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Microbial Contamination of Handmade Sauce Used by Street Food Vendors in Jashore, Bangladesh

M. Hossain [1*✉], B.K. Dey [2]

1. Department of Microbiology, Jashore University of Science and Technology, Jashore 7408, Bangladesh
2. Department of Fisheries and Marine Bioscience, Jashore University of Science and Technology, Jashore 7408, Bangladesh

HIGHLIGHTS

- Total viable bacterial cells in the sauce samples ranged from 1.2×10^3 to 4.2×10^9 Colony Forming Unit/g.
- Loads of Enterobacteriaceae and *Escherichia coli* were unacceptable in 13.33 and 10% of the samples, respectively.
- Overall 80% of the samples were contaminated with *Salmonella* spp.
- The consumption of contaminated street foods is of great concern in Bangladesh.

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Acronyms and abbreviations
PS=Plum Sauce
TS=Tomato Sauce
CFU=Colony Forming Unit

ABSTRACT

Background: Contaminated handmade street foods are often claimed to occur food-borne diseases, especially in developing countries. Therefore, considering the public health issue, this study was conducted to assess the microbial contamination of handmade sauce used by street food vendors in Jashore, Bangladesh.

Methods: A total of 30 samples of Plum Sauce (PS) and Tomato Sauce (TS) were collected from Jashore district, Bangladesh. The quantitative microbial tests were done by dilution plate technique. Identification of particular bacterial group or species was performed using selective media. All the data related to microbial count were subjected to ANOVA test using SPSS version 21.0.

Results: All the sauce samples contained viable Enterobacteriaceae cells; whereas 80% and 83.33% of the total samples were found to be contaminated with *Salmonella* spp. and *Escherichia coli*, respectively. Total viable bacterial cells found in the samples ranged from 1.2×10^3 to 4.2×10^9 Colony Forming Unit (CFU)/g. In addition, total Enterobacteriaceae and *E. coli* counts ranged from 30 to 2.0×10^7 and from 0 to 7.0×10^5 CFU/g, respectively. Although PS samples contained a higher amount of Enterobacteriaceae and *E. coli* compared to TS, no significant difference ($p>0.05$) was found.

Conclusion: The consumption of street foods is of great concern in Bangladesh. Making the vendors aware of sanitary practices is too crucial that could be achieved through training of the vendors at the root level of the country. Furthermore, it is necessary to monitor the street foods frequently by the national authorities.

Introduction

Selling handmade foods by the side of roads is a common traditional practice in many countries in the world (Ahmed et al., 2017). Consumption of street-side foods is dramatically increasing day by day because of their easy

* Corresponding author. ✉ hossainmik37@gmail.com
ORCID ID: https://orcid.org/0000-0002-7093-8876

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access, attraction, flavor, and appearance. Although street foods are popular in most regions of the world mainly due to the cheap price, there are some safety concerns about their hygiene and safety (Alimi, 2016).

In Bangladesh, handmade street-side foods like hog pulp pickles, tomato pickles, and carrot pickles are too popular (Faruque et al., 2010). A report revealed that approximately 62-78% of all the shops in Bangladesh occurred by the roadsides sell such kind of products; of which 58-66% shops are located by the sides of open drains, sewerage, manholes, and dustbins; and a 94% of which serve drinking water from the municipal tap water (Faruque et al., 2010) that is frequently claimed as contaminated (Parveen et al., 2008). Sometimes the vendors store the drinking water in capless plastic drums where the water comes into contact with open-air, indicating further possible contamination of water (Faruque et al., 2010; Khairuzzaman et al., 2014). All the circumstances addressed above are likely to occur cross-contamination of foods.

