Original Article

Bacteriological analysis of street-vended sliced watermelon (Citrullus lanatus) from selected sites in Adama City, Ethiopia

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Abstract

Background: Feeding of fruits, including watermelon, is a vital source of vitamins and essential micronutrients. Watermelon and Pineapple are the most sliced fruits that are highly purchased in Adama. Despite the health benefits obtained from daily consumption of fruits, they are at risk of microbial contamination mainly owing to their vending and handling approaches. The public health concern of food-borne infections related to street-vended fruits is becoming serious. This study was aimed at determining the commonest bacterial isolates associated with sliced watermelon sold by street vendors in Adama City, Ethiopia.

Methods: A cross-sectional study consisting of a total of 24 sliced watermelon samples was randomly collected from four selected sites of the city, namely: Mebrat Haile, Post Office, Franko, and Bole sites, in April-May 2023. For bacterial isolation and identification, standard bacteriological methods were used. The samples were serially diluted with distilled water, and bacteriological analysis was conducted to determine aerobic mesophilic bacteria on plate count agar (PCA), Enterobacteriaceae on MacConkey, coliforms on violet red bile agar (VRBA), and Salmonella and Shigella on Salmonella and Shigella agar (SSA) selective media. Results: The results of this study showed that watermelon samples were contaminated from 2.59×10^6 to 2.86×10^7 mean plate counts for aerobic mesophilic bacteria, 3.4×10^5 to 1.59×10^6 for Enterobacteriaceae, 1.65×10^6 to 2.74×10^6 for coliform count, and 1.2×10^5 to 3×10^5 CFU/ml of Salmonella and Shigella count. Three out of four standard biochemical tests showed positive for the presence of E. coli, Salmonella species, and Shigella species. Our findings revealed that samples from the Post Office site and the Bole site were the most and the least contaminated, respectively.

Conclusions: On the whole, mean aerobic counts and mean coliform counts were not statistically significant among vendors and sites in selected areas. The bacterial analysis showed that the hygienic condition of sliced watermelon sold in Adama City Street was very poor, demanding strict attention from

Introduction

Fruits may be sold to consumers as whole fruits or as fresh-cut fruits on the street. The street food trade is a growing sector in several developing nations today. It has been reported that more than 20 kinds of fruits are sold as minimally processed, of which watermelon (Citrullus lanatus) is among the frequently vended and consumed in the world (1). Watermelon belongs to the Cucurbitaceae family.

In the past couple of years, a considerable increase in the consumption of sliced fruits has been reported in Africa, including Ethiopia, due to their fair price in slices, expediency, freshness, easy accessibility, and dietary significance (2, 3). Its expansion has also been linked with urbanization and the need of urban populations for both employment and food (4).

Epidemics of food-borne infections in several third-world nations are frequently associated with multifaceted factors. Studies have demonstrated that the problem of toilet facilities and clean water supply, as well as low sanitary and hygienic conditions, have a strong link to possible outbreaks of food-borne infections. Moreover, poverty-related factors like socioeconomic and standard of living conditions, poor health coverage, and related issues have been implicated for the stated infections (5, 6, 7).

Studies showed that frequent feeding of fruits and vegetables not only improves the health of an individual but also decreases the chance of exposure to chronic diseases (8). Their relatively low calories have been another benefit of fruits and vegetables for weight control.

Despite their health benefits, there have been an increasing possible health risks associated with the feeding of fresh-cut ready-to-eat fruits. Previous studies reported an increase in outbreaks of human infections linked with the feeding of contaminated fruits and vegetables (9). The bacteriological quality of street-vended fruits becomes important since the food is the major source and transmission of food-borne infections and food intoxications (10, 11). Most recent study showed that fruits and vegetables harbor diverse medically important bacterial pathogens, including Salmonella species, Escherichia coli O157:H7, Shigella species, and Bacillus cereus (12). It has been evident that food-borne infections have become more common over time and have now become a serious public health problem globally, accounting for over 600 million people exposed to the risk annually (7).

