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ORIGINAL ARTICLE

# Isolation of fecal *Escherichia coli* from fresh strawberries (*fragaria ananassa*) in Iran

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## ABSTRACT

**Background and Objectives:** Microbial safety of the fresh fruits is a global concern. The production of strawberry (*Fragaria ananassa*) in Iran has grown 4.5% from 2016 to 2019. Little information is available about the microbiological quality of strawberry produced in Iran. The objective of this investigation was to assess the fecal *Escherichia coli* (FEC) contamination of fresh strawberries of south of Kerman province where is the centers of production and supply of strawberries to other parts of Iran.
**Materials and Methods:** In this cross-sectional descriptive study, the FEC of a total number of 109 strawberry samples, which were collected from green-houses of strawberry of Kerman province during three months, were enumerated using the most probable number (MPN) as described by Iranian National Standards Organization, protocol No.2946, and interpreted using the latest statute released by Iran Veterinary Organization, Executive order number 2946, released in 2011.
**Results:** MPN of FEC counted in strawberries ranged between <0.3 (n=37) and >110 (n=19) per gram (g) having a mean, mode, and median value of 250.3, <0.3, and 9.4 MPN/g, respectively. More than one-fourth of the samples (28.44%) were polluted with FEC at a level of >100 MPN/g.
**Conclusion:** Our findings may be resulted from the sanitary quality of the farm and strawberries of the study area, which indicated that the microbial safety of the strawberries in this survey was not satisfactory, alarming public health.

**Keywords:** Contamination; *Escherichia coli*; Fragaria; Fruit; Iran; Public health

## INTRODUCTION

Important parts of a nutritious and healthy diet are fresh fruits, which are often eaten without cooking or further processing. The demand for fresh fruits with an acceptable microbial safety has significantly increased worldwide (1, 2).

The risk of microbiological contamination does not absolutely remove despite the fact that conventional sanitation techniques have been employed for cleaning and processing of fresh fruits (1). So, the fresh fruits may play a role as vehicles to transfer the pathogens to humans (3, 4). For this reason, the microbiologists try to develop a plan of action or policy designed to decrease the prevalence of the pollution of fresh fruits and consequently foodborne outbreaks, worldwide (1). In this respect, in 2007, an expert committee was assembled by World Health Organization (WHO) and Food and Agriculture Organization (FAO) to establish priority commodities of concern in terms of microbiological hazards associated with fresh fruits. The committee considered multiple factors, including historical outbreaks, potential for contamination, exposure levels and poten-

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tial for control, prevalence and severity of disease and trade and economic impacts. As a result, the highest level of preference was allocated to leafy vegetables and leafy herbs, followed by berries, green onions, melons, sprouted seeds and tomatoes (5).

Iran, where the growing season is long, exported and imported many fresh fruits and vegetables. The production of strawberry (*Fragaria ananassa*) in Iran has grown 4.5% from 2016 to 2019 (6). The south of Kerman province (Lat: 30° 17' 2.1732" N; Lang: 57° 5' 0.1068" E), the largest province of Iran, is one of the centers of production and supply of strawberries to other parts of Iran. The area under greenhouse strawberry cultivation in Kerman is mostly in Jiroft, Anbarabad and Kahnooj cities. Harvesting of greenhouse strawberries in this area has been started in early January and has been continued until the end of May. The strawberries have been sent to the markets of Tehran, Isfahan, Fars, East and West Azarbaijan provinces, Iran.

One of the well-known members of Enterobacteriaceae family, *Escherichia coli (E. coli)* have been taken into account for the study because of its participation in foodborne outbreaks (2, 4, 5). *E. coli* (i. e, thermotolerant coliforms or generic *E. coli*) is an indicator of recent human or animal fecal contamination. At local farms, *E. coli* is likely to be beneficial for verification of good hygienic practices (GHP) and good agricultural practices (GAP) applied to berries. Moreover, evaluation of food contamination to fecal contaminants helps us to prevent the food-borne diseases (5, 7-9).

