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PREVALENCE OF MULTI-DRUG RESISTANT BACTERIA IN SELECTED STREET FOOD AND WATER SAMPLES

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Abstract

Present study was conducted to determine the bacteria and their multi-drug resistance pattern of Velpuri and water of Velpuri shop of different areas of Dhaka city. A total of 74 bacteria were isolated of which 26 isolates were subjected for further study. Eleven and 15 isolates from 26, were found Gram-positive and Gram-negative bacteria, respectively. Three isolates of Gram-positive bacteria were found rod shaped and spore formers which were identified as *Bacillus* spp. while eight isolates were found round shaped and non-spore formers and identified as *Staphylococcus, Streptococcus, Planococcus, Micrococcus*. In case of Gram-negative bacteria, *Alcaligenes, Escherichia, Pseudomonas, Enterobacter, Proteus, Klebsiella, Yersinia* were found to be associated with the samples. Among 26 isolates *Pseudomonas* and *Planococcus* were found to be dominating genera. Besides provisional identification, four selected isolates were further confirmed through molecular characterization based on 16S rDNA sequence analysis. Antibiotic sensitivity test results revealed that isolated bacteria were resistant against common antibiotics like penicillin G (80.77%), vancomycin (61.53%) and rifampicin (57.70%). Among the isolates *Pseudomonas, Enterobacter cloacae, Eshcherichia coli, Klebsiella, Proteus morganii, Yersinia enterocolitica* were found to be multi-drug resistant which is very much alarming for the consumers.

Introduction

The poverty level of community, the demographic expansion and a continuous urbanization factors in various developing countries have led to the emergence of a novel and most dominant form of restaurants known as “street foods”. Generally, street foods are ready-made foods sold by salesmen or by peddlers moving from one location to the other (Canet 1997). Due to its relatively low cost, street foods represent one of the most cost efficient forms of feeding among low earning people, students, craftsmen, and high school going students (Chauliac *et al.* 1998).

Increase in the world wide consumption of ready to eat (RTE) food product have resulted in increase in food borne illness associated with these products (Sivapalasingam *et al.* 2004).

Due to the excessive and inappropriate use of antibiotics there has been a gradual emergence of populations of antibiotic resistant bacteria, which pose a global public health problem (Komolafe 2003). Velpuri is one of the popular snacks of Dhaka. It is crispy deep fried bread which is usually served with spicy fillet made up of pulse, cucumber, onion and dressing. The dressing is made up of tamarind juice, pepper and black salt. Considering all these facts the present study was undertaken to determine the bacteria associated with Velpuri and water of the Velpuri shop in different areas of Dhaka city and their multi-drug resistance pattern of the associated bacteria.

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Materials and Methods

Samples were collected from different sites of Dhaka city *viz.,* Moghbazar (MB), Dhaka Medical College (DMC), Bangladesh University of Science and Technology (BUET), Kalabagan (KB), Mirpur-1(MP-1), Teacher Students Centre (TSC), Jagannath University campus (JU), Chankharpul (CP), Curzon Hall (CH), Science Annex (SA) and Udayan School (US). Samples were collected in sterile containers and brought to the laboratory immediately for bacteriological analysis. Microbiological analysis were carried out by ten-fold serial dilution (Greenberg *et al.* 1998) and plated on nutrient agar, MacConkey agar, *Salmonella-Shigella* (SS) agar, and Cetrimide agar media were used for isolation of bacterial strains. The morphological, cultural and biochemical studies of the selected isolates were done following standard laboratory manuals (Sneath *et al.* 1986, Krieg and Holt 1984, SAB 1957, Collins and Lyne 1984, APHA 1998). Antibiotic susceptibility test was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plate (Hudzicki 2012). After incubation at 37°C for 24 hrs, zone diameter around the disc was measured and isolates were classified as susceptible (S), intermediately resistant (I) and resistant (R). Nine antibiotics *viz*., penicillin (10 U), neomycin (30 µg), gentamycin (10 µg), streptomycin (10 µg), doxycyclin (30 µg), ciprofloxacin (5 µg), vancomycin (30 µg), rifampicin (5 µg), polymyxin B (300 U) were tested.