Although efforts have been made to improve the safety of the food supply, the occurrence of food-borne infections coupled with the unhygienic handling of street-vended fruits remains a significant health concern in both developed and developing countries (6, 13, 14). Despite favorable weather conditions for possible contamination of street-vended watermelon and its seasonal widespread availability in Adama City, there are limited research reports on the distribution of food-borne infections related to street-vended fruits in general and watermelon in particular. Therefore, this study was aimed at assessing the bacterial contamination of street-vended sliced watermelon sold in Adama City in Ethiopia.

Methods and materials

Study Area and   Study Design

A cross-sectional study was conducted in Adama city, located at 8.54°N 39.27°E with an altitude

of 1712 meters above sea level, 99 km southeast of Addis Ababa, the capital city of Ethiopia. The city is generally characterized by hot weather with a mean annual maximum temperature of 30 °C and a mean annual rainfall fall 371 mm. According to the population projection of 2022, the total population of the city was estimated to be 456,900 (15).

Sample Collection

A total of 24 sliced watermelon samples were randomly collected from street vendors located at four spot sites of Adama City, such as Mebrat Haile area, Post Office area, Franko area, and Bole area in April-May 2023. The four selected sites are situated on the main roads of the city and are heavily populated and hotspots for several street vendors. Hence, those four sampling sites were purposively selected. All sliced samples were transported aseptically to the laboratory of the Applied Biology department, Adama Science and Technology University by using polyethylene bags. The samples were kept in a refrigerator at 4°C until further processing.

Bacteriological Methods

Serial Dilution and CFU Calculation

Serial dilution was done by taking 25g of each sample, sliced with a sterile knife, for the bacteriological quality. One gram of watermelon sample was homogenized in 9 ml of 0.1% buffered peptone water (BPW) for preparation of a homogenate suspension using a stomacher bag as demonstrated before (16). Then, from each serial dilution, an aliquot of 0.1 ml was spread on plate count agar, blood agar, and MacConkey agar plates for total viable aerobic count and coliform count. The bacteria were inoculated in duplicates, and the plates were kept at 33–37°C for 24–48 h (16). The counted colonies were expressed in CFU per ml and converted to log CFU per ml. The CFU was determined by the following formula developed by Brugger et al. (17).

$$CFU = \frac{no.\,colony}{Dilution\,factor \times vol.\,in\,ml} \times 10^1$$

Bacteria Identification

Bacterial isolates were identified by subculturing the primary culture into pure culture colonies. Then, using pure culture plates, the bacterial identification was done based on the morphology of colonies, followed by Gram staining and further biochemical testing using indole, catalase, urease, and triple sugar iron tests (16).

Enumeration of Bacterial Colony

Enumeration of bacterial colonies was performed for aerobic mesophilic bacteria, enterobacteria, total coliforms, and Salmonella and Shigella using plate count agar, MacConkey agar, violet red bile agar, and Salmonella and Shigella agar, respectively. Following incubation, colony counts ranging from 30 to 300 were considered.

Data Analysis

The data generated were properly managed and entered into Microsoft Excel and analyzed using SPSS version 20 (IBM, Armonk, NY, USA). Accordingly, descriptive statistics were computed. Mean bacterial loads were calculated and compared with respect to sites of data collection. Statistical significance was set at a p-value of <0.05.