The Most Probable Number (MPN) method is a statistical, multi-step assay consisting of presumptive, confirmed and completed phases. In the assay, serial dilutions of a sample are inoculated into broth media. Analysts score the number of gas positive (lactose fermentation) tubes, from which the other 2 phases of the assay are performed, and then uses the combinations of positive results to consult a statistical table, to estimate the number of organisms present (5, 9, 10). MPN values are particularly useful when low concentrations of organisms (<100/g) are encountered in such materials as milk, food, water and soil where particulate matter of the matrix may interfere with obtaining accurate colony counts. MPN values are useful to count "viable" organisms and only viable organisms are enumerated by the MPN determination (9).

Several studies in Iran and abroad investigeted the microbial safety of fresh vegetebles and fruiuts. However, according to our literature review, no report was found regarding the rate of contamination of strawberries with fecal *E. coli* in Iran.

In Tehran, Zare Jeddi et al. (11) investigated the microbial and fungal quality of vegetables associated with low processing and consumable sprouts such as bean sprouts. Denis et al. (5) studied the bacterial contamination (i. e., pathogenic bacteria or fecal *E. coli*) of fresh fruits including cantaloupe, vegetables, onions, berries, and vegetables in Canada. From 2012 to 2013 in Belgium, Delbeke et al. (8) studied the presence of shigatoxin-producing *E. coli* in berries, the risk factors of farms of berries, and the possible prevention methods. Ortiz-Solà et al. (12) studied the microbiological quality of fresh and frozen strawberries obtained from marketplaces and fields in Spain, during 2017 and 2018. The samples were assayed for total aerobic mesophilic microorganisms, molds and yeasts, total coliforms, *E. coli*, *Salmonella* spp., *Listeria monocytogenes* and Norovirus. Mukherjee et al. (13) conducted a microbiological analysis of fresh fruits and vegetables, including tomatoes, leafy greens, lettuce, green peppers, cabbage, cucumbers, broccoli, strawberries, apples, and seven other types of commodities produced by organic and conventional farmers in Minnesota to determine the coliform count and the prevalence of *E. coli*, *Salmonella*, and *E. coli* O157:H7. Bohaychuk et al. (14) analyzed Lettuce, spinach, tomatoes, carrots, green onions and strawberries for *E. coli, Salmonella*, *E. coli* O157:H7 and *Campylobacter* spp. Johannessen et al. (15) examined the presence of *E. coli* in the strawberries harvested from Norwegian farms.

For these reasons, the objective of the present study was to investigate the rate of contamination of fresh strawberries with thermotolerant coliforms to find out if strawberries host thermotolerant coliforms or not.

## MATERIALS AND METHODS

**Study area.** Jiroft (Lat: 28° 40' 7.79" N; Long: 57° 44' 7.79" E) is located in the south of Kerman Province. The sub-province of Jiroft is bound by those of Kermān (north), Bam (east), ʿAnbarābād and Kahnuj (south), and Bāft (west). It is comprised of three districts, eleven rural districts, and three towns (Darb-e Behešt, Jebāl-e Bārez, and Jiroft, which is the administrative center of the sub-province). Jiroft is considered the most suitable region in the entire Kerman Province for agriculture. Thanks to its variant climate zones, it produces both warm and cold weather crops (16).

**Ethics approval and consent to participate.** This work was made possible through collaboration of farmers in Jiroft city, Iran, since they kindly approve the permission to collect "Strawberries (*Fragaria ananassa)*" from their farms. The collection of plant material complies with relevant institutional guidelines and legislation.

**Sample collection.** Strawberries (*Fragaria ananassa*) were taken from 18 producers of strawberry (i. e., 18 greenhouses) located in Jiroft, Kerman. The greenhouse was intermittently sampled during the fruiting and picking season in 2020 during three months. Strawberry samples consisted of 5 whole fresh, ripe berries, which were picked with gloves and collected in plastic bags (mean weight: 95 ± 14 gr).

**Sample preparation.** All samples were transported on ice in a cool box, stored at 4°C, and analyzed within 24 h. In the laboratory, strawberry samples were cut into smaller particles with a blender and subsamples of 25 ml were included for next step.

