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Original Research Article

Assessment of Bacteriological Quality of Street Food and Their Antibiotic Profiling

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The microbial contamination of street foods has become an important public health issue in developing countries like India because of widespread food borne diseases. Thus, the aim of this study was to determine the bacteriological quality of street foods. A total of 120 samples (104 were street food samples and 16 were dish washing water samples) was collected and analyzed for total viable count (TVC). The isolation of pathogens was carried out on selective and differential media and was further identified on the basis of morphology and biochemical tests. Similarly, antibiotic profiling was performed by disk diffusion method. The results suggest that the highest TVC was found to be in panipuri ($4.5\pm1.72\times10^5$ cfu/ml) followed by samosa ($3.8\pm0.97\times10^5$ cfu/g), bhelpuri ($3.2\pm0.77\times10^5$ cfu/g) and poha ($3.1\pm0.78\times10^5$ cfu/g) while kachori samples showed least count ($2.8\pm0.97\times10^5$ cfu/g). Similarly, the TVC of dish washing water (DWW) samples was $5.32\pm1.66\times10^5$ cfu/ml. Out of 120, 68 samples (56.67%) were found to be contaminated with *S. aureus* followed by *E. coli* (50%), *Enterobacter* sp. (36.67%), *P. aeruginosa* (26.67%) whereas *Salmonella* sp. was found in 20 samples (16.67%). The antibiotic susceptibility results suggest that *S. aureus*, *E.coli* and *Enterobacter* sp. showed 55%, 29.63% and 15.38% resistance to aztreonam while *E. coli* and *Enterobacter* sp. showed 7.41 % and 23.08% resistance to vancomycin and gatifloxacin respectively. These findings indicate considerable rate of contamination in street foods and DWW. The identified foodborne bacteria and antibiotics resistance isolates could pose a public health problem in that locality. Therefore, regular inspection, health education and training of vendors on food handling and safety practices are recommended.
Keywords: Bhelpuri; kachori; panipuri; samosa; street foods.

INTRODUCTION

In developing countries like India food sold by street vendors is the major source of food- borne diseases (Kibret *et al*, 2013), however these food are appreciated mostly for their unique flavour and for their convenience (Nyenje *et al*, 2012). In any case, their microbiological safety is not always sure. The main sources contributing to microbial contamination of such food are infrastructure, preparation and storage, cooking, cleaning and serving utensils, quality of water and personal cleanliness of food handlers (Muinde *et al*, 2005); (Ghosh *et al*, 2007). Other sources of contamination include place and surface of food preparation, flies and dust on uncovered food items, lack of facilities for drainage of waste water and garbage disposal, dishwashing cloths, contaminated raw materials and water, inaccessibility and lack of consumable water, time inadequate reheating of cooked food and improper and unsanitary food handling by vendors (Islam *et al*, 2015).

A general lack of awareness about the epidemiological consequence of many streets vended foods, poor information of street vendors in basic food safety measures and poor public awareness of the hazards posed by certain foods has extremely hampered the deployment of a precise scientific approach to this very serious issue (Rane, 2011). Therefore, the conditions of street food preparation and vending raise many worry about the customer's well-being (Barro *et al*, 2006).

The potential for the contamination of street foods with pathogenic microorganisms has been well documented and several disease outbreaks have been traced due to consumption of contaminated street foods (Temesgen *et al*, 2016). The street foods are contaminated with enteropathogens such as *Escherichia, Salmonella,* and *Enterobacter* along with toxin producing bacteria such as *Staphylococcus* species. Apart from this, food may also be contaminated with *Pseudomonas* and other species. Street vended chats frequently related to diarrhoeal diseases because of their improper handling and serving practices. Besides in the current years, a significant increase in antibiotic resistance has been observed, mostly in developing countries (Guchi *et al*, 2010); (Tassew *et al*, 2010), because of self-medication and general public are unaware of the effects antibiotics hold. Thus it is essential to monitor the antibiotic susceptibility of food-borne pathogens (Mazumdar *et al*, 2014).