Sewage and contaminated water have been claimed to occur frequent microbial food contamination with *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and *Escherichia coli* (Bonetta et al., 2016; Flemming et al., 2017; Odonkor and Addo, 2018). Unhygienic practice during peeling, slicing, handling, trimming, packaging, etc. can result in bacterial contamination of foodstuffs (Barro et al., 2006). It seems that some Bangladeshi street vendors pay no attention to importance of personal hygiene during food handling (Faruque et al., 2010). Furthermore, the handmade street-side foods in Bangladesh are mostly kept in a temperature ranging from 0 to 50 ^{o}C that facilitates the growth of a variety of mesophilic pathogenic bacteria, including *E. coli*, *Staphylococcus aureus*, *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp., and *S. typhi* in the foods (Barro et al., 2006; Mensah et al., 2002).

It is estimated that approximately 30 million cases of food-borne illnesses happen annually in Bangladesh (Khairuzzaman et al., 2014). Even the country nowadays is facing an increasing prevalence of food-borne diseases. Therefore, considering the public health issue, this study was conducted to assess the microbial contamination of handmade sauce used by street food vendors in Jashore, Bangladesh.

Materials and methods

Sample collection

Two sub-districts namely Jashore Sadar and Chaugachha of Jashore District, Bangladesh (Figure 1) were included in this study. A total of 30 samples comprising 15 Plum Sauce (PS) and 15 Tomato Sauce (TS) were collected from 30 different selected places (one sample from each site) in those selected sub-districts during March 2015. Also, all the 30 vendors were interviewed to know whether they received any food hygiene training. Samples were obtained from vendors, put into sterile containers, and then kept in an icebox. Immediately after collection, the samples were transported to the Laboratory of Microbiology, Jashore University of Science and Technology, Jashore, Bangladesh.

Enumeration of total bacteria and Enterobacteriaceae

Overall bacterial cell enumeration was done by following dilution plate technique that had been described by Buchanan and Gibbons (1974). At first, 1 g of each sample was homogenized with 9 ml of sterilized distilled water. Thereafter, 1 ml homogenized sample was serially diluted for 10 times (10^{-1}-10^{-10}). From each dilution tube, 0.1 ml liquid was spread on to the Nutrient Agar (Oxoid, UK) plate. The inoculated plates were incubated at 37 ^{o}C for 48 h and then, the bacterial colonies were enumerated. The plates giving a range between 30 and 300 colonies were considered to take into account. Enumeration of Enterobacteriaceae was performed by following the same techniques, but Violet Red Bile Glucose Agar (Oxoid, UK) was used instead of Nutrient Agar; and the round purple colonies surrounded by a purple halo were considered to be Enterobacteriaceae colonies (FDA, 2002). For total bacteria and Enterobacteriaceae, presence of more than 10^3 and 10^4 Colony Forming Unit (CFU)/g sauce sample, respectively has been considered as unsatisfactory markers as recommended by CFS (2014).

Detection of Salmonella spp.

Detection of *Salmonella* spp. was performed in accordance with Buchanan and Gibbons (1974) where Bismuth Sulfite Agar (Oxoid, UK) was used. The dilution step was the same as previously described in the section of "Enumeration of total bacteria and Enterobacteriaceae". For instance, however, the dilution was performed up to 5 times (10^{-1}-10^{-5}) instead of 10 times. *Salmonella* colonies were detected depending on their morphological characteristics and confirmed through further tests including biochemical tests. For *Salmonella* spp., presence of 0 (zero) CFU/25 g sauce has been set as an unsatisfactory marker based on CFS (2014).

Enumeration of E. coli

E. coli was enumerated by using membrane filtration technique (0.45-micron cellulose nitrate filter paper) as previously described by Vergine et al. (2017). The

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dilution procedure was the same as previously described in the section of "Enumeration of total bacteria and Enterobacteriaceae". For instance, however, 6-fold dilution was performed instead of 10-fold. Briefly, 1 ml liquid from each dilution was mixed with 9 ml of sterilized distilled water, and followed by filtration. The filter paper was placed on MacConkey Agar (Oxoid, UK), and incubated at 44 ^{o}C for 24-48 h. *E. coli* colonies were detected depending on their morphological characteristics and confirmed through further tests including biochemical tests. For *E. coli*, the presence of more than 10^2 CFU/g sauce has been considered as an unsatisfactory marker according to CFS (2014).

