



Bacterial contamination of cockroaches in different wards of hospital, restaurant and home

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ABSTRACT

Cockroaches are very capable of mechanically transmitting harmful microorganisms, which is seen to be a severe hazard to the general public's health. The purpose of this study was the evaluation of cockroach bacterial contamination in various locations throughout Babylon. 300 cockroaches were caught from different wards of the hospital, restaurants, and houses. Using PBS buffer, the external surface of the cockroaches was washed to collect bacteria. Standard phenotypic methods were used to identify and classify bacteria. Afterward, the bacterial resistance to different antibiotics was investigated using the Kirby-Bauer disk diffusion susceptibility test. The 200 (66.6 %) *American cockroaches* including 56 (18.7 %) *Blattella germanica* and 44 (14.6 %) *Blatta orientalis* were identified. Noteworthy, 96.6 % of cockroaches were infected with different bacteria. *Bacillus* strains, coagulase-negative *Staphylococci* (CoNs), and *Escherichia coli* were the most frequent among the isolated bacteria. On average, the highest antibiotic resistance was detected to cefotaxime, ampicillin, cephalothin, and kanamycin. On the other hand, the isolated bacteria showed high sensitivity to gentamicin, nitrofurantoin, tetracycline, trimethoprim/sulfamethoxazole (SXT), and chloramphenicol. high antibiotic resistance in bacteria isolated from different wards of the hospital and the high potential of transmission of these bacteria by cockroaches is a serious warning for the health of society.

1. Introduction

Insects are one of the more crowded phyla of arthropods, which are economically, health-wise, and medically important [1]. Cockroaches belong to the Blattaria order. Approximately 4000 species of cockroaches have been classified so far, of which less than 1 % are pests [2]. Cockroaches live in warehouses, bakeries, baths, hospitals, and even ships and ports. They hide in narrow and dark

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cracks during the day and leave their shelter for food at night [3].

Periplaneta americana is commonly found in areas with moderate to warm climates. They are considered a great hazard to public health because they live in sewage and carry parasites and human diseases. American cockroaches are one of the largest cockroaches belonging to the Blattodea order, reaching up to 1.5 inches in length with an average weight between three and 10 g. Common food sources are pests and reptile carcasses [4,5].

Blattella germanica, known as the Croton bug, is a small beetle with an average length of between 0.43 and 0.63 inches. The body color changed from brown to almost black. Closely similar to Asian cockroaches. Despite being a German cockroach, the Asian cockroach is attracted to light and can fly like a butterfly [6,7].

Blatta orientalis is a large species of cockroach, typically known as a waterbug. The average size of grown males is 0.71–1.14 inches, while mature females are between 0.79 and 1.06 inches. The body is shiny, ranging in color from dark brown to black. Females seem slightly different than males (wider bodies). It appears wingless at a casual glance, but it is brachial and non-functional just below its head. Male wings are longer and cover three-quarters of the abdomen in brown. None of them can fly [8,9].

These insects are medically important and are a potential danger to public health due to their habit of regurgitating a part of the eaten food, excreting feces in living places, having weak bites, especially between the fingers, and producing a bad smell, especially for American cockroaches. Also, the secretions and crusts caused by the skin replacement contain allergens, which cause many acute respiratory diseases, itching, and dermatitis [10].

Serological assessments of cockroach body secretion and feces particles detected multiple allergen materials. Cockroaches account for the second-leading cause of asthma after allergies caused by dust. The capability of cockroaches to run quickly and move from one place to another increases the transmission of pathogens and also allows them to escape from pesticides [11].

The feces of cockroaches contain compounds of tryptophan derivatives that contain mutagenic xanthuric acid, kynurenic acid, and 8-hydroxyquinadic acid. The role of cockroaches in spreading pathogenic bacteria in the hospital environment and causing nosocomial infections is too important. Cockroaches are typically infected with pathogenic bacteria from leprosy, bubonic plague, dysentery, urinary tract infections, abscesses, and food poisoning compounds. Cockroaches, especially American, German, and Oriental cockroaches, are considered the most important carriers of approximately 150 bacterial species, 90 protozoa species, 60 species of yeast, 45 species of roundworms, 12 species of pathogenic molds, and several hookworms and whipworms [2].

Cockroaches are omnivorous. They feed on various foods and even human excrement, transferring a large number of harmful microorganisms and spreading diseases such as typhoid, leprosy, dysentery, etc. These microorganisms live in the intestines of cockroaches for a long time and are finally transferred to another organism [12].