The popular street foods such as poha, samosa, kachori, panipuri and bhelpuri are spicy and used as snacks and are sold by vendors at most of the places in India. People of all age group and classes consume these street foods because of its availability and reasonable price. However, there are many reports on foodborne diseases because of contaminated street foods. Hence, this paper aimed to assess the bacterial quality the street food and also to determine their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Sample Collection

Total 120 samples were collected from various places of Amravati region, Maharashtra state, India. Out of 120, 104 samples were street food samples include poha (20), samosa (20), kachori (20), panipuri (24) and bhelpuri (20) while 16 were DWW samples. All the samples were collected aseptically in pre-sterile poly-bags and sterile bottles, kept in ice boxes and were labelled. Samples were then transferred to the laboratory and stored at 4^0C until analysis. Theses samples were taken out of the freezer and were analyzed when the temperature of the samples were at room temperature.

Microbiological Analysis

For microbial analysis, 1 g of each solid sample and 1 ml of each liquid sample were serially diluted in saline (0.85% NaCl w/v) separately. The total viable counts were determined by spreading 0.1 ml from last dilution on nutrient agar plates followed by incubation at 37^0C for 48 hrs. The TVC was reported as colony forming units per gram of solid food sample (cfu/g) or per millilitre of liquid sample (cfu/ml). Similarly, selective and differential media such as Mannitol salt agar, Cetrimide agar and MacConkey agar were used for isolation of pathogenic bacteria. The isolated bacteria were further subjected to Gram's staining to characterize their morphology and biochemical tests (catalase, oxidase, indole, MR-VP, citrate, urease, TSI and motility) for their identity.

Antibiotic Susceptibility Tests of Isolates

The antibiotic profiling was performed by Kirby-Bauer diffusion method on Muller-Hinton agar according to Clinical and Laboratory Standard Institute guidelines Kirby *et al*, 1996); (CLSI, 2014). In short, the isolated bacterium was suspended in a nutrient broth separately and incubated for 30 min to make it comparable with the 0.5% McFarland standard. After incubation, a sterile cotton swab was dipped into the suspension and bacteria were inoculated onto the Muller-Hinton agar**.** Antibiotic discs were placed by using disc dispenser and the plate was incubated at 37^0C for 24 hrs. The antibiotics used in this study included ciprofloxacin (5mcg), oflaxacin (5mcg), doxycycline hydrochloride (30mcg), vancomycin (30mcg), azithromycin (15mcg), gatifloxacin (5mcg), sparfloxacin (5mcg) and aztreonam (30mcg) of Hi-Media Laboratories, Bombay, India. The isolates were characterized as susceptible or resistant, based on the diameter of zone of inhibition and results interpreted according to Clinical Laboratory Standards institute.

RESULTS AND DISCUSSION

In the present study, total 120 samples were analyzed for TVC and bacterial pathogens. TVC was used to determine the general bacterial load of the food sample and is a valuable tool to monitor the food process. This may reflect the cleanliness level of food handling and retail storage (Collins *et al*, 1989). The present results showed that TVC of panipuri samples was highest ($4.5 \pm 1.72 \times 10^5$ cfu/ml) followed by samosa ($3.8 \pm 0.97 \times 10^5$ cfu/g), bhelpuri ($3.2 \pm 0.77 \times 10^5$ cfu/g) and poha ($3.1 \pm 0.78 \times 10^5$ cfu/g) whereas kachori ($2.8 \pm 0.97 \times 10^5$ cfu/g) showed the lowest TVC (Table 1). Similarly, the TVC of DWW was found to be $5.32 \pm 1.66 \times 10^5$ cfu/ml (Table 2). The high TVC of street food may be due to the food items were generally prepared much before the time of selling and stored at room temperature that might have provided a suitable environment for the growth of the bacteria (Das *et al*, 2010). The present results of TVC were in agreement with earlier reports of street foods (Kumar *et al*, 2006). The present observation showed that out of the 120 samples, 68 samples (56.67%) were found to be contaminated with *S. aureus* followed by *E. coli* contamination in 60 samples (50%), *Enterobacter* sp. contamination in 44 samples (36.67%), *P. aeruginosa* contamination in 32 samples (26.67%) whereas *Salmonella* sp. contamination was found in 20 samples that account 16.67% (Figure 1).

