

Original Article

The First Report of Drug Resistant Bacteria Isolated from the Brown-Banded Cockroach, *Supella longipalpa*, in Ahvaz, South-western Iran

Babak Vazirianzadeh ¹, *Rouhullah Dehghani ², Manijeh Mehdinejad ³, Mona Sharififard ⁴, Nersi Nasirabadi ³

¹Department of Medical Entomology, College of Health and Infectious and Tropical disease Research Centre, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

²Department of Environmental Health, College of Health and Social Determinants of Health (SDH), Research Center, Kashan University of Medical Sciences, Kashan, Iran

³Departments of Medical Microbiology, College of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

⁴Departments of Medical Entomology, College of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

(Received 7 July 2012; accepted 30 June 2013)

Abstract

Background: The brown-banded cockroach, *Supella longipalpa* is known as a carrier of pathogenic bacteria in urban environments, but its role is not well documented regarding the carriage of antibiotic-resistant pathogenic bacteria in Iran. The aim of this study was to determine the resistance bacteria isolated from the brown-banded cockroach in Ahvaz, south west of Iran.

Methods: Totally 39 cockroaches were collected from kitchen area of houses and identified. All specimens were cultured to isolate the bacterial agents on blood agar and MacConky agar media. The microorganisms were identified using necessary differential and biochemical tests. Antimicrobial susceptibility tests were performed for isolated organisms by Kirby-Bauer's disk diffusion according to NCLI guideline, using 18 antibiotics.

Results: From the 39 collected *S. langipalpa*, 179 bacterial agents were isolated, 92 of alimentary ducts and 87 of external body surfaces. Isolated bacteria from cockroaches were identified as *Enterobacter spp.*, *Klebsiella spp.*, *Citrobacter spp.*, *Escherichia coli*, *Salmonella spp.*, *Proteus spp.*, coagulase negative staphylococci, *Serratia marcescens*, *Staphylococcus aureus*, and *Bacillus* species. The pattern resistance rates were determined for gram negative bacilli and gram positive cocci regarding 18 antibiotics.

Conclusion: The brown-banded cockroach can be involved in the spread of drug resistant bacteria and increases the possibility of contacting human environment to drug resistant bacteria. Therefore, the potential of removing this insect should be improved. This is the first original report of drug resistant bacteria isolated from the brown-banded cockroach of Iran.

Keywords: *Supella longipalpa*, Drug Resistant Bacteria, Iran

Introduction

Cockroaches are the most abundant and important pest insects that inhabit various public places such as hospitals, food manufacturing sites and kitchens (Ebellling 1978, Burgess and Chetwyn 1979). Cockroaches have been considered as transmitters and spreaders of pathogenic bacteria in hospitals and households or residential areas (Lamiaa Bouamama

2005). Cockroaches feed indiscriminately on garbage and sewage and so have copious opportunity to disseminate human pathogens. In addition, their nocturnal and filthy habits make them ideal carriers of various pathogenic microorganisms (Allen 1978, Graczyk et al. 2005, Pai et al. 2005).

So far numerous pathogenic bacteria, in-

cluding *Salmonella spp.*, *Shigella spp.*, *Campylobacter spp.*, *Pseudomonas aeruginosa* and *K. pneumonia* have been isolated from cockroaches (Cotton et al. 2000).

Antibiotic resistance is a serious public-health problem, reduced effectiveness of antibiotics results in greater patient mortality rates, prolonged hospitalization and increased healthcare costs. The economic impact of antibiotic resistance has been estimated between \$5 and \$24 billion annually in the United States alone (Hall 2004). Insects associated with food animals, especially cockroaches not only are important nuisance pests but also potential vectors of animal and human pathogens. Organic waste in and around animal production facilities provide excellent habitats for the growth and development of these insects. Because of their habitat preferences, unrestricted movement, mode of feeding, and attraction to residential areas, cockroaches have a great potential to disseminate fecal bacteria, including human and animal pathogens and antibiotic resistant strains (Zurek and Gorham 2008, Graczyk et al. 2001).

The brown-banded cockroach, *Supella longipalpa*, is a small species of cockroach, measuring about 10 to 14,mm long. Cockroaches like *S. longipalpa* which live in drier parts of houses can harbor and transmit the different bacteria within and between premises because of small bodies. This leads to spread the bacteria which may be pathogenic and antibiotic resistance, potentially (Manweiler 1998, Gibson and Hunter 2009).

