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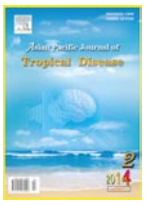

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Antibiotics susceptibility patterns of bacteria isolated from American and German cockroaches as potential vectors of microbial pathogens in hospitals

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ABSTRACT

Objective: To identify the cockroach species, their bacterial flora and antibiotics susceptibility patterns of these bacteria in Shiraz.

Methods: In the present descriptive study, only two species of cockroaches were recognized. The washing solutions from the digestion systems and surfaces of 156 American and German cockroaches were cultured. The latter was found to be the commonest (89.7%) in all places.

Results: Overall, 18 species of bacteria were isolated and identified by standard culture methods. The most frequent bacterium isolated from both species of cockroaches in all places was *Pseudomonas* (41.7%). The second and third commonest bacteria were *Enterobacter* (39.7%) and *Klebsiella* (32.7%), respectively.

Conclusions: The antibiogram profiles showed full (100%) resistance of *Klebsiella*, *Citrobacter*, *Acinetobacter* and *Proteus* to amoxicillin and ampicillin at both hospitals, while *Pseudomonas* showed resistance (95.7%) to cephalothin. Thus it is concluded that German and American cockroaches carry multidrug resistant bacteria in two hospitals which raises alarm for stricter control measures.

1. Introduction

An extensive diversity of arthropods including insects is known to carry many human pathogens which have increasing public health importance in Iran[1-3]. Some like the cosmopolitan cockroaches (Dictyoptera: Blattaria) are closely integrated with human dwellings and adapted to reproduce within domestic or peridomestic environments[4-6]. About 4 000 species of these are recognized but <1% are synanthropic pests[7]. Cockroaches live in dark, warm, wet and inaccessible filthy places, coming out to search for nutrition and water. As they usually move to food storage places as well as sewerage systems and other facilities mostly associated with discarded waste, they are potential carriers of pathogenic bacteria[8]. Cockroaches are omnivorous and voracious feeders. Due to their special feeding habits, they are often responsible for dispersal of so many pathogens which later will be taken by human ingestion.

Numerous cockroaches, particularly the domestic and peridomestic *Blattella germanica* (*B. germanica*) and *Periplaneta americana* (*P. americana*), respectively, usually infest hospital wards, partly as a result of inefficient slow removal of medical wastes in Iran[10], and partly because such environments offer optimal refuges for their hiding and proliferation. At hospitals, cockroaches could be effective carriers of infectious agents especially to patients in intensive care, neonatal units, care facilities and nursing homes[1,9].

Furthermore, many published documents have globally indicated the association of cockroaches with many infectious agents responsible for nosocomial infections,

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spread of allergens and drug resistant microbes[10-13].

Comparing with the role recognized clinically as pathogenic carriers of various viruses, bacteria, fungi, protozoa and helminthes, the medical importance of cockroaches is far greater. Even though it has been practically difficult to prove the direct role of cockroaches in transmission of pathogens to humans, such insects are crucial in nosocomial infections which carry microorganisms, and their clinical role in the spread of bacteria cannot be neglected[1].

P. americana and *B. germanica* can move stilly at night among hospital sections, patients' places, and food-handling locations, mostly via cracks in walls, and their ability to transport of multidrug resistant infectious agents is a major concern for many health workers. In studies on isolated bacteria from hospital cockroaches, it has been shown that they could act as potential vectors of drug resistant bacteria[14]. They are potential to transmit some food-borne pathogens. They may thus spread some multidrug resistant bacteria at hospitals and food preparation and distribution centers. Antibiotics susceptibility of bacteria associated with hospital cockroaches thus possesses an increasingly significant challenge to health-associated systems.