Table 1: Total viable count of different street food samples

Street food samples	TVC ($\times 10^5$cfu/ml or g)*
Poha (n=20)	3.1±0.78
Bhelpuri (n=20)	3.2±0.77
Kachori(n=20)	2.8±0.97

Street food samples	TVC ($\times 10^5$cfu/ml or g)*
Samosa(n=20)	3.8±0.97
Panipuri(n=24)	4.5±1.72

*In parenthesis, data represents the mean (±) standard deviation of total viable count of samples. Each sample was analyzed in triplicate.

Table 2: Total viable count of dish washing water samples

Street food samples	TVC ($\times 10^5$cfu/ml or g)*
DWW (n=16)	5.32±1.66

*In parenthesis, data represents the mean (±) standard deviation of total viable count of samples. Each sample was analyzed in triplicate.

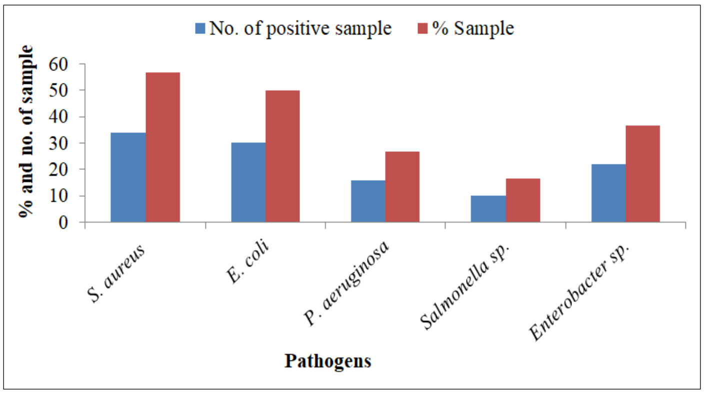

Figure 1: Percent samples contaminated with pathogens

Figure 2: Percent contamination of *S. aureus* in different samples

The microbial analysis results of the food samples revealed that highest contamination of *S. aureus* was found in panipuri (66.67%) followed by bhelpuri (60%), samosa (60%) and kachori (40%) samples while poha showed least contamination (20%) of *S. aureus* (Figure 2). The presence of *S. aureus* was an indication of contamination from the skin, mouth or nose of food handlers through coughing and sneezing. This contamination can be introduced into the street foods during handling, processing or vending. This might be one of the reasons for higher load of *S. aureus* which are responsible for food poisoning (Chavan *et al*, 2015). Our findings were in agreement with the earlier studies who reported the presence of *S. aureus* and *Salmonella* sp. from street foods (Kharel *et al*, 2016); (Bryan *et al*, 1997); (Mosupye *et al*, 1999).