One of the most important public health problems is fighting multi-resistant bacteria. The resistance of microorganisms to antibiotics is encoded by different genes transferred by horizontal gene transfer between bacteria. Cockroaches have the potential to mechanically transfer antibiotic-resistant bacteria from contaminated to non-contaminated areas and vice versa, causing serious challenges for public health [13–15].

Therefore, it's important to evaluate the abundance of cockroach populations and their ability to transmit pathogenic bacteria. Also, the lack of sufficient data on the degree of bacterial contamination of their external surfaces and the prevention of the transmission of various infections could be an important challenge in a clinical setting. To this end, the present research was conducted to evaluate the bacterial contamination of the cockroach population in hospital, residential, and restaurant environments.

2. Materials and methods

2.1. Cockroaches collection

This is a descriptive-analytical study conducted between 2019 and 2021. A total of 300 cockroaches were caught by hand and then transferred to the laboratory using sterile gloves. To prevent cross-contamination with cockroaches, each sample was carried in a separate bottle. Each sample was placed at 4° centigrade below zero for 5 min to anesthetize. The species of cockroaches was determined according to their characteristics (size, color, presence or absence of wings, etc.) [16].

2.2. Bacterial detection

At first, the external surface of the cockroaches was washed using sterile physiological serum. At first, the external surface of the cockroaches was washed with sterile physiological serum. Then, the solution obtained from washing the external surface of the cockroach was centrifuged at 2500 rpm for 4 min. The sediment was cultured on EMB and Blood agar media (Merck Germany) and then incubated at 37 °C overnight. In the following, for each colony, all the differential tests were performed to separate the bacteria up to the genus. To isolate gram-negative bacteria, tests such as (gram staining, fermentation of sugars, and motility tests such as TSI (Merck), SIM (Merck), and Simon citrate (Merck) were done. Noteworthy, different tests such as gram staining, oxidase, and catalase tests, sensitivity to antibiotics, fermentation of sugars (mannitol), etc. were used to isolate Gram-positive bacteria [17,18].

2.3. Antimicrobial susceptibility test

The Kirby-Bayer disc diffusion method was used to determine the antimicrobial sensitivity status of the samples. Notably, *Pseudomonas aeruginosa* ATCC 27853 was used as a control strain. From each strain, a suspension with turbidity equal to half a McFarland unit was prepared and transferred to Mueller-Hinton Agar medium (Merck). Afterward, different antibiotic discs include ampicillin

(30 µg), tetracycline (30 µg), kanamycin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1.25–23.75 µg), gentamicin (30 µg), nitrofurantoin (300 µg), cephalothin (30 µg) and cefotaxime (30 µg) provided by MAST, England, were placed on each plate and incubated at 37 °C. After 24 h, the growth inhibition zone diameter was measured. The diameter of the inhibition zone of bacteria for each antibiotic was classified as resistant, semi-sensitive, and sensitive according to CLSI guidelines [19–21].

2.4. Statistical analysis

The relationship between cockroaches and their infections with pathogens in different locations was analyzed using Kruskal-Wallis and ANOVA tests. GraphPad Prism v.8.3.0 and SPSS v.20.0 software (SPSS Inc. Chicago, IL, USA) were used for statistical analysis, and a P-value ≤ 0.05 was considered significant.

3. Results

3.1. Cockroach collection and bacterial isolation

In general, 300 cockroaches were collected in this study, and 290 different strains of bacteria were identified on their external surfaces. Of these, 44, 56, and 200 cockroaches were caught from restaurants, homes, and hospitals, respectively. Fig. 1 and Table 1 show the distribution of bacteria in isolated places. 200 (67 %) samples were American cockroaches, and the frequency of *Blattella germanica* and *Blatta orientalis* was 56 (19 %) and 44 (14 %), respectively.

The number of bacteria isolated from cockroaches was 290 (96.6 %), the isolated bacteria included *Escherichia coli*, *Bacillus* spp., *Escherichia coli*, *Klebsiella* spp., *Acinetobacter* species, coagulase-negative Staphylococci (CoNs), *Enterococcus* spp., *Proteus* spp., *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. CoNs, *Escherichia coli*, and *Bacillus* strains were the most frequently isolated species. There is a significant relationship between the collection places of cockroaches and the rate of bacterial infection (P value < 0.05).