In an earlier report, some 19 species of bacteria were isolated from American, German and oriental cockroaches that were collected from some hospitals in Tehran while 25 various species of medically-significant bacteria were separated from American and German cockroaches in hospitals of central Tehran[15]. Despite the implication of these cockroaches in the dispersal of pathogenic bacteria to people, no data exists on the abundance and species of bacteria carried by the cockroaches in different hospitals of Shiraz, south of Iran. The major aim of the present investigation was to identify pathogenic bacteria and their antibiotics susceptibility on and in these insects caught from two public education hospitals and a student dormitory in Shiraz.

2. Materials and methods

2.1. Sample areas

Adults *P. americana* and *B. germanica* were collected at three locations: two education hospitals (marked F and Q, for privacy and ethical rules) and a student dormitory (for control) that they lie within 3 500 m of center of Shiraz. At night the cockroaches were collected just by hand catch wearing sterile gloves and using forceps and torches. To show some indication of the relative level of infestation at each location, the same investigators made collections on the basis of time of catching by hand. Each adult insect was put in a separate sterile screw-capped vial. In the lab, each insect was anaesthetized using chloroform solution and examined carefully under a low-power dissecting microscope to identify it about its species level.

2.2. Preparation of bacterial suspensions

For isolation of bacteria, each cockroach was washed in 5 mL sterile physiological saline within vials for 2 min. These suspensions were cultured in trypticase soy broth (Merk, LOT:VM 42595 22) as separate samples. To prevent contamination of alimentary tracts, it is necessary to use 70% ethanol before alimentary tracts extraction. The culture media was incubated at 37 °C for 24 h.

2.3. Bacterial diagnosis

Each incubated sample was then separately cultured on plates of (i) blood agar, (ii) xylose-lysine-desoxycholate agar, and (iii) eosin methylen blue agar. The genus of bacterial growth on the plates were recognized by colonial appearance, Gram staining, oxidative fermentation test, lysine decarboxylase, Simmons' citrate test, urease, lactose fermentation on triple sugar iron agar, the methyl red, Voges-Proskaeur tests, deoxyribonuclease, sulphide and indole production and motility test[16].

2.4. Antibiotics susceptibility tests

Mentioned isolated bacteria are examined by disk agar diffusion test to evaluate their antibiotics susceptibility patterns. Several antibiotics such as: amoxicillin, ampicillin, carbenicillin, cephalothin, ciprofloxacin, gentamicin, imipenem, and ticarcillin for Gram-negative bacteria, and novobiocin, penicillin and vancomycin for Gram-positive bacteria were used. The antibiotics outcomes were subsequently tabulated.

2.5. Data analysis

The frequencies of cockroaches from different collection sites, bacterial flora (externally or internally and Gram-positive or Gram-negative), and their antibiotics susceptibility outcomes were compared applying *t*-tests. The prevalences of carriers of each separated bacterial species were compared between German and American cockroaches in *Chi*-square tests. Statistically significant difference was reported when $P<0.05$.

3. Results

3.1. Cockroach infestation

Totally, 156 adult insects were collected. The two hospitals and a student dormitory were all reported to be infested with *P. americana* and *B. germanica* in different quantity. Hospital Q and the student dormitory each accounted for 40.4% (n=63) of the entire cockroach collection, whereas only 19.2% (n=30) of the collection came from hospital F. The numbers of cockroaches caught at hospital Q and the student dormitory were thus similar ($P>0.05$). Just two kinds of cockroaches were reported that the collected number of *B. germanica* was about nine times as many as *P. americana*. Student dormitory was identified to be infested with the predominant species (*B. germanica*). Of the species collected in the hospitals, 93.6% were caught from non-clinical areas

and 6.4% from clinical areas ($P<0.05$).

3.2. Bacterial isolation

Almost 65% of pathogenic bacteria that isolated from cockroaches were caught from hospital Q and the student dormitory. The prevalence of pathogenic bacteria was similarly high in *B. germanica* (98%). The most frequent species of bacteria (>82%) both internally and externally on American and German cockroaches at all three sampled places in Shiraz was *Pseudomonas*. At all locations, the gastrointestinal tract as well as the external surfaces were maximally (>82%) infected with at least one species of bacteria (Figure 1).