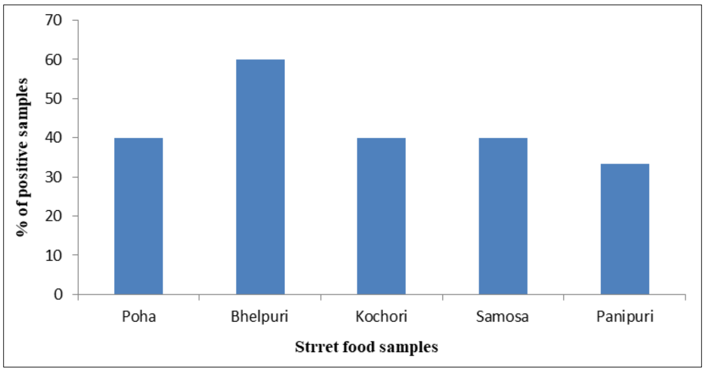

Figure 3: Percent contamination of *E. coli* in different samples

The highest contamination of *E. coli* was found in bhelpuri (60%) followed by poha (40%), kachori (40%) and samosa (40%) while panipuri sample showed least contamination (34.34%) as showed in Figure 3. The presence of total coliform in street-vended foods can be linked to contamination resulted from poor hygienic practice of food handlers, inappropriate processing, incomplete heating, use of contaminated water during preparation and washing or secondary contamination via contact with contaminated equipments such as chopping boards, knives, and serving wares (Benkerroum *et al*, 2004); (Weil Q *et al*, 2006); (Derbew *et al*, 2013). *E. coli*, *Salmonella* sp. and *S. aureus* were reported in some common street foods such as kachori and samosa from India (Kharel *et al*, 2016). Among all the samples, the highest contamination of *P. aeruginosa* was found in 60% samples of each bhelpuri, kachori and samosa followed by poha (20%) whereas it was not recovered from panipuri samples (Figure 4). The present results were similar to previous reports from street food (Mazumdar *et al*, 2014).

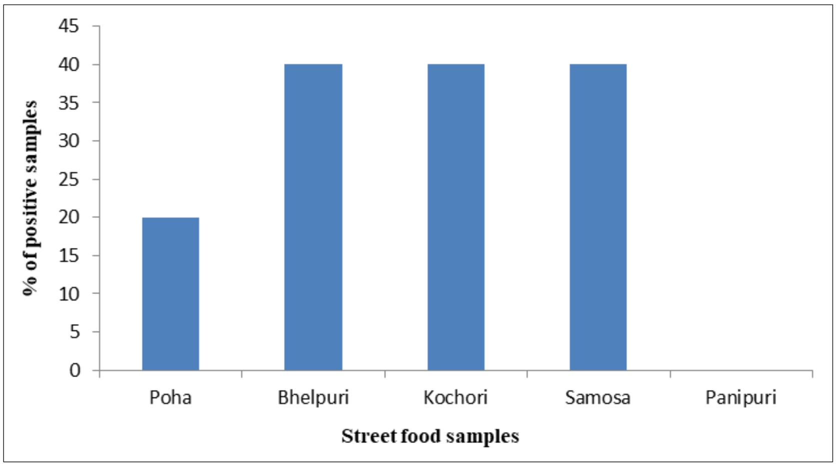

Figure 4: Percent contamination of *P. aeruginosa* in different samples

The present results reveal that maximum *Salmonella* sp. contamination was found to be in bhelpuri samples (40%) followed by kachori (20%) and samosa (20%) while poha and panipuri sample were free from *Salmonella* sp. contamination (Figure 5). The previous studies also reported the isolation of *Salmonella* from street food (Kharel *et al*, 2016). Similarly, (Das *et al*, 2010) reported high loads of bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas* sp. from panipuri and bhelpuri which were correlated with our findings. Out of the total samples, 80% bhelpuri samples were found to be contaminated with *Enterobacter* sp. followed by panipuri (34.44%), samosa (20%) and poha (20%) whereas kachori sample was free from *Enterobacter* sp. contamination (Figure 6). In the present study, we found a high load of *Enterobacter* sp. that was in contrast with (Muzumdar *et al*, 2014). The presence of *Salmonella* sp. and *Enterobacter* sp. were maybe associated with poor hygienic practice and use of contaminated water during preparation and (Derbew *et al*, 2013).