3.2. Antibigram susceptibility result

The results of the antibiogram indicated that the bacteria isolated from different wards of the hospital were more resistant to different antibiotics than the bacteria isolated from restaurant and home environments. This difference was statistically significant (P value < 0.05). Figs. 2 and 3 show the results of antimicrobial susceptibility in Gram-negative and Gram-positive bacteria.

4. Discussion

In this study, three species, including *Periplaneta americana*, *Blatta orientalis*, and *Blattella germanica*, are detected. *Periplaneta*

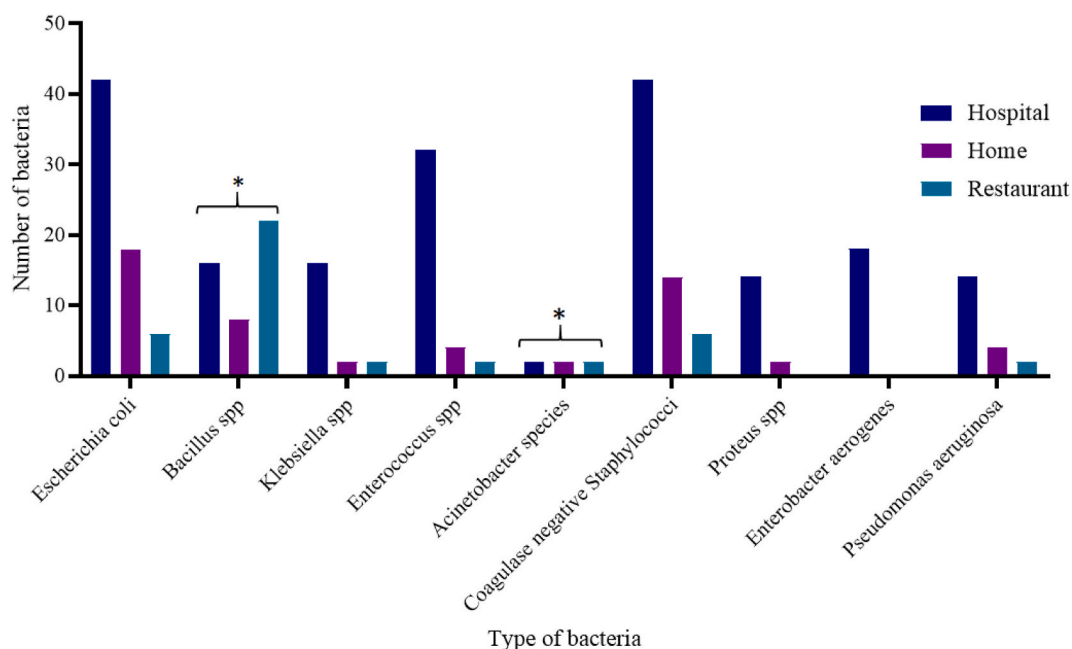


Fig. 1. Frequency of bacteria isolated from different areas (*p < 0.05).

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

| Bacteria | Cockroaches | | | Total |
|---|-------------------------------------|-----------------------------------|------------------------------------|-----------|
| | <i>Periplaneta americana</i> No (%) | <i>Blattella germanica</i> No (%) | <i>Blattella orientalis</i> No (%) | |
| <i>Escherichia coli</i> | 50 (75.7)* | 8 (12.1) | 8 (12.1) | 66 (100) |
| <i>Bacillus</i> spp | 16 (34.8) | 14 (30.4) | 16 (34.8) | 46 (100) |
| <i>Klebsiella</i> spp | 10 (50) | 6 (30) | 4 (20) | 20 (100) |
| <i>Enterococcus</i> spp | 28 (78.9)* | 2 (5.2) | 6 (17.7) | 36 (100) |
| <i>Acinetobacter species</i> | 4 (66.6) | 0 (0.0) | 2 (33.3) | 6 (100) |
| Coagulase-negative <i>Staphylococci</i> | 20 (32.2) | 20 (32.2) | 22 (33.3) | 62 (100) |
| <i>Proteus</i> spp | 8 (50) | 4 (25) | 4 (25) | 16 (100) |
| <i>Enterobacter aerogenes</i> | 9 (50) | 2 (11.1) | 7 (38.8) | 18 (100) |
| <i>Pseudomonas aeruginosa</i> | 12 (60.0) | 6 (30.0) | 2 (10.0) | 20 (100) |
| Total | 159 (54.4) | 62 (21.2) | 71 (24.3) | 290 (100) |

americana was the most frequent cockroach (67 %), followed by *Blattella germanica* (19 %) and *Blatta orientalis* (14 %). Zarchi et al. conducted an experiment in Tehran hospitals. Their result noted that 65.6 % of the cockroaches belong to *Periplaneta americana*, followed by the frequency of the German cockroach (12.1 %), and 22.3 % were reported as the frequency of the oriental cockroach, which is consistent with the results of this study [22]. Other studies also announced that American and German cockroaches were the most frequent [23,24].

