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Bacteriological Quality Assessment of Selected Street Foods and Antibacterial Action of Essential Oils Against Food Borne Pathogens

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Abstract

The present investigation was aimed to study the microbiological quality of three popular street foods namely, Panipuri, Dahibara and Chaat. In total 25 samples were collected in and around from different vendors in Baripada, Odisha, India. Informal observations of the vendors were also carried out. All the samples were subjected to coliform analysis, determination of total aerobic bacterial load and isolation of pathogenic bacteria on selective media. 86.66% of the samples were coliform positive. Apart from E. coli, other food borne pathogens like Shigella dysenteriae, Salmonella spp., Streptococcus faecalis, Bacillus cereus, Enterobacter spp., Pseudomonas spp. were also isolated. It was reported that pathogens like E. coli and B. cereus could survive for a longer period in these food samples. All the isolates showed high multiple antibiotic resistance index ranged between 17.64 - 47.05. Susceptibility of these pathogens against ten different essential oils was evaluated and oils showing better antibacterial activities were used to determine their Minimum Inhibitory Concentrations (MIC) against these bacterial pathogens. Both Clove and Japanese mint oils could kill the pathogens (E. coli) in the food samples within 48hrs..

Key words: Street food; Contamination; Safety, Bacteriological quality, Food borne pathogens; Essential oils; Antibacterial properties; Baripada, India.

Introduction

Street foods or fast foods have been defined by FAO as "Ready to eat foods & beverages prepared and/or sold by vendors especially in streets & other public places for immediate consumption". These foods are well appreciated by consumers, mostly by urban workers because of their taste, low cost, nutrient value & ready availability for immediate consumption. It includes fast foods, junk foods, snacks, beverages, meals, salads, sliced fruits & drinks for a wide variety of people (WHO,2009). The most popular local street foods in India are panipuri, bhelpuri, chaats, dahipuri, dahibara, jellebi, pavbhaji, idli, dosa, bara, pakoda, samosa, icecream, lassi, pizza, sandwich etc. Among these panipuri, bhelpuri, chaats, dahibara are commonly preferable by all level of consumers. Expansion of these foods range from regional to global and are exposed to maximum sources of contamination by various pathogenic microorganisms. Food borne illness of microbial origin is not only a national problem but is a major international health problem associated with street foods (WHO 2009). The contamination of street foods is mostly affected due to unhygienic water, improper washing of utensils, improper handling and packaging, low quality of raw materials used, unhealthy preparation & serving and improper storage practices. In addition, several intrinsic and extrinsic factors such as, pH, moisture content, water availability, temperature, gas, (CO_2, O_2) which create a favourable environment for growth & multiplication of microorganisms. Street vended foods are prepared & either sold for immediate consumption or consumed later without further processing or preparation (Dawson and Canet, 1991). Further, these foods are generally available wherever there is heavy flow of people like market complexes, festive crowds and in such areas where there is limited sanitary facilities of running water, garbage disposal & clean toilets. In addition to this, use of simple facilities like wheel barrows, trays, mats, tables & make shift stalls increase the risk of food contamination. Microbial studies on street vended foods in American, Asian & African countries have revealed increased bacterial pathogens in food which documented outbreaks of illnesses in human community (Mahale *et al*. 2008). This condition has given rise to many concerns to know about the quality, safety, sanitary standards & hygiene of street vending operations. Application of common preservative practices to street foods becomes limiting, because of their short lifetime.

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Considering the views above, an attempt has been made to investigate the bacteriology of most popular street foods (Panipuri, Dahibara and Chhat) sold at different localities of Baripada town in Odisha, India. Further, an attempt was made to improve the quality of these street foods by application of different essential oils of plant origin.

Material and Methods

Samples were collected from vendors at different localities i. e. at market complexes, near schools, colleges and University areas in Baripada Town, Odisha, India. . Data regarding time of preparation, water (borewell/Open well/ municipal supply) used, serving and washing practices, members of family involved in preparation of food, house type of vendors and availability of toilets, educational qualification of vendors were collected maintaining total confidentiality. Samples were collected in sterile containers and transported to the laboratory and processed within one hour of collection. Five samples from each (Panipuri, Dahibara, Chaat) were collected. However, both Panipuri and Dahibara samples were fractionated to both liquid(Sour soup of panipuri and curd of Dahibara) and soild (potato masala used in Panipuri and Bara of Dahibara) parts, in total amounting to twenty five samples.

