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Isolation and Identification of some Pathogenic Bacteria from the External Surfaces and Digestive Tract of the American Cockroach (Periplaneta americana)

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Abstract

The American cockroach (Periplaneta americana) A common pest in city environments, its potential role in the transmission of pathogenic microorganisms is known. This research aims to isolate and identify bacterial species found on exoskeleton and inside the gastrointestinal tract of this insect. The samples were collected from residential buildings using sterile trapping methods. The exterior surfaces and gut were emptied under sterile conditions to obtain bacterial samples. The samples were cultured on general and selective food media, then subjected to microscopic examination after gram dye, and biochemical tests to determine Bacterial species. Blood agar, central MacConkey agar, Mannitol salt agar medium, Salmonella-Shigella agar medium, Eosin Methylene Blue agar, and Nutrient agar medium the results showed the presence of several types of bacteria, including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus. These findings point to the potential role this insect may play in mechanically transporting bacteria in human environments. Background: Periplaneta americana, commonly known as the American cockroach, is increasingly recognized as a vector for pathogenic and antibiotic-resistant bacteria in both residential and healthcare settings. This study aimed to isolate, identify, and analyze the bacterial load present on the external surfaces and in the digestive tracts of these cockroaches. Methods: A total of 25 P. americana specimens were collected from human dwellings in urban areas. Bacterial isolation was performed from both the external surface and gut content using standard microbiological techniques. Gram staining, biochemical testing, and Vitek2 system analysis were employed for identification and characterization of bacterial isolates. Results: A total of 200 bacterial isolates were obtained—100 from external surfaces and 100 from gut contents. Among these, 80% were Gram-negative and 20% Gram-positive. The predominant species were Escherichia coli (45%), Klebsiella pneumoniae (30%), Staphylococcus aureus (15%), and Pseudomonas aeruginosa (10%). The presence of multidrug-resistant organisms, including ESBL-producing and metallo-beta-lactamase-producing strains, highlights the potential of cockroaches to act as reservoirs and transmitters of antimicrobial resistance genes.

Keywords: American cockroach, Periplaneta americana, bacterial isolation, gut, external.

عزل وتحديد بعض البكتيريا المسببة للأمراض من الأسطح الخارجية والجهاز الهضمي للصرصور الأمريكي *(Periplaneta americana)*

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الخلاصة

يُعد الصرصر الأمريكي من الآفات الحضرية الشائعة، ويُعرف بدوره المحتمل في نقل الكائنات الحية الدقيقة الممرضة. يهدف هذا البحث إلى عزل وتشخيص الأنواع البكتيرية الموجودة على السطح الخارجي وفي الجهاز الهضمي لهذه الحشرة تم جمع عينات من الصراصير من مبانٍ سكنية باستخدام طرق اصطياد معقمة. شملت عملية أخذ العينات مسح السطح الخارجي باستخدام محلول ملحي (normal saline) وتشريح الجهاز الهضمي تحت ظروف معقمة. زُرعت العينات على أوساط غذائية عامة وانتقائية، مثل وسط الأغار المغذي، وأغار ماكونكي، وأغار الدم وغيرها. خضعت العزلات للفحص المجهري بعد صبغة غرام، بالإضافة إلى اختبارات كيميائية حيوية، وتم استخدام نظام Vitek-2 Compactلتأكيد التشخيص تم عزل وتشخيص عدة أنواع من البكتيريا، من أبرزهاEscherichia coli ، وKlebsiella pneumoniae، وPseudomonas aeruginosa، و Staphylococcus aureus. لوحظت كثافة بكتيرية أعلى في عينات الجهاز الهضمي مقارنة بالمسحات الخارجية. تُعد معظم الأنواع المعزولة بكتيريا ممرضة أو انتهازية. أجار الدم ، أجار ماكونكي المركزي ، وسط أجار ملح مانيتول ، وسط أجار السالمونيلا الشيجيلا ، أجار إيوسين الميثيلين الأزرق ووسط أجار المغذيات تؤكد نتائج هذه الدراسة وجود بكتيريا ذات أهمية طبية على الصرصور الأمريكي وداخل جهازه الهضمي، مما يشير إلى دوره الرئيسي كناقل ميكانيكي للعدوى في البيئات السكنية. وتبرز هذه النتائج