Results

In this study, aerobic mesophilic bacteria, Enterobacteriaceae, coliform, Salmonella species, and Shigella species were isolated using PCA, MacConkey agar, VRBA, and SSA, respectively, and the growth of the different bacterial isolates is shown in Fig. 1.
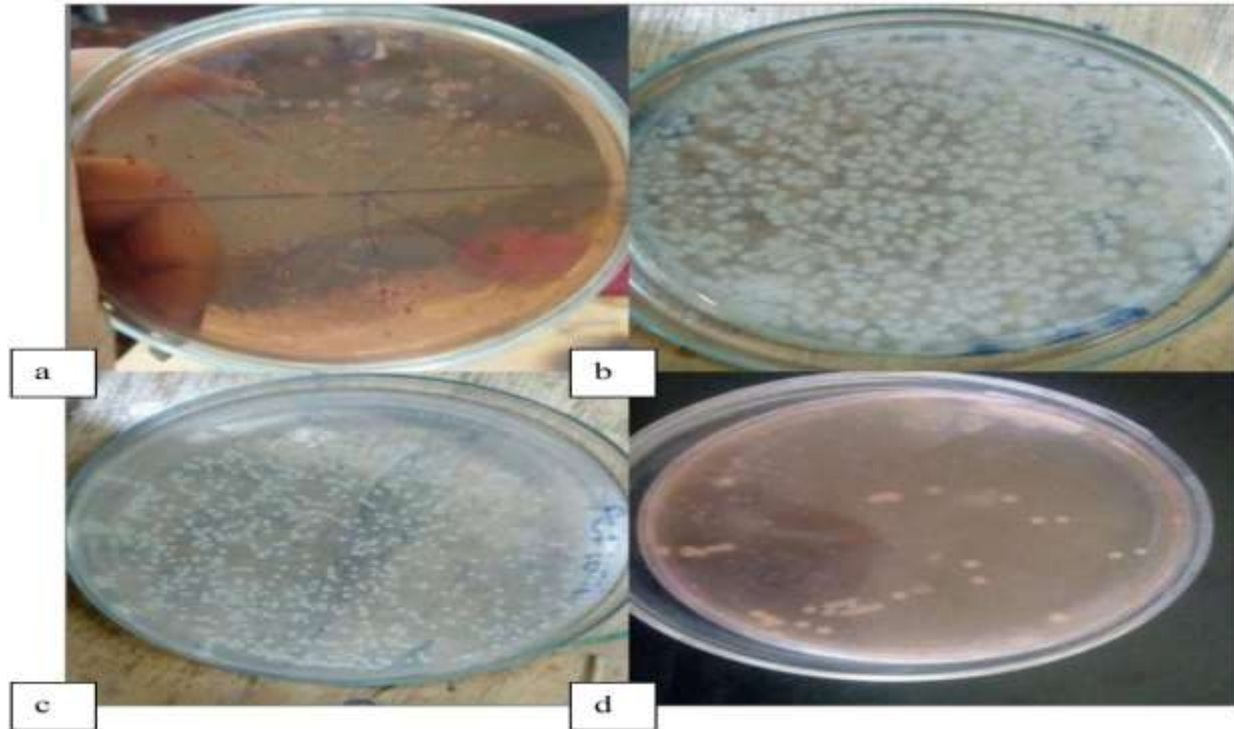
Fig. 1. Appearance of bacterial culture isolated from watermelon sample collected from different sites of Adama City and grown on different media: (a) Enterobacteriaceae grown on VRBA, (b) Aerobic mesophilic bacteria on PCA, (c) Salmonella and Shigella bacteria on SSA, and (d) Aerobic mesophilic bacteria on PCA.

The study revealed that sliced watermelon samples were contaminated with mean total aerobic plate counts from 2.59×10^6 to 2.86×10^6 and a CFU mean of 2.52×10^7 to 2.83×10^7. Plate count colonies of Enterobacteriaceae ranged from 0.34×10^6 to 1.59×10^6 and a CFU mean of 2.35×10^6 to 1.1×10^7. Similarly, plate count colonies of coliforms ranged from 1.65×10^6 to 2.74×10^6, and the CFU mean of 0.65×10^7 to 1.04×10^7.

