vendors*

Face-to-face interviews and visual inspections of local vendors were made using structured questionnaire and checklist to collect data on predisposing factors for bacterial contamination of vegetables and fruits in the study areas. Socio-demographic characteristics of vendors as well as the hygienic and handling-related factors associated with bacterial contamination of fresh fruit and vegetables were collected from randomly selected vendors of over 15 years of age, and from those the required samples (the selected fruit and vegetables) were obtained. In addition, hygienic and handling practices of vendors and the characteristics of vending sites were assessed using observational checklists and closed-ended questionnaires. The required number of samples were collected for around 12 weeks, from first July to September 30, 2021.

#### *Data related to food safety knowledge*

The street vendor awareness on contamination of fresh fruit and vegetables with pathogens, on personal and environmental hygiene, and on proper handling and cleaning was also assessed by face-to-face interviews and visual inspections of local vendors using a structured questionnaire. The questions have three possible answers: *"yes"*, *"no"* and *"do not know"*. Each *"yes"* answer was awarded one point, with the other answers being awarded 0 points. The total questions have a maximum of 100 points, where a score of less than 50 was considered to indicate a low level of food safety knowledge, 50–75 denoted a satisfactory level, and better than 75 was considered good.

### Laboratory sample collection and transportation

A total of 242 samples containing four vegetable and two fruit types that are frequently consumed in the area were purchased weekly from randomly selected vendors in three selected markets. Variable numbers of samples: Lettuce ($n$=42), Cabbage ($n$=42), Green pepper ($n$=42), Tomato ($n$=40), Avocado ($n$=38), and Mango ($n$=38) were collected based on their abundance in markets. Nearly one sample of each vegetable and fruit type was collected per week per site for 12 weeks. Each sample was placed in a sterile polythene bag, properly labeled, and immediately transported to the Medical Microbiology Laboratory of Jimma University for bacteriological analysis. All samples were processed within 24 h after collection.

### Microbiological analysis

#### *Sample preparation and processing*

We performed the microbiological analysis according to standard procedures. Briefly, 25 g of each vegetable sample and fruit sample was properly collected, trimmed, and transferred into a sterile sample bag containing 225 mL of Buffered Peptone Water (BPW) (CM1049) and incubated at 37 °C for 20 h to obtain a pre-enrichment homogenate. For selective enrichment of *Salmonella* spps., 0.1 mL and 1 mL of pre-enrichment culture were added to 10 mL of Rappaport Vassiliadis soya (RVS) broth and 10 mL of Muller Kauffmann tetrathionate-novobiocin (MKTTn) broth, and the plates were incubated at 41.50 °C and 37 °C for 24 h, respectively. Using a 10 µl microbiological loop, each selected enrichment homogenate sample was streaked on Xylose Lysine Deoxycholate (XLD) and Brilliance *Salmonella* Agar (OXOID, CM1092B+SR0194). The plates were allowed to solidify, inverted, and incubated at 37 °C for 24 h for colony formation. *Salmonella* spps. were isolated and identified based on distinctive morphological properties and using appropriate biochemical tests such as Triple Sugar Iron (TSI) agar test, urease test, and Sulphite-Indole-Motility (SIM) tests as recommended by EN ISO 6579-1:2017/Amd 1:2020 and as described by Hendriksen, R.S. (2003) [15, 16].

#### *Escherichia coli* O157: H7 detection

About 1 mL of pre-enrichment homogenate samples were added into a tube containing 9 mL of Modified Tryptone Soya Broth with Novobiocin (CM0989+SR0181) and incubated for 18 h at 41.5 °C for selective enrichment [17, 18]. For selective isolation, 0.1 mL of selective enrichment samples were sub cultured on Cefixime-Tellurite Sorbitol MacConkey Agar (C-T SMAC) and on Cefixime-Rhamnose SMAC Agar (CR-SMAC) (CM1005+SR0191) and incubated for 24 h at 37 °C. Non-sorbitol fermenter, transparent/colorless with a weak pale brownish *E. coli* O157:H7 suspect colonies were further confirmed with indole production tests and *E. coli* O157:H7 antisera latex agglutination test kits (DR0620M), following the manufacturer's instructions. *Escherichia coli* O157 (CCUG 29889) and *Escherichia coli* (ATCC 25922) were

used as positive and negative controls for reproducibility of Sorbitol MacConkey agar plates, respectively.

