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ANTIBIOTIC RESISTANT PATTERNS OF BACTERIAL ISOLATES FROM READY-TO-EAT (RTE) STREET VENDED FRESH VEGETABLES AND FRUITS IN DHAKA CITY.

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Abstract

Fresh vegetables and fruits like cucumber, carrot, green mangoes and water used in the shops were collected from different vendors of Dhaka metropolitan city, Bangladesh, were analyzed for microbiological quality. Lactose fermenter (LF) bacterial load of cucumber, carrot, mangoes and washing water were found to be 4.3×10^3 to 9.45×10^5 cfu/g, 9.3×10^3 to 7.2×10^6 cfu/g, 28 to 305 cfu/g and 8.5×10^2 to 1.57×10^7 cfu/ml, respectively. Non-lactose fermenter (NLF) bacterial load of cucumber, carrot, green mangoes and washing water were found to be 6.1×10^2 to 2.7×10^5, 8.95×10^2 to 3.8×10^6, 0.0 to 570 and 4.0×10^2 to 3.9×10^6 cfu/ml, respectively. Overall enteric bacterial load of cucumber, carrot, green mangoes and washing water varied from 6.5×10^3 to 1.22×10^6, 1.02×10^4 to 1.1×10^7, 22 to 870 and 1.3×10^3 to 1.96×10^7 cfu/ml, respectively. The sold carrot was found to be the good vehicles for enteric bacteria as the highest count was observed associated with the carrot. Seven enteric bacteria like *Klebsiella*, *Enterobacter*, *Salmonella paratyphi* A, *Hafnia*, *Escherichia coli*, *Alcaligenes*, *Proteus* were associated with the present samples. The presence and abundance of enteric bacteria clearly showed the food hazard from microbiological point of view. Multiple drug resistance was observed which is very much alarming to the consumer.

Key words: Enteric bacteria, lactose fermenter, non-lactose fermenter, antibiotic resistance.

Introduction

In recent times ready to eat (RTE) fresh vegetables and fruits are often popularly consumed by young group of people of different profession. Street foods (ready-to-eat foods sold in the informal sector) form an important and well established sector of the food industry in Ghana. These are readily available, inexpensive, nutritionally balanced and also provide a source of income for the vendors (Ekanem 1998). Increases in the world-wide consumption of RTE food produce have resulted in increases in food-borne illness associated with these products (Sivapalasingam *et al.* 2004). In cities like Mumbai, with the growing population eating fresh fruits and vegetables has become very popular (Poorna 2001).

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The bacterial population of salad vegetables is diverse and has been studied (Khan *et al.* 1994). Food borne parasites have received little attention in developing countries (Francis *et al.* 1999). As a rule, these organisms infest vegetables while still in the field, and are usually transmitted by contaminated wash water and spread by ineffective hygiene practices (Beuchat 1996). Special attention has been paid to those vegetables which are usually eaten raw, since they can act as vehicles for transmitting pathogenic enteric diseases (Ayres *et al.* 1980, ICMSF 1981). Studies on street vended foods in USA, Asia and a few African countries have revealed high bacterial counts and presence of food borne bacterial pathogens (Mosupuye and Holy 1999, Bryan *et al.* 1997, FAO 1997). A number of studies have assessed the microbiologic conditions of RTE vegetables available in supermarkets, street markets, and grocery shops, as well as in self-service and fast-food restaurants (Soriano 2000).

In developing countries where environmental sanitary conditions are poor, gasteroenteric diseases like dysentery, diarrhoea, enteric fever etc. are generally transmitted through water and food items. Considering above facts fresh vegetables sold by vendors were selected and the present study was under- taken to determine the bacteriological quality of RTE raw foods like cucumber, carrot and green mangoes sold by vendors in Dhaka city.

Materials and Methods

Ten different areas of Dhaka city such as residential area, public rushed area were selected for study. The sampling sites were Chankharpool, Curzon Hall area, Bango Bazar, Shahbagh bus stoppage, Farmgate, Kawran Bazar, Motijheel and Mohammadpur. RTE raw foods like carrot, cucumber, green mango were collected in sterile polythene bags. Water was also collected from the vendor in sterile plastic bottles.