أهمية اتخاذ تدابير فعالة في مجال مكافحة الآفات والنظافة العامة للحد من المخاطر الصحية المرتبطة بانتشار الصراصير الخلفية: يُعترف بشكل متزايد بأن صرصور "بيريبلانيتا أمريكانا"، المعروف باسم الصرصور الأمريكي، ناقل للبكتيريا المسببة للأمراض والمقاومة للمضادات الحيوية في كل من البيئات السكنية ومراكز الرعاية الصحية. هدفت هذه الدراسة إلى عزل وتحديد وتحليل الحمولة البكتيرية الموجودة على الأسطح الخارجية وفي الجهاز الهضمي لهذه الصراصير الطريقة جُمعت 25 عينة من صرصور "بيريبلانيتا أمريكانا" من مساكن بشرية في المناطق الحضرية. عُزل الصرصور من السطح الخارجي ومحتوى الأمعاء باستخدام تقنيات ميكروبيولوجية قياسية. استُخدمت صبغة غرام، والاختبارات الكيميائية الحيوية، وتحليل نظام "فيتيك 2" لتحديد وتوصيف العزلات البكتيرية النتائج :تم الحصول على 200 عزلة بكتيرية - 100 من الأسطح الخارجية و100 من محتويات الأمعاء. من بين هذه العزلات، كانت 80% منها سلبية الغرام و20% موجبة الغرام. كانت الأنواع السائدة هي الإشريكية القولونية (45%) والكلبسيلة الرئوية (30%)، والمكورات العنقودية الذهبية (15%) والزائفة الزنجارية (10%). ويُبرز وجود كائنات مقاومة للعديد من الأدوية، بما في ذلك سلالات مُنتجة لإنزيم بيتا لاكتاماز المُستَهْلَك (ESBL) وميتالو بيتا لاكتاماز، قدرة الصراصير على العمل كمستودعات وناقلات لجينات مقاومة مضادات الميكروبات .

1. Introduction

The largest among common household cockroaches, this cockroach can be easily found in commercial and large buildings such as restaurants, grocery stores, bakeries and any place where food is prepared and stored. Mass migrations of the common Americana. The American cockroach, Periplaneta americana (Linnaeus), is the largest of the most common domestic cockroach species, with an average length of 4 cm. It is found in residential and commercial buildings. The cockroach is primarily found in steam warming burrows or buildings Expansive organization. The American cockroach is moment as it were to the German cockroach in terms of populace thickness [1]. The American cockroach has spread all over the world by exchange, primarily found in storm cellars and sewer channels, the American cockroach is once in a while found in homes, in any case the disease can happen after overwhelming downpours. It can create into colossal numbers, with more than 5,000 cockroaches some of the time found in person sewer gaps[2].

This cockroach is spread all over the world, especially in tropical and subtropical regions, and is one of the species that migrate easily through shipments of goods and furniture in the form of egg bags (ootheca) or whole or nymphs and adapts to complex urban environments, lives in residential buildings, stores, food institutions, and health facilities, and can be easily moved from one place to another or from one sad to another through sewage pipes and the abundance of its spread is attributed to its ability to reproduce rapidly and withstand diverse environmental conditions, Which is difficult to fight[3]. This insect has a mutation minus incomplete metamorphosis) Where the egg and nymph and the full adult pass their life cycle. In adulthood, The American cockroach lays (12-16) eggs in a membranous sac called ootheca. Eggs hatch after an incubation period depends on temperature and relative humidity to the first nymphs where the nymphs (6-14) melt before turning into adults. It was noted that the American cockroach has (9-14) nymph phase when it is raised alone at room temperature, this intra-species difference in the number of nymph phases is a common phenomenon in under mutated insects and is affected by environmental and genetic factors[4].

knowing that cockroaches are an important factor in the spread of many pathogens, especially those that are located in the human gut, which is more important in transporting these pathogens mechanically compared to house flies, however, it is difficult to consider cockroaches as a real factor in the transfer of pathogens because they can be transmitted by other means [5].