The outcome of another study also indicated that in clinical environments, the number of American cockroaches was higher, and American cockroaches were most abundant in non-clinical environments. This result was not statistically significant [25].

In the current study, a total of nine genera of bacteria were detected in cockroaches. CoNs and *E. coli* were the most frequent; additionally, these bacteria were most commonly detected from home samples, and *Bacillus* species were the most commonly detected from restaurant samples. This study announces that cockroaches can disseminate various microorganisms, which is consistent with reports of other similar studies [26]. Chitzasi and his team performed a study of the frequency of cockroaches in different places in Mashhad City between 2013 and 2014. The most frequent strains isolated from cockroaches in hospitals, dormitories, and homes were *Enterococcus*, *Enterobacter aerogenes*, and *Klebsiella oxytoca* [27].

In a study conducted by Lin and colleagues, *E. coli*, *P. aeruginosa*, *Salmonella*, and *S. aureus* were most commonly identified in cockroaches [28]. In another survey conducted by Chaichanawongsaraj et al., *E. coli* and *Klebsiella* spp. had the highest frequency, which is isolated from cockroach bodies. The results also reported that this insect can be a common vector of microorganisms [29].

The study of Fakoorziba et al. on *Blattella germanica* and *Periplaneta americana* showed that these insects have a high potential for carrying pathogenic bacteria. A total of 25 different genera of bacteria were isolated in this study. Of this, 22 genera belong to Gram-negative bacteria. The Enterobacteriaceae family, which includes *Proteus*, *Citrobacter*, *Aerobacter*, and *Klebsiella*, was detected in cockroaches. Furthermore, *Bacillus* was isolated from the outer surface of cockroach bodies [30].

The most frequent strain isolated from different sites in the current study was *E. coli*, which is the most common pathogen in the Enterobacteriaceae family. *E. coli* is responsible for the most common pathogen of the urinary tract and about 90 % of urinary tract

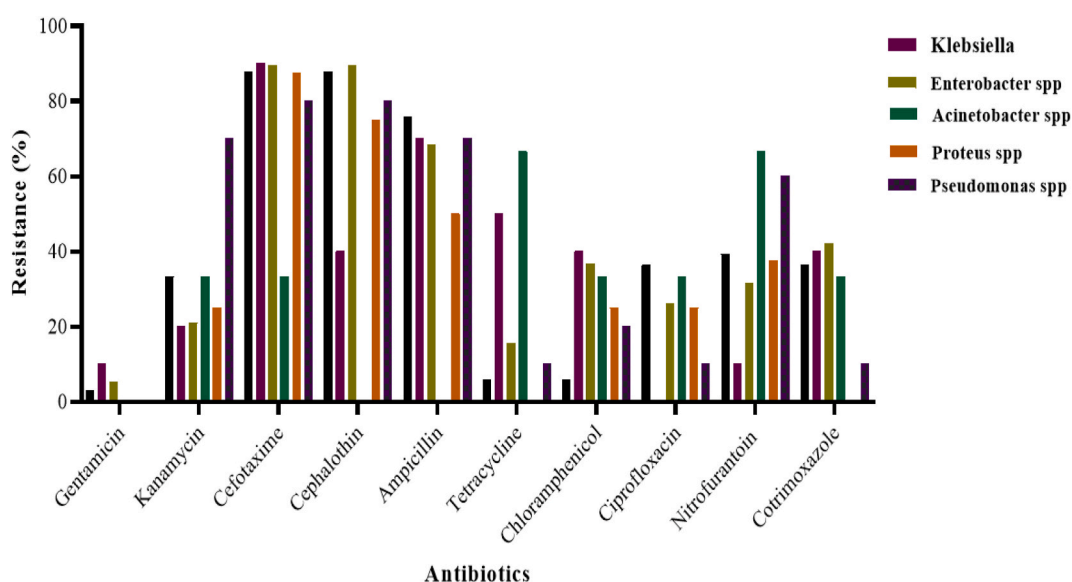


Fig. 2. Antibiotic resistance profile of Gram-negative bacteria.