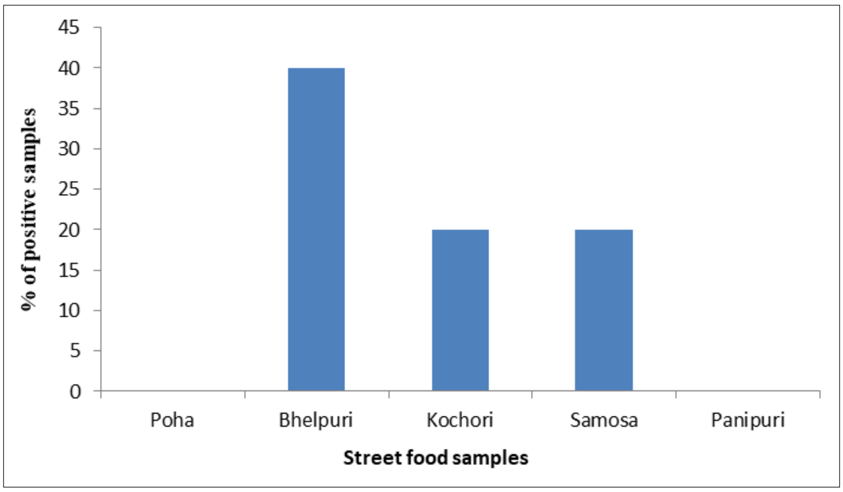

Figure 5: Percent contamination of *Salmonella* sp. in different samples

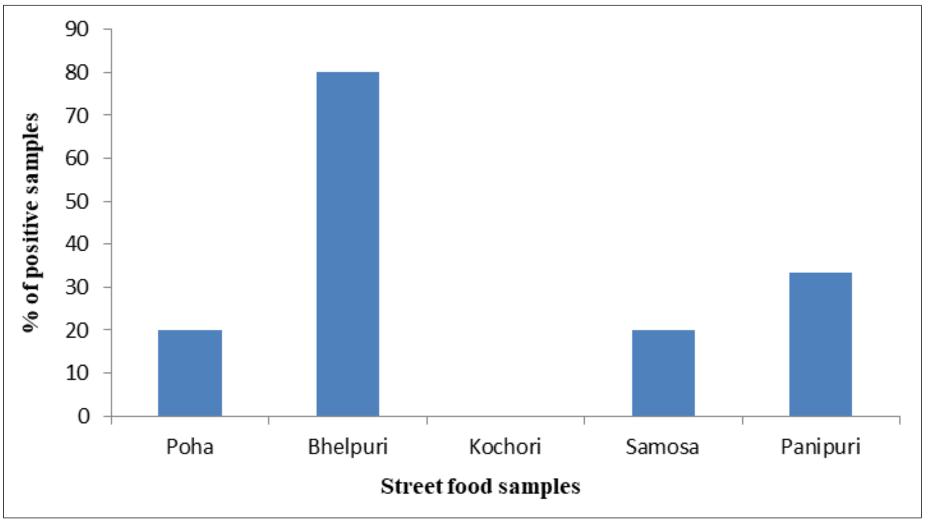

Figure 6: Percent contamination of *Enterobacter* sp. in different samples

The microbial analysis results of the DWW reveals that all the samples were contaminated with *S. aureus* and *E. coli* (100%) followed by *Enterobacter* sp. (75%) while least contamination of *P. aeruginosa* (25%) and *Salmonella* sp. was (25%) detected (Figure 7). In most of the cases, running water is not accessible at vending sites. Therefore, hands and utensil washing are normally done in one container and sometimes without cleanser. This might be the reason for a high load of pathogens in DWW in our study. Many studies reported that those bacteria from dirty dishwashing water and other sources can hold fast to utensil surfaces and constitute a threat of contamination during food vending (Mosupye *et al*, 1999); (Bhaskar *et al*, 2004).