Statistical analysis

All the data related to microbial count were subjected to one-way Analysis of Variance (ANOVA) using SPSS version 21.0. The significance level was set at 0.05.

Results

In this survey, among all the 30 interviewed vendors, not a single vendor was recorded having any training on hygiene practice for food preparation. The common ingredients used in the preparation of all the foods are smashed mustard seeds, salt, sugar, mustard oil, powdered red chilli, and water, where turmeric powder was used in all TSs but not in PSs.

As shown in Table 1, total viable bacterial cells found in the samples ranged from 1.2×10^3 to 4.2×10^9 CFU/g. In addition, total Enterobacteriaceae and *E. coli* counts ranged from 3×10^1 to 2.0×10^7 and from 0 to 7.0×10^5 CFU/g, respectively. The presence of *E. coli* and *Salmonella* spp. were recorded in 83.33% (25 out of 30) and 80% (24 out of 30) of the total samples. Loads of Enterobacteriaceae and *E. coli* were more than acceptable limits in 13.33 and 10% of the samples, respectively.

Eleven out of 15 TS samples and 14 out of 15 PS samples were contaminated with *E. coli*. Furthermore, for *Salmonella* spp., among 15 TS and 15 PS samples, the number of contaminated ones was 11 as well as 13, respectively. None of studied bacterial parameters were significantly different (p>0.05) in TS and PS samples (Table 1).

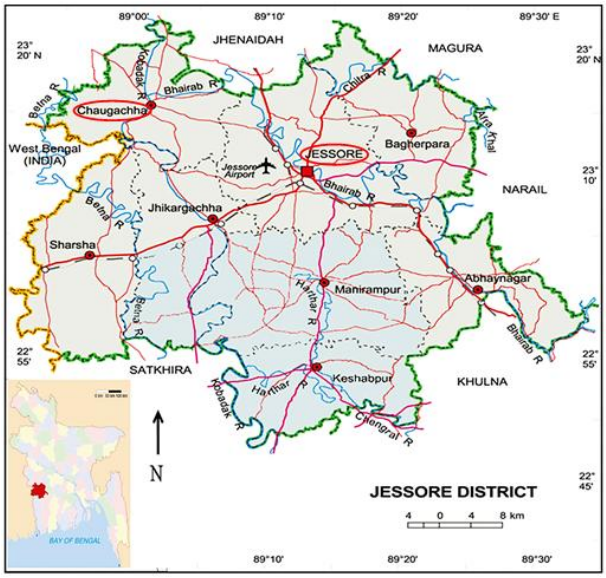

Figure 1: Map of Jashore district (previous name: Jessore) in Bangladesh, highlighting Jashore Sadar and Chaugachha with red oval shapes

Table 1: Microbial counts of the handmade sauce samples collected from Jashore district, Bangladesh

Sample	Size	Bacterial load (CFU/g)		Enterobacteriaceae (CFU/g)		*E. coli* (CFU/g)	
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Tomato sauce	15	1.6×10^3 to 2.0×10^8	$1.8\times10^7\pm5.3\times10^7$	3×10^1 to 3.0×10^5	$3.4\times10^4\pm9.0\times10^4$	0 to 2.0×10^3	$1.6\times10^2\pm5.1\times10^2$
Palm sauce	15	1.2×10^3 to 4.2×10^9	$5.4\times10^8\pm1.3\times10^9$	5.6×10^2 to 2.0×10^7	$1.6\times10^6\pm5.1\times10^6$	0 to 7.0×10^5	$8.7\times10^4\pm2.3\times10^5$
Total	30	1.2×10^3 to 4.2×10^9	$2.8\times10^8\pm9.2\times10^8$	3×10^1 to 2.0×10^7	$8.3\times10^5\pm3.7\times10^6$	0 to 7.0×10^5	$4.3\times10^4\pm1.7\times10^5$