There are insufficient data on the potential health impact of this species in the resident areas of Iran. More infestation with this species has recently been reported from urban environments of Iran, but its role is not well documented regarding the carriage of antibiotic-resistant pathogenic bacteria in Iran. This is the first original report of drug resistant bacteria isolated from the brown-banded cockroach of Ahvaz and Iran.

The aim of this study was to determine the

resistance bacteria isolated from external surfaces and digestive tract of the brown-banded cockroaches (*S. longipalpa*) which were collected from kitchen area of houses of Ahvaz, south west of Iran.

Materials and Methods

A total of 39 cockroaches were collected from kitchen area of houses, Ahvaz SW Iran. The samples were transported to the medical entomology laboratory of Ahvaz Jundyshapur University of Medical Sciences (AJUMS) and placed in the sterile dishes in freeze-temperature for 10–15min to anaesthetize them. The cockroaches were identified after immobilization by freezing, using keys of Department of Entomology and Nematology University of Floridaas and Cochran (Cochran 1999).

Afterward, two samples were taken from each cockroach, one of external surface body with sterile cotton swabs and the other one directly from alimentary duct after dissecting the insects. All specimens were cultured to isolate the bacterial agents on blood agar and Mac Conky agar media (Himedia India and Merck Germany). The microorganisms were identified using necessary differential and biochemical tests.

Cultured media were incubated at 37 °C for 24 hours. The various bacteria growth on the agar media were identified by colonial morphology on blood agar plates, Gram stains characteristics. In addition, other necessary biochemical tests including: oxidase, catalase, motility, coagulase, indole, methylred, voges proskauer (MRVP), gelatine hydrolysis, gas from glucose, H₂S production and acid produced from various sugar according to standard microbiological procedures were also used for identification (Forbes et al. 2002, Jorgensen et al. 2005).

Antimicrobial susceptibility testing was performed for isolated organisms by Kirby-Bauer's disk diffusion according to Clinical and

Laboratory Standards Institute (CLSI) guideline. Antibiotic disks were used and the susceptibility pattern of isolated from samples to commonly used antibiotics was then reported. The antibiotic disks (padten Teb, Tehran, Iran) comprised ampicillin (10µg), cephalothin (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), chloramphenicol (30µg), gentamicin (10µg), tetracycline (30µg), trimethoprim-sulfamethoxazole (25µg), ceftazidime (10µg), amikacin (30µg), nitrofurantoin (300µg), nalidixic acid (30µg), cefalexin (10µg), imipenem (10µg), vancomycin (30µg), cefotaxime (10µg), kanamycin (30µg), and penicillin (10µg) guidelines to determine susceptibility of UTIs agents (CLSI 2002, Mpuchane 2006).

Results

From 39 collected *S. langipalpa*, 179 bacterial agents were isolated, 92 of alimentary ducts and 87 of external body surfaces (Table 1). Isolated bacteria from cockroaches were identified as *Enterobacter spp.*, *Klebsiella*

spp., *Citrobacter spp.*, *E. coli*, *Salmonella spp.*, *Proteus spp.*, coagulase negative staphylococci, *S. marcescens*, *Staphylococcus aureus*, and *Bacillus* species.

The pattern resistance rates were determined for gram negative bacilli and gram positive cocci regarding 18 antibiotics. Among all the isolates Gram negative bacilli, from kitchen area of houses, ampicillin, cephalothin, ceftazidime, nitrofurantoin, nalidixic acid, trimethoprim-sulfamethoxazole, cefalexin and tetracycline, resistance rates were above 52.4% and cefotaxime expressed the highest susceptibility among all the isolates from the kitchen area of houses. Among all the isolates Gram positive cocci, from kitchen area of houses, ampicillin, amikacin penicillin, ceftazidime, nitrofurantoin, nalidixic acid, trimethoprim-sulfamethoxazole, cefalexin, cefotaxime and tetracycline, resistance rates were above 53.8% and ciprofloxacin expressed the highest susceptibility among all the isolates from the kitchen area of houses (Table 2).