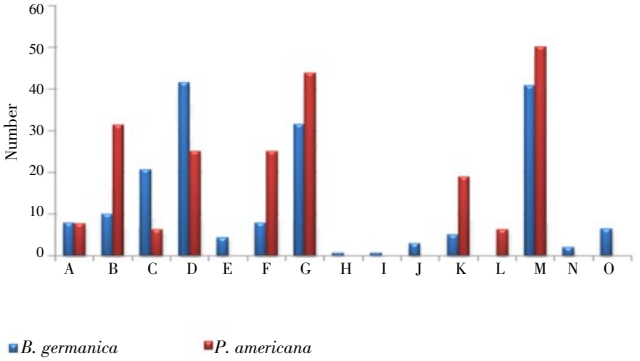

Figure 1. The numbers of bacterial isolates from *P. americana* and *B. germanica* from two public hospitals and a student dormitory in Shiraz, Iran. A: *Acinetobacter*; B: *Bacillus*; C: *Citrobacter*; D: *Enterobacter*; E: *Enterococus*; F: *Escherichia coli* (*E. coli*); G: *Klebsiella*; H: *Moraxella*; I: *Morganella*; J: *Nonenterococus*; K: *Proteus*; L: *Providentia*; M: *Pseudomonas*; N: *Shigella*; O: *Staphylococcus saprophiticus* (*S. saprophiticus*).

3.3. Antibiograms

The antibiogram profiles showed full (100%) resistance of *Klebsiella*, *Citrobacter*, *Acinetobacter* and *Proteus* bacteria to amoxicillin and ampicillin at both hospitals, while *Pseudomonas* showed tolerance (95.7%) to cephalothin. Resistance of isolated bacteria from cockroaches to selected antibiotics at all locations showed that *Pseudomonas*, *Enterobacter* as well as *Klebsiella*, *Citrobacter* and *Proteus* were resistant to cephalothin (95.7%), ampicillin (94.6%), and amoxicillin (100%) and ampicillin (100%), respectively. It showed that *Acinetobacter* had full resistance to cephalothin, amoxicillin and ampicillin. The prevalence of bacterial resistance in studied locations was as follow: at hospital F, full drug resistance of *Pseudomonas aeruginosa* to ampicillin, amoxicillin, and cephalothin was observed. *Pseudomonas* at hospital Q was resistant to ticarcillin (73.3%), ciprofloxacin (67.0%), carbenicillin (67.0%), imipenem (67.0%), ampicillin (67.0%) and cephalothin (80.0%). In this study, *Enterobacter* at hospitals F and Q was fully resistant to amoxicillin and ampicillin. While at student dormitory, it was shown that *Enterobacter* was resistant to ampicillin (84.6%) and amoxicillin (61.5%). *Klebsiella* exhibited full resistance to amoxicillin and ampicillin at hospitals F and Q. *Klebsiella* was resistant to amoxicillin (60%) and ampicillin (100%) at dormitory. At hospital F and student dormitory, *Acinetobacter* demonstrated full resistance to amoxicillin, ampicillin and cephalothin, while at hospital Q, it had full resistance to amoxicillin and ampicillin, but only 50% resistance to cephalothin was discerned. *Enterococus* was fully resistant to penicillin and vancomycin separately at hospital Q and only shown resistance to penicillin at dormitory (Table 2). The most resistant bacteria at hospital F isolated from German and American cockroaches were *Pseudomonas* and *Acinetobacter* which had drug resistance to at least four antibiotics. Similarly, the most resistant bacteria at hospital Q isolated from these cockroaches were *Pseudomonas* (resistant to at least 7 antibiotics) as well as *Acinetobacter* and *Enterococcus* was resistant to a lower number of antibiotics (Table 2).

Table 1

The numbers and percentages (%) of medically important bacteria separated from the external surfaces and alimentary tracts of German and American cockroaches collected at two hospitals and a student dormitory in Shiraz, Iran. *n* (%).