Four conventionally identified isolates were subjected to molecular identification based on 16S rDNA sequence analysis for further confirmation. The following primer pairs were used- 5'-16S rRNA: CCAGACTCCTACGGGAGGCAGC, 3'-16S rRNA: CTTGTGCGGGCCCCCGT CAATTC for the partial amplification of 16S rRNA gene. Supernatant of heat lysed cell suspension was used as the source of template DNA during PCR amplification of 16S rRNA gene. The PCR reaction was performed following an initial denaturation at 95ºC for 5 mins., denaturation at 94ºC for 1 min., annealing at 55ºC for 30 sec., extension at 72ºC for 1 min., and final extension was at 72ºC for 10 mins. After completion of cycling program, the reactions were held at 4ºC. The amplified products were separated electrophoretically on 1% agarose gel. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system (Microdoc DI-HD, MUV21-254/365, Cleaver Scientific). The sequence generated from automated sequencing of PCR products were analyzed through NCBI-BLAST database (http://blast. ncbi.nlm.nih.gov/) and rRNA BLAST (http://bioinformatics.psb.ugent.be/cgi-bin/rRNA/ blastform.cgi) programs to find out possible similar organism through alignment of homologous sequences.

Results and Discussion

During this study 74 discrete bacterial colonies were primarily selected and finally 26 bacterial isolates were studied in detailed for identification and their multi-drug resistance pattern. Considering morphology, Gram reaction and major biochemical tests and following Bergey's Manual of Systematic Bacteriology (Sneath *et al*. 1986) isolated bacteria were conventionally identified. Among Gram-positive three isolates (W-7, V-3, V-13) were *Bacillus*, four isolates (W-9, W-10, V-9, V-11) were *Planococcus*, two isolates (V-10, V-12) were *Streptococcus*, one isolate (T-3) was *Micrococcus* and the remaining one (W-8) was the member of *Staphylococcus*. The isolated Gram-negative members were *Alcaligenes* (W-1, W-3, W-5, W-6), *Pseudomonas* (W-2, W-4, W-5, V-7, V-8), *Klebsiella* (V-2, V-3), *Yersinia* (V-4), *Enterobacter* (V-5), *Proteus* (V-6), *Escherichia* (T-1).

The relative abundance of the isolated bacteria associated with Velpuri and water of the Velpuri shop was shown in Table 1. The Gram-positive *Planococcus* was the dominating member (36%) followed by Gram-negative *Pseudomonas* (33%). *E. coli*, *Proteus*, *Yersinia* and

Enterobacter were found to be less frequent in comparison to other members. Four isolates (W-2, W-4, V-2 and V-5) were selected for molecular identification. DNA from all the four isolates was

Table 1. Bacterial abundance in Velpuri and water.

Name of organisms	Number of occurrence	Abundance (%).
Gram-positive bacteria		
Planococcus	4	36
Bacillus	3	27
Streptococcus	2	18
Micrococcus	1	9
Staphylococcus	1	9
Gram-negative bacteria		
Pseudomonas	5	33
Alcaligenes	4	26
Klebsiella	2	13
Yersinia	1	6
Enterobacter	1	6
Proteus	1	6
Escheichia coli	1	6

subjected to PCR amplification and the products were separated in 1.0 % agarose gel through electrophoresis. In the gel approximate size of the amplified DNA band was observed as 600 bp (Fig. 1). The amplified DNA was sequenced and the bacterial isolate W-2 was identified as *Pseudomonas mendocina*, the isolate W-4 was identified as *Pseudomonas* sp. the isolate V-2 was identified as *Klebsiella pneumoniae* and the isolate V-5 was identified as *Enterobacter cloaceae* through NCBI-BLAST and rRNA BLAST analysis. All the four isolates matched with their conventional identification (Table 2).

Table 2. Conventional and molecular identification of four selected isolates.

Isolate name	Conventional identification	Molecular identification				
		Scientific name	Strain	Identity match (%)	Max. coverage score	E-value
W-2	*Pseudomonas mendocina*	*P. mendocina*	13-2	100	913	0.0
W-4	*Pseudomonas* sp.	*Pseudomonas* sp.	ZJY-484	99	989	0.0
V-2	*Klebsiella pneumoniae*	*K. pneumoniae*	IW4	96	896	0.0
V-5	*Enterobacter cloaceae*	*E. cloaceae*	TRC-322	83	183	1e-42

The microbial load and the presence of the bacterial pathogens in foods are a good indication of the food quality and the potential health risk they pose to consumers (Rosmini *et al*. 2002). *Staphylococcus aureus* was isolated from water samples, which are the most predominantly virulent human *Staphylococcus* pathogens causing a wide range of diseases (Wertheim *et al.* 2005).

Table 3. Culture and sensitivity test of the isolated Gram-negative bacteria.

