Original Article

Bacterial Contamination of Collected Cockroaches and Determination Their Antibiotic Susceptibility in Khorramabad City, Iran

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Abstract

Background: Cockroaches are one of the most important carriers of pathogenic microorganisms. Therefore, the presence of cockroaches in public places, especially in hospitals, homes, and restaurants, is dangerous, and threatens the health of society, people, and the environment. The aim of this study was evaluation of bacterial contamination of cockroaches and the sensitivity of these bacteria to various antibiotics, captured from Khorramabad City, Iran

Methods: This descriptive cross-sectional study was performed on 150 cockroaches collected from hospital environments, homes, and restaurants in Khorramabad. The outer surface of the cockroaches was washed with physiological saline. The suspension was centrifuged for 5 minutes at 2000rpm. Isolation and identification of bacteria was performed using phenotypic methods. Antibiotic susceptibility testing was performed by disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guideline.

Results: A total of 100 American cockroaches (66.66%), 28 *B. germanica* (18.66%) and 22 *Blatta orientalis* (14.66%) were identified. In total, 97.33% of the collected cockroaches were infected with bacteria. The most bacterial infection of the cockroaches was *Escherichia coli*, coagulase-negative Staphylococci and *Bacillus* respectively. The overall results of the antibiogram test showed that the identified bacteria were resistant to cephalothin, ampicillin, cefotaxime, and kanamycin antibiotics, semi-sensitive to ciprofloxacin and sensitive to tetracycline, gentamicin, nitrofurantoin, Trimethoprim/sulfamethoxazole, and Chloramphenicol.

Conclusion: Infection of cockroaches with pathogenic bacterial agents in hospital, residential, and restaurant environments, as well as the observation of bacterial resistance to some common antibiotics is worrying.

Keywords: Cockroaches; Bacteria; Antibiotic

Introduction

Insects are one of the most populous classes of arthropods, which are important in terms of economic, health, and medical (1). Cockroaches belong to the order Blattaria that so far about 4,000 species of cockroaches have been identified, less than 1% of which are considered pests. Cockroaches live in warehouses, bakeries, baths, hospitals, and even ships and harbors, hiding in dark, narrow crevices and crevices during the day and leaving their shelters at night to feed (2). Today, perhaps few places can be found that are safe from the presence of cockroaches, and therefore increases their potential as a harmful health factor (3).

This insect is important in medicine due to its habit of vomit (puking) a portion of the food eaten, defecating in the living environment, having weak bites, especially between the fingers, and producing a foul odor, particularly in the American cockroach, which is potentially dangerous to human health. Also, secretions and scabs from changing their skin contain aller-

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gens that dermatitis, itching, and many acute respiratory diseases are their complications (4, 5). Laboratory studies by using serological methods on cockroach secretions and fecal particles have isolated and identified several allergens from them. Cockroaches are the second important cause of asthma after dust allergies (6, 7). The ability of cockroaches to quickly run and move increase their ability to escape the control and transmission methods of pathogens (8).

Cockroach feces contains compounds such as xanthurenic acid and quinolinic acid, all of which are derivatives of tryptophan and have mutagenic and carcinogenic properties (9). The importance of cockroaches in the spread of nosocomial bacteria and the incidence of nosocomial infections in patients is greater than previously thought. Cockroaches are naturally infected with the pathogenic bacteria that cause leprosy, plague, bloody diarrhea, urinary tract infections, pimples, abscesses, and food poisoning. Cockroaches are infected with approximately 150 species of bacteria, 60 species of yeast, 90 species of protozoa, 45 species of ringworm, 12 species of pathogen fungi, and several hookworms and whipworms. The most important and prominent carriers of these bacteria are *Blattella germanica*, *Periplaneta americana*, and *Blatta orientalis* (2).

In Iran twelve species of this family have already been recognized. In terms of the number of species, the family Ectobiidae (Blattellidae) is the largest order of cockroaches in the world and eight species of this family have been reported from the country. The German roach, *B. germanica*, the major pest of residential dwellings, belongs to family Blattellidae as well (10).

Cockroaches are omnivores and due to feeding on a variety of foods and even human waste, they can spread diseases such as typhoid, leprosy, dysentery, etc. by transmitting large numbers of harmful microorganisms. These microorganisms live for a long time in the intestines of cockroaches and are eventually transferred to another organism (11-13). One of the most important challenges that the world is facing in the fight against bacteria is their increasing antibiotic resistance. The resistance of microorganisms to antibiotics is coded by different genes that are also capable of horizontal transfer between bacteria. Cockroaches can mechanically transfer antibiotic-resistant bacteria from hospital environments to non-hospital areas and vice versa, causing serious problems for public health (14).

