

Original Article

Prevalence and Antibiotic Resistance of Gram-Negative Pathogenic Bacteria Species Isolated from *Periplaneta americana* and *Blattella germanica* in Varanasi, India

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Abstract

Background: Cockroaches are among the medically important pests found within the human habitations that cause serious public health problems. They may harbor a number of pathogenic bacteria on the external surface with antibiotic resistance. Hence, they are regarded as major microbial vectors. This study investigates the prevalence and antibiotic resistance of Gram-negative pathogenic bacteria species isolated from *Periplaneta americana* and *Blattella germanica* in Varanasi, India.

Methods: Totally, 203 adult cockroaches were collected from 44 households and 52 food-handling establishments by trapping. Bacteriological examination of external surfaces of *Pe. americana* and *Bl. germanica* were carried out using standard method and antibiotics susceptibility profiles of the isolates were determined using Kirby-Bauer disc diffusion methods.

Results: Among the places, we found that 54% had cockroach infestation in households and 77% in food-handling establishments. There was no significant difference between the overall bacteria load of the external surface in *Pe. americana* (64.04%) and *Bl. germanica* (35.96%). However the predominant bacteria on cockroaches were *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. However, *Kl. pneumoniae* and *Ps. aeruginosa* were the most prevalent, drug-resistant strains were isolated from the cockroaches with 100% resistance to sulfamethoxazole/trimethoprim and ampicillin. For individual strains of bacteria, *Escherichia coli* was found to have multi-resistance to four antibiotics tested, *Citrobacter freundii* four, *Enterobacter aerogenes* and *Proteus mirabilis* to three.

Conclusion: Cockroaches are uniformly distributed in domestic environment, which can be a possible vector for transmission of drug-resistant bacteria and food-borne diseases.

Keywords: *Periplaneta americana*, *Blattella germanica*, Gram-Negative, Bacteria, Antimicrobial resistance

Introduction

Cockroaches have become most vital pest, are commonly found in association with man in areas where food is prepared and stored, such as restaurants, kitchens, bakeries, and grocery stores (Rivault et al. 1993a, Vythilingam et al. 1997). These insects live in different environments, like sewage pipes, latrines, garbage, wall slits, baseboards and filthy places, as they are attracted by food, organic waste

and fluids regularly discharged in such sites (Graczyk TK et al. 2001, William J. Bell et al. 2007, Allotey J et al. 2009). There are two cockroach species commonly found infesting in domestic area, these are the German cockroach (*Blattella germanica*), and the American cockroach (*Periplaneta americana*) (Rivault et al. 1993a, Gratz 1999, Graczyk et al. 2005, Mpuchane et al. 2005, Bell et al. 2007). The

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German cockroach is more prevalent inside the home while the American cockroaches are common around the home and associated with water drainage systems and water pipes (Gratz 1999, Graczyk et al. 2005, Mpuchane et al. 2005, Bell et al. 2007, Allotey et al. 2009). Cockroaches have always been associated with different sites of domestic environments, by food, organic waste and fluids that are regularly discharged in such sites (Rivault et al. 1993b, Mpuchane et al. 2005).

Recently more than 40% of cockroach populations are found in domestic areas, because of uncontrolled of domestic sewage, poor sanitation and environment pollution which makes ideal environment for increase the cockroaches, population (Gratz 1999, Graczyk et al. 2005). Furthermore, their feeding mechanisms and filthy breeding habits make them ideal carriers of various pathogenic microorganisms (Chaichanawongsaroj et al. 2004, Graczyk et al. 2005). More than 100 species of bacteria have been isolated from or passed through cockroaches, which were carried on their rough body parts, cuticle, gut, vomits and feces (Cruden and Markovetz 1987, Allotey et al. 2009, Cloarec et al. 1992).

Cockroaches are mechanical carriers or perfect vector for harbouring and transmitting virulent bacteria associated with food born infections. Some of them demonstrate resistance to antibiotics (Paul et al. 1992, Rivault et al. 1993, Mpuchane et al. 2005). Transmission could occur by cockroach regurgitation or fecal pellet deposition into human foodstuffs (Cochran 1982, Bell et al. 2007). Although recently several studies of cockroaches in domestic environments have shown the presence of microorganisms spread on their external surfaces, such as *Enterobacter* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and several other potential pathogens (Oothuman et al. 1989, Le Guyader et al. 1991, Prado et al. 2002, Graczyk TK et al. 2005, Saitou K et al. 2009).