Table 1. Frequency of isolated bacteria from Cockroaches

Isolated Bacteria from Cockroaches	Alimentary Tract No. (%)	External Surface No. (%)	Total No. (%)
<i>Enterobacter aerogenes</i>	3(3.26)	2(2.30)	5(2.79)
<i>Enterobacter cloacae</i>	3(3.26)	3(3.45)	6(3.35)
<i>Enterobacter agglomerans</i>	9(9.78)	9(10.35)	18(10.10)
total	15(16.30)	14(16.10)	29(16.20)
<i>Klebsiella pneumonia</i>	12(13.04)	13(14.94)	25(14.00)
<i>Klebsiella oxytoca</i>	2 (2.17)	1(1.15)	3(1.70)
total	14(16.30)	14(16.10)	28(15.64)
<i>Citrobacter freund</i>	9(9.78)	7(8.05)	16(8.94)
<i>Escherichia coli</i>	13(14.13)	11(12.64)	24(13.41)
<i>Salmonella para A</i>	3(3.26)	2(2.30)	5(2.79)
<i>Serratia marcescens</i>	4(4.35)	4(4.60)	8(4.50)
<i>Proteus mirabilis</i>	5(5.43)	6(7.00)	11(6.2)
<i>Proteus vulgaris</i>	2(2.17)	1(1.15)	3(1.70)
total	7(7.61)	7(8.10)	14(7.82)
coagulase Negative Staphylococci	11 (11.96)	9(10.35)	20(11.17)
<i>Staphylococcus aureus</i>	3 (3.26)	4(4.60)	7(3.91)
<i>Bacillus cereus</i>	9(9.78)	10(11.50)	19(10.61)
<i>Bacillus subtilis</i>	3(3.26)	4(4.60)	7(3.91)
total	12(13.04)	14(16.10)	26(14.53)
Total	92(52.57)	87(47.43)	179(100)

Table 2. Percentages of resistant of isolated bacteria from Cockroaches

Antibiotics Resistance	Gram negative bacilli	Gram positive cocci
Ampicillin	86.8	100
Cephalothin	69.5	44.3
Ceftriaxone	42.4	45.5
Ciproflexoxacin	24.7	19.6
Chloramphenicol	47.3	-
Gentamicin	44.2	38.8
Tetracycline	59.4	63.4
Trimethoprim-sulfamethoxazole	53.6	57.1
Ceftazidime	100	85.3
Amikacin	25.9	53.8
Nitrofurantoin	70.1	-
Nalidixic acid	52.4	74.9
Cefalexin	67.9	63.2
Imipenem	-	-
Vancomycin	-	-
Cefotaxime	13.5	85.3
kanamycin	36.5	-
Penicillin	-	100

Discussion

The biology and ecology of *S. longipalpa* make it an ideal mechanical vector of bacteria to animal and human. Brown-banded cockroaches are found in homes, apartments, hotels, and hospitals. They are less frequently found in stores restaurants and kitchens. They are frequently transported in furniture and will spread rapidly through an entire building. Brown-banded cockroaches are generally found on ceilings, high on walls, behind picture frames, and near motors of refrigerators and other appliances. The results of this study indicated that *Supella longipalpa* could play a great role as a mechanical carrier of bacteria. In this study, all 39 cockroaches were found to carry the 9 genera of medically important bacteria.

The most frequent bacteria isolated from *S. angipalpa* coming from external bodies of this cockroaches was *K. pneumonia* followed by *E. coli* and *E. coli* was the most frequent coming of alimentary tract of *S. langipalpa* followed by *K. pneumonia* in the present study was. This is very similar to the studies of Vahabi et al. in Iran and Bouamama et al. in Morocco (Bouamama et al. 2010, Va-

habi et al. 2011). However, the most biodiverse isolated bacteria during the current investigation was totally related to *Enterobacter sp.*: *E. aerogenes*, *E. cloacae* and *E. agglomerans*, with 16.20% of all species. This is important when *Enterobacter spp.* are known as food spoilage bacteria and are considered as cockroach transmitted bacteria in food poisoning.

In this study, most of the bacteria isolated were medically important, including *Enterobacter spp.*, *Klebsiella spp.*, *Citrobacter spp.*, *E. coli*, *Salmonella spp.*, *Proteus spp.*, coagulase negative Staphylococci, *S. marcescens*, *Staphylococcus aureus*, and *Bacillus* species. These findings agree with the results of Chaichanawongsaroj et al. (2004) in Thailand, which showed presence *Enterobacter spp.*, *Klebsiella spp.*, *Citrobacter spp.*, *E. coli*, *Salmonella spp.*, *Proteus spp.*, *Serratia spp.*, species on the *Periplaneta americana* and *Blatta orientalis* collected from Hospital, Food-handling establishments and human dwellings (Bennett 1993, Chaichanawongsaroj et al. 2004, Salehzadeh et al. 2007).

