

ISOLATION AND IDENTIFICATION OF SOME GRAM-NEGATIVE BACTERIA FROM COCKROACHES COLLECTED FROM HOSPITAL, RESTAURANT AND SLUM AREAS OF DHAKA CITY, BANGLADESH

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Abstract

The study was conducted to identify the bacteriological quality from different species of cockroaches in three study areas in Dhaka city. A total of 15 cockroaches was collected from three different selected study areas, viz. Penang Restaurant of Bakshi Bazar, Sir Salimullah Medical College Mitford Hospital and Urban slum of Kamalapur, Dhaka. Using detection keys, the cockroaches were identified as *Periplaneta americana* (53.33%), *Blatella germanica* (40%) and *Blatta orientalis* (6.67%). All of 15 cockroaches were used to isolate bacteria from external body surface and alimentary tract. A total of 48 isolates was obtained from these three species of cockroaches. Out of them 29 (60.42%) isolates were obtained from *P. americana*, 17 (35.42%) from *B. germanica*, and 2 (4.16%) from *B. orientalis*. Three Gram negative bacteria, viz. *Klebsiella* sp., *Escherichia coli* and *Pseudomonas* sp. were isolated from alimentary canal and external surface. On the external body surface 71.43% isolates were *E. coli*, 21.43% were *Pseudomonas* sp. and 7.14% were *Klebsiella* sp. In case of alimentary tract, 55.89% isolates were *Pseudomonas* sp., 23.52% were *E. coli*, and the rest 20.59% were *Klebsiella* sp. Comparison between study areas showed that the food handling area yielded maximum number (41.67%) of isolates and the urban slum yielded minimum number (25%) of isolates. In food handling isolates, 45% were *Klebsiella*, whereas *E. coli* and *Pseudomonas* sp. were 40% and 15%, respectively. In Hospital, *E. coli* was the most common (50%), followed by *Klebsiella* (37.5%) and *Pseudomonas* sp. (12.50%). In urban slum, 41.67% isolates were *Klebsiella* sp., 41.67% were *Pseudomonas* sp. and 16.66% were *E. coli*. This study shows that cockroaches carry several Gram-negative bacteria on their body surface and alimentary tract and play a role in bacterial transmission to human.

Key words: Cockroaches, Gram-negative bacteria, Isolation, Dhaka City.

INTRODUCTION

Cockroaches are among the most notorious pests of premises, which not only contaminate food by leaving the droppings and bacteria that can cause food poisoning, but also they transmit bacteria, fungi and other pathogenic microorganisms in infested areas (Czajka *et al.* 2003, Salehzadeh *et al.* 2007). Two most significant pest cockroaches universal as well as in Bangladesh are the German cockroach (*Blatella germanica*) and the American cockroach (*Periplaneta americana*). The German cockroach is more common inside homes particularly in kitchens while the later is found around the home close to water pipes and drainage systems (Akbari *et al.* 2014). They live close to human dwellings and are important carriers of etiological agents belonging to all groups of potential pathogens: viral, bacterial, protozoan and helminthes (Agbodaze and Owusu 1989, Fotedar *et al.* 1991, Cloarec *et al.* 1992, Pai *et al.* 2003). Numerous pathogens counting 32 species of bacteria (including *Salmonella* and *Shigella* species), 15 species of fungi and moulds, seven helminths (intestinal parasites), two protozoans, and one virus were found in or on cockroaches or in their feces (Pai *et al.* 2005, Zarchi and Vatani 2009). In Bangladesh, *Salmonella*, *Shigella*, *S. aureus*, *B. cereus*, and *E. coli* were isolated from cockroaches (Paul *et al.* 1992).

Periplaneta americana harbored more bacteria species than *B. germanica* (Rivault *et al.* 1993, Prado *et al.* 2006, Pai *et al.* 2003). The range of problems caused by the presence of cockroaches varies between hospitals, food-handling establishments, public institutions, and multi-family dwellings (Rivault *et al.* 1993). In hospitals, it was found that cockroaches can act as potential vectors in the epidemiology of nosocomial infections, especially in the transmission of drug-resistant bacteria (Fotedar

et al. 1991, Cotton *et al.* 2000). *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* sp. and several other potential pathogens were isolated from cockroaches collected from hospitals (Guyader *et al.* 1989, Oothuman *et al.* 1989).

The role of cockroaches as mechanical vectors and reservoir for pathogens and their drug resistance is unknown. The goal of this study was to isolate and identify Gram negative pathogenic bacteria associated with guts and cuticle of the cockroaches in the selected areas by using classical microbiological methods.

