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BACTERIA LADEN STREET FOOD (CHATPATI) AND THEIR MULTIPLE ANTIBIOTIC RESISTANCE INDEX

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Abstract

The bacteria associated with street food Chatpati and their multi-drug resistance pattern were investigated. The highest bacterial load of solid part of Chatpati was recorded on NA as 1.07×10^5 - 9.7×10^5 cfu/g followed by 1.55×10^4 - 4.05×10^5 cfu/g on EMB. In case of liquid part (Spicy tamarind) maximum load was 3.1×10^2 - 6.4×10^4 cfu/ml on SS agar. The bacterial isolates were provisionally identified as *Escherichia coli*, *Klebsiella* sp., *Enterobactor* sp., *Micrococcus variens*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Salmonella paratyphi A*. Multiple antibiotic resistance index (MRI) was found between 14.28 and 71.43%. *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp. and *Salmonella paratyphi A* showed the highest MRI percentage. Bacterial high loads and the presence of many enteric bacteria indicated significant food contamination. The presence of multi-drug resistant bacteria is very much alarming for the city dwellers habituated with the popular snacks like Chatpati.

Introduction

Street foods such as ready-to-eat foods (RTE), beverages and processed food are sold at stationary locations or by mobile vendors in streets and open places. The street foods provide a source of affordable nutrients to the majority of the people specially the low earning group in the developing countries (Muzaffar *et al.* 2009). Street foods are frequently associated with diarrhoeal diseases due to their improper handling and serving practices (Barro *et al.* 2006). Microbial contamination of RTE sold by street vendors and hawkers has become a major health problem for the consumers (Tambekar *et al.* 2008).

In developing countries, drinks, meals and snacks sold by street food vendors are widely consumed by millions of people and a considerable percent of consumers have been suffering from disease like dysentery, diarrhea, enteric fever etc. (Ali *et al*. 2011, Das *et al.* 2011, Rath and Patra 2012). In addition, multi-drug resistance of food borne microorganisms made the food safety situation more vulnerable in public health (Khairuzzaman *et al.* 2014). Approximately, 30 million people in Bangladesh are suffering from food borne illnesses each year (FAO 2012). In Bangladesh Chatpati in addition to Phuchka, Jhalmuri, Velpuri, Panipuri, Puri, Samosa, Singara, Beguni, Chop etc. is very common and popular item (Rahman *et al.* 2014). Food borne illnesses caused by microorganisms are a major national and international health problem and an important cause of death in developing countries (Garode and Waghode 2012). Considering street foods and food borne pathogens an attempt was made to study the bacteria associated with Chatpati and their multiple antibiotic resistance index.

Materials and Methods

In total eight Chatpati samples were collected from different vendors in and around Dhaka University campus. Samples were categorized into two parts *viz*. the liquid tamarind soup part and

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the solid part of chickpea, mashed potato etc. The liquid and the solid part were collected separately in sterile PET (Polyethylene terephthalate) bottles and polythene bags. The samples were labeled properly and brought to the laboratory as soon as possible and analyzed within one hr of collection. Collected samples were preserved in a refrigerator at 4°C before and after the microbiological analysis. The pH of the samples was measured by a pH meter (Jenway 3310, U.K.). Nutrient agar (NA) medium was used for the enumeration and isolation of aerobic heterotrophic bacteria while MacConkey agar (Difco), *Salmonella-Shigella* (SS) agar and Eosine Methelene Blue (EMB) agar media were used for the enteric bacteria. Serial dilution technique (Clesceri *et al.* 1998) was used for the isolation of microorganisms. Plating in duplicated plates was made for each diluted sample. The plates were then incubated at 37°C in an incubator (Memmert GmbH + Co Kg 8540 Sehwabach) for 24 hrs. After incubation colonies were counted by a colony counter (Digital colony counter, DC-8 OSK 100086, Kayagaki, Japan). Following standard manuals Gram stain and essential biochemical tests were performed. Characterization and identification of the isolates were made through standard microbiological methods (Sneath *et al.* 1986, WHO 1987).