Ten gm of each RTE food (cucumber, carrot and green mangoes) samples were taken in sterile conical flask containing 90 ml sterile water. In case of water 1 ml of sample water was added in sterile conical flask containing 99 ml sterile water. After mixing thoroughly decimal serial dilution was conducted for enumeration and isolation of bacterial colony. MacConkey agar medium was used for enteric bacterial analysis. The plates were then incubated for 24 hrs at 37°C. Colony grown on the plates were counted with the colony counter. Bright red colonies were considered as lactose fermenter (LF) while non-lactose fermenters (NLF) were whitish in colour. Total count of bacteria was determined simply by counting of LF and NLF. Following standard manuals Gram stain and essential biochemical tests were performed. Haemolysis was carried out with blood agar base (Difco). Melted blood agar medium with 5% sheep blood was poured in sterilized Petri dishes (Schuch *et al.* 1992). After solidification the medium was inoculated with 24 hrs old culture and incubated at 30°C. After incubation haemolytic reactions were recorded.

Culture and sensitivity test was carried out to reveal the drug resistance pattern of the isolates. Seven common antibiotic disks (Oxoid Ltd.) *viz.* polymixin B (300 μg), rifampicin (5 μg), neomycin (30 μg), vancomycin (30 μg), streptomycin (10 μg), gentamycin (12 μg), penicillin G (10μg) were tested against isolated bacterial strains.

Results and Discussion

Bacterial counts of RTE vended fresh vegetables, fruits and the water samples are shown in Table 1. The RTE fresh vegetables, fruits and washed water showed wide variation in enteric bacterial load. Lactose fermenter bacterial load of cucumber, carrot, green mangos and washing water were found to be 4.3×10^3 to 9.5×10^5, 9.3×10^3 to 7.2×10^6, 28 to 305 cfu/g and 8.5×10^2 to 1.57×10^7 cfu/ml, respectively. NLF bacterial load of cucumber, carrot, green mangoes and washing water were found to be 6.1×10^2 to 2.7×10^5, 9.0×10^2 to 3.8×10^6, 0.0 to 570 cfu/g and 4.0×10^2 to 3.9×10^6 cfu/ml, respectively. Total enteric bacterial load of cucumber, carrot, green mangoes and washing water varied from 6.5×10^3 to 1.2×10^6, 1.0×10^4 to 1.1×10^7, 22 to 870 cfu/g and 1.3×10^3 to 2.0×10^7 cfu/ml, respectively.

Among the RTE vended fresh vegetables and fruits carrot was found to be good carrier of enteric bacteria as it showed the highest count (7.2×10^6 cfu/g). On the other hand green mangoes were found to be little bit better to the consumer as it was associated with less number (22 - 870 cfu/g) of enteric bacteria. This could be a due to acidic nature of the green mango.

Isolated bacterial isolates were all Gram negative, short rod and non spore former. Physiological and biochemical tests showed that the isolated organisms comprised of seven genera *viz.* *Escherichia* (5.6%), *Enterobacter* (16.7%), *Klebsiella* (5.6%), as LF and *Hafnia* (5.6%), *Proteus* (11.1%), *Alcaligenes* (16.7%) and *Salmonella* (22.2%) as NLF (Table 2 and 3). *Salmonella* (22.2%) was found to be dominating over others.

Table 1. Bacterial count of RTE street vended fresh vegetables, fruits (cfu/g) and used water (cfu/ml).

