

ISOLATION OF GRAM-NEGATIVE BACTERIA FROM COCKROACHES TRAPPED FROM URBAN ENVIRONMENT

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Abstract. Three different areas -- hospital, food-handling establishments and human dwellings, were surveyed for pathogenic gram-negative bacteria carried on the cuticles of cockroaches. Fifty species of bacteria were identified from all cockroaches. *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter cloacae* were the most frequently found. Pathogenic and potentially pathogenic bacteria represented 58% of all bacteria identified. The numbers of pathogenic and potentially pathogenic bacteria were similar in hospital areas and food-handling establishments, while, human dwellings possessed a poorer bacterial flora. *E. coli*, *K. pneumoniae* and *E. cloacae* were dominant species in hospital areas, while in food-handling establishments and human dwellings, *E. coli*, *K. pneumoniae* and *C. freundii* predominated. Therefore, cockroaches can play a role in bacterial transmission, due to the bacteria carried on their cuticles.

INTRODUCTION

Cockroaches are among the medically important pests in urban environments that cause serious public health problems. They have been found to harbor a number of pathogenic and potentially pathogenic bacteria which were carried either on the cuticle or in the gut (Cloarec *et al*, 1992). The bacterial loads were up to 14 million on the bodies, and 7 million in each of their fecal droppings (Bennett, 1993). These insects are considered important disease vectors transmitted by both mechanical and biological routes.

Cockroaches are likely to be encountered in favorable environmental conditions with a ready source of food. The range of problems caused by the presence of cockroaches vary 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 the transmis-

sion of drug-resistant bacteria (Fotedar *et al*, 1991; Cotton *et al*, 2000). *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp and several other potential pathogens have been isolated from cockroaches collected from hospitals (Le Guyader *et al*, 1989; Oothuman *et al*, 1989). Cockroaches have been associated with an outbreak of dysentery (Burgess and Chetwyn, 1981). *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). Transmission could occur by cockroach regurgitation or fecal pellet deposition into human food-stuffs (Cochran, 1982). Thus, these insects also play a role in transmitting food-borne diseases. Their presence reduces the perception of human well-being and sanitary standards.

The aim of this investigation was to compare the species richness and relative abundance of gram-negative bacteria carried by cockroaches between several urban environments, including hospital, food-handling establishments (canteens and restaurants), and multi-family dwellings.

MATERIALS AND METHODS

Sampling

Thirty cockroaches were collected over a period of 3 months (March 2001-May 2001) from

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three types of areas around the building of the Faculty of Allied Health Science, Chulalongkorn University, Bangkok, Thailand. The areas studied were as follows.

Hospital. Cockroaches were caught around the patient building and canteens of Chulalongkorn Hospital.

Food-handling establishments. Insects were trapped around several canteens and restaurants at Siam Square.

Human dwellings. Insects were usually caught in kitchens, bathrooms and toilets.

Cockroaches were caught in food-baited pit-fall traps. Traps were deposited at night, collected the next morning, and transported to the laboratory for further processing.

Bacterial isolation and identification of enterobacteriaceae from external surfaces

Trapped cockroaches were frozen at 0°C for 10 minutes. Each cockroach was collected in a sterile test tube and identified using standard taxonomic keys (Herms, 1950). After identification, 2 ml of sterile 0.85% normal saline was added to the tube and the cockroach was thoroughly shaken for 2 minutes. 0.01 ml of the wash was cultured on blood agar and MacConkey agar and incubated overnight at 37°C. Identification of gram-negative bacteria was achieved by use of standard methods (API System, bioMerieux, France).

