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OCCURRENCE OF GRAM-NEGATIVE BACTERIA IN COCKROACHES COLLECTED FROM DIFFERENT AREAS OF DHAKA CITY

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Abstract: The presence of Gram-negative bacteria in cuticle and alimentary tract of cockroaches collected from four selected areas of Dhaka city was investigated. Using detection key, a total of 12 cockroaches were identified as *Periplaneta americana* (58.33%), *Blattella germanica* (33.33%) and *Blatta orientalis* (8.33%). From these three species 54 different bacterial isolates were obtained. Out of them, 33 were obtained from *P. americana* (14 from external and 19 from gut), 9 from *B. orientalis* (5 from external and 4 from gut) and 12 from *B. germanica* (5 from external and 7 from gut). Bacterial isolates obtained from external surface of the cockroaches belonged to four different Gram negative bacterial species (*Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp. and *E. coli*), six bacteria have been obtained from alimentary tract (*Klebsiella* spp, *Pseudomonas* spp., *Enterobacter* spp., *Escherichia coli*, *Salmonella* spp. and *Shigella* spp.). *Pseudomonas* spp. were the most predominant bacteria isolated from external surface (33.34%) while *Klebsiella* spp. were found to dominate in alimentary tract (23.33%). *P. americana* carried the highest number (33) of bacterial isolates and *B. orientalis* carried the minimum (9). This study shows that cockroaches carry several bacteria on their external body surface and alimentary tract and can play a role in bacterial transmission to humans. Therefore, the existence of cockroaches in human dwellings is a serious public health problem.

Key words: Cockroaches, Gram-negative bacteria, Dhaka city

INTRODUCTION

Cockroaches (Blattidae, Orthoptera) are among the medically important pests in urban environment that cause serious public health problem (Cloarec *et al.* 1992). They are highly prevalent in residents, restaurants, medical and financial institutions, particularly in areas with unsanitary and insalubrious conditions and are often found in intimate association with human beings (Oothuman *et al.* 1989, Boase 1999, Hamid and Shahnaz 2012). They spread filth and ruin food and their chewing type of feeding mechanism and filthy breeding habits make them efficient vectors and transmitters of several potential pathogens (Gupta 1997).

Cockroaches have been found to harbour diverse pathogenic bacteria, different protozoa, pathogenic worms, fungi and viruses on either the cuticle or in the gut (Cloarec *et al.* 1992, Khrustalyova 1994). They can carry up to 14 million bacteria on the body and 7 million in each of their fecal droppings

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(Bennett 1993). Human pathogens like *Salmonella typhi*, *Shigella dysenteriae* and toxigenic strains of *Escherichia coli* can be retained in the gut of cockroaches for up to several days (Stek 1982).

Studies have shown that of the 25 different species of medically important bacteria isolated from *Blattella germanica* and *Periplaneta americana* collected from public hospitals and a residential house in central Tehran, Iran, *Klebsiella* spp. were the predominant bacteria (Tilahun *et al.* 2012)

Climatic condition of Bangladesh is very favorable for the survival of cockroaches. In urban areas the density of population is high and this gives poor hygienic condition, allowing cockroaches to infest every premises. Out of 13 different species of cockroaches, only three species (*Periplaneta americana*, *Blatta orientalis* and *Blattella germanica*) are commonly found in houses, offices and restaurants in Bangladesh (Safe Way Pest Control 2011).

Despite the abundance of cockroaches in different localities of Dhaka city, there is scanty information about their role as mechanical transmitters of pathogenic bacteria. This study therefore aims at investigating the carriage of Gram-negative bacteria in cockroaches collected from Dhaka city.

MATERIAL AND METHODS

The study was conducted in Entomology Laboratory, Department of Zoology and Microbiology Laboratory, Department of Microbiology, University of Dhaka. For the collection of the cockroaches four selective locations were chosen. The locations were: 1 No. Mess and Bikalpa Mess of Shahidullah Hall, Dining hall of Fazlul Huq Muslim Hall and Dhaka Medical College Hospital, Dhaka, Bangladesh.

Cockroaches were collected by traps and hand picking using sterile hand-gloves (Paul *et al.* 1992). Trap was made by disposable plastic bottles and lures. Collected cockroaches were placed into sterile tubes individually. These tubes were transferred to laboratory immediately after capturing and the cockroaches were killed by using chloroform. The identification of cockroach was done according to Imms (1965), Borror *et al.* (1981) and Kabir *et al.* (1981).

