Original Article

Comparing the Bacterial Flora of Insectary and Filed Strains of *Supella longipalpa* (Blattaria: Ectobiidae) and their Antibiotic Resistant Pattern in Qom Province, Central Iran

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Abstract

Background: Cockroaches play a role in the mechanical transmission of microbial pathogens. This study was designed to determine the isolated bacteria and their susceptibility to conventional antibiotics from brown-banded cockroach, *Supella longipalpa*, in Qom province, central Iran.

Methods: In this cross-sectional study, brown-banded cockroaches were bred in an insectary and caught from hospitals of Qom. The samples were taken from the surface and alimentary canal and then cultured on microbial culture media; next, grown specimens were identified using differential culture media. Finally, after performing diagnostic tests and identifying the bacteria species, their susceptibility to various antibiotics was evaluated.

Results: A total of 120 adult cockroaches from the insectary and hospitals were included in the study. Ten bacterial genera were found; nine were Gram-negative and one was Gram-positive. The genus *Klebsiella*, *Enterobacter*, *Staphylococcus*, *Citrobacter* and *Hafnia* were isolated only from the hospital strain. On the other hand, *Pseudomonas* and *Escherichia* from both groups and other species such as *Salmonella*, *Proteus* and *Shigella* were isolated only from laboratory strains. The highest antibiotic resistance among Gram-positive cocci and Gram-negative bacilli were 100.0% and 98.1% for ampicillin and Ceftazidime, respectively.

Conclusion: The results of this study showed that various pathogenic bacteria harbored by brown-banded cockroaches. Moreover, it was found that most of these bacteria belong to the *Enterobacteriaceae* family, which can be pathogenic and thus threaten human health.

Keywords: Brown-Banded cockroaches; Microbiome; Alimentary canal; Body surface; Pathogens

Introduction

Cockroaches are the most common and ubiquitous health pests in many houses and buildings, whose widespread in human places has a long history that causing great dissatisfaction due to dirty habits and unpleasant odors (1–5). Brown-banded cockroaches, *Supella longipalpa* (Fabricius 1798) (Blattaria: Ectobiidae), are the smallest adult cockroaches, 10 to 14 mm in length, that live in dry areas of houses, and they are capable of transmitting various bacteria that may act as antibiotic-resistant pathogens (6). These cockroaches are found in houses, apart-

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ments, hotels, and hospitals. They often live inside homes' furniture, and they are transported to another building or house through these routes. In buildings, they are usually found inside elevators, false ceilings, behind the snatch, near the refrigerator's motor, freezers, and other appliances (7).

Bacterial agents can be detected in different internal and external organs of cockroaches, and they can play a role as carriers or vectors of these agents to humans and other animals (8). Therefore, contact with the external surface of cockroaches or the defecation of these insects on food, utensils, and hospital equipment pose human with infection. The importance of cockroaches may be much greater than what is generally recognized (9). Thus, the presence of cockroaches in human dwellings is a prominent public health issue and more importantly in sensitive environments like hospitals. Some bacterial genera such as *Salmonella, Shigella, Staphylococcus* and *Streptococcus* cause typhoid fever, dysentery, arthritis, pimples and abscesses, intestinal diseases, etc (10). Also, *Pseudomonas, Proteus, Serratia* and *Escherichia coli* lead to urinary and respiratory infections that can be transmitted to humans through cockroaches. Other important bacteria causing plague or leprosy have been isolated from cockroaches (11). One group of bacteria that grows in simple environments belongs to the family Enterobacteriaceae, which is found in nature on plants and animals (9). These bacteria can lead to urinary tract infections, infectious poisoning, inflammation of the stomach or intestines, jaundice and infections of the abdominal cavity, pneumonia or wound infections (12,13). In the last decade, over-prescription or incorrect prescription of antibiotics has led to the emergence of antibiotic resistance to a significant extent (14,15). Microbial antibiotic resistance is a growing public health problem that complicates and increases the cost of medical treatment to $ 30 billion per year (16). The mean bacterial contamination of cockroaches was calculated to be 67.9% (17). Insect evolution along with some bacterial endosymbionts has been known as a crucial and interactive relation in the successful survival of both bacteria and insects known as symbiosis. They are endosymbiotic bacteria almost in all cockroaches having a role in recycling nitrogen from urea and ammonia to produce amino acids (18).

The presence of symbiotic bacteria in the digestive truck of insects has increased their survival. For example, it has been proved that the presence of *Wolbachia* bacteria in flies has provided protection against some viral infections (19,20). Some insect gut microbes have the ability to secondary detoxify metabolism, (21) and protect the host by providing immunity against pathogens (22–24). In addition to the importance of studying pathogenic microorganisms in identifying the potential of cockroaches in their transmission, the identification of symbiotic bacteria (endosymbionts) in order to use new control methods such as paratransgenesis is of great importance (10, 24–26).