Antibiotic sensitivity test was carried out against seven common antibiotics *viz.* streptomycin (10 µg); gentamycin (10 µg); rifampicin (5 µg); vancomycin (30 µg); polymixin B (300 µg); penicillin g (10µg); neomycin (30 µg) by disc diffusion method and multiple antibiotic resistant index (MRI, %) was determined using the formula (Mahapatra *et al.* 2006) :

$$\text{MRI\%} = \frac{\text{No. of antibiotics to which pathogen showed resistance}}{\text{No. of antibiotics used}} \times 100$$

Results and Discussion

The pH of liquid samples observed to be highly acidic (pH 2.8 – 3.6) whereas solid samples ranged in between 4.5 – 6.0 (Table 1). The higher acidity of liquid samples could be due to tamarind as one of the major ingredients. The bacterial load of Chatpati was shown in the Table 1.

Table 1. pH and bacterial load of collected samples.

Sample No	pH		Bacterial load (cfu/g of solid part and cfu/ml of liquid part) on							
			Nutrient agar		MacConkey agar		SS agar		EMB agar	
	Solid part	Liquid part	Solid part	Liquid part	Solid part	Liquid part	Solid part	Liquid part	Solid part	Liquid part
1	5.96	3.42	7.1×10^4	2.35×10^4	Nil	1.8×10^4	Nil	6.4×10^4	4.1×10^4	3.05×10^4
2	6.00	2.90	9.7×10^5	3.8×10^2	Nil	Nil	Nil	6.0×10^3	2.45×10^5	Nil
3	4.54	3.21	2.65×10^5	1.6×10^4	Nil	Nil	Nil	Nil	4.05×10^5	4.0×10^3
4	5.93	3.60	4.1×10^5	1.15×10^4	2.1×10^5	1.45×10^5	1.55×10^3	3.1×10^2	Nil	Nil
5	4.65	3.01	1.07×10^5	2.0×10^4	Nil	Nil	Nil	4.95×10^4	Nil	Nil
6	5.67	3.11	7.95×10^5	1.35×10^5	4.05×10^3	Nil	Nil	3.4×10^2	1.55×10^4	2.95×10^4
7	4.93	2.81	4.85×10^4	2.0×10^3	1.8×10^4	Nil	1.05×10^3	Nil	Nil	Nil
8	4.50	3.26	2.55×10^4	3.4×10^2	Nil	Nil	Nil	Nil	1.65×10^4	Nil

The highest bacterial load (1.07×10^5 - 9.7×10^5 cfu/g) was recorded on NA followed by 1.55×10^4 - 4.05×10^5 cfu/g on EMB. The similar findings were also observed by other workers (Das *et al.* 2010, Tambekar *et al*. 2008). In case of liquid part the highest bacterial load (3.1×10^2 - 6.4×10^4 cfu/ml) was recorded on SS agar followed by 3.8×10^2 - 1.35×10^5 cfu/ml on NA. The results revealed that the solid fractions were more contaminated in comparison to the liquid parts. It could be due to the presence of tamarind juice as one of the major ingredients. High contamination might be due to unhygienic preparation and contaminated water. In most cases supply of potable water is not available at vending sites and thus hand and dish washing are usually done in buckets and sometimes without soap. The use of raw vegetables also contributes to the bacterial load (Tambekar *et al*. 2008). Moreover, the contamination may come from the utensils, raw materials, or transport methods used as well as lack of aseptic handlings. Vendors usually prepare and serve the food in bare and unwashed hands could be the most probable sources of contamination (Khairuzzaman *et al*. 2014).