#### Antimicrobial susceptibility test

Antimicrobial susceptibility testing was conducted by the Kirby Bauer disk diffusion method according to the guidelines of clinical laboratory standards [19]. The media was prepared according to the instructions of the manufacturer. Briefly, bacterial suspensions were prepared in tubes containing 0.9% (w/v) phosphate-buffered saline with turbidity adjusted to 0.5 McFarland standard. Using a sterile cotton swab, bacterial suspension was streaked uniformly on the surface of Muller-Hinton agar. Antibiotic disks (from Oxoid (Basingstoke, England) including ciprofloxacillin (CIP-5 µg), Gentamycin (GM-10 µg), Ceftriaxone (CRO-30 µg), ceftazidime (CZT-30 µg), cefotaxime (CXT-30 µg), trimethoprim-sulfamethoxazole (SXT-1.25/23.75$\mu$g), meropenem (MEM-10 µg), ampicillin (AMP-10 µg), amoxicillin-clavulanate (AMC-20/10µg) and chloramphenicol (CAF-30 µg) were placed on the surface of cultures. Finally, the diameter of the inhibition zone around the disks was measured after incubation of the plates at 37 °C for 24 h.

#### Phenotypic detections of ESBL production

The screening for ESBL producers was done by using a disc of ceftazidime (30 µg) and cefotaxime (30 µg). The zone of inhibition ≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime was considered as potential ESBL producers as recommended by CLSI [19]. The combined disc test was used for phenotypic confirmation of ESBL producers. The combination of ceftazidime and cefotaxime alone and in combination with clavulanic acid (10 µg) were used for the confirmation of ESBL-producing isolates. An increase in the zone of inhibition ≥ 5 mm for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was interpreted as positive for ESBL production. *E. coli* ATCC 25,922 and *Salmonella* Typhimurium ATCC 14,028 were used as reference strains for quality control. The strains were obtained from the Ethiopian Public Health Institute.

#### Data analysis and presentation

Data obtained from laboratory procedures were summarized and analyzed using a Microsoft Excel data sheet. Prevalence is expressed as the percent positive samples from the total samples tested. The numbers and proportions of positive samples and resistance isolates were presented using statistical tables.

## Result

#### Socio-demographic characteristics and hygienic practices of vendors

During the visits, a total of 83 vegetable and fruit vendors were checked to see if they had a formal training certificate on food safety and on their vegetable and fruit handling practices by using observational checklists. About 71 (85.5%) of them were female, and 32 (38.6%) were 25–34 years old. Most vendors 23 (27.7%) didn't complete their primary school, and none of them had any short course training on safe handling and processing of vegetables and fruits (Table 1).

In this study, only 30.0% of vegetable and fruit vendors had good knowledge on safety practices and sources of contamination. None of the vendors transported vegetables to the market or sold them under controlled

**Table 1** Socio-demographic characteristics of vegetables and fruit vendors in peri urban kebeles of Jimma town, southwest Ethiopia, 2021

| Characteristics | Category/Status | Number (%) |
|---|---|---|
| Gender | Male | 12 (14.5) |
| | Female | 71 (85.5) |
| Age in year | 15–24 | 17 (20.5) |
| | 25–34 | 32 (38.6) |
| | 35–44 years | 21(25.3) |
| | 45 and above years | 13 (15.7) |
| What is your highest level of education | No formal education | 23 (27.7) |
| | Primary | 42 (50.6) |
| | Secondary | 17 (20.5) |
| Have you ever received any short course training on food safety and on hygiene? | Yes | 83 (100.0) |
| | No | 0 (0.0) |
| Years of experience in selling vegetables and fruits | < 1 | 21(25.3) |
| | 1–2 | 27 (32.5) |
| | 3–5 | 22 (26.5) |
| | > 5 | 12 (14.5) |
| Vendor's knowledges of food safety | Good | 25 (30.0) |
| | Poor | 58 (70.0) |

temperature conditions. None of the vendors were washing and disinfecting their hands after handling money before handling vegetables again. About 36.1% of vendors placed their vegetables and fruits on dirty leaf materials, and 73.5% of them displayed openly without any protections during sale, which poses risk conditions for cross-contamination of fruits and vegetables. Washing of vegetables before display was practiced by 38.6%, among which 31.2% used stream water. In addition, 43.4% of them didn't use separate packing materials for each type of fruit and vegetable. Moreover, most vegetable and fruit vendors indicated that inadequate supply of clean water and lack of clean, protected vendor sites posed a challenge towards maintaining hygiene (Table 2).