Isolation and identification of Gram-negative bacteria from cockroach samples: The external body surface of the cockroaches was washed using 5 ml sterile physiological saline for two minutes, and the wash was taken as external body homogenate sample. After external body washing, the cockroaches were soaked in 90% ethanol for 5 minutes to decontaminate their external surfaces and dried, followed by washing with sterile saline to remove traces of ethanol. The alimentary tract of cockroach was aseptically dissected out using sterile

entomological dissecting needles under a dissecting microscope. The instrument was dipped in ethanol and flamed between dissections. The excised gut was homogenized in 5 ml of sterile normal saline water (Tachbele *et al.* 2006).

A total of 24 samples consisting of 12 external body surface and 12 gut homogenates were analyzed. All the collected homogenate samples were inoculated by using spread plate technique onto MacConkey agar, xylose lysine deoxycholate agar (XLD), eosin methylene blue agar (EMB), the selective media used for isolation of common pathogenic Gram-negative bacteria. Growths on all plates were observed and the morphological characteristics of colonies (size, shape, elevation, color, consistency, opacity, pigmentation) were noted. Then isolated colonies were sub-cultured into nutrient agar medium.

Gram staining was made to determine the size, shape, arrangement and Gram reaction of the isolated organisms according to the method described by Pelczar *et al.* (1993). The identification of the isolates was done by performing various biochemical tests, which included indole test, citrate utilization test, Kligler's iron agar (KIA) test, motility, indole, urease (MIU) test. All the tests were performed according to the standard protocol as described in Bergey's Manual of Systematic Bacteriology (2001).

RESULTS AND DISCUSSION

A total of 12 cockroaches were collected and examined for their bacteriological quality. Out of them 58.33% was *Periplaneta americana,* 33.33% *Blattella germanica* and 8.33% was *Blatta orientalis* (Fig. 1). The maximum numbers of cockroaches were collected from Bikalpa Mess, Shahidullah Hall (33.33%) and the minimum were collected from Dining hall, Fazlul Huq Muslim Hall (16.67%) (Table 1). *Periplaneta americana* and *Blattella germanica* were isolated in larger numbers. This is in conformity with the results of Zarchi *et al.* (2009) who reported *Periplaneta americana* and *Blattella germanica* as the most active cockroaches in hospitals of Tehran city, Iran. Also, in a research conducted by Pai *et al.* (2005) in China, *Periplaneta americana* and *Blattella germanica* were isolated as the most common species.

A total number of 54 bacterial isolates were obtained from external body surface and alimentary tract homogenates of 12 cockroaches enrolled in the study. Based on the cultural, morphological and biochemical tests the isolates were presumptively identified as *Klebsiella* spp. 14 (26%), 13 *Pseudomonas* spp. (24%), *Enterobacter* spp. 11 (20%), *E. coli* 9 (17%), *Salmonella* spp. 4 (7%) and *Shigella* spp. 3 (6%) (Table 2, Figs 2, 3). Out of 54 bacterial isolates, 33 were obtained from *P. americana* (14 from external and 19 from gut), 9 from *B. orientalis* (5 from external and 4 from gut) and 12 from *B. germanica* (5 from

external and 7 from gut) (Table 3). Alimentary tract and external surface samples yielded 30 and 24 isolates, respectively.

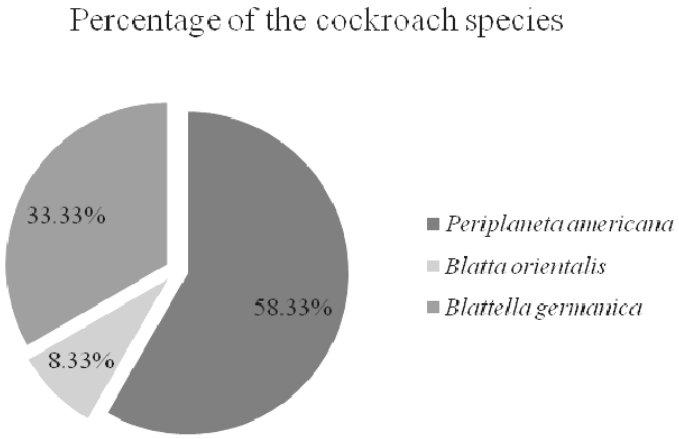

Fig. 1. Percentage of the cockroach species collected from the study areas.

Table 1. Distribution of cockroaches in study areas

Study area	*Periplaneta americana*		*Blatta orientalis*		*Blattella germanica*		Total	
	Number	%	Number	%	Number	%	Number	%
1 No. Mess, SH	3	42.86	0	0	0	0	3	25
Bikalpa Mess, SH	2	28.57	1	100	1	25	4	33.33
Dining hall, FH	2	28.57	0	0	0	0	2	16.67
DMCH	0	0	0	0	3	75	3	25
Total	7	100	1	100	4	100	12	100

The most predominant species of bacteria obtained from external surface of cockroaches in this study was *Pseudomonas* spp. (33.34%), and the least dominant was *E. coli* (16.67%). In the alimentary tract, *Klebsiella* spp. (23.33%) was most frequently isolated while *Shigella* spp. was least frequent (10.0%) (Table 4). These Gram-negative bacilli have been most frequently isolated from cockroaches in several other studies (Fotedar *et al.* 1991, Cloarec *et al.* 1992; Rivault *et al.* 1993). Lin *et al.* (2008) reported isolation of *E. coli, Pseudomonas* sp., *Salmonella* sp. and *Enterobacter* sp. from cockroaches.

