

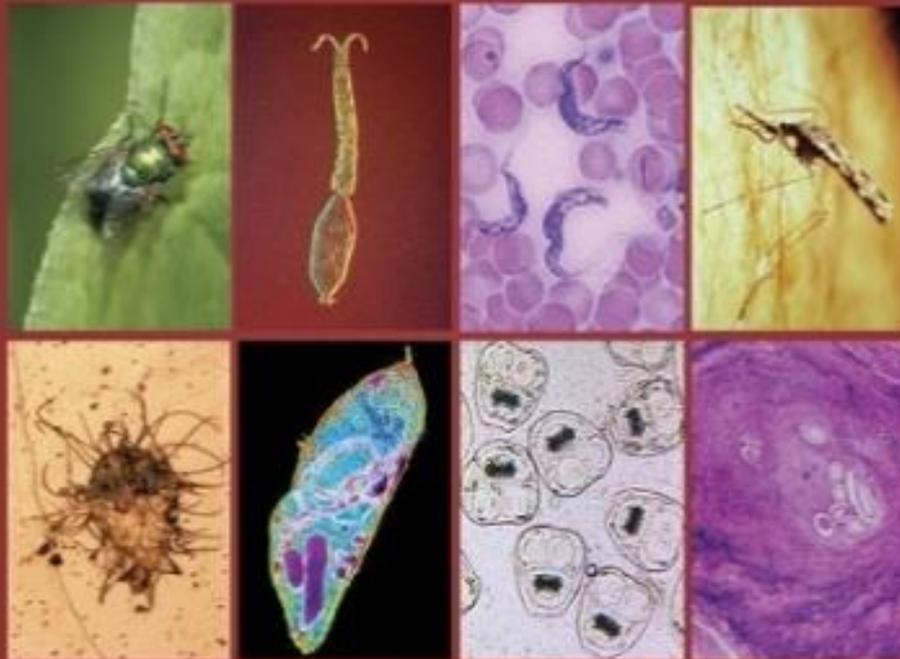


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## The Potential Role of the American Cockroach (*Periplaneta americana*) as a Vector of Enterobacteriaceae in Kuwait

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### ABSTRACT

Due to cockroaches' filthy habits and habitats, they are incriminated to mechanically disseminate a wide range of food-borne pathogens. This study was designed to examine the American cockroach (*Periplaneta americana*) as a carrier of bacteria and explore their risk to human health in Kuwait. From the 6 governorates of Kuwait, 600 cockroaches were collected for isolating and quantifying bacterial load using macroscopical, microscopical, and biochemical methods. A total of 20 bacterial isolates were determined by this study of which *Escherichia coli* was the most prevalent and *Acinetobacter baumannii* was the second dominant and as far as we are aware this is the first identification of this species on the American cockroach. *Pseudomonas luteola* and *klebsiella pneumoniae* were recognized as the least frequent bacteria. Several bacterial isolates were identified as highly pathogenic to humans such as *Salmonella choleraesuis* and *Serratia marcescens* and others. This study, which is regarded as the first study carried out in Kuwait, accentuated that the American cockroach is graded as a potential disseminator of life-threatening pathogenic agents and its public health implications should be highly considered. Hence, effective preventive and control measures are needed.

### INTRODUCTION

Cockroaches are cosmopolitan species that scavenge a wide range of nutrients varying in nutritional value and composition. The adaptation of cockroaches to different unsanitary habitats, such as human waste disposal and sewage systems, enables them to disseminate a wide variety of deadly pathogens (Guzman and Vilcinskis, 2020). They serve as reservoirs for potentially pathogenic bacteria, in particular members of the family *Enterobacteriaceae*, *Mycobacterium*, and *Staphylococcus* (Guzman and Vilcinskis, 2020).