2. Materials and Method:

2.1. Isolation and purification of bacteria from external surfaces

The bacteria on the exterior of the body of the cockroaches confined the bunch by setting them in sterile plastic holders and after that including 10 millilitres of sterile physiological saline, shaking the cockroaches well for two minutes. The cockroaches were taken out of the tubes, and the remaining fluid containing the Bacteria was removed by centrifuge at a speed of 2000 r/min for 10 minutes, the floating upper liquid was disposed of and the sediment containing the bacteria was taken for the purpose of growing it on plates containing the appropriate nutrient media (blood aquarium medium). Blood agar, central MacConkey agar, Mannitol salt agar medium, Salmonella-Shigella agar medium, Eosin Methylene Blue agar, and Nutrient agar medium the dishes were then incubated in the incubator in aerobic conditions at 37 ° C for 24 hours. Bacterial isolates purification was performed by replanting single colonies of which are in the same growing media[6].

2.2 Isolation and purification of bacteria from the inner intestine.

The cockroaches were dismembered and after that the internal insides of the cockroaches were confined and their surfaces washed with 70% ethyl for 5 minutes, at that point cleared out to dry at room temperature in sterile conditions. At that point I washed with sterile physiological saline for 2-3 minutes to evacuate follows of liquor. The insides of the cockroaches were dissected under an anatomical magnifying lens employing a sterile creepy crawly dismember slicer unit. The rebellious were plunged in ethanol and sanitized with fire between each cutting. 10 ml of sterile physiological saline was included to the expelled digestive system and blended well, For the reason of getting the microscopic organisms, the fluid containing the bacteria was expelled by a centrifuge at a speed of 2000 cycles / minute for 10 minutes, the floating upper liquid was discarded and the precipitate was taken and grown on dishes containing food media (Blood agar, MacConkey agar, Mannitol salt agar, Salmonella-Shigella agar medium)., Eosin Methylene Blue agar medium, Nutrient agar medium). Dishes are incubated in the incubator in air conditions with a temperature of 37 ° C and for 24 hours. Purification of bacterial isolates was carried out by replanting individual colonies on the same culture media as above[6].

2.3 Bacterial Identification.

Bacterial isolates were diagnosed based on the characteristics of colonies growing in solid culture media such as blood agar base, MacConkey agar and chocolate agar, the microscopic characteristics of Cram-colored pigmented bacterial cells, and the results of biochemical tests. The diagnosis of bacterial isolates was confirmed using the Vitek2 device.

3. Results and Discussion:

3.1 Bacterial Isolation and Purification

Bacteria were isolated and purified from the outer surfaces and inner intestines of the cockroaches collected, 100 bacterial isolates were obtained, of which (80%) were negative for Gram stain, while 20 isolates (20%) were Gram stain (Fig. 1-1). While 100 bacterial isolates were obtained from the inner intestines of cockroaches, 80 (80%) were negative for Gram stain, while 20 were Isolation (20%) positive for Gram stain (Fig. 1).

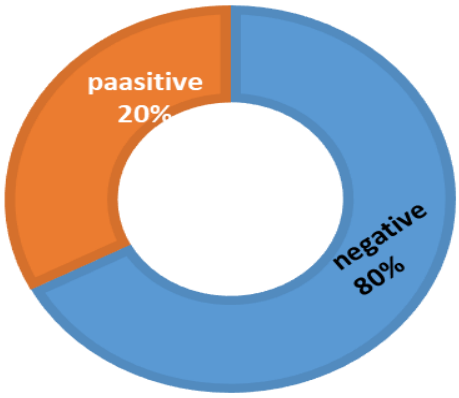

Figure -1 Proportion of Gram stain and negative bacterial isolates isolated from the outer surfaces of cockroaches.