MATERIAL AND METHODS

Sample collection site

The present study was conducted in Entomology Laboratory, Department of Zoology and Microbiology Laboratory, Department of Microbiology, University of Dhaka. For the collection of cockroaches three selective locations were chosen. The locations were Penang Restaurant of Bakshi Bazar, Sir Salimullah Medical College Mitford Hospital (SSMCMH) and Kamalapur slum at Dhaka, Bangladesh.

Cockroach collections

Cockroaches were collected from the study areas with traps and hand picking. The trap was made with disposal plastic bottles and lures. For microbial investigation, cockroaches were collected using sterile screw-capped 250 ml jars and sterile hand-gloves following Paul *et al.* (1992). The collected cockroaches were placed into the sterile tubes individually. The tubes were transferred to the lab immediately after capturing and were killed using chloroform. The cockroaches were identified according to Kabir *et al.* (1981).

Bacteriological analysis of the cockroaches

The external body surface of the collected cockroaches was washed with vortexing in 10 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 five minutes to decontaminate their external surface and dried, followed by washing with sterile saline to remove traces of ethanol. The alimentary tract of cockroaches was aseptically dissected out using autoclaved-sterilized entomological dissecting needles under a dissecting microscope. The instrument was dipped in ethanol and flamed between dissections. The excised gut was homogenized in 10 ml of sterile normal saline water following Tachbele *et al.* (2006).

A total of 15 cockroaches, five from each site, containing 30 samples (15 external body surface and 15 gut homogenates) was analyzed. The samples were tested for the presence of common enteric Gram negative pathogens, for this purpose one loop full homogenate sample was cultured onto MacConkey agar medium by using streak plate technique, followed by incubation at 37°C for 24 hours. Growth on all plates was observed and the characteristics of the isolate colonies were noted. To obtain pure culture, isolated colonies from the selective medium were sub-cultured on Nutrient agar (NA) medium.

Identification of bacterial isolates by biochemical tests

The identification of the bacterial isolates was done by performing Gram staining and biochemical tests. Gram staining was performed to determine the size, shape, arrangement, and Gram reaction of the isolated organisms. The steps were followed as described by Pelczar *et al.* (1993). The test included Citrate utilization test, Catalase test, Motility test, Urease test, Indole test and Kligler's Iron Agar test. All the tests were performed according to the standard protocol as described in Bergey's Manual of Systematic Bacteriology (Garrity 2001). The isolates that were presumptively identified as *E. coli* were

grown on Eosine Methylene Blue (EMB) agar, which is a selective medium for *E. coli*, where *E. coli* gives their characteristic metallic sheen. Similarly, *Pseudomonas* sp. was further grown on Cetrimide agar, the selective medium for this bacterium.

RESULTS AND DISCUSSION

Periplaneta americana was the most common species in all of the study areas (53.33%), followed by *B. germanica* (40.00%) and *B. orientalis* (6.67%) (Table 1). This result indicates that these three species of cockroaches were the most active in the study areas and this result is consistent with the findings of Pai *et al.* (2005) in some selected areas in China. Fifty percent *Periplaneta americana* were found in Penang restaurant, 12.50% in SSMCMH and 37.50% in Urban slum, Kamalapur. *B. germanica* were only found in SSMCMH (66.67%) and Urban slum, Kamalapur (33.33%); while *B. orientalis* were found only in Penang restaurant (Table 1)

Table 1. Percentage of total cockroaches collected from the study areas.

Study area	<i>Periplaneta americana</i>	<i>Blatella germanica</i>	<i>Blatta orientalis</i>	Total (%)
Penang Restaurant, Bakshi Bazar	4	0	1	5 (33.33)
Sir Salimullah Medical College Hospital	1	4	0	5 (33.33)
Urban Slum, Kamalapur	3	2	0	5 (33.33)
Total (%)	8 (53.33)	6 (40)	1 (6.67)	15 (100)

The numbers of bacterial isolates collected from Penang restaurant were 20 (seven from external body surface and 13 from gut), 16 from SSMCH (five from external body surface and 11 from gut) and 12 from Urban slum, Kamalapur (two from external body surface and 10 from gut). The total number of bacterial isolates collected from *P. americana* was 29 (nine from external body surface and 20 from gut), 17 from *B. germanica* (five from external body surface and 12 from gut) and 2 from *B. orientalis* only (found only from gut) (Table 2). No growth found in the samples of external body surface homogenate samples of any of the cockroaches.

Table 2. Distribution of the isolate colonies.