Bacteria	Name of antibiotics and inhibition zone area (mm)								
	RD (5µg)	CN (10µg)	N (30µg)	PB (100 U)	P (10U)	VA (30µg)	S (10µg)	DO (30µg)	CIP (30µg)
Alcaligenes sp.	11 ± 1.6 **R**	22±1.6 S	23±1.4 S	12±2 I	0 **R**	0 **R**	22±2.4 S	13±0.8 I	32±2 S
Pseudomonas mendocina	11 ± 1.6 **R**	25±3.2 S	26±2.4 S	17±1.6 S	0 **R**	0 **R**	25±3 S	0 **R**	27±1.2 S
Alcaligenes sp.	11 ± 1.2 **R**	27±2.8 S	22±1.4 S	15±1.7 S	0 **R**	0 **R**	26±0.8 S	14±2.6 I	26±2.1 S
Pseudomonas sp.	4 ± 5.1 **R**	23±2.9 S	19±3.8 S	16±1.8 S	0 **R**	0 **R**	22±3 S	13±4.4 I	26±4.3 S
Pseudomonas viridiflava	0 **R**	22±0 S	24±3.8 S	13±0.9 S	0 **R**	0 **R**	18±0.8 S	0 **R**	28±2.1 S
Alcaligenes sp.	17 ± 1.6 I	24±2.4 S	27±3.8 S	15±4 S	0 **R**	0 **R**	22±2.1 S	17±3.5 S	21±1.3 S
Alcaligenes sp.	14 ± 0.7 **R**	24±1.6 S	22±3.8 S	16±2.6 S	0 **R**	0 **R**	22±2.1 S	17±1.2 S	33±3.2 S
Klebsiella pneumonia	6 ± 4.1 **R**	22±2 S	19±3.8 I	13±2.4 S	0 **R**	0 **R**	16±4.4 S	14±2.8 I	26±1.8 S
Klebsiella sp.	6 ± 4.3 **R**	19±4 S	17±3.8 I	15±1.6 S	0 **R**	0 **R**	21±1.2 S	13±1.6 I	29±5.3 S
Yersinia enterocolitica	6 ± 4.3 **R**	21±6 S	17±3.8 S	13±4.8 S	0 **R**	0 **R**	23±3 S	15±0.4 I	31±4.5 S
Enterobacter cloacae	9 ± 0 **R**	17±1.6 S	16±3.8 I	13±3 S	0 **R**	0 **R**	17±2 S	13±0.4 **R**	26±4.5 S
Proteus morganii	4 ± 4 **R**	19±3.4 S	20±3.8 S	14±3.5 S	0 **R**	0 **R**	22±6 S	14±2 S	30±3.5 S
Pseudomonas syringae	0 **R**	23±2 S	18±3.8 S	13±1.4 S	0 **R**	0 **R**	20±1.6 S	0 **R**	0 **R**
Pseudomonas viridiflava	0 **R**	21±0.8 S	21±3.8 S	14±1.4 S	0 **R**	0 **R**	19±2.3 S	0 **R**	28±2.6 S
Escherichia coli	8 ± 2.8 **R**	20±2.8 S	18±3.8 S	14±0.9 S	0 **R**	0 **R**	21±4.7 S	14±3.2 S	30±6.4 S

"±" = Standard deviation, R = Resistant, I = Intermediate resistant, S = Susceptible. Penicillin (10 U) = P Neomycin (30µg) = N, Gentamycin (10µg) = CN, Streptomycin (10 µg) = S, Doxycyclin (30 µg) = DO, Ciprofloxacin (5 µg) = CIP, Vancomycin (30 µg) = V, Rifampicin (5 µg) = RD, Polymyxin B (300 U) = PB.

Table 4. Culture and sensitivity test of the isolated Gram-positive bacteria.

Bacteria	Name of antibiotics and inhibition zone area (mm)								
	RD (5µg)	CN (10µg)	N (30µg)	PB (100 U)	P (10 U)	VA (30µg)	S (10µg)	DO (30µg)	CIP (30µg)
Bacillus schlegelii	32 ± 4	28±3.5	26±1.4	16±2	45±4.7	21±1.2	20±7.3	26±3.3	38±4.5
	S	S	S	S	S	S	S	S	S
Staphylococcus aureus	48 ± 1.6	32±2.3	26±4	22±3	46±4.1	27±1.6	21±2.3	32±1.2	28±2.6
	S	S	S	S	S	S	S	S	S
Planococcus citreus	41 ± 5.7	27±3.6	27±2.6	19±1.2	40±5.1	25±1.6	40±0.4	24±1.2	29±4
	S	S	S	S	S	S	S	S	S
Planococcus citreus	36 ± 2	24±3.7	24±1.2	19±2	38±5.7	21±1	24±0.9	24±1.6	27±2.3
	S	S	S	S	S	S	S	S	S
Planococcus citreus	13 ± 4.2	17±3.2	18±3	14±2	0	0	16±3.5	16±0.4	23±4.7
	R	S	S	S	**R**	**R**	S	S	S
Streptococcus faecium	22 ± 0	22±0	18±2.8	16±0.4	21±4.2	17±1.2	22±2.2	18±1.4	23±4
	S	S	S	S	**R**	I	S	S	S
Planococcus citreus	22 ± 1.4	23±2.4	19±0.9	17±0.9	18±4.1	17±1.6	23±3	18±1.5	28±1.2
	S	S	S	S	**R**	I	S	S	S
Streptococcus faecalis	21 ± 2.8	19±4.1	19±1.6	17±1.2	21±4.2	17±1.2	21±0.9	18±2.8	23±1.5
	S	S	S	S	**R**	I	S	S	S
Bacillus subtilis	24 ± 2	30±2.8	28±3	16±2.1	23±4.9	22±2.6	30±3	18±2.8	35±1.5
	S	S	S	S	**R**	S	S	S	S
Bacillus lentus	18 ± 2.1	31±0.4	25±3.7	17±0.8	19±5.7	24±2.8	33±1.7	21±1.2	41±2.6
	I	S	S	S	S	S	S	S	S
Micrococcus variens	38 ± 4	26±3.2	28±4.8	17±2.1	33±6.1	27±2.4	29±3.3	26±0.9	28±4.9
	S	S	S	S	S	S	S	S	S