Due to the abundance of cockroaches and their role in the mechanical transmission of various pathogens, and insufficient knowledge of the level of bacterial contamination on their external surface and prevention of transmission of various infections, and raising the level of public health, this research aimed to evaluate of bacterial contamination of cockroaches and the sensitivity of these bacteria to various antibiotics, captured from Khorramabad City, Iran.

Materials and Methods

Sampling

This is an analytical descriptive cross-sectional study. The study was conducted from March 2019 to March 2020 in Khoramabad, Iran. The study sites included two hospitals: Shohadaye Ashayer and Shahid Madani; and public places (including homes, and restaurants). In this study, 150 cockroaches were captured by hand catch method. After observing the cockroaches, they were transported to the entomology laboratory of Hamadan University of Medical Sciences using sterile gloves. For preventing (cockroaches) mixing contamination with each other, only one cockroach was used in each container. Then each sample was placed at 4 °C for 5 minutes for anesthesia. Species of cockroaches were determined according to the identification key (14, 15).

Bacteria Isolation

Firstly, in the laboratory, the outer surface of the cockroaches was washed with 5mL ster-

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ile normal saline. Then the solution was centrifuged for 5 minutes at 2000g. The sediments that remained at the bottom of the centrifuge tubes were cultured on the various mediums such as Eosin Methylene Blue (EMB) and blood agar (Merck, Germany) (16, 17).

Cultivated mediums were incubated in 37 ^{o}C for 24 hours. In the next step, for each grown colony, all phenotypic differential tests were performed to isolate the bacteria into the genus or species level. For identification of Gram-negative bacteria Gram stain, fermentation of sugars, motility test, Triple Sugar Iron (TSI) (Merck), Sulfide Indole Motility (SIM) (Merck, Germany), and Simmon citrate (Merck) tests were applied. For the identification of Gram-positive bacteria, Gram stain, catalase test, oxidase test, fermentation of sugars (mannitol), and sensitivity to antibiotics were employed (18).

Antibiogram

The resistance of bacterial strains isolated from the cockroaches was investigated using the Kirby-Bauer disk diffusion method (16, 19). A control strain of *Pseudomonas aeruginosa* ATCC 27853 was used for quality control of susceptibility testing.

From the tested organisms, a suspension with turbidity equal to 0.5 McFarland standards was provided for each bacterial strain and transferred to Muller-Hinton agar medium (Merck), and then antibiotic discs including: ampicillin (30µg), tetracycline (30µg), kanamycin (30µg), chloramphenicol (30µg), trimethoprim-sulfamethoxazole (23.75–1.25µg), gentamicin (30µg), ciprofloxacin (5µg), nitrofurantoin (300µg), cephalothin (30µg) and cefotaxime (30µg) that all antibiotic discs were purchased from Mast Group Ltd., (England) and incubated for 24 hours at 37 °C. After 24 hours, the diameter of the growth inhibition zone was measured. The diameter of the growth inhibition zone of the microorganisms for each antibiotic was divided into sensitive, semi-sensitive, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (20). Finally, data were analyzed using SPSS version 20 software package developed by IBM cooperation, using chi-square tests. $P< 0.05$ was regarded as significant. The amount of bacterial contamination as well as the pattern of their resistance to antibiotics is checked with the type and location of collection of cockroaches. The relationship between the place of isolation of cockroaches (home, hospital, and restaurant) with the Frequency of cockroaches and also the dominant species was determined.

Results

The Captured cockroaches and Extracted Bacterial Strains

100, 28, and 22 cockroaches were caught from hospitals, homes, and restaurants, respectively. A total of 146 bacterial colonies or strains were isolated from the 150 caught cockroaches. Table 1 shows the Frequency of bacteria in each location.

Out of 150 cockroaches, 100 (66.6%) were *P. americana,* 28 (18.6%) *B. germanica*, and 22 (14.66%) *Blatta orientalis*. In total, 97.33% of the collected cockroaches were infected with bacteria. The highest bacterial infection of cockroaches was *E. coli*, and then coagulase-negative Staphylococci and *Bacillus* species were the most common bacterial infections. Statistical analysis showed that there is a statistically significant relationship between different places of cockroach collection and the rate of bacterial infection ($p< 0.05$).