**The most probable number (MPN) of thermotolerant coliforms.** Samples were analysed for the MPN (9 tubes method) that thermotolerant coliforms (generic *E. coli*) (7) as an indicator of fecal contamination were counted using the technique published previously (5, 9). Briefly, 25 ml of sample mixture was added in 225 ml of lauryl sulfate broth (LTB, Ibersco, Iran), the presence of coliform was confirmed by 3 tube MPN method using three 10-fold serial dilutions in lauryl sulfate broth (LTB, Ibersco, Iran), followed by incubation at 37°C for 48h. One mL of suspension of LTB broth tubes showing growth and gas production was transferred to tubes containing EC broth (Quelab, India). After incubation at 44°C for 24 h or 48 h, a loopful of gassing EC tubes were inoculated into Pepton water medium (Ibersco, Iran). After incubation at 44°C for 24 h or 48 h, indol test using covax indicator was performed to confirm thermotolerant coliforms. For three tubes each at 0.1, 0.01, and 0.001 g inocula, the MPNs per gram and 95 percent confidence intervals was calculated as previously described by Bacteriological Analytical Manual (BAM) of Food and Drug Administration (9).

## RESULTS

The analysis of thermotolerant coliforms was performed using enumeration methods (most probable number, MPN, or direct plating procedure) with a lower reporting limit of 100 MPN/g, which corresponds to the maximum acceptable concentration of generic *E. coli*, an indicator for fecal contamination in fresh fruits and vegetables, as set by Health Canada (5, 7). We considered the maximum limit of generic *E. coli* in fresh fruits and vegetables to be 100 MPN/g, based on standards in Canada (5). In the present study, 109 samples were investigated to detect the MPN of thermotolerant coliforms among strawberries in southeast Kerman, Iran. Thirty out of 109 samples (28.44%) contained 100 or more MPN/g of thermotolerant coliform (Table 1). According to the above standard, more than one-fourth of the strawberries of the present study were polluted with thermotolerant coliforms (Table 1).

## DISCUSSION

The result of the present study is the report from Iran that counts “fecal” *E. coli* in the strawberries to elucidate whether and to what extent strawberry from the study area (i. e., one of the centers of production and supply of strawberries to other parts of Iran) play a role as a carrier and reservoir of fecal *E. coli*.

Although most strawberries of the present study were polluted with less than 0.3 MPN/g of fecal *E. coli* (Table 1), detection and isolation of any fecal *E. coli* is unacceptable and even, for more than one-fourth of the strawberries of the present study, the number of fecal *E. coli* has exceeded from the microbial safety range of the strawberry which is released by international standard (100 MPN/g) (7), indicating that the product may be not safe for human consumption in the study area. Coliforms, on the other hand, are indicators of water and food quality, and their presence may indicate unsanitary conditions. As a result, since 30 out of 109 samples (28.44%) contained 100 or more fecal coliforms, it seems that unsanitary conditions prevail in strawberry farms in

**Table 1.** Descriptive statistics (MPN/g) of thermotolerant coliforms in collected samples (n=109)

| Minimum | Maximum | Mode[a] | Mean | Median[b] | Percentiles[c] | | |
|---|---|---|---|---|---|---|---|
| | | | | | 25 | 50 | 75 |
| <3 | >1100 | <0.3 | 250.3 | 9.4 | <0.3 | 9.4 | 225 |

a: Most strawberries of the present study were polluted with less than 0.3 MPN/g of thermotolerant coliforms;
b: One-half of the strawberries of the present study were polluted with more than 9 MPN/g of thermotolerant coliforms and other one-half of the strawberries of the present study were polluted with less than 9 MPN/g of thermotolerant coliforms;
c: 50% of strawberries of the present study were polluted with less than 9.4 MPN/g of thermotolerant coliforms while 75% of strawberries of the present study were polluted with less than 225 MPN/g of thermotolerant coliforms

the study area.

In contrast to Johannessen et al. (15) (1.25%; one $\log_{10}$ CFU; strawberry samples from Norwegian farms), Bohaychuk et al. (14) (0%; strawberry samples from Canadian farms), Ortiz-Solà et al. (12) (0%; frozen and fresh strawberry specimens from Spain farms), Yoon et al. (17) (1.2-3.2 $\log_{10}$ CFU per leaf of strawberry from tunnel style strawberry greenhouses and packaging centers in Gyeongnam province, South Korea), Denis et al. (5) (0%; strawberry samples sold at retail in Canada), Delbeke et al. (8) (2.77%; 1 and 3 log CFU/g; strawberry samples from strawberry primary production in Belgium) and Mukherjee et al. (13) (2.7 and 4.2 log MPN/g in organic and conventional produce from Minnesota farms, respectively), we found that more than one-fourth of strawberries contained 100 or more MPN/g of thermotolerant coliform, an indicator of fecal contamination (5).