Study area	<i>Periplaneta americana</i>		<i>Blatella germanica</i>		<i>Blatta orientalis</i>		Total (%)
	External surface	Guts	External surface	Guts	External surface	Guts	
Penang restaurant	C: (14-20) =7	C: (1-11) =11	0	0	0	C:(12-13) =2	20 (41.67)
SSMCMH	C: 32 =1	C: (21-23) =3	C: (33-36) =4	C: (24-31) =8	0	0	16 (33.33)
Urban slum, Kamalapur	C: 47 =1	C: (37-42) =6	C: 48 =1	C: (43-46) =4	0	0	12 (25.00)
Total	9	20	5	12	0	2	48
Grand Total (%)	29 (60.42)		17 (35.42)		2 (10)		48 (100)

Microscopic examination of the isolates after Gram staining showed that all the isolates were Gram negative rods (Fig. 1 and 2). The Gram negative isolates were identified in different biochemical tests which are summarized in Table 3. Fakoorziba *et al.* (2010) isolated around 25 species of medically important bacteria from *P. americana* and *B. germanica*, of which at least 22 were Gram negative.

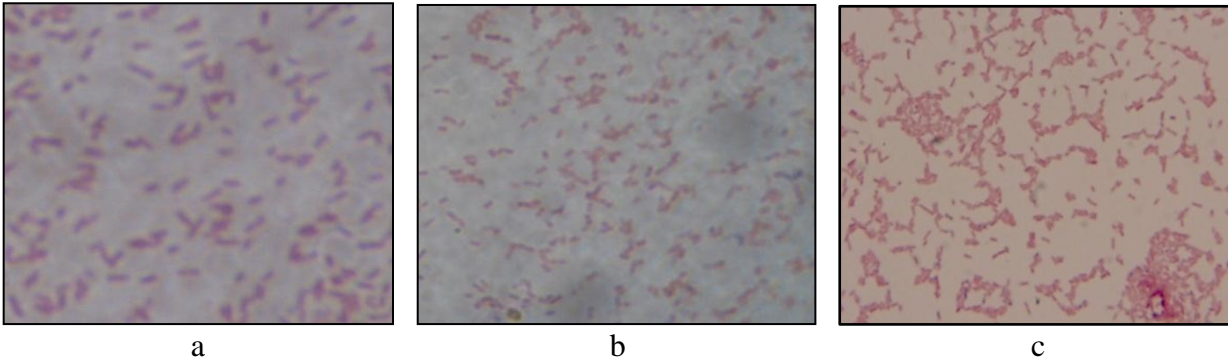


Fig. 1. Microscopic observation: a. *E. coli*, b. *Klebsiella* and c. *Pseudomonas*.

Based on the biochemical tests and growth on selective media it was found that out of 48 isolates, 20 (41.67%) isolates were *Klebsiella* sp., 18 (37.50%) and 10 (20.83%) isolates were *E. coli* and *Pseudomonas* sp., respectively. Wannigma *et al.* (2014) reported that the main bacteria on cockroaches were *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*.

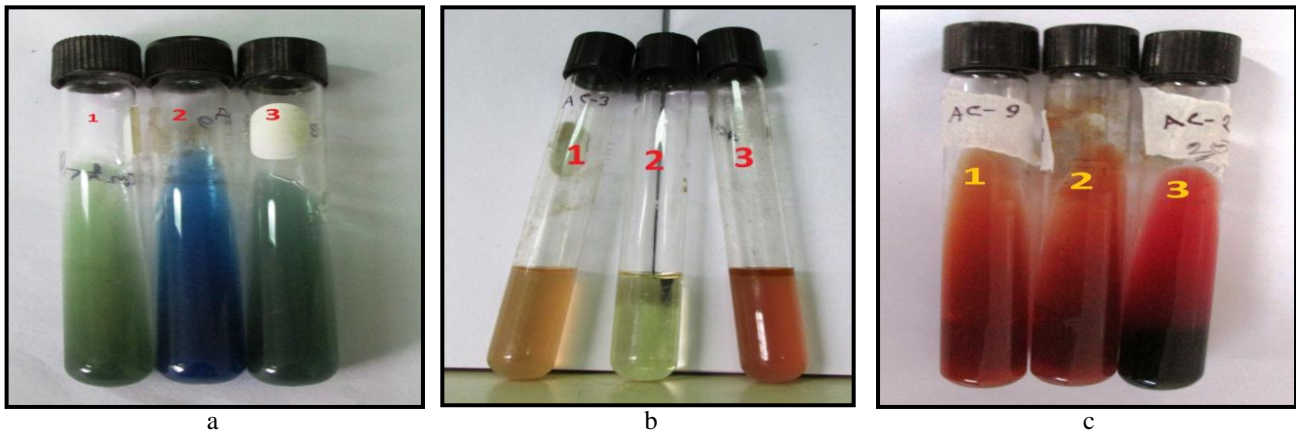


Fig. 2. Biochemical tests used for the identification of isolates: a. Citrate utilization test; b. Indole production test and c. Kligler's Iron Agar test.