The risks associated with cockroaches are widely documented, particularly in residential and communal places with poor standards of hygiene, such as schools and hospitals, (Fakoorziba *et al.*, 2010; Al-bayati *et al.*, 2011). Studies by Menasria *et al.* (2014) and Sarwar (2015) demonstrated that cockroaches transmit many nosocomial contagions, such as drug-resistant *Pseudomonas aeruginosa*, *Shigella*, and pathogenic species of *Salmonella*. Varying hypotheses were postulated regarding the proficiency of cockroaches as pathogenic transmitters. Sarwar (2015) reported that the quantity of a single species of bacteria species on the vector body plays a major role in the prevalence of infection.

In contrast, Nasirian (2017) noted that the potency of bacterial infection depends on the total number of different bacterial species. Another study (Hamu *et al.*, 2014) revealed that the speed of microbial transmission is strongly allied with the vector or host body morphology. Herzner *et al.* (2011) on the other hand, reported that the ability of a cockroach to become an unhygienic scavenger is owing to the germ-infested habitats.

Cockroach infestation is attributed to sanitation standards, occupant activities, and building construction, along with other factors (Shahraki *et al.*, 2013). Despite recent advancements in the structural design of buildings, this insect is still regarded as a human health threat worldwide. In general, most insects possess elaborate immune systems conferring multiple pathways of insecticidal resistance (Perry *et al.*, 2011; Cooper & Eleftherianos, 2017) that portentously increase problems with the eradication of infestations (Rahimian *et al.*, 2019). Nevertheless, some insecticidal agents pose considerable risks to human health (Titlow *et al.*, 2013; Latifi *et al.*, 2015; Rahimian *et al.*, 2019). Moreover, cockroaches are recognized as promising candidates for evaluating pathogen-host interactions (Mpuchane *et al.*, 2006). In addition to their acquisition of effectual adaptive immune components, there are many factors associated with their robust fitness, including their tolerance to thrilling weather conditions and intimidating habitats and their ability to form successful symbiotic relationships with various species of bacteria (Bagde *et al.*, 2013; Sarwar, 2015). For example, the mutualistic intestinal bacteria of cockroaches play a crucial role in regulating their metabolism by maximizing energy extraction and providing robust immunity against pathogenic microorganisms (Schauer *et al.*, 2012). Yun *et al.* (2014) reported that the quantity and types of gut bacteria alter in accordance with the quality of the cockroach's habitat. (Yun *et al.*,

2014). Thus, the diversity of the gut microbial community provides a substantial basis for a better understanding of the biological and ecological role of cockroaches (Schauer *et al.*, 2012).

Although *P. americana* is widely spread in people's habitats in Kuwait, there has been no such study that had assessed their potential public health consequences. Therefore, the current study was designed to identify and quantify bacterial carriages on this insect collected from the 6 governorates of Kuwait states.

## **MATERIALS AND METHODS**

### **Specimen Collection and Preparation:**

In October 2022, adult cockroaches were caught randomly, using hand gloves, from kitchens of houses located in the different governorates of Kuwait (Al-Ahmadi, Al-Asimah, Al-Farwaniyah, Hawalli, Al-Jahrah, and Mubarak Al-Kabeer). The governorates included in this study differ in terms of the population and way of living. Al-Asimah is considered one of the oldest areas in Kuwait, which is inhabited mostly by occupants with a high-quality standard of lifestyle. However, many of the houses in this area have an old construction scheme and sewerage system. Al-Farwaniyah and Hawalli are considered commercial areas with many buildings rented by mostly occupants of different nationalities with a poor quality of life, and a lack of hygiene, and sanitation. Furthermore, these areas suffer from overcrowding, and accumulation of waste. Due to a large number of shops in these areas, there are many wastes that often attract rodents and insects. Mubarak Al-Kabeer, Al-Ahmadi and Al-Jahrah are residential areas inhabited by many occupants. People living in these areas vary in terms of standard of living and the way they maintain hygiene.

Each collection of cockroaches (100 specimens) from each governorate was placed separately in a sterile labelled container and then transferred in an ice box to the National Unit for Environmental

Research and Services (NUERS) at Kuwait University for bacterial analysis.