Among six bacterial isolates, *Klebsiella* and *Enterobacter* were isolated in highest numbers from cockroaches trapped from Bikalapa Mess of Shahidullah Hall, as compared with the others. The dominant species obtained from DMCH were *E. coli* and *Pseudomonas* (Fig. 4).

Table 2. Biochemical tests for presumptive identification of bacterial isolates

Isolate No.	Kligler's Iron Agar test							Presumptive organism
	Lac-tose	Glu-cose	Gas	H_2S	Citrate use	Urease activity	Indole production	
C1, 5, 15, 24, 25, 39, 47, 50, 53	+	+	+	-	-	-	+	*E. coli*
C2, 3, 7, 12, 20, 21, 22, 23, 27, 33, 34, 36, 54	-	-	-	-	+	-	-	*Pseudomonas* sp.
C4, 6, 10, 13, 14, 17, 19, 26, 28, 35, 37, 40, 44, 45	-	+	+	-	+	+	-	*Klebsiella* sp.
C8, 9, 11, 16, 18. 30, 31, 32, 38, 42, 49	-	+	+	-	+	-	-	*Enterobacter* sp.
C29, 43. 46. 51	-	+	-	+	+	+	-	*Salmonella* sp.
C 41, 48	-	+	-	-	-	-	+	*Shigella* sp.

Table 3. Distribution of pathogens isolated from different cockroach species

Isolate	*Periplaneta americana*			*Blatta orientalis*			*Blattella germanica*			Total
	External	Gut	Total	External	Gut	Total	External	Gut	Total	
Klebsiella	4	6	10	2	1	3	1	0	1	14
Pseudomonas	5	4	9	1	0	1	2	1	3	13
Enterobacter	3	4	7	1	1	2	1	1	2	11
E. coli	2	3	5	1	0	1	1	2	3	9
Salmonella	0	1	1	0	1	1	0	2	2	4
Shigella	0	1	1	0	1	1	0	1	1	3
Total	14	19	33	5	4	9	5	7	12	54

Table 4. The frequency of bacteria species isolated from cockroaches external surface and alimentary tract

Bacterial spp.	External surface (%)	Digestive organ (%)
Klebsiella sp.	29.17	23.33
Pseudomonas sp.	33.33	16.67
Enterobacter sp.	20.83	20.0
E. coli	16.67	16.67
Salmonella sp.	0	13.33
Shigella sp.	0	10.0

Most of the bacteria isolated are clinically important and associated with diseases in humans. The pathogenicity of these isolates can be further confirmed by detecting presence of specific virulence genes. These findings agree with the results of other investigations which showed that these bacterial

species on cockroaches collected from hospitals and food handling establishments (Bennett 1993, Chaichanawongsaroj *et al.* 2004, Salehzadeh *et al.* 2007). Zarchi and Vatani (2009) also detected *E. coli, Streptococus* and *Bacillus* as the most isolated bacteria from the hospital cockroaches.

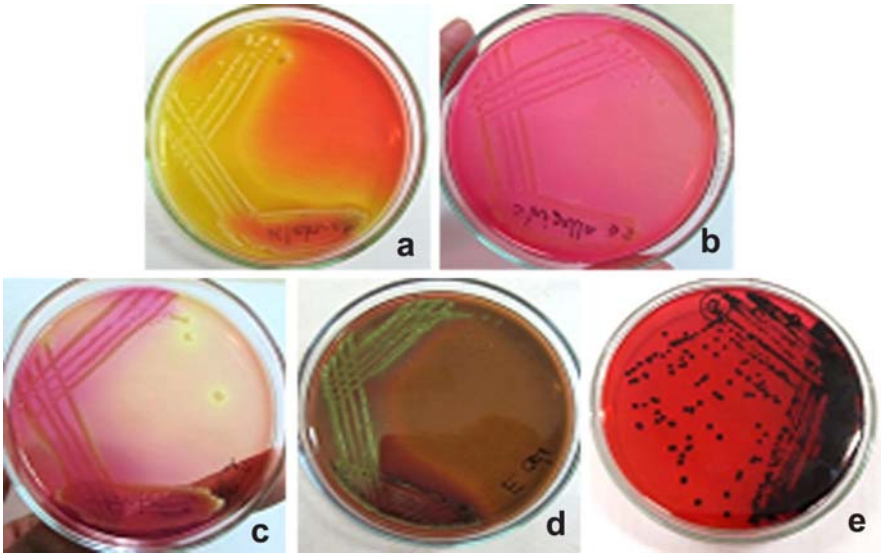

Fig. 2. Growth of representative bacterial isolates on different selective media. (a) *Klebsiella* spp. on XLD agar medium, (b) *Shigella* spp. on XLD agar medium, (c) *Enterobacter* spp. on XLD agar medium, (d) *E. coli* on EMB agar and (e) *Salmonella* on XLD agar.