Table 3. Biochemical test results of different isolates

Study area	Isolate number	Biochemical test							Presumptive identification	
		Catalase test	Lactose	Glucose	Gas	H ₂ S	Citrate use	Urease activity		Indole production
Penang restaurant	C-1,2,3,4,5,8,9,10,18	+	-	+	+	-	+	+	-	<i>Klebsiella</i> sp.
	C-6,7,11,12,14,15,16,17	+	+	+	+	-	-	-	+	<i>E. coli</i>
	C-13,19,20	+	-	-	-	-	+	-	-	<i>Pseudomonas</i> sp.
SSMCMH	C-22,23,24,25,26,27	+	-	+	+	-	+	+	-	<i>Klebsiella</i> sp.
	C-21,28,30,32,33,34,35,36	+	+	+	+	-	-	-	+	<i>E. coli</i>
	C-29,31	+	-	-	-	-	+	-	-	<i>Pseudomonas</i> sp.
Slum, Kamalapur	C-39,40,42,45,46	+	-	+	+	-	+	+	-	<i>Klebsiella</i> sp.
	C-47,44	+	+	+	+	-	-	-	+	<i>E. coli</i>
	C-37,38,41,43,48	+	-	-	-	-	+	-	-	<i>Pseudomonas</i> sp.

From the total isolates the largest percent (41.67%) of isolates were collected from the cockroaches of the Penang restaurant. The Urban slum cockroaches possessed the lowest percentage (25%) of all

isolates. The cockroaches from the SSMCMH contained 33.33% isolates. In case of restaurant the most dominant isolated bacteria was *Klebsiella* sp. (45%) and the least dominant was *Pseudomonas* sp. (15%). But in case of hospital the most dominant isolated bacteria was *E. coli* (50%) and least dominant was *Pseudomonas* sp. (12.5%). On the other hand, *Klebsiella* sp. and *Pseudomonas* sp. were dominant (41.67%) bacterial isolates and the least dominant was *E. coli* in urban slum areas (Table 4). The findings of pathogenic isolates in the cockroaches collected from hospital is consistent with the findings of Zarchi and Vatani (2009) who detected *E. coli* and *Pseudomonas* sp. as the most isolated bacteria from the hospital cockroaches.

Table 3. Distribution of pathogens isolated from three different areas.

Study area	<i>Klebsiella</i> sp.	<i>E. coli</i>	<i>Pseudomonas</i> sp.	Total (%)
Penang restaurant	9 (45%)	8 (40%)	3(15%)	20 (41.67%)
SSMCMH	6 (37.50%)	8 (50%)	2 (12.50%)	16 (33.33%)
Slum, Kamalapur	5 (41.67%)	2 (16.66%)	5 (41.67%)	12 (25%)
Total (%)	20 (41.67)	18 (33.33%)	10 (20.83%)	48 (100%)

From the isolates of external body surface *E. coli* was 71.43%, whereas *Pseudomonas* sp. and *Klebsiella* sp. was 21.43% and 7.14%, respectively. From the gut isolates the highest 55.89% was *Pseudomonas* sp., whereas 23.52% and 20.59% was *E. coli* and *Klebsiella* sp., respectively (Fig. 3). In case of external body surface the same results were obtained by Vahabi *et al.* (2007) who reported that *E. coli* was the most frequent extracted bacterium from cockroach’s body surface in Iran.

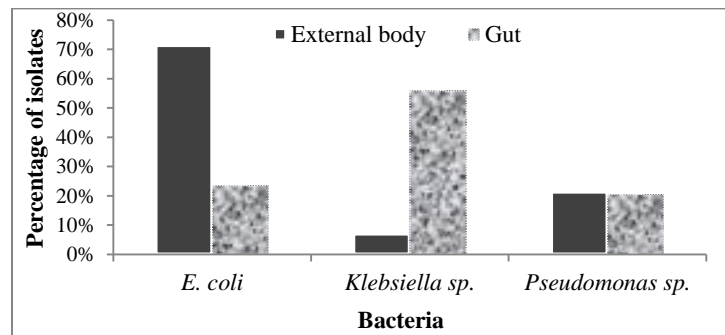


Fig. 3. Percentage of different isolates in external surface and gut.

The isolation of *E. coli*, *Klebsiella* sp. and *Pseudomonas* sp. from the cockroach indicated that domestic pests could cause health problem to humans, as these are potential pathogen. The presence of cockroaches in human dwelling areas is never desirable and must be taken into consideration.

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