### **Isolation, and Identification, of Bacteria from Cockroach's Cuticle:**

Each individual cockroach was picked from each container by forceps and placed in a sterile test tube containing peptone water following aseptic techniques. Then, 1 ml of sterile normal saline (0.9%) was added to the test tube. The tube was thoroughly shaken for 2 minutes to isolate

### **RESULTS**

In total, 600 American cockroaches were collected from the 6 governorates and examined for bacterial isolation. The study showed that the bacterial isolates were 20 species in total and a single cockroach was found positively infected by at least one bacterial species. Our results exhibited that the bacterial diversity was fluctuating among the tested governorates. It was found that the cockroaches obtained from the Al-Asimah governorate displayed the highest diversity with a total of 14 species, followed by Hawalli and Al-Farwaniyah which had 10 and 8 different bacterial isolates, respectively. Moreover, only 7 and 6 bacterial species were recorded from al Ahmadi and Mubarak Al-Kabeer cockroaches, respectively.

Among the 20 bacterial isolates in this study, *E. coli* was found the most prevalent infecting 25.65 % of cockroaches examined (159/600). *Acinetobacter baumannii* was recorded as the second dominant isolate (14 %; 84/600) and it was recorded in all 6 provinces investigated. *Pseudomonas luteola* and *klebsiella pneumoniae* were the least in respect to prevalence and distribution since they were recorded only in Al-Asama province. Also,

the residing microorganisms. The saline was serially diluted ( $10^{-1}$  to  $10^{-7}$ ) and aliquots (0.1 ml each) were then separately inoculated onto freshly prepared nutrient agar plates and incubated overnight at 37°C. Bacterial colonies were identified based on morphological appearance, and microscopic and biochemical examination. The overall bacterial load carried by each insect was counted and expressed in terms of colony-forming units (CFU).

*Pantoea agglomeran* was isolated only from Al-Ahmadi cockroaches (Table 1).

The highest mean load of isolated bacteria belongs to *Escherichia coli* which was recorded in Al-Farwaniyah governorate ( $36.1 \pm 1.2 \times 10^4$ ) and its lowest value was found in Al-Jahrah governorate ( $20.1 \pm 1.1 \times 10^2$ ). The most common bacteria recorded in all the studied governorates were *Acinetobacter baumannii* with a mean load value reaching ( $34.1 \pm 1.3 \times 10^6$ ). The last occurrence bacteria were *Pseudomonas luteola*, *Klebsiella pneumoniae*, and *Chryseobacterium meningosepticum* which were recorded in Al-Asimah governorates exclusively with load values ( $2.1 \pm 0.1 \times 10^6$ ), ( $3.3 \pm 0.9 \times 10^6$ ), and ( $3.2 \pm 0.2 \times 10^5$ ), respectively (Table1). Al-Asimah and Hawalli which are known for their high population and diverse cultures backgrounds and habits, had the highest number of bacterial isolates 15 and 10, respectively and the *Escherichia coli* had the greatest load values in both areas. Despite Mubarak-Al-Kabeer having the least number of isolates, most of their values were found higher compared to other areas in particular *Acinetobacter baumannii* ( $34.1 \pm 1.3 \times 10^6$ ), *Serratia marcescens* ( $27.1 \pm 1.1 \times 10^5$ ) and *Yersinia enterocolitica* ( $29.1 \pm 0.7 \times 10^5$ ).

**Table 1.** Bacterial isolates identified from the cuticle of the American cockroaches in the six governorates of Kuwait during the year 2022 (100 insect / governorate)