Ten grams of solid parts of the samples were dissolved in 90 ml of sterile distilled water and used for bacteriological analysis whereas, the liquid parts were used directly for analysis. pH of the samples were measured directly on the spot at the time of collection by a pH paper as well as in the laboratory through pH meter. Aerobic bacterial load of the samples were determined by ten fold serial dilution, followed by surface viable count through spread plate and streak plate technique on both basal (Nutrient Agar) and selective (MacConkey agar, Eosine Methylene Blue agar, Salmonella Shigella agar, Xylose Lysine Deoxycholate agar and Thiosulphate Citrate Bile Salt sucrose Agar) media, procured from Hi-Media, Mumbai Ltd and prepared as per Manufacturer's instructions. Selected colonies from spread and streak plates were picked, pure cultured and were identified through morphology (colony characters on different Media, Grams staining) and through a battery of biochemical characters (Bhat and Myero 1962 ; Cruickshank et al. 1975; Holt et al. 2000). Presence of coliforms was determined through Most Probable Number (MPN-3 tube method) and confirmation was done followed by streaking on to Eosine Methylene Blue agar plates.

Viability of the pathogens in the food samples [10ml of liquid fractions(sour soup of Panipuri and curd of Dahibara) were taken directly, whereas, 1gm of solid fraction (potato masala) was mixed with 10ml distilled water in screw capped vials and sterilized] was tested at both ambient and at 37^0C by inoculating 100 µl of freshly grown culture of E. coli and Bacillus cereus (as representative pathogens) separately, into the sterilized food items and subcultured onto nutrient agar plates up to 30 days at an interval of 24hrs.

Antibiogram pattern and multiple antibiotic resistance index percentage (MARI%) was determined following the procedure described earler (Mohapatra et al. 2006). Susceptibility of the isolated pathogens was tested against ten edible essential oils[Cinnamon(Cinnamum zeylanicum); Clove (Syzygium aromaticum); Japanese mint (Mentha arvensis); Pepermint (Mentha piperata); Lemon (Citrus limonum); Orange (Citrus aurantium); Tulsi (Ocimum sanctum); Turmeric leaf (Curcuma longa); turmeric rhizome; Ginger (Zingiber officinalis)] by disc diffusion method (Rath et al. 2005). Minimum Inhibitory Concentration (MIC) of the oils (that showed better antibacterial activities through disc diffusion method) against the pathogens was determined through tube dilution method (Rath et al. 2005).

Finally an attempt was made to improve the quality of the food items by adding essential oils into these food samples with an idea that these essential oils with proved antibacterial activities could act as natural food preservatives. In a nutshell, Liquid fractions (sour soup of Panipuri) 10ml in screw capped vials were sterilized to which MIC levels of Clove and Japanese mint oils were added separately. One vial with the sample without oil served as control. Similarly, MIC levels of both the oils were also added to the solid fractions (1gm potato masala in 10 ml distilled water in vials and sterilized) separately and a vial without oil served as control. All the vials were inoculated with 100 µl of freshly grown culture of E. coli and incubated at 370C. After 24 hrs of incubation, aliquots were taken and viable cells were enumerated through plate count method in both test and control sets.

Results

The pH of the liquid samples ranged between 3.0-5.5. Whereas, the pH of the solid fractions, of the samples is reported to be slightly higher and ranged between 4.0-6.5. The higher acidity of liquid samples could be attributable to the addition of Tamarind and Lemon juices to it, however, the above pH values measured in these samples are suitable for growth for food borne pathogens. The aerobic bacterial load in these samples is found to be ranged between 104-107. While comparing the aerobic plate count with socioeconomic status of the vendors(data collected confidentially earlier),it is found that, highest bacterial load are found in the samples collected from those vendors who use water from open wells, involve more people for food preparation and reside in Kachha houses without toilet facilities. Unhygienic surroundings like lack of sewage, improper waste disposal systems, inadequate water supply attract house flies which further increases food contamination (Chumber et al. 2007). Food items are generally prepared much before the time of selling and stored at room temperature that is suitable for multiplication of these pathogens could be another factor contributing to observance of high bacterial load in these food samples. Occurrence of high bacterial load in street foods as observed in this investigation corroborates with the findings of several others (Luca and Torres 2006; Tambeker et al. 2009; Das et al. 2010; Das et al. 2011). It is also observed that the solid fractions of each sample is more contaminated in comparison to the liquid parts. Further, selling structures, use of dirty utensils, even serving food items in plant leaves, serving in bare hands, without head caps, preservation of food in room temperature, ignorance about food sanitation, use of poor quality of raw materials and improper storage at room temperature are the main factors of food contamination. In total, 86.66% of the samples were observed to be coliform positive that resembles with several other investigations (Tambeker et al. 2009; Das et al. 2010; Das et al. 2011) from different parts of the country.