"±" = Standard deviation, R = Resistant, I = Intermediate resistant, S = Susceptible. "±" = Standard deviation , R = Resistant, I = Intermediate resistant, S = Susceptible. Penicillin (10 U) = P Neomycin (30 µg) = N, Gentamycin (10 µg) = CN, Streptomycin (10 µg) = S, Doxycyclin (30 µg) = DO, Ciprofloxacin (5 µg) = CIP, Vancomycin (30 µg) = V, Rifampicin (5 µg) = RD, Polymyxin B (300 U) = PB.

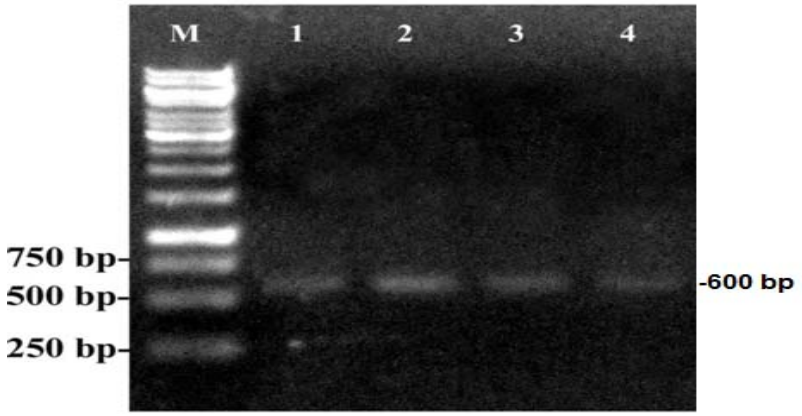

Fig. 1. PCR amplification of part of the 16S rRNA gene. Lane M = 1.0 kb ladder, lanes 1 - 4 represent four different bacterial isolates *viz.* W-2, W-4, V-2 and V-5. In the gel approximate size of the amplified DNA band was 600 bp.

Results of the culture and sensitivity (C/S) test of the Gram negative bacteria were shown in Table 3. Gram-negative bacteria associated with the samples were found to be resistant against common antibiotics like rifampicin, penicillin G and vancomycin. *Pseudomonas syringae* was found to be resistant against five antibiotics tested *viz*. Rifampicin, penicillin G, vancomycin, doxycyclin and ciprofloxacin. In case of Gram-positive bacteria the results were found to be different and most of the Gram positive bacteria were susceptible against common antibiotics tested (Table 4). In a study, Ali *et al*. (2011) observed that bacteria associated with RTE fresh vegetables and fruits most were resistant to penicillin and vancomycin. In the present study similar result was observed. The result clearly indicated that waterborne pathogens are becoming resistant to penicillin and vancomycin. Multidrug-resistant enteric bacteria were isolated from Turkey, cattle, and chicken farms and retail meat products in Oklahoma. A total of 132 isolates of *Klebsiella pneumoniae* were characterized and all isolates were found to be resistant to ampicillin, tetracycline, streptomycin, gentamycin, and kanamycin (Kim *et al*. 2005). In the present study *Klebsiella pneumoniae* was found to be resistant against rifampicin, penicillin G and vancomycin and *Klebsiella* sp.

In this study *Pseudomonas syringae* showed resistance against five antibiotics. Among the isolated bacteria *Pseudomonas, Enterobacter cloacae, Eshcherichia coli, Klebsiella, Proteus morganii, Yersinia enterocolitica* were found to be multidrug resistant. The presence of both Gram-negative and Gram-positive members *viz. E. coli*, *Enterobacter*, *Klebsiella*, *Alcaligenes*, *Staphylococcus*, *Streptococcus* etc. in Velpuri a popular snacks and their multi-drug resistance raises serious food and water safety concerns.

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