Bacterial species	Al-Ahmadi		Al-Asimah		Al-Farwaniyah		Hawalli		Al-Jahra		Mu. Al-Kabeer	
	(%)	Load	(%)	Load	(%)	Load	(%)	Load	(%)	Load	(%)	Load
<i>Escherichia coli</i>	30	25.0 ± 1.1 x 10 <sup>6</sup>	25	29.2 ± 1.5 x 10 <sup>6</sup>	30	36.1 ± 1.2 x 10 <sup>4</sup>	40	30.1 ± 0.4 x 10 <sup>4</sup>	31	20.1 ± 1.1 x 10 <sup>2</sup>	----	----
<i>Acinetobacter baumannii</i>	8	5.0 ± 0.2 x 10 <sup>6</sup>	3	8.2 ± 0.4 x 10 <sup>6</sup>	12	22.5 ± 0.9 x 10 <sup>6</sup>	10	24.1 ± 0.2 x 10 <sup>4</sup>	15	16.2 ± 0.2 x 10 <sup>6</sup>	27	34.1 ± 1.3 x 10 <sup>6</sup>
<i>Acinetobacter haemolyticus</i>	12	19.1 ± 0.5 x 10 <sup>6</sup>	7	4.2 ± 1.1 x 10 <sup>6</sup>	----	----	----	----	10	16.1 ± 0.5 x 10 <sup>6</sup>	----	----
<i>Serratia marcescens</i>	8	1.1 ± 0.2 x 10 <sup>6</sup>	----	----	----	----	6	1.1 ± 1.4 x 10 <sup>6</sup>	7	2.1 ± 0.3 x 10 <sup>6</sup>	17	27.1 ± 1.1 x 10 <sup>5</sup>
<i>Acinetobacter Iwoffii</i>	----	----	6	6.2 ± 0.7 x 10 <sup>6</sup>	8	17.1 ± 0.5 x 10 <sup>6</sup>	10	19.1 ± 0.7 x 10 <sup>5</sup>	8	18.1 ± 0.2 x 10 <sup>6</sup>	----	----
<i>Pantoea agglomerans</i>	10	1.5 ± 0.3 x 10 <sup>6</sup>	----	----	----	----	----	----	----	----	----	----
<i>Enterobacter liquefaciens</i>	12	2.2 ± 0.2 x 10 <sup>6</sup>	----	----	----	----	----	----	3	6.2 ± 0.2 x 10 <sup>6</sup>	11	12.1 ± 0.3 x 10 <sup>6</sup>
<i>Shigella sonnei</i>	20	25 ± 0.5 x 10 <sup>6</sup>	6	20.4 ± 0.3 x 10 <sup>6</sup>	----	----	----	----	----	----	----	----
<i>Proteus mirabilis</i>	----	----	5	5.1 ± 0.4 x 10 <sup>6</sup>	----	----	----	----	5	6.2 ± 0.3 x 10 <sup>6</sup>	----	----
<i>Morganella morganii</i>	----	----	4	5.2 x 10 <sup>6</sup>	----	----	3	2.5 ± 0.3 x 10 <sup>6</sup>	----	----	9	6.1 ± 0.1 x 10 <sup>6</sup>
<i>Klebsiella ozaenae</i>	----	----	7	4.5 ± 0.4 x 10 <sup>6</sup>	----	----	2	1.1 ± 0.2 x 10 <sup>6</sup>	----	----	18	15.1 ± 0.3 x 10 <sup>6</sup>
<i>Hafnia alvei</i>	----	----	4	4.5 ± 0.2 x 10 <sup>6</sup>	7	16.5 ± 0.3 x 10 <sup>6</sup>	----	----	----	----	----	----
<i>Providencia alcalifaciens</i>	----	----	3	1.1 ± 0.3 x 10 <sup>6</sup>	8	16.5 ± 0.1 x 10 <sup>6</sup>	----	----	----	----	----	----
<i>Chryseobacterium meningosepticum</i>	----	----	6	3.2 ± 0.2 x 10 <sup>6</sup>	----	----	----	----	----	----	----	----
<i>Pseudomonas luteola</i>	----	----	2	2.1 ± 0.1 x 10 <sup>6</sup>	----	----	----	----	----	----	----	----
<i>Klebsiella pneumoniae</i>	----	----	5	3.3 ± 0.9 x 10 <sup>6</sup>	----	----	----	----	----	----	----	----
<i>Yersinia enterocolitica</i>	----	----	----	----	10	21.1 ± 0.2 x 10 <sup>6</sup>	4	1.5 ± 1.2 x 10 <sup>6</sup>	21	21.2 ± 0.3 x 10 <sup>6</sup>	18	29.1 ± 0.7 x 10 <sup>6</sup>
<i>Enterobacter cloacae</i>	----	----	----	----	21	16.5 ± 0.2 x 10 <sup>6</sup>	7	6.1 ± 0.4 x 10 <sup>6</sup>	----	----	----	----
<i>Klebsiella oxytoca</i>	----	----	----	----	4	2.2 ± 0.5 x 10 <sup>6</sup>	3	6.1 ± 0.1 x 10 <sup>6</sup>	----	----	----	----
<i>Salmonella choleraesuis</i>	----	----	17	26.2 ± 1.4 x 10 <sup>6</sup>	----	----	15	21.1 ± 0.2 x 10 <sup>6</sup>	----	----	----	----