Table 1- Gram Stain Results of Isolates.

Sample Source	Gram-Positive Bacteria	Gram-Negative Bacteria
External Surface (n=100)	20%	80%
Gut Content (n=100)	20%	80%

The reason for isolating the Gram-negative bacteria in such a high percentage may be due to its high resistance to the environmental conditions in which the American cockroach lives. This resistance is related to the difference in the cellular structure of this group of bacteria compared to the Gram stain bacteria, where the Cram-negative bacteria have an outer membrane, which consists of layers of polyglycolic lipids Lipopolysaccharide and protein molecules, so these bacteria are more resistant[7].

3.2 Bacterial Identification:

Bacterial isolates were diagnosed based on the characteristics of colonies growing in solid culture media, the microscopic characteristics of Gram stain pigmented bacterial cells, and the results of biochemical tests. The diagnosis of bacterial isolates was confirmed using the Vitek2 device.

Table 2- Types of bacteria and sources.

Bacterial Species	% Isolated	Source
Escherichia coli	45%	External and gut
Klebsiella pneumoniae	30%	External and gut
Staphylococcus aureus	15%	External and gut
Pseudomonas aeruginosa	10%	External and gut

Table 3- Next Results of the Vitek2 device.

Type of bacteria	Diseases caused by	Diagnosed
Escherichia coli	Food poisoning, diarrhea, urinary tract infections	Yes
Klebsiella pneumoniae	Pneumonia, urinary tract infections, wound infections	Yes
Pseudomonas aeruginosa	Respiratory tract infections, wound infections, blood infections	Yes
Staphylococcus aureus	Skin infections, food poisoning, bone and joint infections	Yes

Table 4- Distribution of Bacteria Isolated from External and Internal Samples.

Bacterial Species	External Surface (% of isolates)	Gut Content (% of isolates)
Escherichia coli	20%	25%
Klebsiella pneumoniae	12%	18%
Staphylococcus aureus	8%	7%
Pseudomonas aeruginosa	5%	5%
Total	45%	55%

Reliable with this ponder in clinics, it was illustrated that Periplaneta americana can act as a potential vector of hospital-acquired diseases, particularly for the transmission of drug-resistant microbes. These household creepy crawlies moreover have a part in transmitting foodborne infections. The nearness of microbes for these creepy crawlies has been analyzed with seven million in each drop emitted and up to 14 million on their skin. Their nearness decreases human wellbeing and clean measures. Goals This graphic ponder was conducted to affirm that Periplaneta Americana, I do not know what to do Dictyoptera. Blattidae carries pathogenic microscopic organisms in private regions, through bacteriological investigation of the skin of this creepy crawly. Materials and strategies. In this investigate, we caught 25 American cockroaches from human private locales in Ahvaz Governorate amid a long time (2008 and 2009). Cockroaches were collected utilizing sticky traps, vacuum cleaners and coordinate collection. The collected cockroaches were brought to the research facility and distinguished as P. americana. These cockroaches were inspected for the nearness of microbes on their external surfaces utilizing particular standard strategies of bacterial contamination. Comes about. All cockroaches had a bacterial contamination. The development of outside wash congeners brought about within the confinement of E. coli microscopic organisms, protease, Serratia, klebsiella, enterobacterium, Pseudomonas aeruginosa, Staphylococcus aureus, streptococcus, Enterobacter, and micrococcus. Conclusions. The comes about appeared that P. americana microscopic organisms are a supply and potential vector of certain pathogens[8].

The findings from your study on the isolation of bacteria from the external surfaces and digestive tract of the American cockroach (Periplaneta americana) align with recent research highlighting the role of cockroaches as vectors for pathogenic and antibiotic-resistant bacteria. Prevalence of Pathogenic Bacteria in Cockroaches The identification of Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli is in line with previous research. For example, 96.6% of cockroaches in Babylon, Iraq, were discovered to be contaminated with different bacteria, such as Staphylococcus species

and E. coli. Similarly, E. coli and other intestinal infections were found in cockroaches from food-related situations in a study conducted in Pudong, China[9].