During this study 20 isolates were selected for detailed study of which nine were Gram-positive and 11 were Gram-negative (Tables 2 and 3). The isolated Gram-positive bacteria were provisionally identified as *Bacillus*, *Staphylococcus*, *Planococcus*, *Micrococcus*, *Streptococcus*, on the other hand, Gram-negative members identified as the genera of *Alcaligenes*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Escherichia*. Ali *et al*. (2011) observed the presence of *Salmonella paratyphi A*, *Klebsiella*, *Enterobacter*, *Escherichia coli*, *Proteus* sp. and *Alcaligenes* in ready to eat (RTE) food in Dhaka city.

Table 2. Major biochemical test and provisional identification of Gram-positive bacteria.

Isolates	Biochemical test								Provisional name
	DGA	VP	MR	Casein	Gelatin	Citrate	Propionate	NR	
BN1/L1	SA	+	+	+	+	-	-	-	*Bacillus schlegelii*
BN1/L3	FA	-	-	+	+	-	-	-	*Staphylococcus aureus*
EN1/L4	SA	-	+	+	+	-	+	-	*Planococcus citreus*
AF1/L5	SA	_	+	+	+	+	+	-	*Planococcus citreus*
SF1/S12	SA	-	+	-	+	-	-	+	*Micrococcus variens*
SF2/S13	SA	+	+	+	+	-	-	+	*Bacillus subtilis*
BN2/S4	SA	-	-	+	+	-	+	-	*Planococcus citreus*
SF1/S11	FA	-	+	+	+	-	-	+	*Streptococcus faecalis*
BN1/S1	SA	+	+	+	+	-	-	+	*Bacillus subtilis*

+ = Positive result - = Negative result.

Bacterial abundance of the isolates was shown in the Table 4. Among the isolates *Planococcus citreus* and *Bacillus* sp. were the highest (33%). The lowest abundance represented by *Micrococcus variens*, *Streptococcus faecalis* and *Staphylococcus aureus* (11%). The major occurrence of *Planococcus citreus, Alcaligenes* sp., *Escherichia coli, Klebsiella* sp. may be due to poor personal hygiene of the vendors, unhygienic handling of foods, poorly cleaned dishes and use of raw vegetables like cucumber, onion etc. (Das *et al.* 2011). Similar types of bacterial genera were identified in fruits and vegetables in Nigeria (Eni *et al.* 2010).

Street foods are not protected from the various contaminations such as flies, which may carry food borne pathogens, multifunctional hands and also own health status of venders. Potential health risks are associated with contamination of food by *E. coli*, *Salmonella typhi* and *Staphylococcus aureus* during preparation, post cooking and other handling stages (Garode and

Waghode 2012). Barro *et al.* (2006) mentioned that 75% of consumers and 68% vendors' hands *S. aureus* carriers.

Table 3. Major biochemical tests and provisional identification of Gram-negative bacteria.

Isolates	Biochemical test							Provisional name
	Urease	H_2S	Motility	Indole	Oxidase	KOH	Levan	
EN2/S7	-	-	+	-	+	+	-	*Alcaligenes* sp.
AF2/L9	-	-	+	-	+	+	-	*Alcaligenes* sp.
AF2/L10	-	-	-	-	-	+	-	*Salmonella paratyphi A*
SF2/L15	+	-	+	-	-	+	+	*Klebsiella* sp.
BN1/S3	+	-	+	-	-	+	+	*Klebsiella* sp.
EN1/S5	-	-	+	-	-	+	-	*Enterobacter* sp.
AF1/S8	+	-	+	-	-	+	-	*Enterobacter* sp.
BN1/L2	-	-	+	-	+	+	-	*Alcaligenes* sp.
SF2/L12	+	-	+	-	-	+	-	*Escherichia coli*
AF2/S9	+	-	+	-	-	+	-	*Escherichia coli*
SF2/S14	-	-	-	-	-	+	-	*Salmonella paratyphi A*

+ = Positive result - = Negative result.

Table 4. Number of isolates and their percentage of abundance.