### Frequency of isolated bacteria

A total of 242 fresh vegetable and fruit samples were collected from local open markets and examined for bacterial contamination. Of these 242 samples tested, 12.8% (31/242) were contaminated with at least one pathogenic organism, *Salmonella* spp. and *E. coli* O157:H7. Among the vegetables taken from open markets, lettuce 21.4% (9/42) was the most frequently contaminated vegetable, followed by the green pepper 16.7% (7/42), and cabbage 14.3% (6/42). Ten-point five present (10.5%) of avocado and 5.3% (2/38) of mango were contaminated with the pathogenic organisms *Salmonella* spp. and *E. coli* O157:H7 (Table 3).

**Table 2** Hygiene and handling practices of vegetables and fruit vendors in peri urban kebeles of Jimma town, southwest Ethiopia, 2021

| Characteristics | Category/Status | Number (%) |
|---|---|---|
| Where do you get your vegetables from? | Cultivated | 22 (26.5) |
| | Purchase | 61(73.5) |
| How do you package vegetables before transportation? | Unwashed plastic bags | 20 (24.1) |
| | Leafe materials | 30 (36.1) |
| | Washed plastic bags | 17 (20.5) |
| | Clean basket | 16 (19.3) |
| Do you transport vegetables and fruits to the market under refrigeration conditions? | No | 83 (100) |
| | Yes | 0 (00) |
| Do you wash vegetables before selling? | Yes | 32 (38.6) |
| | No | 51 (61.4) |
| If yes, where do you obtain after for washing vegetables? | Stream water | 10 (31.25) |
| | Pipe borne | 8 (25.0) |
| | Well water | 7 (21.9) |
| | Surface water | 4 (12.5) |
| | Municipal water | 3 (9.4) |
| Do you wash your hands with soap? | Yes | 17 (20.5) |
| | No | 66 (79.5) |
| Means of display | Floor | 25 (30.1) |
| | Shelf/table | 28 (33.7) |
| | Packaging material | 30 (36.1) |
| Do you sell all vegetables same day? | No | 48 (57.8) |
| | Sometimes | 35 (42.2) |
| How do you preserve leftovers? | On displaying material | 52 (62.7) |
| | At backyard | 31 (37.3) |
| | In refrigerator | 0 (0.0) |
| Have you stored/displayed vegetables and fruit in sealed containers? | Yes | 22 (26.5) |
| | No | 61 (73.5) |
| Hove you used separate container for each type of vegetable and fruit? | Sometimes | 47 (56.6) |
| | No | 36 (43.4) |
| Is there adequate supply of clean water nearest to your vending area? | Yes | 18 (21.7) |
| | No | 65 (78.3) |
| Did you wash your hands after handling money before handling vegetables again? | Yes | 0 (100) |
| | No | 83 (100) |
| Is your vending site being far from animal's contact? | Yes | 25 (30.1) |
| | No | 58 (69.9) |
| Do your environment around the vending site is far from rubbish and wastewater | Yes | 51 (61.4) |
| | No | 32 (38.6) |

**Table 3** Distribution of *Salmonella* spps and *E. Coli* O157:H7 from fresh vegetable and fruit samples collected from peri urban kebeles of Jimma town, southwest Ethiopia

| Vegetable/ fruit sampled | Number of samples tested | Bacterial species | | Total isolates *N* (%) |
|---|---|---|---|---|
| | | Salmonellaspp. *N* (%) | E. coliO157:H7 *N* (%) | |
| Lettuce | 42 | 7 (16.7) | 2 (4.8) | 9 (21.4) |
| Cabbage | 42 | 5 (11.9) | 1 (2.4) | 6 (14.3) |
| Green pepper | 42 | 6 (14.3) | 1 (2.4) | 7 (16.7) |
| Tomato | 40 | 3 (7.5) | 0 (0.0) | 3 (7.5) |
| Mango | 38 | 2 (5.3) | 0 (0.0) | 2 (5.3) |
| Avocado | 38 | 3 (7.9) | 1 (2.6) | 4 (10.5) |
| **Total** | **242** | **26 (10.7)** | **5 (2.1)** | **31** (12.8%) |