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Discussion

In developing countries, millions of people consume a wide range of various foods sold by the street vendors (Khan et al., 2015). Such types of foods are frequently claimed to contain an unsatisfactory range of pathogenic microbial load (Ahmed et al., 2009; Amare et al., 2019; Campos et al., 2015; Das et al., 2012; Duggan et al., 2012; Khan et al., 2015; Kharel et al., 2016; Kim et al., 2011; Lin et al., 2017; Rashed et al., 2013). The prevalence of food-borne hazards, typically in developing countries, is increasing rapidly with the increasing consumption rate of unhygienic street-side foods (Das et al., 2012).

We found that the collected samples contained a high number of microorganisms ranging from 1.2×10^3 to 4.2×10^9 CFU/g. Similar with our findings, some previous investigations in Dhaka, Bangladesh on several street-side juices reported microbial load ranging from 3×10^2 to 9.6×10^8 (Ahmed et al., 2009), 1.9×10^3 to 2.8×10^7 (Rashed et al., 2013), and 7.7×10^3 to 9×10^8 CFU/ml (Khan et al., 2015). A study in Hawassa city, Nigeria, a high range of total bacterial count (1.7×10^5 to 6.7×10^6 CFU/g) in different types of street foods was detected by Eromo et al. (2016). Also, some street food samples collected from Gondar, Ethiopia were reported to contain total aerobic bacteria up to 6.6×10^4 CFU/g (Amare et al., 2019). Moreover, in another study, conducted on raw and roasted cheese in Northeastern region of South America, the mesophilic microorganism count was reported to be 7.9 and 14.8 log CFU/g (Barreto de Deus et al., 2017). Hence, most results of different research in different parts of the globe are representing a similarity with our findings. The range of total viable bacterial cells recorded (1.2×10^3 to 4.2×10^9 CFU/g) in the present study reflecting an unsatisfactory level of contamination in 100% of the collected samples as the standard for ready-to-eat sausages has been set as low as $<10^3$ CFU/g by the CFS (2014). However, in a study, conducted in Windhoek, Namibia, aerobic plate count represented an unsatisfactory level of 32% of the street-vended ready-to-eat meat samples (Shiningeni et al., 2019).

In some previous studies, several pathogenic bacteria were detected in several types of street foods in Bangladesh and the other countries all over the world such as *E. coli*, *Salmonella* spp. (Ananchaipattana et al., 2016; Campos et al., 2015; Das et al., 2012; Kim et al., 2011; Shiningeni et al., 2019), *Klebsiella* spp., *Enterobacter* spp., *Bacillus* spp., *Enterococcus* spp., *Micrococcus tetragens* (Das et al., 2012; Duggan et al., 2012; Lin et al., 2017; Shiningeni et al., 2019), *Shigella dysenteriae* and *Vibrio* spp. (Das et al., 2012; Khan et al., 2015). *E. coli* can cause urinary and pyogenic infections, septicemia, and diarrhea (Falagas et al., 2007; Samonis et al., 2009). Therefore, along with Enterobacteriaceae, *E. coli* is considered to be a hygiene indicator for food. The unsatisfactory levels of Enterobacteriaceae and *E. coli* in foods are standardized as $>10^4$ and $>10^2$ CFU/g, respectively based on CFS (2014). In this regard, we detected 13.33 and 10% of the total samples to be unsatisfactory based on loads of viable Enterobacteriaceae and *E. coli* cells, respectively. Shiningeni et al. (2019) revealed that 26% and 35% of street ready-to-eat meat products in Namibia were unsatisfactory due to the presence of Enterobacteriaceae and *E. coli*, respectively. Another report in Ethiopia showed that coliform and Enterobacteriaceae counts in several street foods ranged from 2.6×10^3 to 5.1×10^4 CFU/g and from 8.1×10^3 to 6.8×10^4 CFU/g, respectively (Eromo et al., 2016); whereas the Enterobacteriaceae count ranged from 2.2 to 5.2 log CFU/g among street foods distributed in India (Kharel et al., 2016).