Although studies with *Bl. Germanica* showed

that when *Ps. aeruginosa* has been demonstrated to multiply in the gut and excretion of the bacteria continued up to 114 days (Fotedar et al. 1993), also *Salmonella typhi*, *Shigella dysenteriae* and toxigenic strains of *Es. coli* can be retained in the gut of cockroaches for up to several days (Stek 1982). *Periplaneta americana* harboured more bacteria species than the *Bl. germanica* (Fotedar et al. 1992a, Rivault et al. 1993, Prado et al. 2002, Pai et al. 2003).

In hospitals, cockroaches have been considered to be a possible carrier of nosocomial infections, especially the transmission of drug-resistant bacteria (Fotedar et al. 1991, Cotton et al. 2000, Prado et al. 2002, Saitou et al. 2009), also they play a critical role in transmitting food-borne diseases; such as diarrhea, dysentery, cholera, tuberculosis, and typhoid fever (Fotedar and Banerjee 1992, Graczyk TK et al. 2005, Saitou K et al. 2009). Cockroaches are possible vectors for carrying TEM-type beta-lactamases producing Gram-negative bacteria, most often found in *Es. coli* and *Kl. pneumonia*, which showed ampicillin and penicillin resistance (Fotedar R 1991, Cotton et al. 2000, Bradford PA 2001, Paterson DL 2003).

The domestic pollution and poor sanitation gives the favorable condition to growth and development of population of cockroaches in food preparation areas and multi-family dwellings, the diversity of micro-organisms that they are associated and the fact that some of these bacteria highly resistance to recent antibiotics, which is indicates one of the positive health risk for the human beings (Paul et al. 1992, Rivault et al. 1993, Prado et al. 2002, Graczyk TK et al. 2005, Saitou K et al. 2009).

The high population density with poor disposal of left-over's, lack of proper sanitation and ideal temperature conditions in Varanasi City could serve as a source of cockroaches' infestation. With that in mind, this study was an attempt to isolate and identify gram-negative pathogenic bacteria from *Pe. americana*

and *Bl. germanica* in households (Kitchen, living room, toilets and bed rooms) and food-handling establishments (canteens, bakeries, grocery stores, food storage stores, small food processing plants and restaurants), in Varanasi City in India. After isolating bacteria from the trapped cockroaches we determined to find their antibiotic resistance patterns, and also we highlighted spatial distribution, diversity and composition of bacteria in domestic cockroaches.

Materials and Methods

Study area

The city of Varanasi is located in the middle Ganges valley of North India, in the eastern part of the state of Uttar Pradesh, along the left crescent-shaped bank of the Ganges River. The Varanasi Urban Agglomeration consist of seven urban sub-units covers an area of 112.26km². The urban agglomeration is stretched between 82° 56'E - 83° 03'E and 25° 14'N-25° 23.5'N, being located in the indo-gangetic plains of North India (Indian Meteorology Department 2010).

Varanasi experiences a humid subtropical climate with large variations between summer and winter temperatures. Summers are from early April to October, with intervening monsoon. The temperature ranges between 32 °C–46 °C in the summers. Winters in Varanasi from December to February and temperatures below 5 °C are not uncommon. The average annual rainfall is 1,110mm (Indian Meteorology Department 2010).

The catchment's area consists of 29 towns, with total a population of 3,138,670 (Census of India 2001). However, the area under Varanasi municipal council (Nagar Nigam) has a population of 1,100,748 (Census of India 2001). Also approximately 138,000 people in the municipal area live in slums (Census of India 2001). Due to the high population density the city produces about 350 million liters per day of sewer and 425 tons per day

of solid waste (Planning Division, Central Pollution Control Board, Archived, 2006). The solid wastes are disposed in one landfill site and sewage is drains into nearest river (Planning Division, Central Pollution Control Board. Archived 2006).