The aim of this study was to determine the bacterial flora and antibiotic resistance pattern of bacteria isolated from the surface and gastrointestinal tract of brown-band cockroaches, *S. longipalpa*, in laboratory and field conditions.

Materials and Methods

Collecting and rearing of cockroaches

The field cockroaches were captured from three hospitals in the city of Qom, Iran using procedures described previously (27). Briefly, the cockroaches were hand catch by means of a piece of radiology film (10×10 cm), and then they were transferred to a jar with two separable parts. To prevent escapes, the upper inner surface part was lightly greased using petroleum jelly, and then by the end of collecting procedure, the upper part was separated from the lower part. Collected cockroaches were transferred to the entomology laboratory of Qom University of Medical Sciences. The insectary specimens were taken from the cockroach insectarium from School of Public Health, Teh-

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ran University of Medical Sciences. In insectary, the collected cockroaches were transferred to the rearing jars (24×11 cm) and kept at the temperature of 22±3 °C, relative humidity of 55±10%, and a 12 h light–dark period. The cockroaches were offered food (bread, dates) and water (cotton-plugged water vials). Samples were collected from second generation.

Dissecting cockroaches and sampling

To prevent mixed contamination with each other, cockroaches were kept individually in each container. Before the procedure all surface and instrument of dissecting were disinfected with 70% ethanol. To immobilize, the cockroaches were placed on sterile plates and kept in the freezer for 10–15min, and then they were identified using a morphological key (1). Two samples were taken from each cockroach, the first sample was taken by pulling a sterile cotton swab on dorsal, ventral and plural surfaces of each cockroach, and the second from the alimentary canal following body dissection. On average two microbe isolates were taken from each cockroach. The cockroaches were fixed on solid paraffin using needles, and then by using a scalpel under the binocular loop, their alimentary canal was removed, washed in physiological serum, and then transferred to sterile saline to prepare a homogenized suspension. Prepared samples from the swaps and suspension were cultured on the various mediums.

Bacterial identification

First, the samples were cultured on Blood Agar and MacConkey Agar, and then incubated at 37 °C for 24–48 h under standard conditions (28). Grown colonies of bacteria were evaluated by Gram staining (29). Then, according to the type of Gram-positive or -negative bacteria and existing guidelines, they were evaluated and confirmed using other media and standard biochemical tests including catalase, oxidase, Mannitol Salt Agar, Dnase, Triple Sugar Iron Agar [TSI], Lysine Iron Agar [LIA], Bile Esculin Agar, Sulfide Indole Motility [SIM], Methyl Red [MR]/Voges-Proskauer [VP], citrate, urease, Oxidation/ fermentation [OF], novobiocin susceptibility test [NST], Simmons Citrate Agar [SCA], optochin, Campylobacter (CAMP) and *Salmonella Shigella* Agar. All media were acquired from Merck, Germany. The slide-agglutination technique by commercial antisera (Bahar Afshan Co., Iran) was also done for serogrouping of strains identified as *Salmonella* and *Shigella* species.

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing against isolated bacteria from cockroaches was conducted according to the Kirby-Bauer's disk diffusion based on the Clinical and Laboratory Standards Institute (CLSI) recommendation (30). Based on this guideline, AST was performed on Muller Hinton Agar using antibiotic discs (Padtan Teb, Co., Iran) containing ampicillin (AM, 10 μg), gentamicin (GM, 10 μg), ciprofloxacin (CP, 5 μg), chloramphenicol (C, 30 μg), tetracycline (TE, 30 μg), trimethoprim/ sulfamethoxazole (SXT, 1.25/23.75 μg), streptomycin (S, 30 μg), ofloxacin (OFL, 30 μg), and ceftazidime (CAZ, 30 μg).

Results

Sixty brown-banded cockroaches bred in insectary and 60 cockroaches caught from hospitals of Qom were included in the study. Ten bacterial genera were found in the alimentary canal of brown-banded cockroaches, nine of which were Gram-negative and one Gram-positive. The results showed that *Klebsiella, Enterobacter*, *Staphylococcus*, *Citrobacter* and *Hafnia* from hospitals and *Pseudomonas*, *Escherichia* from both strains and other genera such as *Salmonella*, *Proteus* and *Shigella* were isolated from laboratory strain (Table 1).

The pattern of antibiotic resistance for Gram-negative bacilli and Gram-positive cocci was determined for nine antibiotics. The highest resistance for Gram-negative bacilli was related to Ceftazidime (98.1%), while for Gram-positive cocci it was determined to ampicillin (100.0 %). More results are summarized in Table 2.