From the two pathogenic organisms recovered in this study, *Salmonella* spp. was detected in 10.7% (26/242) of the tested samples, whereas the overall detection rate of *Escherichia coli* O157:H7 was 2.1% (5/242). *Salmonella* spp. recovered in all types of samples, in which the detection rate was 21.4% (9/42) in lettuce samples, 16.7% (7/42) of green pepper, and 14.3% (6/242) of cabbage samples. *Escherichia coli* O157:H7 is detected in 2.8% (2/42) of sampled lettuce and in 2.4% (1/42) of each cabbage and green pepper samples, but not detected in sampled tomato and mango (Table 3).

### Antimicrobial resistance profiles

In this study, 53.8% of *Salmonella* spps were resistant to ampicillin, 42.3% to co-trimoxazole, 46.2% to tetracycline, and 26.9% resistance was observed against ceftriaxone and cefotaxime. In addition, 23.1% and 19.2% of *Salmonella* isolates were resistant to ciprofloxacin and gentamicin, respectively. From the total of five *E. coli* O157:H7 isolates, 2 (40.0%) were resistant against ampicillin, amoxicillin-clavulanic acid, and cotrimoxazole. Only one isolate was resistant to ceftriaxone and cefotaxime, and no resistance was observed against ceftazidime, gentamicin, ciprofloxacin, chloramphenicol, and meropenem (Table 4).

### Multidrug resistance (MDR) profile of isolates

Out of the 26 *Salmonella* isolated, 6 (23.1%) that were isolated from salad showed multidrug resistance, and only one isolate of *E. coli* O157: H7 showed multidrug resistance (Table 5).

**Table 5** Multidrug resistance (MDR) profile of isolates

| Bacterial isolates | Multidrug resistance (MDR) profile *N* (%) | | | Total MDR *N* (%) |
|---|---|---|---|---|
| | R3 | R4 | ≥ R5 | |
| *Salmonella* spp. (*n* = 26) | 3 (11.5) | 2 (7.7) | 1 (3.8) | 6 (23.1) |
| *E. coli* O157:H7 (*n* = 5) | 0 | 1 (20.0) | 0 | 1 (20.0) |
| **Total MDR** | 3 (9.7) | 3 (9.7) | 1 (3.2) | 7 (22.6) |

**Table 6** Frequency of ESBL detection by combination disk-method

| Bacterial isolates | Screening test positive | Confirmatory test positive |
|---|---|---|
| *Salmonella* spp. (*n* = 26) | 7/26 (26.9) | 4/26 (15.4%) |
| *E. coli* O157:H7 (*n* = 5) | 1/5 (20.0) | 1/5 (20.0%) |
| **Total N(%)** | 8/31 (25.8) | 5/31 (16.1%) |

### ESBL- producing isolates

A total of eight isolates (seven *Salmonella* and one *E. coli* O157:H7) fulfilled the screening criteria for ESBL production. Of these, four *Salmonella* and one *E. coli* O157:H7 isolate, with a total of 5/31 (16.1%) isolates, were confirmed as the ESBL producers (Table 6).

## Discussion

Vegetables and fruits are highly prone to contamination by microorganisms through contact with soil, water, and handling during harvest or after-harvest, and infections resulting from contamination of these products are serious health issues, particularly in underdeveloped nations like Ethiopia. In this study, the selected vegetable and fruit samples were taken from street vendors in three kebeles in Jimma town for the detection of pathogenic bacteria such as *Salmonella* spp. and *E. coli* 0157: H7 strain. Accordingly, a total of 12.8% of samples were contaminated with at least one of the pathogenic organisms, *Salmonella* spp. and/or *E. coli* O157:H7. *Salmonella* spps were detected in 10.7% (26/242) of the tested samples, whereas *E. coli* O157:H7 was detected in 2.1% (5/242) of the tested samples. The proportion of *Salmonella* positive samples in this study was lower than the previous study finding in different parts of Ethiopia: Mekelle