Antibiotic Resistance Concerns a rising public health concern is the existence of germs in cockroaches that are resistant to antibiotics. According to a study conducted in Southern Ethiopia, cockroaches taken from hospitals contained metallo-beta-lactamase-producing and extended-spectrum gram-negative bacteria, such as Klebsiella species and E. coli. Additionally, studies have demonstrated that cockroaches can carry genes that cause antibiotic resistance, highlighting their part in the transmission of resistance in human contexts[10].

Cockroach Gut Microbiota and Antimicrobial Activity It's interesting to note that some research has looked into the possibility of cockroach gut bacteria producing antibacterial substances. Cockroach-associated bacteria may play a complex role in both sheltering and possibly battling diseases, as evidenced by the discovery of bacterial species from the stomach of P. americana that show antagonistic action against multidrug-resistant human pathogens[11].

Table 5- Antimicrobial Activity and Source of Cockroach Gut Bacteria

Research Focus	Finding
Antibacterial compound production	Cockroach gut bacteria produce substances active against MDR pathogens
Dual role	Gut bacteria both shelter pathogens and exhibit antagonistic activity
Source	Bacterial species isolated from *Periplaneta americana* gut

3.3 Implications for Public Health

Effective pest control procedures are crucial, particularly in settings like kitchens, food storage spaces, and healthcare facilities, as evidenced by the numerous studies that consistently found dangerous and antibiotic-resistant bacteria in cockroaches. In order to better understand and reduce the hazards associated with cockroach-borne bacterial transmission, public awareness programs and additional study employing molecular approaches are advised[12].

4. Discussion

The present study demonstrates that Periplaneta americana, the American cockroach, carries a significant load of both Gram-negative and Gram-positive bacteria on its external surfaces and within its digestive tract. Of the 200 bacterial isolates obtained (100 from external surfaces and 100 from gut contents), 80% were Gram-negative and 20% were Gram-positive. This finding supports existing literature indicating that Gram-negative bacteria, due

to their outer membrane composed of lipopolysaccharides and proteins, are more resistant to environmental stressors and survive longer on insects' bodies and in harsh habitats[13].

4.1. Role of Cockroaches as Vectors of Pathogenic Bacteria

The findings regarding the prevalence of Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa are consistent with prior reports. Studies from Iraq and China have shown a high rate of contamination among cockroach populations with pathogenic bacteria, especially E. coli and Staphylococcus spp., particularly in domestic kitchens and food-handling areas[14].

Moreover, similar investigations in Ethiopia, Iran, and Nigeria have emphasized that hospital cockroaches harbor multiple clinically significant pathogens, including those responsible for urinary tract infections, pneumonia, and skin diseases. In The 2024 study in Southern Ethiopia reported that cockroaches carried metallo-beta-lactamase (MBL)-producing and extended-spectrum β-lactamase (ESBL)-producing strains of Klebsiella and E. coli, raising public health concerns about their role in the spread of antibiotic-resistant organisms[15].

4.2. Antibiotic Resistance and Public Health Threat

Recent global surveillance (2023–2025) has increasingly associated cockroaches with antibiotic-resistant bacteria, including methicillin-resistant S. aureus (MRSA) and carbapenem-resistant Enterobacteriaceae[16].These multidrug-resistant organisms (MDROs) pose a serious threat, especially in healthcare facilities where cockroach infestations may lead to nosocomial outbreaks. For instance, a hospital ICU outbreak caused by Enterobacter cloacae was directly linked to cockroach presence and only resolved following intensive pest control interventions[17].

In addition, genomic studies have shown that cockroaches serve as reservoirs of resistance genes (ARGs) even in the absence of direct antibiotic exposure. Their gut and body-surface microbiota may contribute to horizontal gene transfer (HGT) of resistance elements in human environments, which aligns with the One Health approach linking human, animal, and environmental health[18].