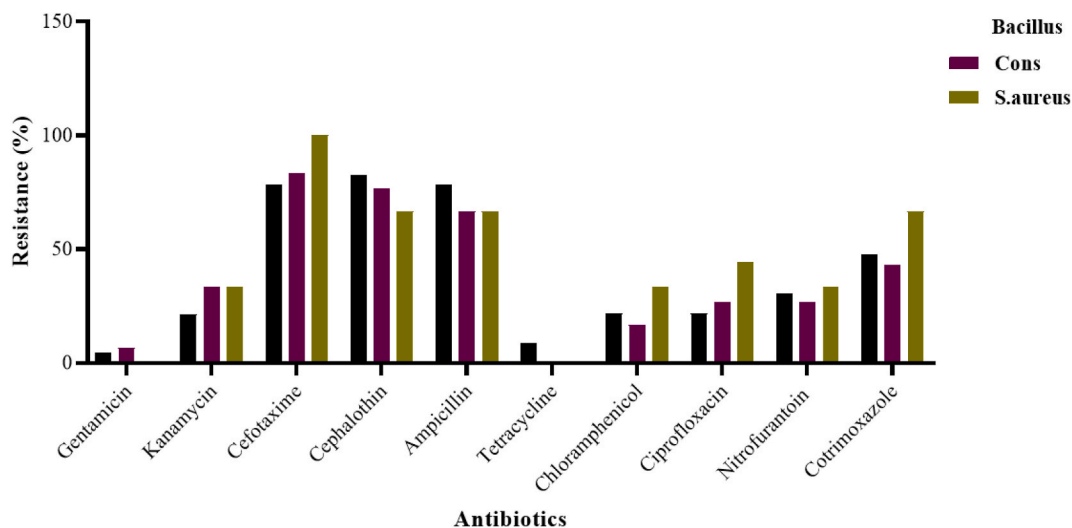


Fig. 3. Antibiotic resistance profile of Gram-negative bacteria.

infections in women. Clinical symptoms of this study are frequent urination, burning while urinating, and hematuria and turbidity of the urine [31].

In addition, *Staphylococcus* was the second species isolated from samples in this study, which accounts for the wide distribution of infections, from a simple skin infection (abscess, sty) to life-threatening conditions such as pneumonia, meningitis, osteomyelitis, endocarditis, and septicemia. One of the most common pathogens that cause wound infections after surgery is *S. aureus*. 500 thousand people are affected by *S. aureus* infections in the United States annually [32–34].

Antimicrobial susceptibility testing indicated the isolated strains had high resistance to cephalothin, ampicillin, and cefotaxime. This resistance is a concern for public health. The outcomes also depicted as isolated have high relative resistance against nitrofurantoin and cotrimoxazole. The organisms were not sensitive to tetracycline, gentamycin, or chloramphenicol, but they were sensitive to kanamycin and ciprofloxacin.

In a Stypukowska-Misiurewicz H. et al. study, cockroaches from nine Warsaw hospitals were evaluated. The results announced that the pathogens carried by the cockroaches are the same isolates that are responsible for nosocomial infection. It is concluded that cockroaches are one of the sources of microbiological hazards for hospitals. This organism is highly resistant to antibiotics [11].

Davari et al. conducted a study on the bacteria living on the outer surface of flies in slaughterhouses and hospitals in Sanandaj City. Antimicrobial susceptibility results indicated that *K. pneumoniae* (43 %), *P. aeruginosa* (37 %), *P. mirabilis* (29.5 %), and *Citrobacter freundii* (28.45) were most commonly identified from samples. The clinical isolates showed high resistance (about 32.5 %) to cephalexin, chloramphenicol, ampicillin, and tetracycline. The highest sensitivity between clinical samples was observed for gentamycin, tetracycline, and chloramphenicol [35].

5. Conclusion

According to the results of this study, it was detected that cockroaches have a high potential for disseminating pathogenic bacteria to other places. They are also able to transfer microorganisms from the hospital environment to non-clinical samples mechanically. So, increasing the level of public hygiene and also planning a control method to fight cockroaches seems crucial.

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Author contribution statement

Abduladheem Turki Jalil: Conceived and designed the experiments and Wrote the paper. Rahman S. Zabibah, Sarmad Jaafar Naser, and Ahmed al-hili: Performed the experiments and contributed reagents, materials, analysis tools, and data. Muna.S Merza, Tahani Alsandook, Mohanad Ali Abdulhadi, and Lubna R Al-Ameer: Analyzed and interpreted the data and Wrote the paper.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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