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## RESEARCH ARTICLE

## Antibiotic resistant bacteria associated with the cockroach *periplaneta americana* in Al-Diwaniya city / Iraq

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### Abstract

Eighty samples of adult American cockroach *Periplaneta americana* were collected from different parts in Al-Diwaniya City which included 40 samples from Al-Diwaniya teaching hospital and 40 samples from houses during period between the beginning of March to the end of June 2014 in order to isolate and identify bacterial pathogens from the external surface and digestive tract of American cockroach and determined the susceptibility of these bacterial strains to different antibiotics.

The present study showed Isolated nine species of bacteria from external surface and digestive tract of American cockroach were: *Klebsiella* (42.56%), *Pseudomonas* (38.61%), *Proteus* (35.34%), *Escherichia coli* (19.84%), *Shigella sonnei* (17.09%), *Salmonella* spp. (14.43%) *Staphylococcus* (13.82%), *Streptococcus* (10.89%) and *Bacillus* (5.13%). bacterial pathogens which isolated from *periplaneta americana* indicate that cockroaches are among the medically important pests found within the human houses and hospitals that cause serious health problems therefore must be combated.

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### Introduction

American cockroaches are found in association with human dwellings and hospitals. They have a worldwide distribution. These domestic pests affect human health in several ways. Their habits make them ideal mechanical carriers of different pathogenic Microorganisms. Numerous bacteria of medical importance have been isolated from cockroaches. American Cockroaches are often found in intimate association with human beings and are present in large numbers around houses or hospitals and in urban areas and villages with poor sanitation and in salubrious conditions<sup>(1)</sup>. Furthermore, their feeding mechanisms and filthy breeding and transmitting pathogenic bacteria<sup>(2)</sup>. American Cockroaches comes in contact with human sewage through sewer systems where they can live, and from there also are able to get into bathrooms and basements<sup>(3)</sup>. Various bacteria may simply be carried on the insect's cuticle or be ingested and sometime later regurgitated or excreted. Moreover, several species of bacteria of public health significance have been isolated from external surface and digestive tract of Cockroaches, such as *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia* and *Pseudomonas aeruginosa*<sup>(4)</sup>. Cockroaches collected in hospitals and households have been found to harbor multi-drug resistant bacteria and hospital Cockroaches with drug resistant *Klebsiella* spp. Have been suggested to play a role in the epidemiology of nosocomial infections<sup>(5)</sup>. In addition a neonatal unit infested with Cockroaches<sup>(6)</sup>. Suffered an outbreak of nosocomial disease due to extend – spectrum B-lactamases- producing *Klebsiella pneumoniae*.

In present study Cockroaches *periplaneta americana* where collected from Al-Diwaniya teaching hospital and many houses in Al-Diwaniya city and bacteria was isolated from the external surface and digestive tract. Afterward, we determined the susceptibility of these isolated bacterial strains to different antibiotics.

### 2. Material and Methods

## 2.1 Collection of cockroaches

Eighty cockroaches (40 from Al-Diwaniya teaching hospital and 40 from houses) were collected in Al-Diwaniya city at night time or in the morning during period between the beginning of March to the end of June 2014. Each cockroach was collected put in a sterile test tube then transported to the laboratory of parasitology in Education College of Al-Qadisiya University.

## 2.2 Preparation of samples

*P. americana* cockroaches anesthetized by freezing at 0°C for 5 minutes. 2ml of sterile normal saline (0.9%) was added to each test tube and the cockroaches were thoroughly shaken for 2 minutes.

## 2.3 Isolation bacteria from external surface of cockroach

A fixed volume (0.01mL) of the saline washing was spread on to plates of selective and non-selective media (Staphylococcus medium, azide blood agar, starch nitrate agar, brilliant green, MacConkey agar, blood agar, nutrient agar and brain heart infusion). The plates were incubated at 30°C for 48 hours. Bacterial count forming units (CFU) were determined and referred per ml.