Table 1: Mean count of aerobic mesophilic bacteria isolated from street-sold sliced watermelon in selected sites of Adama City, Oromia, Ethiopia, 2023

Site	N	Plate 1 (10^4) colonies	Plate2 (10^4) colonies	Plate 3 (10^4) colonies	Mean Plate colonies (10^4)	CFU plate 1	CFU plate 2	CFU plate 3	CFU mean
Mebrat Haile	6	Too many to count	280	280	280+	-	2.8×10^7	2.72×10^7	2.76×10^7
Franko	6	265	262	260	262	2.65×10^7	2.62×10^7	2.72×10^7	2.66×10^7
Post Office	6	Too many to count	289	284	286+	2.89×10^7	2.75×10^7	2.85×10^7	2.83×10^7
Bole	6	267	250	259	259	2.67×10^7	2.5×10^7	2.4×10^7	2.52×10^7

Samples from Post Office and Franko sites showed higher relative contamination when compared to the rest sites (Table 3). Overall, the relative proportion of mean plate colonies and CFU mean isolated from

street-vended sliced watermelon samples of the Post Office site and Bole site were the most and least contaminated of the rest sites (Table 1-3). Total mean count of Salmonella and Shigella bacteria isolated from street-sold sliced watermelon ranged from 1.2x105 to 3x105 CFU/ml of Salmonella and Shigella count. Generally, mean aerobic and coliform counts were not significantly different across vendors and sites in selected areas ($p > 0.05$).

Table 2: Mean count of Enterobacteriaceae isolated from street-sold sliced watermelon in selected sites of Adama City, Oromia, Ethiopia, 2023

Site	N	Plate 1 (10^4) colonies	Plate 2 (10^4) colonies	Plate 3 (10^4) colonies	Mean Plate colonies (10^4)	CFU plate 1	CFU plate 2	CFU plate 3	CFU mean
Mebrat Haile	6	70	55	68	64	$7x10^5$	$5.5x10^6$	$4.5x10^6$	$3.57x10^6$
Franko	6	47	42	46	45	$4.7x10^5$	$4.2x10^6$	$4.1x10^6$	$2.92x10^6$
Post Office	6	180	129	169	159	$1.8x10^6$	$1.29x10^7$	$1.7x10^7$	$1.1x10^7$
Bole	6	34	31	37	34	$3.4x10^5$	$3.1x10^6$	$3.6x10^6$	$2.35x10^6$

Table 3: Mean count of coliform isolated from street-sold sliced watermelon in selected sites of Adama City, Oromia, Ethiopia, 2023

Site	N	Plate1 (10^4) colonies	Plate 2 (10^4) colonies	Plate 3 (10^4) colonies	Mean Plate colonies (10^4)	CFU plate 1	CFU plate 2	CFU plate 3	CFU mean
Mebrat Haile	6	180	178	159	172	$1.8x10^6$	$1.78x10^7$	$1.72x10^6$	$0.7x10^7$
Franko	6	249	228	247	241	$2.49x10^6$	$2.28x10^7$	$2.36x10^6$	$0.9x10^7$
Post Office	6	287	259	276	274	$2.87x10^6$	$2.59x10^7$	$2.62x10^6$	$1.04x10^7$
Bole	6	170	161	165	165	$1.7x10^6$	$1.61x10^7$	$1.55x10^6$	$0.65x10^7$

As indicated in Table 4, standard biochemical tests were performed, and Escherichia coli, Salmonella species, and Shigella species showed positive results for Indole, Triple iron sugar, and catalase tests, whereas they showed negative results for the urease test.

Table 4. Biochemical test results of bacterial isolates from street-sold sliced watermelon in selected sites of Adama city, Oromia, Ethiopia, 2023

Biochemical tests	*Escherichia coli*	*Salmonella* species	*Shigella* species
Indole	+	+	+
Urease	-	-	-
Triple iron sugar	+	+	+
Catalase	+	+	+

Discussion

The present study revealed that sliced watermelon samples were contaminated with mean total aerobic plate counts from 2.59×10^6 to 2.86×10^6 and a CFU mean of 2.52×10^7 to 2.83×10^7. Similar previous studies from Ghana revealed a lower bacterial contamination of watermelon of 8.1×10^5 CFU/ml (18). The present finding is also in contradiction to a study in Mexico, which reported a slightly lower total aerobic count of 4.1×10^5 CFU/ml to 1.6×10^6 CFU/ml in watermelon (19). A study from Nigeria reported a lower mean aerobic count of 1.5×10^2 to 3.6×10^3 CFU/ml in watermelon juice (20).