The results of investigating the relationship between bacterial isolates and cockroaches are shown in Figure 1. Out of 33 *E. coli*, 25 strains were isolated from the *P. americana*, and 15 strains out of 19 *Enterococcus* spp. were isolated from the *P. americana,* which was statistically significant ($p <0.05$). The distribution of other bacteria in cockroaches was uniform.

Sensitivity of Bacteria to Antibiotics

According to the results, it was found that the bacteria isolated from the hospital had a

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higher resistance to antibiotics than the bacteria isolated from homes and restaurants (table 2), which is a statistically significant difference (P< 0.05).

Table 1. Frequency of bacteria isolated from three cockroach species (*P. americana*, *B. germaniaca*, *B. orientalis*) in different parts of Khorramabad, 2019-2020

Bacteria	Collection place			Total
	Hospital No (%) *	Home No (%)	Restaurant No (%)	
Escherichia coli	21 (63.6)	9 (27.3)	3 (9.1)	33 (100)
Bacillus spp.	8 (34.8)	4 (17.4)	11 (47.8)	23 (100)
Klebsiella spp.	8 (80)	1 (10)	1 (10)	10 (100)
Enterococcus spp.	16 (84.2)	2 (10.5)	1 (5.2)	19 (100)
Acinetobacter spp.	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)
Coagulase negative Staphylococci	21 (67.7)	7 (22.6)	3 (9.7)	31 (100)
Proteus spp.	7 (87.5)	1 (12.5)	0 (0.0)	8 (100)
Enterobacter aerogenes	9 (100)	0 (0.0)	0 (0.0)	9 (100)
Pseudomonas aeruginosa	7 (70.0)	2 (20.0)	1 (10.0)	10 (100)
Total	98 (67.1)	27 (18.5)	21 (14.4)	146 (100)

*(p< 0.05)

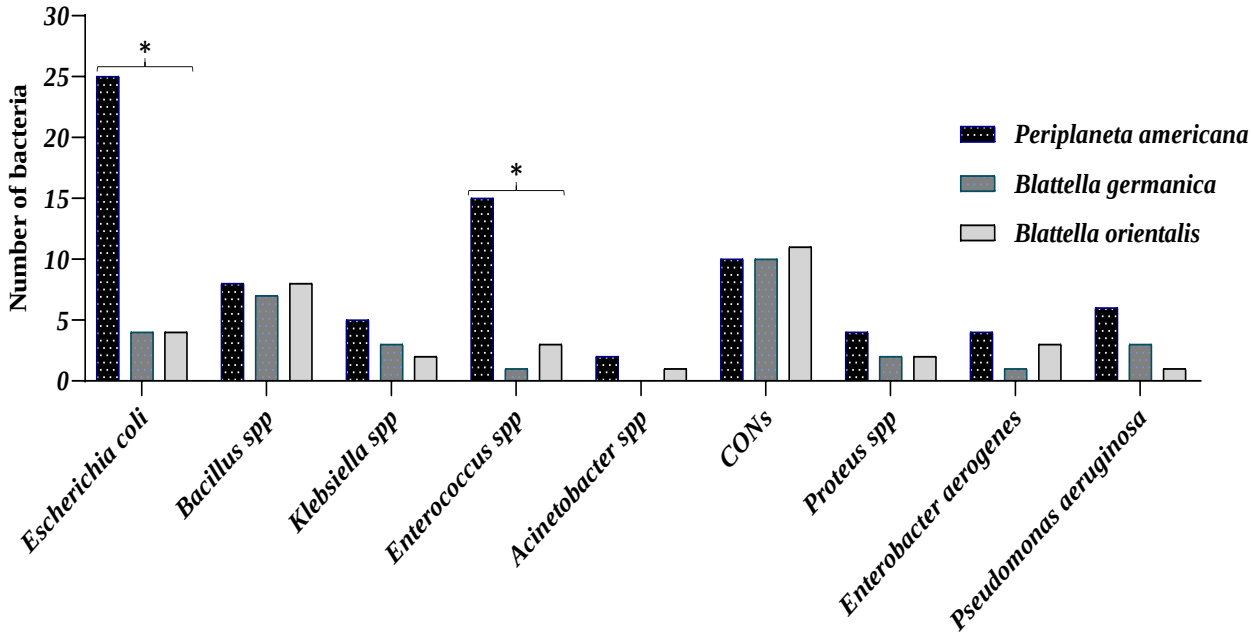

Fig. 1. Relationship between bacterial isolates and cockroach species, *(p< 0.05)