## 2.4 Isolation bacteria from digestive tract of cockroach

After external washing, cockroaches were placed in flask, rinsed with 70% alcohol for 5 minutes (to decontaminate external surface), then transferred to sterilized flake and allowed to dry at room temperature under sterile conditions. Cockroaches were then washed with normal saline for 2-3 minutes to remove traces of alcohol. The digestive tract of each cockroaches was dissected out and macerated aseptically in a sterile pestle and mortar in 2ml of sterile normal saline. A similar way as described previously was done and culture were examined and results were read and colonies identified.

## 2.5 Purification of bacterial isolates

The best growing colonies and the most characteristic ones were picked up by sterile loop and subjected to purification in the same isolation Medium. Agar streak method was used for purification process. A well separated colony from each isolate was picked up on nutrient agar slopes and incubated at  $28.0 \pm 0.1^\circ\text{C}$  for 24 hours. Purity was checked by microscopic examination of the isolate using Gram stain. All cultures were maintained under aerobic conditions.

## 2.6 Identification of bacteria

The best growing colonies and the most characteristic ones were picked up and purified by agar streak method. The identification process was preceded as follow:-

### 2.6.1 Morphological identification

Gram stain; Jensen's modified method was applied using crystal violet as a basic dye and safranin as counter stain<sup>(7)</sup>.

### 2.6.2 Physiological and biochemical identification

Many biochemical reactions were preceded for identification of bacteria according to the keys of (8), (9) and (10). Some of these tests were sensitivity to KCN, Catalase, oxidase, coagulase, acid production from carbohydrates, IMViC, H<sub>2</sub>S production, citrate utilization and growth in triple sugar iron agar medium.

## 2.7 Antimicrobial susceptibility using disc diffusion method (Kirby-Bauer) test

With a sterile cotton applicator, 4-5 well isolated colonies were transferred to a saline solution tube following sterile techniques. The inoculums were calibrated with a 0.05 McFarland standard. Using another cotton-tipped-sterile applicator, the Mueller Hinton agar plate was inoculated, streaking the entire surface of the plate, rotating the plate 60° between streaks and ultimately rimming the plate to ensure confluent growth to the edges. After 2-3 minutes, a mechanical dispenser was used to apply the discs. All plates were incubated at 37°C for 18-24 hours before final reading by using a caliber to measure the zone of inhibition. The size of the zone of inhibition (mm) will determine if the bacterium is resistant or susceptible to different antibiotics based on methods recommended by the CLSI<sup>(11)</sup>. Quality control was carried out according to the recommendations of the CLSI using American Type Culture Collection (ATCC) strains as controls. Nine antibiotics were tested: amikacin (AK), ampicillin (AMP), cefotaxime

(CTX), ceftriaxone (CRO), chloramphenicol (C), ciprofloxacin (CIP), gentamycin (CN), nalidixic acid (NA), tetracycline (TE).

### 3. Results and Discussion

American cockroaches have been considered transmitters and spreaders of pathogenic bacteria in hospitals and houses or residential areas<sup>(12)</sup>. Cockroaches can be a real sanitary hazard as they are known to carry bacteria, fungi, helminthes and viruses as well as their capacity for disseminating bacteria. Cockroaches feed indiscriminately on garbage and sewage and so have copious opportunity to disseminate human pathogen<sup>(6)</sup>. Also their nocturnal and filthy habits make them ideal carriers of various pathogenic microorganisms<sup>(13)</sup>. So far numerous pathogenic bacteria, including Salmonella spp, Shigella spp, Campylobacter spp, Pseudomonas aeruginosa and K. pneumoniae have been isolated from cockroaches. In addition some parasites and fungi have been found in external surfaces or internal parts of body of cockroaches<sup>(14)</sup> and some study have shown that exposure to cockroach antigens may play an important role in asthma-related health problems<sup>(15)</sup>.