During the investigation a total of 126 bacterial strains are isolated and 91.26% of the isolates were identified based on their growth characters on different cromogenic media and through an array of biochemical characters and are assigned to 8 genera viz. *E. coli, Enerobacter, Bacillus Pseudomonas, Salmonella, Shigella, Staphylococcus and Streptococcus* spp (Table1). *E. coli* is observed to be the most dominant genera reported from all the samples followed by *Enterobacter* spp. However, *Shigella dysenteriae* and *Streptococcus faecalis* are reported at a very low percentage. Presence of members of enterobacteriaceae in these street foods may indicate fecal contamination which may be due to insufficient cooking, use of raw vegetables, cross contaminations because of not separating raw and cooked food and contaminated ingradients and hands are important source of contamination and dissemination of fecal-oral transmission of bacteria including coliforms (Burt et al. 2004).

Contamination of most popular fast foods such as Panipuri, Bhelpuri, and Chaat by *Streotococcus faecalis*, *E. coli*, *S. aureus, Klebsiella* and *Pseudomonas* spp, in Bangalore city was reported earlier(Das et al. 2011). The presence of the mesophilic bacilli found in the street vended foods can also be explained in the fact that the ingredients such as wheat and rice flour used in preparation of Indian Chaats contain spores of Bacillus. In accordance to this fact, in our study the members of *Bacillus (*n=25) were identified as *B. cereus* (36%), B. coagulans (32%), B. subtilis (20%), and B. megaterium (8%) based on their growth characters on Hi-chrome Bacillus agar. Presence of spore forming Bacillus spp, more specifically B. cereus in street foods is of great significance since this organism produces heat sensitve and heat stable toxins associated with food poisoning (Bryan et al. 1992). Similarly, 76.1% and 23.9% represented *Salmonella typhi* and *S. typhimurium* respectively, out of 21 Salmonella strains isolated in our study. Incidence of *S. typhi* and *S. typhimurium* in street foods causing various illness world wide was reported (Cardinale et al. 2005 & Tunung et al. 2006).

While studying the viability of *E. coli* and *B. cereus* (taken as representatives of Gram –ve and Gram+ve pathogens respectively) in these food samples it is observed that, both the organisms survived up to 30 days (maximum time period tested) at 37^0C as well as at ambient (room temperature 32±20C). Viability of these organisms for a long period in these samples is an additional factor to contamination of these street foods, as in many cases the vendors store the left out items at room temperature for selling in the next day. From the antibiogram studies it is observed that the strains represented a high degree of multiple antibiotic resistance index (MARI%) ranged between 17.64-47.05(Table 1).

Table 1. **Predominance of bacteria different food samples with the IR multiple antibiotic resistance index.**

Isolate	Occurred in no. of food samples	No. of bacteria isolated (n=126)	Dominance percentage (%)	MAR%	%Susceptibility to essential oils (essential oils n=10)
Bacillus sp.	9	25	19.84	17.64	40.00
E. coli	25	29	23.01	17.64	60.00
Enterobacter sp.	11	28	22.22	29.41	50.00
Pseudomonas sp.	03	05	3.96	29.41	40.00
Salmonella sp.	07	21	16.66	29.41	70.00
Shigella dysenteriae	02	02	1.58	47.05	80.00
Staphylococcus sp	03	04	3.17	23.52	80.00
Streptococcus faecalis	01	01	0.79	23.52	70.00
Unidentified	--	11	8.73	ND	ND