**Table 4** Antimicrobial resistance (AMR) profile of *Salmonella* spps and *E. Coli* O157:H7 isolated from fresh vegetable and fruit samples

| Detected bacterial species | Antibiotics tested and the rate of resistant (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMP (10 µg) | AMC (30 µg) | CTR (30 µg) | CTX (30 µg) | CZT (30 µg) | MRP (10 µg) | GN (10 µg) | CIP (5 µg) | SXT (25 µg) | TE (10 µg) | CAF (30 µg) |
| *Salmonella* spp (*n* = 26) | 14 (53.8) | 9 (34.6) | 7 (26.9) | 7 (26.9) | 5 (19.2) | 0 (0.0) | 5 (19.2) | 6 (23.1) | 11 (42.3) | 12 (46.2) | 6 (23.1) |
| *E. coli O157:H7* (*n* = 5) | 2 (40.0) | 2 (40.0) | 1(20.0) | 1(20.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (40.0) | 1 (20.0) | 0 (0.0) |
| **Total*N* = 31(%)** | 16 (51.6) | 11 (35.5) | 8 (25.8) | 8 (25.8) | 5 (16.1) | 0 (0.0) | 5 (16.1) | 6 (19.4) | 13 (41.9) | 13 (41.9) | 6 (19.4) |

Note: AMP: Ampicillin, AMC: Amoxicillin/clavulanic acid; CTR: Ceftriaxone; CTX: Cefotaxime; CAZ: Ceftazidime; GN: Gentamycin, CIP: Ciprofloxacin, SXT: Trimethoprim-sulfamethoxazole; MRP: Meropenem; TE: Tetracycline; CAF: Chloramphenicol

(22%) [20], Fiche, Oromia (15%) [21], and Arba- Minch (13.3%) [22], and in Nepal (35.2%) [23], but higher than a finding in China (1% & 3%) [24, 25]. Similarly, the detection of *E. coli* O157:H7 in this study was 2.1%, which is slightly higher than a finding in Ethiopia, Addis Ababa (0.51%) [26], Nepal (1.4%) [23], and India (1.3%) [27]. The expected differences in the prevalence of these bacteria might be related to the difference in hygienic conditions of vendors, which could be risky for cross-contamination of fruit and vegetables from different contact surfaces during processing, transportation, and displaying at vendor sites. Poor handling, the sources, transportation, and storage temperature and period of the fruits and vegetables could contribute to the multiplication of bacteria [27]. In addition, different methods of detection, in sample size, the type of sample, and how and when it was collected could contribute to the observed variations.

The detection of *Salmonella* and *E. coli* O157:H7 in 25 gm of foods means that it is risky for consumption. Vegetables grown in soil fertilized by animal manure have a greater chance to be contaminated with *E. coli* O157: H7 [28, 29]. *E. coli* O157: H7 may survive in the soil from 7 to 25 weeks depending on soil types, humidity level, and temperature. Our study revealed that relatively higher proportions of lettuce samples were positive for *Salmonella* spp., followed by green pepper, cabbage, and avocado. In addition, although *E. coli* O157:H7 was also isolated from certain types of fresh vegetables and fruit with relatively low prevalence, this microorganism can cause illness in consumers. This may be potentially hazardous to consumers, especially for individuals with reduced immunity like patients with HIV/AIDS, pregnant women, young children, and old people.

It is well documented facts that a lack of education and training on food safety may contribute to unhygienic practices such as improper handling, processing, and display of fruit and vegetables at the vendor area [30, 31]. In this study, none of the vegetable and fruit vendors have a formal education certificate and/or short course training on food safety, which could be risky for cross-contamination of the fruits and vegetables with *Salmonella* spp. and *E. coli* O157:H7. This condition is serious in our situation because of the widespread practice of raw vegetable and fruit consumption throughout the country. Therefore, the range of activities should be carried out with the appropriate training on knowledge and hygienic practices of fruit and vegetable handlers.

Fruits and vegetables have been identified as vehicles for the transmission of pathogenic antimicrobial-resistant (AMR) microorganisms [32, 33]. In this study, the antimicrobial resistance pattern of isolates was checked with different classes of antibiotics. Accordingly, a total of 8 (6.6%) *Salmonella* spp. and *E. coli* O157:H7 isolates were resistant to two or more classes of antibiotics. More than half (53.8%) of *Salmonella* spps were resistant to ampicillin, 42.3% to co-trimoxazole, and 46.2% to tetracycline. Relatively lower rate, 26.9% of *Salmonella* spps were resistant to each of ceftriaxone and cefotaxime, 19.2% to gentamycin, and 23.1% to each of ciprofloxacin and chloramphenicol. The best efficacies of the above antibiotics were also reported in previous study findings in Ethiopia with different resistance rates [20, 34–36]. In contrast, the higher resistance rate of *Salmonella* spp. against tetracycline, ampicillin, cefotaxime, amoxicillin-clavulanic acid, ciprofloxacin, and gentamycin was reported in Ethiopia (21, 38), China [25], and India [38], in which the rate of resistance varies from 28.8 to 100%. The relatively high rate of resistance against ampicillin, tetracycline, and co-trimoxazole observed in this study might be related to inappropriate and excessive use of these antibiotics in humans and livestock. This study gives insight into the presence of antimicrobial-resistant pathogens in retailed and home-grown fruits and vegetables in our settings.