#### 3.1 Isolation, population and identification of bacteria

Bacteria isolated from the external surface and digestive tract of cockroaches (*Periplaneta americana*), were higher in numbers from houses than hospital, for instance starch medium recorded  $3 \times 10^5$  than  $0.5 \times 10^4$  CFU/ml, blood agar medium recorded  $2 \times 10^4$  than  $4.5 \times 10^5$ ; respectively, (Table 1). Obviously, non-selective media showed higher number of bacteria from both Cockroach digestive tract and external surface than recorded in selective media. A study was carried by<sup>(16)</sup> showed isolated fifty-one species of bacteria on various bacteriological nutritive media. A variety of media used enabled us to isolate large number of bacteria in addition to different types of bacteria resident in or on cockroach. This is in contrast to<sup>(1)</sup> who used 3 types of media viz. MacConkey agar, Chapman agar, and Bile Esculin agar and isolated few types of bacteria. However, our results of bacterial population, in general is concomitant with bacterial population obtained from cockroaches trapped from urban environment by<sup>(17)</sup> and cockroaches (*Diploptera punctata*) by<sup>(18)</sup>. The general trend of bacterial count whether from houses or digestive tract was 1: increase in bacterial population in enrichment media such as blood agar and nutrient agar than other media, 2: increase in gram negative bacteria than gram positive and 3: decrease in bacterial population of cockroach digestive tract from hospital than houses.

**Table (1):- The viable plate count (CFU/ml) of microbial for external surface and digestive tract of *P. americana* isolated from hospital and houses on different types of media.**

Cockroaches		Selective medium				Non-Selective medium			
		1*	2*	3*	4*	5*	6*	7*	8*
hospital	External	$1.5 \times 10^3$	$0.5 \times 10^3$	$0.5 \times 10^4$	$0.5 \times 10^3$	$1 \times 10^3$	$2 \times 10^4$	$2 \times 10^4$	$0.5 \times 10^3$
	Internal surface	$0.5 \times 10^2$	$4 \times 10^2$	$0.2 \times 10^2$	$0.5 \times 10^2$	$0.5 \times 10^2$	$1 \times 10^3$	$0.5 \times 10^2$	$1 \times 10^2$
Houses	External	$3.5 \times 10^4$	$3.5 \times 10^4$	$3 \times 10^5$	$3 \times 10^4$	$3.5 \times 10^4$	$4.5 \times 10^5$	$3.5 \times 10^5$	$1 \times 10^4$
	Internal surface	$2 \times 10^2$	$2 \times 10^2$	$0.1 \times 10^2$	$2 \times 10^2$	$2 \times 10^2$	$2 \times 10^3$	$1.5 \times 10^2$	$2 \times 10^2$

1\*Staphylococcus medium

2\*Azide blood agar

3\* Starch agar medium

4\* Brilliant green bile 2% medium

5\* MacConkey agar medium

6\* Blood agar medium

7\* Nutrient agar medium

8\* Brain heart infusion medium

Among the 362 bacterial isolation from American cockroaches 64 (17.679%) belonged to the group of Gram-positive bacteria 4 (3.1%) in hospital and 21(9.01%) in houses to staphylococci, 2 (1.55%) from external surface and 1 (0.77%) from digestive tract in hospital and 18 (7.72%) from external surface and 2 (0.85%) from digestive tract in houses to streptococci. On the other hand, percentage of *Escherichia coli* bacteria from hospital and houses samples

(from external surface and digestive tract) recorded 2.32%, 4.65%, 4.72%, 8.15% respectively (Table 2). The most frequent bacteria isolated from American cockroaches coming from all samples were *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli*, *Shigella* spp., *Proteus* spp., *Staphylococcus aureus*, *Staphylococcus* spp.

In addition *Streptococcus* sp., and *Listeria seeligeri* were more frequently isolated from houses samples in comparison with hospital samples, (Table 3). Although<sup>(19)</sup> captured 1600 adult cockroaches, they isolated only 12 *Salmonella*, 2 each of *Shigella* and *E. coli* O157, 17 *Staphylococcus aureus* and 24 *Bacillus cereus* from all samples. However, the obtained bacteria were similar to those isolated in this study as samples collected from urban.