Sampling sites	Bacterial load											
	Cucumber			Carrot			Green Mango			Used water		
	LF	NLF	Total	LF	NLF	Total	LF	NLF	Total	LF	NLF	Total
Chankharpool	9.7×10^{3}	1.7×10^{4}	2.7×10^{4}	1.4×10^{5}	6.7×10^{5}	8.1×10^{5}	300	570	870	1.4×10^{6}	1.5×10^{6}	1.9×10^{6}
Bango Bazar	4.3×10^{4}	1.9×10^{4}	6.4×10^{4}	1.3×10^{6}	2.7×10^{6}	4.0×10^{6}	115	205	320	4.7×10^{5}	1.7×10^{5}	6.3×10^{5}
Curzon Hall	3.4×10^{4}	6.5×10^{3}	4.0×10^{4}	5.0×10^{6}	1.9×10^{6}	6.9×10^{6}	135	110	245	6.7×10^{5}	2.3×10^{5}	1.0×10^{6}
Shahbagh	9.1×10^{4}	6.1×10^{2}	9.1×10^{4}	9.3×10^{3}	9.0×10^{2}	1.0×10^{4}	170	60	230	8.8×10^{4}	1.1×10^{5}	1.2×10^{5}
Motijheel	1.8×10^{5}	7.5×10^{4}	2.6×10^{5}	1.1×10^{6}	3.7×10^{5}	1.5×10^{6}	50	110	160	1.6×10^{7}	3.9×10^{6}	2.0×10^{7}
Gulistan	2.6×10^{4}	5.0×10^{3}	3.1×10^{4}	1.0×10^{6}	3.0×10^{5}	1.3×10^{6}	28	5	33	9.7×10^{5}	1.4×10^{5}	1.1×10^{6}
New Market	7.8×10^{4}	1.4×10^{4}	9.2×10^{4}	7.0×10^{6}	1.5×10^{6}	8.5×10^{6}	305	40	355	1.6×10^{3}	4.0×10^{2}	2.0×10^{3}
Kawran Bazar	5.8×10^{5}	1.1×10^{5}	6.9×10^{5}	6.9×10^{6}	2.0×10^{6}	8.9×10^{6}	75	0.0	75	7.9×10^{5}	3.6×10^{5}	1.2×10^{6}
Farmgate	9.5×10^{5}	2.7×10^{5}	1.2×10^{6}	7.2×10^{6}	3.8×10^{6}	1.1×10^{7}	130	15	145	9.9×10^{5}	4.2×10^{5}	1.4×10^{6}
Mohammadpur	4.3×10^{3}	2.2×10^{3}	6.5×10^{3}	1.4×10^{4}	3.5×10^{3}	1.8×10^{4}	18	4	22	8.5×10^{2}	4.5×10^{2}	1.3×10^{3}

Table 2. Biochemical test and provisionally identified bacterial isolates.

Isolates	Biochemical test							Provisional name
	Urea	H_2S	Motility	Indole	Oxidase	KOH	Nitrate reduction	
W-1	+	-	+	-	-	+	+	*Klebsiella*
W-2	-	-	+	-	-	+	+	*Enterobacter*
W-3	-	-	+	-	+	+	+	*Enterobacter*
W-4	+	-	+	-	-	+	+	*Enterobacter*
W-5	-	-	+	-	-	+	+	*Salmonella paratyphi* A
Pw-1	+	-	+	-	-	+	+	*Hafnia*
Pw-2	+	-	+	-	-	+	+	*Escherichia coli*
Pw-3	-	-	+	-	-	+	+	*Alcaligenes*
Pw-4	-	-	+	-	-	+	+	*Salmonella paratyphi* A
Pw-5	+	-	+	-	-	+	-	*Proteus rottgeri*
C-1	-	-	+	-	-	+	+	Unidentified
C-2	-	-	+	-	-	+	+	Unidentified
C-4	-	-	+	-	-	+	+	*Salmonella paratyphi* A
C-5	-	-	+	-	-	+	+	Unidentified
Cu-1	-	-	+	-	-	+	+	*Alcaligenes*
Cu-2	+	-	+	-	+	+	+	*Proteus mongani*
Cu-3	-	-	+	-	-	+	+	*Alcaligenes*
Cu-4	-	-	+	-	-	+	+	*Salmonella paratyphi* A

A higher rate of prevalence was reported in Italy and showed that 68.3% of lettuce samples contained salmonellae (Ercolani 1976).