In the current study, 23.1% of the *salmonella* and 20.0% of the *E. coli* O157:H7 isolates were multi-drug resistant (MDR), which was also reported in the previous finding in Ethiopia (50.0%) [37], and Nepal (13.7%) [23]. The occurrence of drug and multidrug resistance isolates in these food sources means that they could be transmitted to consumers through the food chain, especially in our situation in which unhygienic practices are very prevalent. The frequency of ESBL producers for *Salmonella* was 15.4% and *E. coli* O157:H7 was 20.0% in the present study. It was also reported in a previous study in Nepal in which the prevalence of ESBL producers for *Salmonella* was 7.6% and 13.8% for *E. coli* isolates [23]. A similar study was conducted in Ethiopia and reported that 25.0% of ESBL producers *Salmonella* spp. were detected in vegetables [37]. The emergence of ESBL producers might also be related to inappropriate and excessive use of beta-lactam antibiotics.

#### Limitation of the study

The sample size for the study participants was not calculated. Instead, we used a time interval from first July to September 30, 2021, to get the required number of samples and those vendors from whom the required samples were collected during study period were included in the interview. The findings of this study may be reduced because we included some pathogenic bacteria from selected vegetables and fruits, making it impossible to conclude the true burden of the pathogenic bacteria in the study area. For more informative findings to indicate the specific antibiotic-resistant gene, a molecular technique was not conducted due to resource problems. However, the findings are still significant because the

detection of these pathogenic bacteria in raw vegetables and fruits is concerning.

## Conclusion

This study indicated that the safety aspect of fresh vegetable salads and fruit sold in Jimma town is unacceptable from a microbiological point of view. The presence of drug- and multi-drug-resistant *Salmonella* spp. and *E. coli* O157:H7 isolates was also a concerning problem. In addition, poor hygienic practices of vendors and unhygienic vending sites were observed, which may have great implications for cross-contaminations of vegetables and fruits. Therefore, provision of training and regular inspections to improve the knowledge and practice of vegetable and fruit vendors at roadside and retail shops about safe handling and distribution may have great implications in the prevention and control of the transmission of foodborne infections that might be caused by antibiotic-resistant strains.

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**Author contributions**
AZ, TD, TB, JBM, AH, JY, AA, AW and MA were participated in the study design, and were responsible for recruitment, sampling and for the laboratory analyses. AZ and MA analyzed the data and drafted the manuscript. All the authors have contributed to the manuscript and approved the final version.

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**Data availability**
All the data supporting our findings were incorporated within the manuscript.

## Declarations

**Ethical approval and consent to participate**
Prior to the study implementation, ethical clearance was obtained from Institutional Ethics Board (IRB) of the Institute of Health, Jimma University, (Ref No: IHRPGn /357/2021). Participation was voluntary. Written informed consent was obtained from the sellers/vendors to participate in the study. The questionnaire was anonymous; therefore, any document as a written informed consent that might reveal the identity of the subjects was asked.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interest.

**Author details**
[1]School of Medical Laboratory Sciences, Institute of Health, Jimma University, Jimma, Ethiopia
[2]Gumay Woreda Health Office, Jimma, Ethiopia
[3]Department of Oncology, Institute of Health, Jimma University, Jimma, Ethiopia
[4]Oda Hulle Primary Hospital, Jimma, Ethiopia
[5]Division of Infectious Diseases and Tropical Medicine, University Hospital, Ludwig- Maximilians-Universität (LMU) Munich, Munich, Germany
[6]Department of Bacteriology, Max von Pettenkofer-Institute (LMU), Munich, Germany
[7]German Center for Infection Research (DZIF), Munich, Germany

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