**Table (2): Percentage of main groups bacteria associated with the *P. americana*.**

Group	Cockroach			
	hospital		Houses	
	external surface	digestive tract	external surface	digestive tract
<b>Gram positive bacteria</b>				
<b>Staphylococcus spp.</b>	3.1	-	9.01	1.71
<b>Streptococcus spp.</b>	1.55	0.77	7.72	0.85
<b>Bacillus spp.</b>	-	-	3.43	1.7
<b>Gram negative bacteria</b>				
<b>Escherichia coli</b>	2.32	4.65	4.72	8.15
<b>Shigella sonnei</b>	1.55	3.1	4.29	8.15
<b>Salmonella spp.</b>	3.87	5.42	0.42	4.72
<b>Klebsiella spp.</b>	3.87	23.25	5.57	9.87
<b>Pseudomonas spp.</b>	3.1	20.93	6.43	8.15
<b>Proteus spp.</b>	3.1	19.37	3.86	9.01

**Table (3): Bacterial species isolated from *P. americana*.**

Bacteria	Cockroach	
	hospital	houses
<i>Escherichia coli</i>	+	+
<i>Shigella sonnei</i>	+	+
<i>Neisseria mucosa</i>	+	-
<i>Staphylococcus aureus</i>	+	+
<i>Staphylococcus saprophyticus</i>	+	+
<i>Staphylococcus saccharolyticus</i>	+	+
<i>Streptococcus pyogenes</i>	+	-
<i>Streptococcus faecalis</i>	-	+
<i>Streptococcus durans</i>	-	+
<i>Listeria seeligeri</i>	-	+
<i>Bacillus subtilis</i>	+	+

(+) present

(-) absent

### 3.2 Susceptibility to antimicrobial agents

Many authors isolated multi-drug resistant bacteria from cockroaches especially hospital isolates for instance <sup>(20)</sup> found that two gram-positive and five gram-negative bacteria resistant to ampicillin (13.7% to 100%), chloramphenicol (14.3% to 71.4%), tetracycline (14.3% to 73.3%), and trimethoprim-sulfamethoxazole (14.3% to 57.1%), <sup>(21)</sup> found that among the enterobacteria 96% were resistant to gentamicin, 84% to ampicillin, 75.3% to caphalothin, 66.7% to ampicillin-sulbactam, 50% to aztreonam, 30% to chloramphenicol. and among the coagulase negative *Staphylococcus aureus*, 61% were resistant to oxacillin, and finally <sup>(22)</sup> reported that many bacterial strains were resistant to cefotazime and minocycline.

Our results, in general, indicated that Gram-negative bacilli isolated from cockroach were deemed very susceptible to the antibiotics tested. Ampicillin and ciprofloxacin were found to be active against Gram-negative bacilli strains. In addition the following showed excellent activity, although their effectiveness was not 100% cefotaxime, gentamycin, and tetracycline. On the other hand Gram-positive bacilli from cockroach were significantly more resistant to ceftriaxone, ciprofloxacin and nalidixic acid than other antibiotics and our results is agreement with <sup>(23)</sup>.

**Table (4):S uceptibility test of bacterial isolates.**

Organism	AK	AMP	CTX	CRO	C	CIP	CN	NA	TE
<i>E. coli</i>	I	S	I	S	S	S	I	R	S
<i>S. aureus</i>	S	R	R	I	S	S	R	S	R
<i>S. faecalis</i>	R	R	R	R	R	R	R	R	R
<i>N. mucosa</i>	I	R	S	I	S	R	S	S	S
<i>S. sonnei</i>	S	S	S	I	I	S	S	I	I
<i>S. saprophyticus</i>	R	R	S	R	R	I	R	S	R
<i>L. seeligeri</i>	I	R	I	R	S	R	R	R	R
<i>B. subtilis</i>	R	S	S	R	S	R	R	R	R

AK, amikacin; AMP, ampicillin; CTX, cefotaxime; CRO, ceftriaxone; C, chloramphenicol; CIP, ciprofloxacin; CN, gentamycin; NA, nalidixic acid; TE, tetracycline. S, sensitive; I, Intermediate sensitive; R, resistant.

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