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Table 1. Frequency distribution of bacteria isolated from brown-banded cockroaches using conventional Gram staining and biochemical analysis collected from Qom, 2019

Isolates	Form	Gram Staining	Filed origin		Insectary origin		Total
			Alimentary Tract No. (%)	External Surface No. (%)	Alimentary Tract No. (%)	External Surface No. (%)	
Escherichia coli	Bacillus	Negative	19 (32.2)	14 (23.7)	14 (23.7)	12 (20.3)	59 (24.5)
Pseudomonas aeruginosa	Bacillus	Negative	9 (20.0)	15 (33.3)	8 (17.8)	13 (28.9)	45 (18.7)
***Salmonella* serogroup D**	Bacillus	Negative	0 (0.0)	0 (0.0)	11 (73.3)	4 (26.7)	15 (6.2)
***Salmonella* serogroup A**	Bacillus	Negative	0 (0.0)	0 (0.0)	3 (60.0)	2 (40.0)	5 (2.1)
Shigella sonnei	Bacillus	Negative	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (0.8)
Shigella dysenteriae	Bacillus	Negative	0 (0.0)	0 (0.0)	9 (60.0)	6 (40.0)	15 (6.2)
Proteus vulgaris	Bacillus	Negative	0 (0.0)	0 (0.0)	3 (60.0)	2 (40.0)	5 (2.1)
Proteus mirabilis	Bacillus	Negative	0 (0.0)	0 (0.0)	5 (50.0)	5 (50.0)	10 (4.1)
Klebsiella pneumoniae	Bacillus	Negative	14 (63.6)	8 (36.4)	0 (0.0)	0 (0.0)	22 (9.1)
Klebsiella oxytoca	Bacillus	Negative	3 (75)	1 (25.0)	0 (0.0)	0 (0.0)	4 (1.7)
Enterobacter agglomerans	Bacillus	Negative	4 (80.0)	1 (20.0)	0 (0.0)	0 (0.0)	5 (2.1)
Staphylococcus aureus	Coccus	Positive	4 (57.1)	3 (42.9)	0 (0.0)	0 (0.0)	7 (2.9)
Non-pathogenic staphylococci	Coccus	Negative	6 (25.0)	18 (75.0)	0 (0.0)	0 (0.0)	24 (10.0)
Citrobacter freundii	Bacillus	Negative	9 (52.9)	8 (47.1)	0 (0.0)	0 (0.0)	17 (7.1)
Citrobacter disversus	Bacillus	Negative	2 (25.0)	2 (25.0)	3 (37.5)	1 (12.5)	8 (3.3)
Hafnia alvei	Bacillus	Negative	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	3 (1.2)
Total			72 (29.3)	71 (28.9)	58 (23.6)	45 (18.3)	246 (100)

Table 2. Antibiotic assay test results of the bacteria isolated from brown-banded cockroaches collected in Qom, Iran, 2019. Antimicrobial susceptibility tests against the isolated bacteria from the cockroaches was conducted according to the Kirby-Bauer's disk diffusion based on the Clinical and Laboratory Standards Institute (CLSI) recommendation

Antibiotics	Gram-negative bacilli (%)		Gram-positive cocci (%)	
	Resistance	Susceptibility	Resistance	Susceptibility
Tetracycline	150 (69.8)	65 (30.2)	22 (71.0)	9 (29.0)
Ofloxacin	96 (44.7)	119 (55.3)	16 (51.6)	15 (48.4)
Ampicillin	175 (81.4)	40 (18.6)	31 (100.0)	0 (0.0)
Gentamicin	98 (45.6)	117 (54.4)	12 (38.7)	19 (61.3)
Ciprofloxacin	55 (25.6)	160 (74.4)	6 (19.4)	25 (80.6)
Chloramphenicol	94 (43.7)	121 (56.3)	0 (0.0)	31 (100.0)
Trimethoprim/sulfamethoxazole	121 (56.3)	94 (43.7)	19 (61.3)	12 (38.7)
Streptomycin	160 (74.4)	55 (25.6)	-	-
Ceftazidime	211 (98.1)	4 (1.9)	-	-

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Discussion

In this research, in general, ten genera of bacteria were isolated from 120 individual brown-banded cockroaches. It should be pointed out that these isolates are medically important. As the results showed, the most frequent isolate was *Escherichia coli*, of which 59 isolates were isolated from both hospital (n=33) and insectary (n=26) samples. In a similar study conducted in the west of Iran, same to our study, *E. coli* was the most predominant isolate in both surface and alimentary canal of *Blattella germanica* (31) and *Periplaneta americana* cockroaches (10). A study found that 74.5% of the American cockroaches collected from a hospital were infected with eight types of bacteria. The most common bacterium observed was *E. coli*, same to our study, followed by *Proteus* spp (32). Another study has shown that both *B. germanica* and *P. americana* species of cockroaches carried medically important bacteria, with *Klebsiella* being the most frequently isolated genus similar to our findings (33).