Colony-forming units (CFU) per insect

## DISCUSSION

Several studies worldwide reported the diversity of microbial carriage of the American cockroach (Donkor, 2020; Molewa *et al.*, 2022). Likewise, the present study quantified a substantial variety of bacterial isolates and had the highest number of identified bacteria in comparison with other studies. For example, a study carried out in Iran reported exclusively 7 species on 15 cockroaches (Feizhaddad *et al.*, 2012), while in Nigeria and Morocco, 17 and 10 species were extracted from 240 and 50 cockroaches, respectively (Bouamama *et al.*, 2010; Isaac *et al.*, 2014). Obtaining a high number of bacterial species (20 species) in our study could be attributed to the high number of examined cockroaches (600). From a theoretical perspective, the more cockroach species tested the higher likelihood of bacterial

species being encountered. However, some studies do not comply with this scenario. Solomon *et al.* (2016) identified 18 bacterial species on 1,140 specimens of American cockroaches in Ethiopia, while a significantly greater number of species reaching 30 isolates were recorded from 532 cockroaches in France (Le Guyader *et al.*, 1989).

All examined cockroaches in the present study were found infected by bacteria, in agreement with Moges *et al.* (2016) findings. Moreover, Sayyad *et al.* (2016) documented 74.5% of 98 cockroaches were found infected by medically important bacteria. Vahabi *et al.* (2011) reported 100% of examined American cockroaches and 70% of German Cockroaches (*Blattella germanica*) that were infected with bacteria. In a similar context, Nasirian (2019) reported that the

American cockroach showed the highest contamination rate (21%), in comparison to the German cockroach (10.6%) and other species of cockroaches (15%). Possibly, the body size trait of cockroaches plays a notable role in determining the infection rate as well as the diversity of bacterial species as the larger the surface cuticular area, the greater exposure to contaminants in an antagonistic environment. The level of microbial diversity revealed by the present study might not be exclusively related to cockroaches' body size or the considered sample size but it could be somehow allied to the degree of microbial contamination in the examined environment. Cloarec *et al.* (1992) isolated a variety of bacterial species from 52 samples of German cockroaches infesting low-income flats with highly contiguous floors. Furthermore, it was reported that 80 % of cockroaches collected from places with low-level hygiene standards were contaminated with bacteria (Pai, 2012). Many other factors may play a significant role in making places enormously infectious such as the high population density of roaches which is an important factor in disseminating a range of pathogenic microorganisms and providing an opportunity to contaminate the environment (Vouga and Greub, 2016). Lauprasert *et al.* (2006) noted that most people's diet preferences are meat, rice, fish, bread, and sweets, which are also preferable nutrients for cockroaches, so food leftover (which is a lot) often thrown in the garbage becomes a good nutritional medium not only for the thriving cockroaches but also for microbes' proliferation.

Our results reveal that the diversity of bacterial species in Al-Asimah or Hawalli provinces was higher than that in each of the other provinces due to the high populations living in Al-Asimah and Hawalli provinces in comparison with the other provinces (Public Authority for Civil Information, 2023).