The similar bacteria have been isolated from *P. americana* and *B. germanica* in an-

other study of Botswana (Mpuchane 2006). However, they are known as pathogenic (*Salmonella*, *Shigella*, *B. cereus*), opportunistic pathogens (*Pseudomonas*, *Klebsiella*, *Vibrio*) and food spoilage species (*Pseudomonas*, *Enterobacter*, *Escherichia*, *Erwinia*) they belong to cockroach transmitted bacteria (Mpuchane 2006).

Bacterial species, such as *Klebsiella spp.*, *E. coli*, *Staphylococcus spp.*, *Enterobacter spp.*, *Streptococcus spp.*, *Pseudomonas spp.*, *Proteus spp.*, *Bacillus spp.*, *Neisseria spp.*, *Shigella spp.*, and *Salmonella spp.*, have also been isolated from *P. Americana* and *Polyphega aegyptica* cockroaches by other researchers from Khuzestan (Vazirianzadeh et al. 2011, Kassiri and Kazemi 2012). It is important to say that the above named bacteria have been isolated from housefly, *Musca domestica* in Khuzestan (Vazirianzadeh et al. 2008).

The present study indicates that brown-banded cockroach poses a possible health risk to communities proved that the isolated strains of bacteria were resistant to various antibiotics. It is well-established fact that the resistance to various antimicrobials may be due to presence of some virulence gene, involvement of secretion machinery of multi drug efflux proteins, through mutations in bacterial genome or by gaining additional genes through horizontal gene transfer or by physiology dependent resistance (Mitchell et al. 2004).

One of the most important problems facing global public health today is antimicrobial resistance. The problem is most horrible in developing countries, where the bacterial infections causing human disease are also those in which emerging antibiotic resistance is most evident (Shears 2000, Fathpour et al. 2003, Kalantar et al. 2008). Cockroaches are able to mechanically transfer infectious agents in unsanitary places. Some bacteria that exist in the digestive tract of cockroaches can be also found in environment, that most of them belong to Gram-negative bacilli of Enter-

obacteriaceae family (Cloarec and Rivault 1992) this organisms can cause urinary infections, poisoning, inflammation of the stomach and intestines, and abdominal cavityinfection, pneumonia or wound infections (Le Guyader et al. 1989, Kopanic et al. 1994). Therefore, 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 (Chai-ghanawongsaraj et al. 2004).

Conclusion

Brown-banded cockroaches collected from kitchen area of houses may be involved in the spread of drug resistant bacteria and may increase the potential for human exposure to drug resistant bacteria. It is recommended that suitable steps must be taken to control the cockroaches and monitor the sensitivity pattern of the pathogens transmitted by the cockroaches.

Therefore, the potential of removing this insect should be improved. Parallel to integrated pest management of cockroaches, we must go ahead to produce new antibiotic from natural resources like the filthy habitats of cockroaches which kills them (Lee 2012).

Acknowledgements

The authors gratefully acknowledge personnel of Department of Medical Microbiology, College of Medicine, Ahvaz Jundishapur University of Medical Sciences for providing, their assisting in the study. The authors declare that there is no conflict of interests.

References

Allen BW (1987) Excretion of viable tubercle bacilli by *Blattaorientalis* (the ori-