Sampling and identification of microorganisms

In order to select the target study are a stratified random sampling procedure were used. Ten places (Banaras Hindu University and Lanka, Durgakund and Bhelpura, Gadowalia, Sigra, Cantonment Railway station, City Railway Station, DLW, Sunderpur, Ganga ghats around area, Kotwalia) were sampled from the Varanasi City according to the geographical layout of the area. In the months of April and May 2011, 203 cockroaches were collected from 44 households (Kitchen, living room, toilets and bed rooms) and 52 food-handling establishments (canteens, Bakeries, Grocery stores, food storage stores, small food processing plants and restaurants), in Varanasi City. These households and food-handling establishments were selected randomly, in addition the occupants were did not use pesticides in the recent. Cockroaches were caught in food-baited pitfall traps describe by Patterson (1989). Traps were kept on the floor under bead, pantry, cupboards, storage racks and bench for two or three consecutive nights. Each trapped cockroaches was placed in a sterile test tube and was subsequently taken to the laboratory. Trapped Cockroaches were frozen at 0 °C for 5min, and then species of cockroaches were identified under an Olympus SZX12 Dissecting Stereo Microscope according to Harwood and James (Harwood et al. 1979).

Bacterial isolation, identification and enumeration of enterobacteriaceae from external surfaces

Two milliliters of sterile normal saline (0.9 %) were added to test tube containing one cock-

roach, and the tube was thoroughly shaken for 2 minute to isolate micro-organisms from the external surface. Aliquots (0.01ml) of the washed were then separately inoculated onto the blood agar and MacConkey agar plates and incubated overnight at 37 °C. Bacterial colonies of were identified by morphological appearance, macroscopic examination, gram staining, and biochemical tests according to national standard method describe by Health Protection Agency (Health Protection Agency 2007, 2010). The overall load of bacteria carried by each insect was counted and expressed as number and also as a colony forming unit (CFU).

Antibiotic susceptibility tests

The antibiotic susceptibility of *Ps. aeruginosa*, *Kl. pneumoniae*, *Es. coli*, *Proteus mirabilis*, *Citrobacter freundii*, *Enterobacter aerogenes* species to 12 important antibiotics were tested by the Kirby-Bauer disk diffusion method according to the recommendations of the Clinical Laboratory Standards institute (CLSI 2010). These antibiotics (Becton Dickinson, Cockeysville, MD, USA) included ampicillin (10g/ disk), gentamicin (10g/ disk), ciprofloxacin (5g/ disk), ofloxacin (5g/ disk), chloramphenicol (30g/ disk), tetracycline (30g/ disk), sulfamethoxazole/ trimethoprim (25g/ disk), cephalothin (30g/ disk), ceftazidime (30g/ disk), imipenem (10g/ disk), piperacillin (100g/ disk), and cefoperazone (75g/ disk).

Statistical analysis

Data on the concentration of the Gram Negative bacteria was entered into Excel and transformed into log₁₀ Colony-Forming Units (CFU) of both Cockroach species. Rates were compared using the chi square test. P< 0.05 was considered to be statistically significant.

Results

Cockroach infestation

Among the 44 households studied, 28(54%) had cockroaches infestation: 16(57%) with *Pe.*

americana, 5(18%) with *Bl. germanica*, and 7 (25%) with both species. Totally 79 adult cockroaches were collected from households (Fig. 1): 52 *Pe. americana*, 27 with *Bl. germanica*. In households, most of the *Pe. americana* were collected from (66.7%) kitchen, also 16.4% in bed room, 9.8% in dining room and less than 3% in toilet and living room. As well as the higher rate of *Bl. germanica* was found in the storage room (41.3%), sleeping room (25.1%) and kitchen (22.6%). The occurrence of *Bl. germanica* in dining room and living room was 4.3 and 6.7%, respectively.