	Name of organisms	Number of occurrence	Percentage
Gram-positive	*Planococcus citreus*	3	33
	Bacillus sp.	3	33
	Streptococcus faecalis	1	11
	Staphylococcus aureus	1	11
	Micrococcus variens	1	11
Gram-negative	*Alcaligenes* sp.	3	27
	Klebsiella sp.	2	18
	Escherichia coli	2	18
	Salmonella paratyphi A	2	18
	Enterobacter sp.	2	18

To find out MRI the isolates were tested against some common antibiotics *viz*. streptomycin, gentamycin, rifampicin, vancomycin, polymixin B, penicillin G, neomycin and the results was shown in Table 5. The MRI percentage of the isolates ranged in between 14.28 and 71.43. *Escherichia coli*, *Klebsiella* sp., *Salmonella paratyphi A* and *Enterobacter* sp. showed the highest percentage (71.43) of MRI. Ali *et al*. (2011) reported *Enterobacter* sp. showed highly resistance to gentamycin, vancomycin, penicillin G and rifampicin. Das *et al*. (2011) reported that *Salmonella paratyphi* showed the 72.4% MRI. In another work Rath and Patra (2012) reported the highest

Table 5. Antibiotic sensitivity pattern of the isolates.

Organism	Antibiotics		MRI%
	Sensitive[a] to	Resistant to	
Staphylococcus aureus (1)[b]	S-10(16), CN-10(21.5), RD-5(29), VA-30(23), N-30(26), PB-300(19), P-10(31)	-	0
Bacillus schlegelii (1)	S-10(23), CN-10(29.5), RD-5(23), VA-30(20), N-30(20.5), PB-300(12.5), P-10(27)	-	0
Planococcus citreus (3)	S-10(17), CN-10(23), RD-5(26.5), VA-30(12), N-30(19), PB-300(17)	P-10	14.28
Streptococcus faecalis (1)	S-10(26), CN-10(16), N-30(17.5), PB-300(19.5), P-10(22)	RD-5, VA-30	28.57
Bacillus subtilis (2)	S-10(30), CN-10(31.5), RD-5(22), VA-30(23), N-30(21), PB-300(16.5), P-10(27)	-	0
Micrococcus variens (1)	S-10(26), CN-10(25), RD-5(31), VA-30(21), N-30(28), PB-300(17.5), P-10(19.5)	-	0
Escherichia coli (2)	S-10(20), CN-10(18)	RD-5, VA-30, N-30, PB-300, P-10	71.43
Klebsiella sp. (2)	S-10(18), CN-10(21)	RD-5, VA-30, N-30, PB-300, P-10	71.43
Salmonella paratyphi A (2)	CN-10(15), PB-300(14)	S-10, RD-5, VA-30, N-30, P-10	71.43
Enterobacter sp. (2)	S-10(21), CN-10(16)	RD-5, VA-30, N-30, PB-300, P-10	71.43
Alcaligenes sp. (3)	S-10(26), CN-10(29), N-30(22)	RD-5, VA-30, PB-300, P-10	57.14

[a]Values in parentheses represent zone of sensitivity in mm and [b] represent number of occurrence; MRI = Multiple antibiotic resistance index. [S-10 = Streptomycin (10 µg), CN-10 = Gentamycin (10 µg), RD-5 = Rifampicin (5 µg), VA-30 = Vancomycin (30 µg), N-30 = Neomycin (30 µg) PB-300 = Polymixin B (300 µg) and P-10 = Penicillin G (10 µg)].

MRI in *Shigella dysenteriae* followed by *Enterobacter* sp., *Staphylcoccus* sp. and *Streptococcus faecalis*, which is very much similar to our present study. The presence of *E. coli*, *Salmonella*, *Klebsiella*, *Enterobacter* etc. and the multiple drug resistant bacteria associated with Chatpati indicated significant health hazardous. Therefore, it could be concluded that Chatpati sold in the streets is not safe for the consumers and needs immediate public awareness both for consumers and the vendors.

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