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Table 2. Antibiotic resistance pattern of bacteria isolated from cockroaches; the number of sensitive, resistant, and intermediate resistant bacteria to different antibiotics are listed as numbers for each bacterium

Antibiotics	Response	Bacteria									Total
		E. coli	*Bacillus*	*Klebsiella*	*Enterobacter* spp	*Acinetobacter* spp	*Cons*[1]	*Proteus spp*	*S. aureus*	*Pseudomonas* spp	
Gentamicin	S	25	16	7	15	2	25	6	7	9	112*
	I	7	6	2	3	1	3	2	2	1	27
	R	1	1	1	1	0	2	0	0	0	6
Kanamycin	S	7	3	5	2	0	6	4	5	3	35
	I	16	13	3	13	2	14	2	1	5	69
	R	11	7	2	4	1	10	2	3	2	42
Cefotaxime	S	2	3	0	1	0	3	1	0	1	11
	I	2	2	1	1	2	2	0	0	1	11
	R	29	18	9	17	1	25	7	9	8	123*
Cephalothin	S	3	2	2	0	3	4	2	3	1	20
	I	1	2	4	2	0	3	0	0	1	13
	R	29	19	4	17	0	23	6	6	8	112*
Ampicillin	S	2	2	1	2	2	6	2	2	2	21
	I	6	3	2	4	1	4	2	1	1	24
	R	25	18	7	13	0	20	4	6	7	100*
Tetracycline	S	27	14	9	13	1	27	5	8	8	112*
	I	4	7	0	3	0	3	3	1	1	22
	R	2	2	1	3	2	0	0	0	1	11
Chloramphenicol	S	20	7	3	9	1	19	3	2	6	70
	I	11	9	3	3	1	6	3	4	2	42
	R	2	5	4	7	1	5	2	3	2	31
Ciprofloxacin	S	7	9	8	9	1	8	4	3	0	49
	I	14	7	2	5	1	14	2	2	9	56
	R	12	5	0	5	1	8	2	4	1	38
Nitrofurantoin	S	10	6	9	7	0	6	4	5	2	49
	I	10	10	0	6	1	16	1	1	2	47
	R	13	7	1	6	2	8	3	3	6	49
Cotrimoxazole	S	17	10	4	8	1	15	5	1	7	68
	I	4	2	2	3	1	2	3	2	2	21
	R	12	11	4	8	1	13	0	6	1	56

1. Coagulase-negative Staphylococci
S: susceptible, I: intermediate, R: resistant, *($p< 0.05$)

Discussion

In the present study, we identified three species of *P. americana*, *B. germanica* and *B. orientalis* that the most common of them were American cockroaches, which included 100

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cockroaches (67%), followed by German cockroaches 28 (19.4%) and *B. orientalis* 22(14/8%).

In a study conducted by Zarchi et al. in Tehran hospitals, they showed that 65.6% of cockroaches were American, 12.1% German, and 22.3% Oriental, which is statistically consistent with the number of American cockroaches in the present study (21). Also in other similar researches in the provinces of Iran (Zanjan, Mazandaran, and Esfahan), *P. americana* and German cockroaches were identified as two dominant species (22, 23). The results of Pai et al. studies showed that in clinical settings (environment) most cockroaches are German type and in non-clinical places, most cockroaches are American type but this difference was not statistically significant (24).

The most common in hospital samples were coagulase-negative Staphylococci and *E. coli*. The most bacteria found in the house samples were *E. coli* and coagulase-negative Staphylococci. *Bacillus* spp. was the most common bacterium found in restaurant samples. This study showed that the prevalence of bacterial infection in cockroaches caught in hospitals, houses, and restaurants in Khorramabad were 97.33%.

As in some reports, the role and importance of cockroaches as carriers of bacteria, fungi, and pathogens in humans has been confirmed (25). This study also showed that cockroaches can transmit a variety of bacteria. In the study of Chitsazi et al. in Mashhad during the years 2010–2011, the most isolated bacteria were related to *Enterococcus* from cockroaches in Mashhad hospital, and the most frequent of cockroaches caught from student dormitories were related to *Enterobacter aerogenes* and most abundant of isolated cockroaches from houses are related to *Klebsiella oxytoca* (26).