A total 52 food- handling establishments studied, 40(77%) had cockroaches infestation (Fig. 2): 21(53%) with *Pe. americana*, 8(20%) with *Bl. germanica*, and 11(28%) with both species. Totally 124 adult cockroaches were collected from food-handling establishments: 52 *Pe. americana*, 46 with *Bl. germanica*. In the higher rate of *Pe. americana* were collected from bakeries (36.3%) and grocery stores (42.5%). The collection rate of *Pe. americana* obtained in food storage stores, restaurants and small food processing plants were 12.7, 2.3 and 6.2%, respectively. *Bl. germanica* was highly founded in the food storage stores (28.3%), small food processing plants (18.1%), restaurants (7.6%), also bakeries (16.3%) and grocery stores (29.7%).

Bacterial isolation

Among the 203 cockroaches consisting of *Pe. americana* and *Blattella germanica* collected from the households and Food-handling establishments, eight different pathogenic species of gram-negative belonging to glucose non-fermenting Gram-negative rods and enterobacteriaceae were identified from the cuticles and gust of the cockroaches analyzed (Table 1). Moreover, 197 (97.04%) cockroaches of *Pe. americana* and *Blattella germanica* were found to carry one or more species of bacteria on the external surface. There was no significant different between

the overall bacteria load of the external surface in *Pe. americana* (64.04%) and *Bl. germanica* (35.96%), ($P > 0.05$).

The bacterial species were most frequently in food-handling establishments, however few bacterial species were identified from samples in households. Among these bacterial pathogens, four bacterial species appeared frequently, isolated in highest numbers from cockroaches trapped around the food-handling establishments, as compared with the households (Table 1). These bacteria were *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae*, which are potential pathogens. Although there was no significant difference between the overall bacteria load of the external surface in cockroaches found in the food-handling establishments (60.08%) and households (39.92%), ($P > 0.05$) (Table 1).

Antibiotic Resistance

The results of the antibiotic sensitivity tests

showed that, among the 6 gram-negative pathogenic bacteria species studied, had resistance to 8 of 12 antibiotics (66.66%) were tested (Table 2). Including *Ps. aeruginosa* and *Proteus mirabilis* had resistance to 7 of 12 antibiotics tested, *Kl. pneumoniae* and *Enterobacter aerogenes* to 4 and extended-spectrum beta lactamase (ESBL), *Escherichia coli* to 7 and *Citrobacter freundii* to 6 (Table 2). However, *Kl. pneumoniae* and *Ps. aeruginosa* were the most prevalent, drug-resistant strains were isolated from the cockroaches with 100% resistance to sulfamethoxazole/trimethoprim and ampicillin. For individual strains of bacteria, *Es. coli* was found to have multi-resistance to four antibiotic tested, *Citrobacter freundii* four, *Enterobacter aerogenes* and *Proteus mirabilis* to three (Table 2). An overall sensitivity to imipenem, cefoperazone, ciprofloxacin and ofloxacin also was observed.

Table 1. Gram-negative bacteria isolated from *Periplaneta americana* (n= 130) and *Blatella germanica* (n= 73) collected in Households and Food-handling establishments

Bacteria	Cockroaches											
	<i>Periplaneta americana</i>						<i>Blatella germanica</i>					
	Households (n= 52)			Food-handling establishments (n= 78)			Households (n= 27)			Food-handling establishments (n= 46)		
	No*	%	Mean Load*	No*	%	Mean Load*	No*	%	Mean Load*	No*	%	Mean Load*
<i>Klebsiella pneumoniae</i>	7.0	13.5	4.2 x 10 ⁷	17.0	21.8	3.9 x 10 ⁸	7.0	25.9	4.1 x 10 ⁷	11.0	23.9	3.7 x 10 ⁸
<i>Escherichia coli</i>	9.0	17.3	3.5 x 10 ⁶	14.0	17.9	4.0 x 10 ⁷	5.0	18.5	3.9 x 10 ⁶	8.0	17.4	4.1 x 10 ⁶
<i>Enterobacter aerogenes</i>	7.0	13.5	3.2 x 10 ⁵	5.0	6.4	3.1 x 10 ⁴	3.0	11.1	3.9 x 10 ⁶	6.0	13.0	3.9 x 10 ⁶
<i>Enterobacter cloacae</i>	5.0	9.6	3.6 x 10 ⁴	11.0	14.1	3.5 x 10 ⁶	2.0	7.4	3.0 x 10 ⁵	5.0	10.9	3.4 x 10 ⁶
<i>Salmonella spp</i>	6.0	11.5	3.4 x 10 ⁶	5.0	6.4	3.3 x 10 ⁵	4.0	14.8	3.8 x 10 ⁵	5.0	10.9	4.1 x 10 ⁵
<i>Citrobacter freundii</i>	5.0	9.6	4.0 x 10 ⁴	9.0	11.5	4.3 x 10 ⁵	3.0	11.1	3.9 x 10 ⁵	4.0	8.7	4.2 x 10 ⁴
<i>Proteus mirabilis</i>	6.0	11.5	3.1 x 10 ⁴	7.0	9.0	3.4 x 10 ⁵	2.0	7.4	3.1 x 10 ⁴	3.0	6.5	3.6 x 10 ⁵
<i>Pseudomonas aeruginosa</i>	7.0	13.5	4.3 x 10 ⁶	10.0	12.8	4.0 x 10 ⁸	1.0	3.7	4.1 x 10 ⁶	4.0	8.7	3.8 x 10 ⁵
Total	52.0	100.0		78.0	100.0		27.0	100.0		46.0	100.0	