According to a similar work carried out in Jashore city, Bangladesh, the coliform count in unpacked food samples was reported to be 4.7×10^4 CFU/g (Al-Fuad et al., 2018). But, in our study the range of Enterobacteriaceae (3×10^1-2.0×10^7 CFU/g) and *E. coli* (0 -7.0×10^5 CFU/g), count extremely varied among the samples. Moreover, in the current research, overall 6.67% samples were found to contain quite a high ($>5\times10^5$ CFU/g) number of viable *E. coli* cells, indicating a high risk of disease. However, the potency of health hazard is appeared to be correlated with the consumption rate of contaminated foods and the growth phase of microbes in the foods.

In this work, the mean value of Enterobacteriaceae and *E. coli* counts in PS samples were found to be higher compared to TS samples, although no significant difference was found. However, eating PSs may be riskier than TSs due to their higher mean value of Enterobacteriaceae and *E. coli* contamination. The reason behind the higher Enterobacteriaceae and *E. coli* content in PSs is yet to be unraveled; however, turmeric powder in TSs is known as an antimicrobial agent (Ali et al., 2006; Gupta et al., 2012) that possibly can reduce the number of viable bacteria. Moreover, correlations among pH, storage time, temperature, and the degree of initial contamination yet to be known.

In the current investigation, it was revealed that as much as 80% of total food samples contained *Salmonella* spp. indicating a serious concern upon consumption of Bangladeshi street foods. Islam et al. (2015) identified *Salmonella* spp. in 50% of various food samples including sauces collected from street vendors in Dhaka city, Bangladesh. In a study in Thailand, 4% of each three categories of collected ready-to-eat street meat products were found to be contaminated with *Salmonella* spp. (Ananchaipattana et al., 2016). According to El Rahman et al. (2018), the prevalence of *Salmonella* spp. values

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ranged from 4 to 8% in different sandwich samples from Alexandria province, Egypt, showing a much lower rate of contamination compared to the present study. In addition, Bereda et al. (2016) stated that 19.7% of street food samples from Jigjiga city, Eastern Ethiopia were contaminated with *Salmonella* spp. In a study in Florida, USA, among several ready-to-eat food samples collected from tourist sites, 71.4% (10 out of 14) of the samples were found to be *Salmonella* positive (Okumus et al., 2019), that is close to the result of our study.

Importantly, we assessed only *Salmonella* spp. and *E. coli*, but some other pathogenic Gram-positive and/or Gram-negative bacteria types might present in the collected samples, increasing the risk of street food consumption. Several authors already have indicated the existence of pathogenic microorganisms along with *Salmonella* spp. and *E. coli* in foods (Duggan et al., 2012; Khan et al., 2015) like *Enterobacter* as well as *Citrobacter* (Falagas et al., 2007; Samonis et al., 2009). Furthermore, antibiotic resistance of those pathogens may result in more severe infections. In Bangladesh, generally, personal hygiene is not maintained properly by the vendors during vending food products (Faruque et al., 2010). Furthermore, vendors often use dirty processing-utensils (Alimi, 2016; Rane, 2011) and water (Barro et al., 2006; Nwachukwu et al., 2008) which could result in microbial contamination.

Conclusion

The results of this study revealed that the consumption of street foods is of great concern in Bangladesh. The microbial quality of sauces used for the preparation of street foods must be improved to reduce of health risk in consumers. In this regard, making the vendors aware of sanitary practices is too crucial that could be achieved through training of the vendors at the root level of the country. Furthermore, it is necessary to monitor the handmade street foods frequently by the national authorities.

Author contributions

M.H. designed the study framework; M.H. and B.K.D. conducted the experiment, analyzed the data, and wrote the manuscript. Both authors read and approved the final manuscript.

Conflicts of interest

Both authors declared that there was no conflict of interest.

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