Except for Denis et al. (5) and Delbeke et al. (8), other researchers have reported "*E. coli*" and/or "coliforms" count in strawberries and/or other fruits/vegetables. Besides the type of sample, study area and study design, the difference between our results and the results of other researchers can be due to differences in the preharvest processing. Most crops grow in the "natural" environment and therefore can be sensitive to pollution that comes from a variety of sources. Examples of these resources could be soil, the type of water and water temperature used for irrigation, the treatment of water, which used for irrigation, the distance between strawberry fields to the nearest toilet, the submergence of strawberry fields (8) and wildlife population (18).

Plants can be grown in either soil or soilless cultivation as well as in either highly protected environments or open fields (i.e., open area, enclosed area or greenhouse farms) (8). Berries are no exception to this rule, and when harvested during the fruiting season, usually by hand, placed directly in the packaging for sale after picking, care must be taken that the hygiene issues related to harvesting the product is observed such as the use of clean gloves and personal hygiene of workers (8).

Whether the farmer/legislator observe/meet the recommendations and guidelines for GAP (i. e., Code of Good Practice for Fresh Fruits and Vegetables, Microbiological Guidance for Produce Suppliers to Chilled Food Manufacturers, Risks of Pathogens in Ready to Eat Fruits, Salads through the production process, and The Global Gap Standard for Integrated Farm Management) needs further investigation to explain the cause of the contamination of strawberry in the study area. Calls for Hazard Analysis Critical Control Point (HACCP) on farms may help strengthen GAP methods and reduce the potential for crop contamination (19).

Meteorological data including the average daily temperature and dust deposition as well as successive and consecutive fruiting seasons can describe our results (8). Especially in the rainy season, farms of animal and poultry, which may be located near the strawberry farm, may lead to patrolling of wild or domestic animals, play a source of contamination, and as a result, can describe our findings (8).

Olaimat and Holley (20) reported that some strains of bacteria are more capable to colonize at the surface of crop than others, and that biofilm formation, tissue damage, plant species, and host maturation may play a role for the survival of the pathogen. Moreover, pathogens such as *E. coli* serotype O157:H7 and *Salmonella* species are able to penetrate into the vascular system of the plant. As a result, the access of chemical disinfectants is unlikely (5, 19).

Failure to apply the preventive measures can be considered as a description of the results of the present study, which requires further study (19). The preventative measures to reduce strawberry contamination, such as drip irrigation, avoidance of organic fertilizers, use of mulch foils, gloves for workers, or the use of refrigeration and decontamination/disinfection processes is questionable to explain our findings. Moreover, the effectiveness of disinfection methods such as chemical washing and spraying, radiation treatment and biological methods is highly varied and also the presence of common non-pathogenic bacteria on the surface of the product, contamination with other pathogen, type of treatment, surface to be treated, type of disinfectant, duration and the degree of exposure to the disinfectant may play a role to vary the contamination rate of the crop product (19). There is a deficit in scientific information to write a valid result in leveling the effectiveness of these methods (19).

Finally, maybe as pitfall of the present study, it is important to note that *E. coli* screening is appropriate as an indicator organism but the screening should be performed regularly and with the respect to the other farm risk-factors and/or pathogens (i. e., *Salmonella* spp.) (8). In addition, determination of the potential capability of the isolates of the present study to cause food-borne diseases will be generative (5). Moreover, to reveal the quantity or amount of strawberries presented in the market in the study area will help to infer more accurately that the amount of fecal *E. coli* in strawberries is “high” or “low” requiring ordinary screenings for strawberries in market places.

## CONCLUSION

In conclusion, our findings indicate the contamination of strawberries of the study area with fecal *E. coli* at a level that alarms a risk for public health. These findings suggest that activities related to the healthy production of strawberries should be initiated by the farmer and performed on the farm. The role of legislator to implement relevant policy to provide a safe product throughout the food chain, especially at the farm level, should not be overlooked.

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