It should be noted that in two individual specimens the number of isolated bacteria from the alimentary tract was more than the external surface. The second most frequent isolate was *Pseudomonas aeruginosa* which was detected in both insectary and hospitals origins. As it is presented in table 1, some isolates such as *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Citrobacter freundii* were detected only in hospital specimens. On the other hand, some other isolates like *Shigella dysenteriae* and *Proteus mirabilis* were isolated from insectary specimens. Generally, in both sample groups, it can be claimed that more bacteria were isolated from the alimentary tract than the external surface.

In accordance with the present research, in a similar study, bacteria were isolated from the alimentary tract and external surface of *S. longipalpa*; the researchers reported that *K. pneumonia* and *E. coli* were the two most frequent bacteria (34). It should be pointed out that food is one of the major ways of carrying pathogens that infect humans, and food-borne diseases are a global serious health issue (35). It is worth to mention, in the current study food preparing for insectary strain was performed under insectary condition, thus transmission of same isolates may occur through this route. Cockroaches are apparently considered as potential mechanical carriers for food-borne germs due to their filthy behaviors, and their cuticle is able to carry pathogens (36). Microorganisms can even be ingested and then excreted or regurgitated by cockroaches (37). A wide range of bacteria such as *Pseudomonas aeruginosa, Klebsiella oxytoca, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Escherichia coli* has been reported as the causative agent of food-borne diseases in several studies (38–40).

In the present study, *E. coli* and *P. aeruginosa* were the most predominant species, which have been reported to be food-borne pathogens. For instance, Tachbele et al. (33), who studied cockroach-associated food-borne bacterial pathogens from a few hospitals and restaurants in Addis Ababa, Ethiopia, reported that *B. germanica* was the most detected cockroach in both the hospitals and restaurants, and the bacterial isolates were *Salmonella* spp, *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*; the researchers expressed that *B. germanica* was able to ingest *Salmonella* contaminated food and excrete viable bacteria in its feces. Therefore, it can be explained that the bacteria isolated from the two samples in the current research can be considered as food-borne pathogens in the different wards of the hospitals. Similarly, a previous study conducted by our team showed *E. coli* and *P. aeruginosa* were the most prevalent bacterial isolates detected in houseflies (*Musca domestica*) (41).

In our study, the highest resistance for Gram-negative bacilli was observed for Ceftazidime, while a previous study reported resistance to cephalothin, ampicillin, cefotaxime, and kanamycin antibiotics in *P. americana*, *B. germanica*, and *Blatta orientalis* (42).

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Naturally, as Table 1 shows, cockroaches in hospitals are more prone to result in food-borne diseases due to carrying more pathogenic bacteria compared to insectary strains. Furthermore, cockroaches have been known as carriers of nosocomial infections in hospitals and health centers (36,43). Among the bacteria isolated in the present study, *S. aureus*, *E. coli*, and *P. aeruginosa* can cause nosocomial infection more than others (44). Nevertheless, nosocomial infections are highly spread by cockroaches, there is no direct relationship between the eradication of cockroaches and lower infection cases (43). Antibiotic resistance is a very serious health problem across the globe, and it is estimated that annually 700,000 deaths happen due to infections caused by bacterial antimicrobial-resistant (45,46). It has been documented that cockroaches can harbor antibiotic-resistance bacteria (47, 48). The results obtained in the current study depicted that detected isolates were resistant to several antibiotics. Our results accord with those reported in a similar study (34). Also, in another study, it was determined that 38 and 6 species of Gram-negative and -positive bacteria, respectively, which were resistant to different antibiotics, were isolated from *B. germanica* (49).

In this study, the hospital specimens were kept in insectary for two generation, presumably the microbiome of the specimens would be changed after two generations in insectary, and they could not represent the complete flora of the hospital specimens. Although it was a limitation of this study, however, still there were a significant variation between the hospitals and insectary specimens' microflora.

Conclusion

This study showed that brown-banded cockroaches either collected in the hospital or insectary carried important pathogenic bacteria, which mostly were high antibiotic-resistant against conventional antibiotics. Thus, this insect species could take a role in the expansion of antibiotic-resistant bacteria through colonies of pathogenic bacteria, especially in susceptible environments like hospitals. Therefore, employing control measures for cockroach populations including brown-banded cockroaches in hospitals, not only decline the chance of transmitting pathogens directly or through food or other bridge carriers to the admitted patient or hospital personnel, but also confine the expansion of antibiotic-resistant bacteria through their bodies.

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Ethical considerations

The Ethical Committee of the Faculty of Medicine of Iran University of Medical Sciences has approved the procedures required for the study. Code No: IR.MUQ.REC.1398.029.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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