ND: Not determined

Table 2. Minimum inhibitory concentration(MIC) of the oils against the pathogens

Pathogen/Isolate	MIC (µl/ml)			
	Clove	Japanese mint	Lemon	Ginger
B. cerueus	3.9	3.9	250	250
E. coli	1.9	15.6	250	250
Enterobacter sp.	1.9	1.9	250	250
Pseudominas sp	3.9	7.8	250	250
Salmonella sp.	3.9	31.2	250	250
Shigella dysenteriae	3.9	62.5	125	250
Staphylococcus sp.	1.0	0.97	3.9	3.9
Streptococcus faecalis	3.9	15.6	250	250

TABLE 3: Antibacterial efficacy of Japanese mint and Clove essential oils against *E,coli* in model food systems

	Viable count CFU/gm/ml					
	Sour Soup			Potao Masala		
Incubation Time	JM	CL	Control	JM	CL	Control
24 hr	Nil	Nil	2.3×10^{3}	Nil	1.9×10^{5}	2.3×10^{6}
48 hr	Nil	Nil	2.3×10^{3}	Nil	0.6×10^{2}	4.4×10^{8}

JM : Japanese mint oil; CL:Clove oil

Highest MAR index % of 47. 05 is reported in case of *Shigella dysenteriae* and least MAR index % in case of *Bacillus cereus* and *E. coli* i. e. 17.64. The strains were resistant to one or more of the 17 antibiotics tested. This prompted us to study the antibacterial potential of ten essential oils (as there is a growing demand about use of natural preservatives in food items among the consumers), primarily through disc diffusion method and we observed a high degree of susceptibility of these pathogens towards the oils. Interestingly, *S. dysenteriae* that showed highest MARI%, also, represented highest degree of susceptibility (80%). Low degree of susceptibility towards the oils was reported in case of *B. cereus* and *Pseudomonas* sp. (40%). Minimum inhibitory concentration (MIC) of the four oils (Lemon, Ginger, Japanese mint and Clove, that showed better antibacterial activities in the previous experiment) against the pathogens was studied through two fold tube dilution method. The MIC values ranged between 1.9-250 µl/ml and lowest MIC was reported with Clove oil followed by Japanese mint (Table-2).

From the MIC studies it is evident that both Clove and Japanese mint oils were effective at a low concentration and the activities were reported to be bactericidal as no growth of the pathogens was observed when one loop of the oil pathogen mixture sub-cultured onto Nutrient agar plates from the MIC dilution tubes. In several studies literature suggests the effect of essential oils against microorganisms even at less than 1µl/ml(Mohapatra et al., 2006 & Rath et al. 2007) , though higher concentrations are also reported (Rath 2007).

Further, we designed an experiment to study effect of these two essential oils (Clove and Japanese mint) directly supplementing (at MIC levels) to both liquid and solid parts of the food samples. Both the oils have the ability to kill the pathogens in the food samples within 24-48 hrs of incubation (Table3). The killing ability was more rapid in liquid in comparison to solid fractions. Singh et al., (2002) reported the antibacterial action of essential oils depends on the bacterial load and more effective in food products that have a lower level of bacterial contamination. Similar findings were also reported in our case, i. e. the effectiveness of both the oils were observed significantly in case of liquid fractions as lower bacterial load was reported in it, in comparison to the solid parts. Further, the better action of the oils in liquid samples could be attributable to low pH, less nutrition value and addition of spices (ginger, garlic, coriander leaves, with proved antibacterial activities) to the liquid portions could have accelerated the antibacterial action of these oils synergistically.

Conclusion

Through this study we have established the presence of pathogenic bacteria in selected street food samples collected in and around Baripad Town, Odisha, India, also have established the possible reasons of contamination of these food samples and persistence of pathogens in it for a long period under natural conditions. Pathogens isolated in this study, reported high multiple antibiotic resistance indices which is of great concern. Further, the susceptibility of these pathogens towards essential oils is proved through assay methods as well as directly supplementing to the street foods. Since there is a growing demand about natural food preservatives and also looking into the edibility, non toxicity and antibacterial properties of these essential oils, it is suggestive to explore these natural products as food preservatives in various food industries against costly toxic chemical preservatives.

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