*One insect may simultaneously harbour more than one species of bacteria.

*Mean Concentration of Gram Negative Bacteria (CFU)

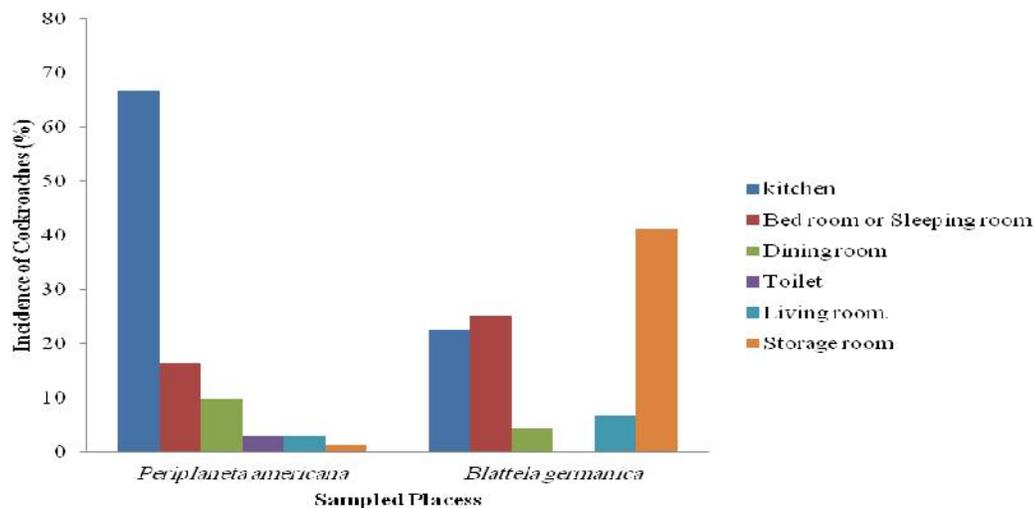


Fig. 1. Distribution (%) of the cockroaches infestation at households

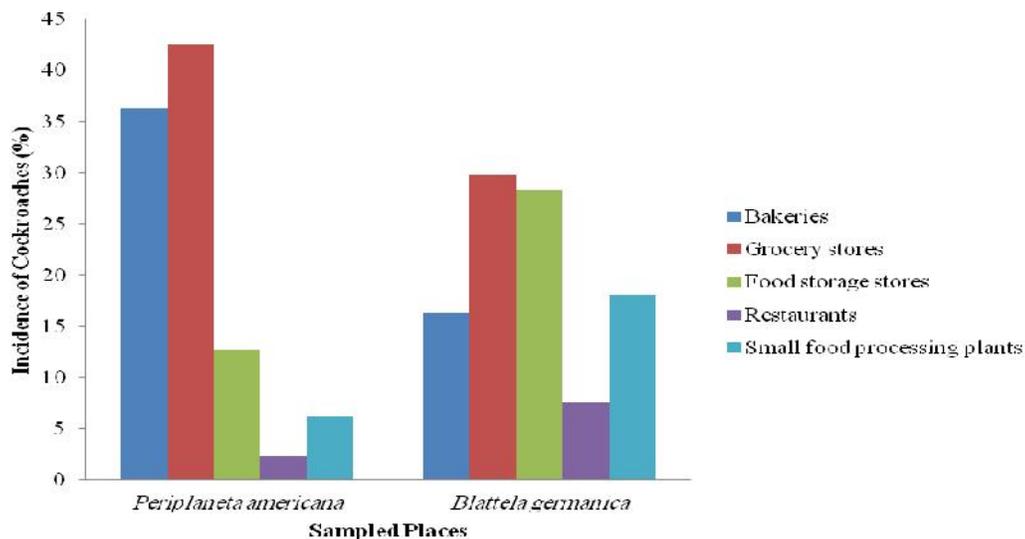


Fig. 2. Distribution (%) of the cockroaches infestation at food-handling establishments

Table 2. Antimicrobial resistance of gram-negative bacteria isolated from cockroaches collected in households and food-handling establishments

Gram-negative bacteria	Antimicrobial resistance (%)											
	AMP	PIP	CP	CRP	TE	SXT	CAZ	CFP	IMP	GM	CIP	OFX
<i>Pseudomonas aeruginosa</i> (n=22)	100	0	60.1	50	51.6	100	0	0	0	28.6	0	0
<i>Klebsiella pneumonia</i> (n=42)	100	0	0	0	52.3	95.4	0	0	0	24.5	0	0
<i>Escherichia coli</i> (n=36)	58.4	13.7	53.4	10.3	82.1	22.3	8.6	0	0	0	0	0
<i>Citrobacter freundii</i> (n=21)	46.7	7.3	0	6.3	24.1	23.4	15.3	0	0	5.6	0	0
<i>Enterobacter aerogenes</i> (n=21)	78.6	0	0	0	50.1	91.3	0	0	0	17.5	0	0
<i>Proteus mirabilis</i> (n=18)	66.3	21.3	20.1	12.4	27.4	37.8	0	0	0	7.3	0	0

Abbreviations: AMP: ampicillin, PIP: piperacillin, CP: cephalothin, CRP: chloramphenicol, TE: tetracycline, SXT: sulfamethoxazole/ trimethoprim, CFP: cefoperazone, CAZ: ceftazidime, IMP: imipenem, GM: gentamicin, CIP: ciprofloxacin, OFX: ofloxacin.

Discussion