Table 6- Role of Cockroaches in the Transmission of Antibiotic-Resistant Bacteria

Aspect	Description
Associated MDROs	Methicillin-resistant Staphylococcus aureus (MRSA), Carbapenem-resistant Enterobacteriaceae (CRE)
Healthcare Impact	Outbreaks in hospitals and ICUs; e.g., Enterobacter cloacae outbreak linked to cockroach infestation
Reservoir Role	Harbor antimicrobial resistance genes (ARGs) in gut and on body surface
Gene Transfer Mechanism	Facilitate horizontal gene transfer (HGT) of resistance elements between microbes in the environment
Antibiotic Exposure Requirement	Resistance genes found even without direct antibiotic exposure, indicating environmental persistence
One Health Implication	Highlights the importance of integrated approaches connecting human, animal, and environmental health in addressing antibiotic resistance

4.3. Cockroach Gut Microbiota: Potential for Antimicrobial Discovery.

Interestingly, some bacterial isolates from the gut of P. americana have been reported to produce antimicrobial compounds with activity against multidrug-resistant human pathogens. A 2025 study found that gut bacteria from cockroaches synthesized streptophenazines and actinomycins, which showed strong antagonistic activity against MRSA and Pseudomonas aeruginosa[19]. This dual role suggests that while cockroaches are vectors of disease, their microbial communities may also be a promising source for novel antibiotics.

Table 7- Source for novel antibiotics

Compound	**Source**	**Target Pathogens**	**Activity**
Streptophenazines	Cockroach gut bacteria	MRSA, *Pseudomonas aeruginosa*	Strong antagonistic activity
Actinomycins	Cockroach gut bacteria	MRSA, *Pseudomonas aeruginosa*	Strong antagonistic activity

4.4. Environmental and Domestic Risk

In residential and food storage environments, cockroaches contribute to microbial contamination of surfaces, utensils, and food. Research indicates that a single cockroach may carry up to 14 million bacteria on its external surface and excrete millions more in its feces, potentially contaminating food and fomites in kitchens and storage areas[20].Their ability to move freely between unsanitary sites (e.g., drains, toilets) and human dwellings increases their potential to spread harmful microorganisms.

4.5. Recommendations and Control Measures

Given their demonstrated role in the transmission of infectious and drug-resistant organisms, it is critical to implement strict pest control programs in hospitals, homes, and food-handling areas. This includes:

- Integrated pest management (IPM) using physical, chemical, and biological controls.
- Routine monitoring and microbial surveillance of cockroach populations.
- Public education about proper sanitation and the health risks posed by cockroaches.
- Advanced molecular techniques, such as 16S rRNA gene sequencing and metagenomics, to identify emerging pathogens and resistance genes.

5. Conclusion

According to this study, the American cockroach (Periplaneta americana) serves as a major mechanical vector for a number of opportunistic and pathogenic bacteria, including as Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Cockroaches taken from residential settings had these bacteria extracted from their exterior surfaces and digestive tracts, with the gut samples having a significantly greater bacterial load. Due to structural characteristics like an outer membrane rich in lipopolysaccharides, Gram-negative bacteria are more likely to survive in hostile settings, as evidenced by their preponderance in both exterior and internal isolates. These characteristics increase their resilience to disinfectants and desiccation, which helps explain why they persist on insect carriers. These conclusions are supported by recent research showing that cockroaches carry and spread virulent and multidrug resistant bacterial strains, which may exacerbate foodborne and hospital-acquired diseases. Because of the potential for antimicrobial resistance to spread, their presence in kitchens, sewers, and healthcare facilities is a major public health problem. Given P. Americana's extensive range and tenacity, immediate and ongoing pest management measures are necessary. Campaigns for public health should highlight the dangers of cockroach infestations and encourage hygienic procedures in locations where food is handled and garbage is disposed of. Future studies should also use molecular diagnostic techniques to precisely identify bacterial species and resistance genes, improving our comprehension of their epidemiological significance.

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