In this study, *E. coli* was found to be the most dominant bacteria isolated in

regard to its prevalence and quantity (mean bacterial load count). The latter came in accordance with several studies done by Feizhaddad *et al.* (2012), Sayyad *et al.* (2016), and Solomon *et al.* (2016). This was not surprising since *E. coli* is renowned as a fecal bacterium dominating many habitats such as soil water and plants and is normally found in the intestines of humans and animals. In humans, some *E. coli* strains cause gastrointestinal illnesses ranging from aqueous diarrhoea and bloody diarrhoea to haemorrhagic colitis (Ckekabab *et al.*, 2013). Nonetheless, the prevalence of *E. coli* is considered a strong indicator of waste and sewage contaminations and it can endure environmental fluctuations. Our study showed that *Acinetobacter baumannii* was the second dominant species among others, and as far as we are aware this is the first identification of this species on the American cockroach. Nasirian (2019) reported that *Acinetobacter baumannii* was isolated from the German cockroaches but not from the American cockroaches. This bacterial species is considered a major opportunistic human pathogen and implicated as a causative agent of pneumonia, and meningitis and it occurs sometimes in skin-infected soft tissue (Peleg *et al.*, 2008). Other human pathogenic species isolated in this study were: (1) *C. Chryseobacterium meningosepticum*, which is an emerging pathogen among immunocompromised adults and is known to cause meningitis, particularly in premature neonates (Kassiri *et al.*, 2014) and (2) *Yersinia enterocolitica*, which is recognized to cause enterocolitis, acute bloody diarrhoea, and pseudoappendicitis. Furthermore, the epidemic bacteria *Salmonella* and *shigella spp.*, pose major public health concerns worldwide as they are known to be foodborne pathogens incriminated to be the causative agent of food poisoning and diarrhoea (Teshome *et al.*, 2019).

Some of the bacterial species recorded by the present study, including

*Salmonella*, *Shigella*, and *E. coli* have been also recorded by other studies carried out in Kuwait. These species were previously isolated from the stool of diarrhoeic patients admitted to hospitals in Kuwait (Chugh, 2009; Albert *et al.*, 2009). In addition, Al-Harbi *et al.* (2017) isolated many bacterial species, including *Klebsiella pneumoniae*, *K. oxytoca*, and *Pseudomonas spp.*, as contaminants in public sites *e.g.*, fitness clubs, gas stations, hospitals, and supermarkets. In the first survey of seven Middle Eastern countries including Kuwait, Alothman *et al.* (2020) reported that most of the hospitalized patients with an infection (98.2%) were receiving antimicrobial drugs against *Acinetobacter baumannii*, *Enterobacter cloacae*, *K. pneumoniae*, and other *Klebsiella spp.* They reported that these bacteria have attained high levels of resistance to quite a lot of antimicrobial drugs.

Cockroaches are the most household notorious pests. They scavenge in the kitchens, storerooms, and bathrooms seeking food and shelter. In Kuwait, American cockroaches are widespread, and many people are apprehensive about their manifestation in households due to their repulsive and annoying characteristics. Cockroaches can survive in practically any environment such as moist areas and sewage systems. Mullins (2015) reported that improper sewer construction, pipe networks, and waste composition play a substantial role in sustaining cockroach infestations in residential areas. In Kuwait, most of the sewer pipe infrastructures were renovated and replaced by new piping systems with one-way valves that can prevent cockroaches and rats from re-entering once they move out. Despite that, cockroaches are still widespread and problematic, and their infestation is challenging for many people. Consequently, people always put a great effort into combating cockroaches using different types of insecticides, but unfortunately, most of them are not aware of their medical importance as vectors for

pathogens with multiple health risks. Our study, which is the first in Kuwait, could pay the attention of people and public authorities to the threats held by this insect. We know that it is impossible to eradicate cockroaches from homes, but following regular and continuous cleaning schemes and using certified insecticides under professional supervision may minimize their massive uncontrolled infestation.

## CONCLUSION

Cockroaches are one of the world's most resilient pests and controlling them requires a prolonged investigation to understand their survival capability in hostile habitats. Therefore, the present study was designed to examine the pathogenicity of domestic household American cockroaches. This pest is able to mechanically carry highly resistant pathogenic bacteria and constitutes an important reservoir for human health-threatening microbiota. Thus, the Kuwaiti Ministry of Health is obligated to implement health awareness programs concerning public health risks associated with cockroaches in residential areas and occupied buildings.

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