In Bangkok, several species of bacteria were also isolated from cockroaches in residential and hospital areas. *Escherichia coli* and *Klebsiella pneumoniae* were most common in the home, and *E. coli* and *Enterobacter cloacae* were most common in the hospital, which was consistent with our study of the prevalence of *E. coli* (27). In a study by Lin et al, the most isolated bacteria from cockroaches were *E. coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Staphylococcus aurous* (28). In another study by Chaichanawongsaroj et al. *E. coli* and *Klebsiella* spp. were commonly isolated from nosocomial cockroaches and showed that the insect, as a carrier of this bacterium, was involved in the transmission of these bacteria (27). The study by Fakoorziba et al. in 2010 on *P. americana* and *B. germanica* showed the high potential of these organisms to carry pathogenic bacteria, which isolated 25 different species of important medical bacteria that 22 species were gram-negative bacteria. In this study, bacteria from the family Enterobacteriaceae of the genera *Proteus*, *Citrobacter*, *Aerobacter*, *Klebsiella* and *Bacillus* were isolated from the outer surface of cockroaches (29). Akbari and colleagues conducted a study to determine aerobic bacterial community of American cockroach guts and found 11 bacterial species including: *E. coli*, *Shigella flexineri*, *Citrobacter freundii*, *E. vulneris*, *Enterobacter cloacae*, *Yersinia pseudotuberculosis*, *Y. intermedia*, *Leclericia adecarboxylata*, *Klebsiella oxytoca*, *K. planticola*, and *Rahnella aquatilis*. Some of the isolated bacteria of that study were consistent with the present study (13).

Also in the present study, *Staphylococcus* was the second bacterium that was abundantly identified in the studied cockroaches, this bacterium causes a wide range of infections from simple skin infections (Such as decoctions, boils, scabs, eyelashes, and abscesses) to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, and septicemia. *Staphylococcus aureus* is one of the five most common causes of nosocomial infections, especially post-surgical wound infections. Each year, 500,000 people in hospitals in the United States become infected with *S. aureus* (30–32).

The results of the antibiogram test showed that the identified bacteria were resistant to the antibiotics Cephalothin, Ampicillin, Cefotaxime,

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and Kanamycin that this bacterial resistance was worrying, and they were also relatively resistant to the antibiotics Nitrofurantoin and Co-trimoxazole. The cultured bacteria were also semi-sensitive to the antibiotics kanamycin and ciprofloxacin and were sensitive to the tetracycline, gentamycin, and chloramphenicol antibiotics.

In a study by Stypułkowska-Misiurewicz et al. and colleagues, they examined cockroaches in nine Warsaw hospitals and found that the bacteria carried by their bodies were the same ones (species) responsible for nosocomial infections. Therefore, they stated that there was a microbiological risk of cockroaches in the hospital environment and it was proved that some bacteria in the body of cockroaches are responsible for nosocomial infections, in which there is also antibiotic resistance (5). Davari et al. conducted a study with the aim of investigating the frequency of resistant and sensitive bacteria isolated from houseflies, their findings showed, *K. pneumonia* had the highest bacterial count with 43% and *P. aeruginosa* 37%, *Proteus mirabilis* 29.1%, and *Citrobacter freundii* were reported 4.28%. Among all hospital samples, resistance to cephalexin, chloramphenicol, ampicillin, and tetracycline was high at 32.5%. The result was that gentamicin showed the highest sensitivity among all hospital specimens (33). In our study, the highest sensitivity to tetracycline, gentamicin, and chloramphenicol antibiotics was reported.

Considering that many diagnostic tests were needed to determine the bacterial strain, and this required a lot of financial resources, so we decided to stop the differentiation tests at the species level in some isolates.

Conclusions

According to the results of this study, it was found that cockroaches can be very effective in transmitting pathogenic bacteria and they can also mechanically transfer bacteria from hospital environments to the outside of the hospital and vice versa, which is very important. In addition, according to the findings of this research, it can be assumed that cockroaches play a major role in the spread of antibiotic resistant bacteria. The next point is that cockroaches in hospital environments were more contaminated with bacteria, one of the reasons for this is that people who visit hospital centers are usually sick and carry various pathogens. Therefore, raising the level of health and combating cockroaches seems very necessary.

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Ethical considerations

Since human samples and animal studies were not used in this study, there were no ethical restrictions.

Conflict of Interest statement

The authors declare there is no conflict of interests.

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