According to the International Commission on Microbiological Specifications for Foods (ICMSF), mean aerobic counts of ready-to-eat foods have been estimated to be acceptable, tolerable, and unacceptable if they range from 0 to 10^3, 10^4 to 10^5, and $\geq 10^8$, respectively (21). In the present findings, the magnitude of contamination may possibly be somewhat tolerable as per the recommended standards, but is approaching the unacceptable border. The presence of aerobic mesophilic bacteria can be attributed to several factors, such as improper handling and processing, the use of dirty processing utensils like knives and trays (22). It has been reported that monitoring the hygienic quality of products during processing and distribution involves aerobic plate count (23).

Plate count colonies of Enterobacteriaceae ranged from 0.34×10^6 to 1.59×10^6 and a CFU mean of 2.35×10^6 to 1.1×10^7. Samples from Post Office and Franko sites showed higher relative gross contamination when compared to the rest sites (Table 3). Overall, the relative proportion of mean plate colonies and CFU mean isolated from street-vended sliced watermelon samples of the Post Office site and Bole site were the most and least contaminated of the rest sites, which appears to be due to the relative density' of the population in the former

(Table 1-3). It has been reported that the presence of Enterobacteriaceae count in watermelon samples has been attributed to their being natural flora of the fruit, which may be introduced into the fruits under improperly operated processes (24).

In this study, plate count colonies of coliforms isolated from street-vended watermelon ranged from 1.65×10^6 to 2.74×10^6 and a CFU mean of 0.65×10^7 to 1.04×10^7. Similar studies conducted in different parts of the world have shown coliform and E. coli were isolated from freshly sliced street-vended watermelon samples (25, 26). Studies report that besides its physical proximity to soil contamination during cultivation, unhygienic handling and distribution to customers of watermelons have chiefly been intensifying bacterial contamination (27).

Total mean count of Salmonella and Shigella bacteria isolated from street-sold sliced watermelon ranged from 1.2×10^5 to 3×10^5 CFU/ml. Out of four standard biochemical tests, three showed positive presumptive tests for the presence of E. coli, Salmonella species, and Shigella species. Previous studies reported the contamination of watermelon by varieties of bacterial species, mainly E. coli, Salmonella, and Shigella (28, 29, 30, 31).

In general, mean aerobic and coliform counts did not show statistical significance did not show statistical significance did not show statistical significance did not show statistical significance among the vendors and sites in selected areas ($p > 0.05$). The consumption of vended foods is a risk for food-borne infections owing to the difficulty in ascertaining the hygienic processes of the fruits, mainly after harvesting, during processing, and before packaging (32).

The study focused on the isolation of mesophilic aerobic bacteria, enterobacteria, coliforms, and Salmonella and Shigella species associated with street-vended sliced watermelon in Adama City. The total count of bacteria, including coliforms, was within marginal acceptable ranges with the potential to adversely affect the consumers'

health. Limited samples from a few vending sites in selected sites of Adama City and the inability to use sensitive diagnostics appear to be among the limitations of the present study.

Conclusion

This study revealed that the street-vended sliced watermelons that were taken from four different sites of Adama city (Mebrat Haile, Post office, Franko, and Bole) were contaminated by varying degrees of bacteria such as Enterobacteriaceae, aerobic mesophilic bacteria, Salmonella species, and Shigella species. Post Office site and Franko site were the most contaminated, while Bole site was the least contaminated of the rest sites, which appears to be due to the relative density of the population in the former sites. Overall, the bacterial analysis showed that the hygienic condition of sliced watermelon sold in Adama City Street was very poor. Thus, the city administration, along with the health office, should work on public awareness on street-vended fruits, including watermelon, in controlling such unhygienic practices.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Conflicts of interest

The authors declare that there is no conflict of interest.

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