Cockroaches are among the most notorious pests, having nocturnal and filthy habits, which do not only contaminate food by indiscriminate deposition of faecal materials and cause food poisoning but also transmit bacteria and other pathogenic microorganisms in infested areas (Kopanic 1994, Czajka et al. 2003, William J et al. 2007). These are of major concern for human health due to their capacity to act as a potential mechanical vector for transmitting more than 50 disease causing microorganisms (Cloarec et al. 1992, Rivault et al. 1993a, William J et al. 2007). In this study, there were 8 species of human pathogenic gram-negative bacteria isolated from the external bodies of two species of cockroaches (*Pe. americana* and *Bl. germanica*) from the domestic area in Varanasi city.

The bacterial species most frequently identified from cockroaches are gram-negative bacilli, especially in the family enterobacteriaceae (Fotedar et al. 1991, Cloarec et al. 1992, Rivault et al. 1993). In agreement with those studies, we also collected seven different species of bacteria belonging to family enterobacteriaceae, *Kl. pneumoniae*, *Es. coli*, *En. aerogenes*, *En. cloacae*, *Salmonella* spp., *Citrobacter freundii*, and *Proteus mirabilis*, carried by the cockroaches from households and food-handling establishments. These species can cause urinary tract and wound infections, typhoid, diarrhoea, pneumonia, gastroenteritis and respiratory infections, among others diseases in humans (Graczyk et al. 2005, Williams et al. 2010).