Bacteria	Student dormitory		Hospital Q				Hospital F				Total	*P*
	B. germanica (*n*=63)		*P. americana* (*n*=14)		*B. germanica* (*n*=49)		*P. americana* (*n*=2)		*B. germanica* (*n*=28)			
	AT	ES	AT	ES	AT	ES	AT	ES	AT	ES		
Pseudomonas	33 (52.4)	13 (20.6)	4 (28.6)	3 (6.1)	3 (6.1)	15 (30.6)	1 (50)	1 (50)	2 (7.1)	1 (3.6)	65 (41.7)	0.001
Enterobacter	10 (15.9)	15 (23.8)	0	4 (28.6)	20 (40.8)	12 (24.5)	0	0	2 (7.1)	6 (21.4)	62 (39.7)	0.055
Bacillus	1 (1.6)	3 (4.8)	0	5 (35.7)	0	8 (16.3)	0	1 (50)	1 (3.6)	1 (3.6)	19 (12.2)	0.029
Klebsiella	4 (6.3)	13 (20.6)	2 (14.3)	4 (28.6)	15 (30.6)	9 (18.4)	0	1 (50)	2 (7.1)	6 (21.6)	51 (32.7)	0.171
Citrobacter	7 (11.1)	5 (7.9)	1 (7.1)	1 (7.1)	5 (10.2)	3 (6.1)	0	0	9 (32.1)	3 (10.7)	30 (19.2)	0.021
Shigella	0	1 (1.6)	0	0	0	1 (2)	0	0	1 (3.6)	0	3 (1.9)	0.822
Proteus	1 (1.6)	5 (7.9)	1 (7.1)	1 (7.1)	0	1 (2)	1 (50)	1 (50)	1 (3.6)	0	10 (6.4)	0.766
Acinetobacter	0	2 (3.2)	0	0	2 (4.1)	2 (4.1)	1 (50)	1 (50)	3 (10.7)	2 (7.1)	12 (7.7)	0.015
Providentia	0	0	0	1 (7.1)	0	0	0	0	0	0	1 (0.6)	0.476
Morganella	0	0	0	0	0	0	0	0	1 (3.6)	0	1 (0.6)	0.121
E. coli	0	5 (7.9)	4 (28.6)	0	2 (4.1)	0	0	0	0	4 (14.3)	15 (9.6)	0.711
Enterococcus	3 (4.8)	3 (4.8)	0	0	0	0	0	0	0	0	6 (3.8)	0.010
Moraxella	0	0	0	0	1 (2)	0	0	0	0	0	1 (0.6)	0.476
Non *Enterococcus*	2 (3.2)	0	0	0	0	2 (4.1)	0	0	0	0	4 (2.6)	0.613
S. saprophiticus	4 (6.3)	2 (3.2)	0	0	2 (4.1)	0	0	0	1 (3.6)	0	9 (5.8)	0.254

ES: external surfaces; AT: alimentary tracts.

Table 2
The percentage of antibiotics resistances of isolated bacteria from American and German cockroaches in two hospitals and a school dormitory of Shiraz, Iran.

Antibiotics	Student dormitory (%)								Hospital Q (%)								Hospital F (%)							
Bacteria	Gm	Cf	Ipm	Am	Amx	Cp	Cb	Tic	Gm	Cf	Ipm	Am	Amx	Cp	Cb	Tic	Gm	Cf	Ipm	Am	Amx	Cp	Cb	Tic
Pseudomonas	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	60.0	87.0	67.0	67.0	67.0	80.0	67.0	73.3	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0
Enterobacter	0.0	100.0	0.0	84.6	61.5	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0
Klebsiella	0.0	100.0	0.0	100.0	60.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0
Citrobacter	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0
Acinetobacter	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	50.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0
Enterococcus	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0

Tic: Ticarcillin; Cb: Carbinicillin; Cp: Ciprofloxacin; Amx: Amoxycillin; Am: Ampicillin; Ipm: Imipenem; Cf: Cephalotin; Gm: Gentamicin.