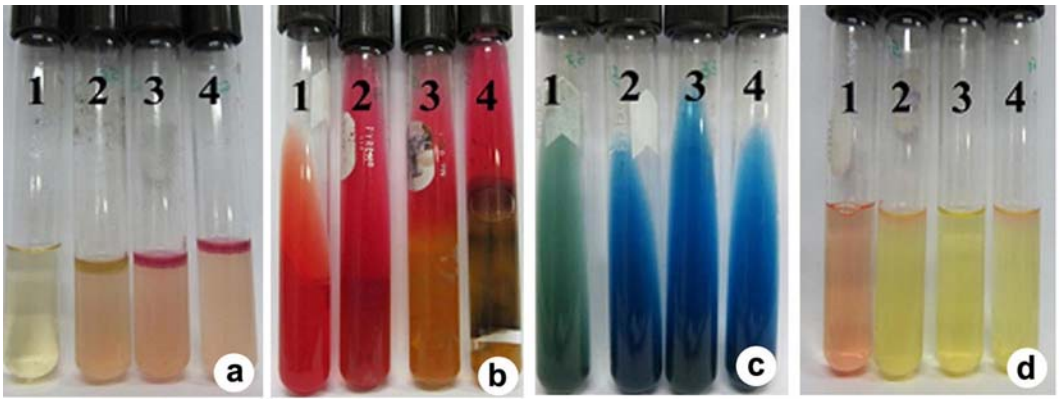

Fig. 3. Representative of biochemical tests used for identification of isolates. (a) Indole test: 1: Negative control; 2: *Klebsiella* spp.; 3: *E. coli*; 4: *Shigella* spp. (b) Kligler iron agar test: 1. Negative control; 2: *Pseudomonas* spp.; 3: *Shigella* spp; 4: *Salmonella* spp. (c) Citrate utilization test: 1: Negative control; 2: *Pseudomonas* spp.; 3: *Shigella* spp.; 4: *Salmonella* spp. (d) Urease test: 1. Negative control; 2: *Pseudomonas* spp.; 3: *Shigella* spp. and 4: *Salmonella* spp.

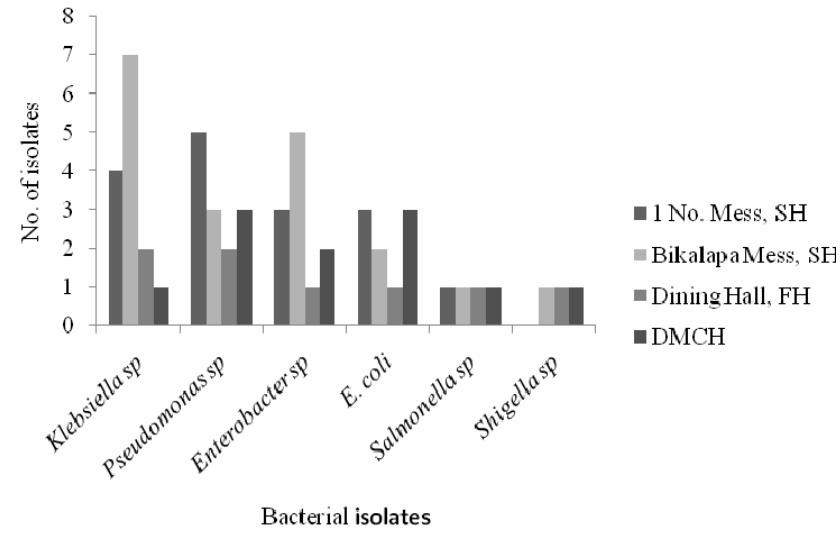

Fig. 4. Distribution of bacterial isolates in the various study areas.

The presence of cockroaches in an environment rich with pathogenic bacteria, like hospitals is highly dangerous as it might enable transmission of life threatening diseases. Presence of cockroaches carrying pathogenic bacteria in food-processing establishments is also alarming and imposes great threat to public health. These data also indicate to the lack of hygiene in hospitals and food processing areas in Dhaka.

This study suggests that the cockroach is potential source of transmission of life threatening diseases. Regular control of this pest by strict implementation of pest control regulations is mandatory in Bangladesh to ensure good public health.

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