However, we found that makeup of the microbial population of the *Pe. americana* was considerably higher than the *Bl. germanica*, but there was no significant different between the overall bacteria load on the external surface in these two species of cockroaches. It may depend with their size and as well as the sanitation condition of domestic environment (William et al. 2007). However

the predominant bacteria on cockroaches were *Kl. pneumoniae*, *Es. coli*, *En. aerogenes*, and *Ps. aeruginosa*, as same with other numerous investigations (Rivault et al. 1993a, Mpuchane 2005, Pai et al. 2005). These results coincide with those found by Pai et al. (2005), there were no relative differences between the species of bacterial strains found in *Pe. americana* and *Bl. germanica*. It was assumed that, cockroaches were mechanically transmitting pathogens obtained in the environment and were capable of traveling while harboring these bacteria.

Cockroaches are associated with an outbreak of food-poisoning, gastro-enteritis and dysentery (Mackerras and Mackerras 1949, Rueger and Olson 1969, Burgess and Chetwyn 1981, S Mpuchane 2005). In urban area of Varanasi, we found that distribution of these bacteria among the households and food-handling establishments was no significantly different. However, most of the *Pe. americana* collected from kitchen, bakeries and grocery stores, where as higher rate of *Bl. germanica* founded in the storage room, small food processing plants and restaurants. These results are in conformance with those found by Mpuchane et al. (2005). Cockroaches, especially species that come in contact with faeces like German cockroaches may transmit bacteria responsible for food poisoning (Cochran 1982, Rivault et al. 1993b). They are attraction to human and animal faeces, rotting food, secretions from corpses, sputum, pus, and the like gives them a well-earned disgust factor among the general public (Rivault et al. 1993b, S Mpuchane et al. 2005, William et al. 2007, Sudershan et al. 2009). These finding suggest the, these bacteria species are uniformly distributed among the both places and especially this two cockroaches species are importance of transmitting food-borne diseases.

Cockroaches may act as reservoirs for antibiotic resistant virulent bacteria found on do-

mestic environment, which may cause serious health problems for Human beings (Pai et al. 2005, Ahmad 2011). Similar with this, in our study 6 gram-negative bacteria strains, obtained from the households and food-handling establishment environments, showed resistant to antibiotics, ampicillin, tetracycline and sulfamethoxazole/ trimethoprim.

Although, we have found that lower activity of ampicillin and tetracycline against these bacterial strains, *Kl. pneumoniae*, *Ps. aeruginosa* and *Es. coli*, may essentially due to the natural resistance of the bacterial species (Navarro Risueño et al. 2002). Moreover, the antibiotic resistant strain of *Kl. pneumoniae* bacteria that cause pneumonia has been found in patients and cockroaches in a New Delhi hospital (Fotedar 1991). Also, the less susceptibility of *Es. coli* and *Citrobacter freundii* against ceftazidime was may due to the extended-spectrum b-lactamases enzyme activity (Emery et al. 1997). But the result of the test was not agreement with Pai HH (2005).

However, the bacteria isolated from the cockroaches were relatively resistant to gentamicin. The results of were not in agreement with Pai et al. (2005), but it coherence with those found by Marinésia (2006). Also recently we have found that *Ps. aeruginosa* isolated from wound infection patients at different ward of Sri Sundaralala Hospital, in a Banaras Hindu University, showed resistant to all commonly use antibiotics (Anupurba S 2006, Basu S 2009). This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes, inadequate use of antibiotics and the low permeability of the bacterial cellular envelopes (Poole 2004, Basu 2009).

We observed that activity of imipenem and ciprofloxacin against Gram-negative bacteria, showed 100% sensitivity. The results are in accordance to those found by Pai et al. (2005).

Based on our study, cockroaches are uniformly distributed among the places of house-

holds and food-handling establishments in Varanasi area. We also determined that both species of *Pe. americana* and *Bl. germanica*, have equal potential to carry the pathogenic bacteria as a vector in their external surface. Moreover, bacteria species were studied showed relative resistance to antibiotic tested in our study. It may possible threat for human health.

Conclusion

Most of the domestic area in Varanasi City, especially food-handling establishments and human dwellings, are highly polluted with domestic pollutants, which play a key role for the transmission of a wide range of pathogenic bacteria and also provide ideal conditions for the breeding and multiplication of cockroach.

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