RESULTS

Ninety cockroaches were caught, consisting of *Periplaneta americana* and *Blatta orientalis*. Fifty different species of gram-negative bacilli were identified from the cuticles of the cockroaches analyzed (Table 1). The bacterial species were found predominantly in food-handling establishments and hospital areas, while fewer bacterial species were identified from samples in human dwelling areas. Four bacterial species appeared frequently, *Klebsiella pneumoniae*, *Escherichia coli*, *Citrobacter freundii*, and *Enterobacter cloacae*, which are potential pathogens. The other pathogenic and potentially pathogenic bacterial species identified included *Enterobacter agglomerans*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Citrobacter diversus*, *Salmonella* spp, *Serratia marcescens*,

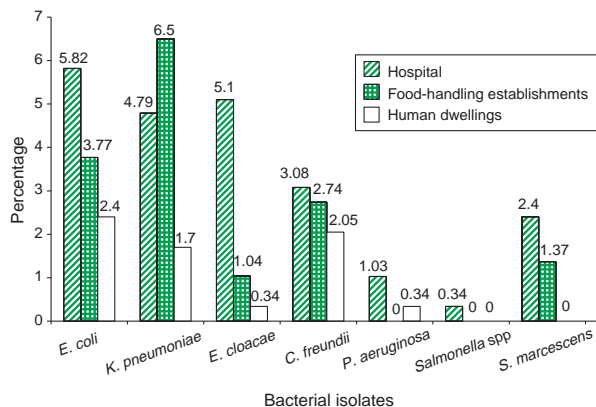


Fig 1—Important pathogenic bacteria isolated from hospital, food-handling establishments, human dwellings.

Proteus vulgaris, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*, *Edwardsiella tarda*, and *Morganella morganii*. All these pathogenic bacteria represented 58% of all identifications. *Enterobacter* spp (8.9%), *Klebsiella* spp (10.3%), and *Escherichia* spp (4.8%) were the most frequently found from samples in hospital, food-handling establishments, and human dwellings, respectively.

Among common bacterial pathogens, *Escherichia coli*, *Enterobacter cloacae* and *Citrobacter freundii* were isolated in highest numbers from cockroaches trapped around the hospital, as compared with the others (Fig 1). *Klebsiella pneumoniae* was isolated in greater numbers from samples of food-handling establishments. The three dominant hospital species were *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*. In food-handling establishments and human dwellings, *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* were the most frequently found. *Salmonella* spp was identified only from cockroaches trapped in the hospital area.

DISCUSSION

Cockroaches always carry a great variety of bacterial species collected from the environment in which they live. The fewer the bacterial species there are in the environment, the fewer the bacteria cockroaches will carry, as shown in our data for human dwellings. The bacterial species most

Table 1
Occurrence of different bacterial species identified from the different urban environments studied (hospital, food-handling establishments, and human dwellings).