- ental cockroach) following ingestion of heat-fixed sputum smears: a laboratory investigation. *Trans R Soc Trop Med Hyg.* 81: 98–99.
- Bennett G (1993) Cockroaches as carriers of bacteria. *Lancet.* 341: 732.
- Burgess NRH, Chetwyn KN (1979) Cockroaches and the Hospital Environment. *Nursing Times*, February. 5–7.
- Bouamama L, Sorlozano A, Laglaoui A, Lebbadi M, Aarab A, Gutierrez J (2010) Antibiotic resistance patterns of bacterial strains isolated from *Periplaneta americana* and *Musca domestica* in Tangier, Morocco. *J Infect Dev Ctries.* 4(4): 194–201.
- Chaichanawongsaroj N, Vanichayanarak K, Pipatkullachat T, Polrojpanya M, Somkiatcharoen S (2004) Isolation of gram-negative bacteria from cockroaches trapped from urban environment. *Southeast Asian J Trop Med Public Health.* 35(3): 681–684.
- Cloarec A, Rivault CF (1992) Cockroaches as carriers of bacteria in multi-family dwellings. *Epidemiol Infect.* 109: 483.
- Cochran DG (1999) World Health Organization communicable diseases prevention and control WHO pesticide evaluation scheme (WHOPES), 85.
- Cotton MF, Wasserman E, Pieper CH, Van Tubbergh D, Campbell G, Fang FC, Barnes J (2000) Invasive disease due to extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal unit: the possible role of cockroaches. *J Hosp Infect.* 4: 13–17.
- Ebell W (1978) *Urban Entomology*. University of California, California Press, Berkeley.
- Fathpour H, Emtiazi G, Ghasemi E (2003) Cockroaches as reservoirs and vectors of drug resistant *Salmonella* spp. *Fresenius Environ Bull.* 12: 724–727.
- Forbes BA, Sahm DF, Weissfeld AS (2002) *Bailey and Scott's diagnostic microbiology: A textbook for isolation and identification of pathogenic microorganisms*, 11th ed, The Mosby Inc., St. Louis.
- Gibson CM, Hunter MS (2009) Inherited Fungal and Bacterial Endosymbionts of a Parasitic Wasp and Its Cockroach Host *Microb Ecol.* 57: 542–549.
- Graczyk TK, Knight R, Tamang L (2005) Mechanical transmission of human protozoan parasites by insects. *Clin Microbiol Rev.* 18: 128–132.
- Graczyk TK, Knight R, Gilman R, Cranfield M (2001) The role of non-biting flies in the epidemiology of human infectious diseases. *Microbes Infect.* 3: 231–235.
- Hall BG (2004) Predicting the evolution of antibiotic resistance genes. *Nat Rev Microbiol.* 2: 430–435.
- Jorgensen JH, Turnidge JD, Washington JA (1999 and 2005) Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron E J, Pfaller MA, Tenover FC, Tenover R H, editors. *Manual of clinical microbiology*. 7th ed. Washington, American Society for Microbiology. 1526–1543 and 221–236.
- Kassiri H, Kazemi S (2012) Cockroaches [*Periplaneta americana* (L.), Dictyoptera, Blattellidae] as Carriers of Bacterial Pathogens, Khorramshahr County, Iran. *Jundishapur J Microbiol.* 5(1): 320–322.
- Kalantar E, Motlagh M, Lordnejad H, Reshamansh N (2008) Prevalence of urinary tract pathogens and antimicrobial susceptibility patterns in children at 55 hospitals in Iran. *Iranian J Clin Infect Dis.* 3(3): 149–154.
- Kopanic RJ, Sheldon B, Wright CG (1994) Cockroaches as vector of *Salmonella*: laboratory and field trials. *J Food Protect.* 57: 125–132.
- Le Guyader A, Rivault C, Chaperon J (1989) Microbial organisms carried by brown-banded cockroaches in relation to their

- spatial distribution in a hospital. *Epidemiology Infection*. 102: 485–492.
- Lee S, Siddiqui R, Khan NA (2012) Animals living in polluted environments is potential source of antimicrobials against infectious agents. *Pathog Glob Health*. 106(4): 218–223.
- Manweiler SA (1988) Two parasites of the brownbanded cockroach: a competition matrix. University of California, Berkeley.
- Mitchell J, Tali De-Medina, Yehuda C (2004) Epidemiological interpretation of antibiotic resistance studies: what are we missing? *Nature Rev Microbiol*. 2: 979–983.
- Pai HH, Chen, WC, Peng CF (2005) Isolation of bacteria with antibiotic resistance from household cockroaches (*Periplaneta americana* and *Blattella germanica*). *Acta Trop*. 93: 259–65.
- Shears P (2000) Antimicrobial resistance in the tropics. *Tropical Doctor*. 30: 114–116.
- Salehzadeh A, Tavacol P, Mahjub H (2007) Bacterial, fungal and parasitic contamination of cockroaches in public hospitals of Hamadan, Iran. *J Vect Borne Dis*. 44: 105–110.
- Vazirianzadeh B, Mehdinejad M, Dehghani R (2011) Identification of bacteria which possible transmitted by *Polyphagaegyptica* (Blattodea: Blattidae) in the region of Ahvaz, SW Iran. *Jundishapur J Microbiol*. 2(1): 36–40.
- Vazirianzadeh B, Shams Solary S, Rahdar M, Hajhossien R, Mehdinejad M (2008) Identification of bacteria which possible transmitted by *Muscadomestica* (Diptera: Muscidae) in the region of Ahvaz, SW Iran. *Jundishapur J Microbiol*. 1(1): 28–31.
- Zurek L, Gorham JR (2008) Insects as vectors of food borne pathogens. In *Wiley Handbook of Science and Technology for Homeland Security*. Edited by: Hoboken V JG, N J John. Wiley and Sons,inc. New York.