4. Discussion

In the present research, two cockroach species were collected in public hospitals of Shiraz, with *B. germanica* being more common than *P. americana*. German cockroaches are relatively small species among other cockroach species. Reproductive capacity enables a small population of *B. germanica* to change to a major infestation after a short time[17]. The most common bacterium, *Pseudomonas*, was found to be resistant to several antibiotics.

It was found that cockroaches are common at public hospitals and student residences in Shiraz. These insects carry a wide variety of multidrug resistant bacteria both internally and externally which could facilitate their spread to other wards. Based on the results of the present study, German cockroaches were very frequent (89.7%), while the American cockroaches were less abundant (10.3%). Pai *et al.* found that German cockroach was dominant in clinical areas and American cockroach was common in non-clinical areas[14]. Similar studies elsewhere found the highest frequency of German cockroach at hospitals[1,18,19]. These show the breeding potential of the German cockroach, and effects of environmental factors and the use of pesticides causing drug resistance which subsequently increases their prevalence in the tested environments. But in general, German and American cockroaches are two predominant species in the hospital environments. Indeed, cockroaches captured at dormitory, hospitals or other places habitually contain a very large number of micro-organisms[20,21].

The results indicated that the prevalence of infection at hospital F was respectively related to *Citrobacter*, *Klebsiella*, *Enterobacter*, *Escherichia coli* and *Pseudomonas*. While the most prevalent bacteria at burn hospital Q were respectively related to *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Bacillus* and *Citrobacter*. According to previous studies, the most prevalent bacteria at burn centers were *Pseudomonas*. At burn hospital Q, the most prevalent bacteria were, however, *Enterobacter* and *Klebsiella* which are were also part of the human intestinal normal flora.

Fakoorziba *et al.* found that at Tehran hospitals *Klebsiella* were the most common bacterial species[1]. Among the most common bacteria isolated from German cockroaches, *Pseudomonas*, *Serratia* and *Enterococcus* accounted for a high prevalence at hospitals. These results showed that these insects have the sustenance capability of *Salmonella* bacteria in their digestive tracts. These results revealed that the role of these insects in environmental pollution might endanger human health[15,22].

Generally there was no significant difference between the bacteria on the outer surface and the digestive system of cockroaches ($P>0.05$). The outer surface and digestive system had the same species of bacteria in cockroaches, which was consistent with other studies[14,19]. High prevalence of *Enterobacter* and *Klebsiella* in nosocomial infections can be attributed to fecal contamination. Bacteria carried by cockroaches in the kitchen, home and garden of hospitals is a direct reason for transferring nosocomial infections.

Drug resistance of *Pseudomonas aeruginosa* separated from these species suggests in a way that there are different patterns of drug resistance at the two hospitals. Drug resistance to antibiotics such as carbinicillin, ticarcillin, ciprofloxacin and imipenem at hospital Q was demonstrated. It means the isolation of *Pseudomonas* with multiple resistances to antibiotics. But at the public hospital F in the city, *Pseudomonas* resistance to selective drugs has not been discerned. This could be due to long-term treatment of drug-resistant cases at burn hospital. The slum dwelling conditions of hospital Q would definitely exacerbate the disease treatment cases. The transmision of multidrug-resistant bacteria at burn hospital may be done by cockroaches[23,24]. However, drug resistance to other species of bacteria was not different at the other two hospitals and student residences.

According to the results of this and other previous studies, the primary role of cockroaches in carrying nosocomial pathogens and mechanical transmission of drug-resistant bacteria is ascertained. It is recommended that local health workers, including doctors and skin burn specialists involved in the prescription of antibiotics at hospitals, should take great precautions. It is very important to pay attention to such issue so as to prevent the development of drug resistance in patients with prolonged course of recovery.

Conflict of interest statement

We declare that we have no conflict of interest.

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