Bacterial strains	Hospital	Food-handling establishments	Human dwellings	Total	<i>Bacterial strains</i>	Hospital	Food-handling establishments	Human dwellings	Total
<i>Enterobacter</i>					<i>Cedecea</i>				
<i>E. cloacae</i>	15	3	1	19	<i>C. davisae</i>	3	1	4	8
<i>E. agglomerans</i>	6	3	0	9	<i>C. neteri</i>	0	1	0	1
<i>E. sakasakii</i>	2	6	1	9	<i>C. lopagei</i>	0	1	0	1
<i>E. gergoviae</i>	1	3	1	5	<i>Pseudomonas</i>				
<i>E. asburiae</i>	2	3	1	6	<i>P. matophilia</i>	0	1	0	1
<i>E. aerogenes</i>	0	3	1	4	<i>P. aeruginosa</i>	3	0	1	4
<i>Enterobacter</i> spp	0	0	2	2	<i>P. pseudoalkaligenes</i>	0	5	0	5
<i>Escherichia</i>					<i>Pseudomonas</i> spp	4	1	1	6
<i>E. coli</i>	17	11	7	35	<i>Edwardsiella</i>				
<i>E. vulneris</i>	0	1	0	1	<i>E. tarda</i>	0	1	0	1
<i>E. hermanii</i>	1	0	0	1	<i>E. ictaluri</i>	2	1	1	4
<i>E. balntae</i>	0	7	7	14	<i>Providencia</i>				
<i>Klebsiella</i>					<i>P. rustigianii</i>	1	0	0	1
<i>K. pneumoniae</i>	14	19	5	38	<i>Providencia</i> spp	0	0	3	3
<i>K. rhinoscleromatis</i>	1	3	1	5	<i>Ewingella</i>				
<i>K. oxytoca</i>	0	3	0	3	<i>E. americana</i>	6	1	0	7
<i>K. terrigena</i>	0	3	0	3	<i>Rahnella</i>				
<i>K. ozanae</i>	0	2	0	2	<i>R. aquatilis</i>	1	0	0	1
<i>Klebsiella</i> spp	2	0	2	4	<i>Kluyvera</i>				
<i>Citrobacter</i>					<i>K. ascorbata</i>	2	0	1	3
<i>C. freundii</i>	9	8	6	23	<i>Kluyvera</i> spp	0	3	0	3
<i>C. diversus</i>	0	2	1	3	<i>Hafnia</i>				
<i>Citrobacter</i> spp	5	1	0	6	<i>H. alvei</i>	0	2	0	2
<i>Salmonella</i>					<i>Moraxella</i>				
<i>Salmonella</i> spp	1	0	0	1	<i>M. urethralis</i>	0	2	0	2
<i>Serratia</i>					<i>Morganella</i>				
<i>S. marcescens</i>	7	4	0	11	<i>M. morganii</i> ss.	0	1	0	1
<i>S. liquefaciens</i> gr	1	0	0	1	<i>morganii</i>				
<i>S. odotifera</i>	0	1	0	1	<i>Obesumbacterium</i>				
<i>Serratia</i> spp	1	0	0	1	<i>Obesumbacterium</i>	1	0	1	2
<i>Proteus</i>					<i>Enteric</i> gr				
<i>P. vulgaris</i>	0	2	0	2	<i>Enteric</i> gr 63	5	0	0	5
<i>P. mirabilis</i>	6	5	4	15	<i>Enteric</i> gr 63	0	1	0	1
<i>P. penneri</i>	1	4	0	5	No. of species identified in each area	28	37	21	50
<i>P. myxofeaceins</i>	0	1	0	1	Total occurrence of bacteria	120	120	52	292

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). These species can cause urinary tract infections, sepsis, gastroenteritis, urinary, biliary and peritoneal infections, pneumonia, or wound infections (Roth and Willis, 1957; Le Guyader *et al*, 1989; Burgess *et al*, 1973).

The importance of cockroaches as potential vectors of potentially pathogenic bacteria varies

in each area. The presence of cockroaches in a bacteria-rich environment, especially with pathogenic and potentially pathogenic bacteria, is more serious than in a bacteria-poor environment. Areas where people have low level immunity should be aware of bacterial contamination from cockroaches. However, it appeared from our data that the numbers of pathogenic and potentially pathogenic bacteria carried by cockroaches trapped from hospital and food-handling estab-

lishments were similar. However, the bacterial species in the hospitals appeared more resistant to antibiotics, which is considered more serious (Fotedar *et al*, 1991).

In food-handing establishments, cockroaches could be dangerous if the bacteria they carry are pathogenic. Cockroaches were found to be associated with an outbreak of dysentery in Northern Ireland. The causative organism was *Shigella dysenteriae* serotype 7 (Burgess and Chetwyn, 1981). *Salmonella* species also can be carried by cockroaches and have been found to cause a gastro-enteritis outbreak (Mackerras and Mackerras, 1949). *Escherichia coli* is a key-stone species in environmental surveillance as an indicator of fecal contamination. Thus, the potential of cockroaches to transmit food-borne diseases should not be ignored or simply rejected, but afforded further investigation.

The presence of cockroaches in human dwelling areas is never desirable, and must be taken into consideration. Although many of the pathogenic species are only opportunist, or potential, pathogens, and the presence of immunodepressive people is rarer in homes than hospitals, it is also a risk factor for human health and reduced sanitation level.

Cockroaches can, therefore, present a real hazard for human health because they can carry several pathogenic and potentially pathogenic bacteria, including other microorganisms. Pest control regulations differ between public establishments and private dwellings. Elimination of cockroaches from sensitive areas, such as hospitals, is essential. In food-handling establishments and human dwellings, cockroaches must also be controlled, to maintain acceptable hygiene standards.

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