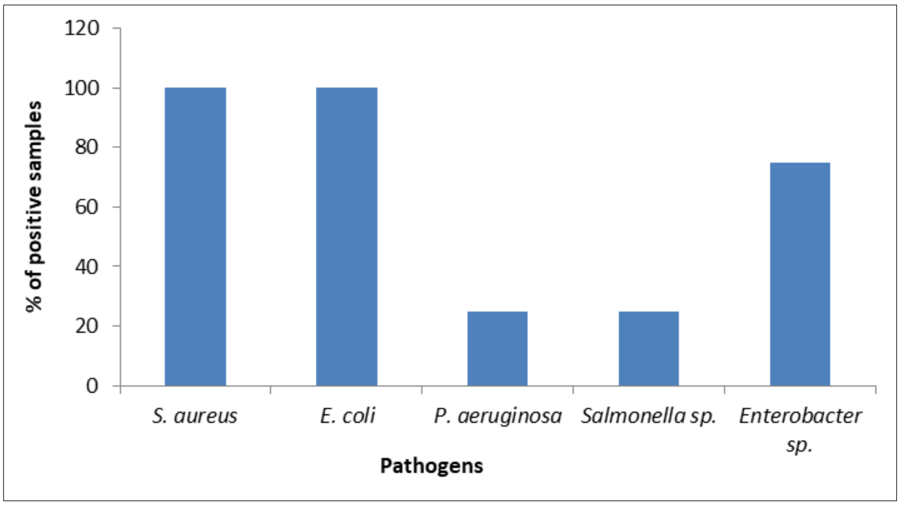

Figure 7: Percent contamination of DWW by bacteria

Table 3: Antibiogram of pathogens isolated from street food

Isolates	Total No. of isolates	Antibiotics resistance (%)							
		CIP	OF	SPX	GAT	AT	AZM	VA	DO
S. aureus	40	0	0	0	0	55	0	0	0
E. coli	54	0	0	0	0	29.63	0	7.41	0
P. aeruginosa	30	0	0	0	0	0	0	0	0
Salmonella sp.	12	0	0	0	0	0	0	0	0
Enterobacter sp.	26	0	0	0	23.08	15.38	0	0	0

Ciprofloxacin (CIP), oflaxacin (OF), doxycycline hydrochloride (DO), vancomycin (VA), azithromycin (AZM), gatifloxacin (GAT), sparfloxacin (SPX) and aztreonam (AT).

The development of antibiotic resistance among bacterial pathogen is of global concern today in clinical studies (Mwambete *et al*, 2015). In the present investigation, it was observed that all the bacterial isolates were sensitive to ciprofloxacin, oflaxacin, sparfloxacin, azithromycin and doxycycline hydrochloride. However, most of them showed resistance to aztreonam that includes *S. aureus* (55%), *E. coli* (29.63%) and *Enterobacter* sp. (15.38%) whereas *P. aeruginosa* and *Salmonella* sp. were susceptible to it (Table 3). Among all the isolates, *E.coli* and *Enterobacter* sp. showed resistance to vancomycin (7.41%) and gatifloxacin (23.08%) respectively. The present findings were in agreement with Sharma and Mazumdar (2014) who reported that all the *S. aureus* isolates were sensitive to ciprofloxacin. However, the risk level of infection by these organisms becomes higher when it turns out to be antibiotic resistant (Ilangovan *et al*, 2016). Hence, the presences of such pathogen in street food as reported in our study are also alarming.

CONCLUSION

The present findings reveal that street foods and DWW was contaminated with bacteria such as *S. aureus, E. coli, Enterobacter* sp., *P. aeruginosa* and *Salmonella* sp. that might be a potential hazard to human health. To avoid such risks, consumer awareness regarding the freshness, quality and hygienic environment of the street foods are needed. At the vending site, a provision of clean potable water is must for street food preparation, cleaning utensils and used dishes. Similarly, by educating and training of vendors about hygienic conditions and food handling and lastly, the concerned Government authorities should periodically check and monitor the preparatory conditions of the stalls in order to maintain the quality of the street foods.

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CONFLICTS OF INTEREST

Author has no conflicts of interest to declare.

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