Adu-Gyamfi and Nketsia-Tabiri (2007) reported *Escherichia coli*, *Enterobacter* spp. and *Klebsiella* spp. from both morning samples of macaroni. In present study *Enterobacter* ssp. and *E. coli* were also present in the RTE foods.

Mosupuye and von Holy (1999) reported the presence of *Salmonella* in street foods in South Africa. Viswanathan and Kaur (2001) showed presence of *Salmonella, Serratia, Enterobacter*, *Staphylococcus aureus*, faecal *E. coli* and *P. aeruginosa* in vegetables and fruits. The present study also showed the presence of *Salmonella, Enterobacter* in RTE vended street fresh vegetables and fruit samples.

WHO (2002) reported that *Salmonella* sp. causes salmonellosis and typhoid fever and *E. coli* O157:H7 causes severe illness and deaths, especially among children in several countries.

Table 3. Number of isolates and their percentage.

Name of Organisms	Number of occurrence	Percentage
Alcaligenes	3	16.7
E. coli	1	5.6
Enterobacter	3	16.7
Hafnia	1	5.6
Klebsiella	1	5.6
Proteus rottgeri	1	5.6
Proteus mongani	1	5.6
Salmonella	4	22.2
Unidentified	3	16.7

The presence of *E. coli* in the samples, which were also positive for faecal coliforms, indicated faecal contamination of the RTE foods analysed. These results are similar to those on RTE vegetables (Kaneko *et al.* 1999, Nguz *et al.* 2005) and lettuce served in restaurants (Soriano *et al.* 2000).

The isolates were studied for their sensitivity to some common antibiotics. A good number of isolates showed more or less resistant towards antibiotics tested (Table 4).

Table 4. Culture and sensitivity test of the isolates.

Strain	Name of antibiotics and inhibition zone area (mm)						
	P-10	CN-120	VA-30	S-10	RD-5	PB-300	N-30
Klebsiella	R	9.5	R	6.5	R	8.0	7.5
Enterobacter	R	R	R	8.0	R	7.0	8.5
Enterobacter	R	6.0	2.5	R	4.5	6.0	7.5
Enterobacter	R	18	R	8.5	R	5.5	13
Salmonella paratyphi A	R	6.0	R	8.5	R	8.0	8.0
Hafnia	R	5.0	R	9.0	R	5.0	7.5
Escherichia coli	3.0	12	R	10	1.5	9.0	8.0
Alcaligenes	2.5	8.0	R	7.0	R	6.0	7.0
Salmonella paratyphi A	R	15	R	8.0	0.5	5.5	9.0
Proteus rottgeri	R	14	R	8.0	R	8.0	11
Unidentified	R	9.0	R	10	R	8.0	6.0
Unidentified	1.0	R	R	8.0	R	6.0	5.0
Salmonell paratyphi A	R	14	4.0	9.0	2.0	9.0	9.0
Unidentified	R	R	R	10	R	6.5	9.0
Alcaligenes	1.0	7.5	2.5	7.0	1.0	8.0	7.0
Proteus mongani	R	10	R	6.0	0.5	8.0	7.0
Alcaligenes	R	17	R	5.0	1.0	8.0	11
Salmonella paratyphi A	R	8.0	R	9.0	R	7.0	8.0

P-10 = Penicillin G (10 μg), CN-120 = Centamycin (120 μg), VA-30 = Vancomycin (30 μg), S-10 = Streptomycin (10 μg), RD-5 = Rifampicin (5 μg), PB-300 = Polymixin B (300μg) and N-30 = Neomycin (30 μg).

Penicillin and vancomycin were found to be more resistant in comparison to other antibiotic tested. Although hemolysis of the isolated bacterial isolates were found to be negative but multiple drug resistance could be the health hazards to the consumer.

Conclusion

The presence of potential food borne pathogens and multiple drug resistance in ready to eat street vended fresh vegetables and fruit raises serious food safety concerns and calls for urgent action. In addition to the provision of infrastructure and enforcement of laws and codes of practice on street foods, emphasis should be placed on educating vendors on simple preventative steps of keeping food hygienically safe.

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