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Determination of Carrier Parts of Some Microorganisms in Body of the American Cockroach *Periplaneta americana* in Some Parts of Mosul City

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Abstract

Compared to other organisms, insects are found in all the world. The diversity and evolutional success of insects depend on its relation with useful microorganisms. These microorganisms are found mostly in the intestines and epidermis of the insect. A Cockroach is one of the most important medical insects. All of the species of this insect live in dirty environments especially sewers. There are two species of disease-causing cockroaches: the German cockroach (*Blatella germanica)* and American cockroach (*Periplaneta americana)*. The study aims at defining the role of *P. americana* as a carrier of disease-causing bacteria by isolating and determining these microorganisms from the body of the cockroach. The study included (18) samples of *P. americana*. Each sample was autopsied to get external parts as well as the digestive channel. These specimens underwent a bacteriological study to isolate and diagnose the species of bacteria and which parts carry them. Isolation results showed the diagnosing of (22) isolates of about 13 different species of bacteria, (82%) of isolates came from the external parts of the insect while (18%) isolated from the digestive channel. Most isolates belong to the family Enterobacteriaceae in 68.18%. First place went to *Serratia marcescens* with 36.36% of the isolated species then *Kluyvera intermedia* 13.64% . As for the rest species, all shared a ratio of 4.55%.

Keywords: *Periplaneta americana*, Enterobacteriaceae, *Serratia marcescens*, *Kluyvera intermedia*, Cockroach, Mosul City.

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تحديد الاجزاء الناقلة لبعض الاحياء المجهرية من جسم الصرصر الامريكي *Periplaneta americana* في بعض اجزاء مدينة الموصل

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الخلاصة

مقارنة مع الكائنات الحية الأخرى ، توجد الحشرات في جميع أنحاء العالم. يعتمد التنوع والنجاح التطوري للحشرات على علاقتها بالكائنات الحية الدقيقة المفيدة. وتعد الأمعاء والبشرة الخارجية من اكثر اجزاء الحشرة التي تسكنها الاحياء المجهرية، ان حشرة الصرصر من أهم الحشرات الطبية اذ تتواجد بكافة انواعها في البيئات القذرة خاصة مياه المجاري، يوجد نوعين من حشرة الصرصر الناقلة للمسببات المرضية: الصرصر الألماني (*Blatella germanica*) والصرصر الأمريكي (*Periplaneta americana*). هدفت الدراسة إلى تحديد دور الصرصر الامريكي *P. americana* كناقل للبكتيريا المسببة للأمراض وذلك عن طريق عزل وتحديد هذه الكائنات الدقيقة من اجزاء جسم الصرصر. اظهرت نتائج العزل والتعرف على البكتريا المدروسة لأجزاء (18) عينة صرصرامريكي *P. americana* ، (22) عزلة من حوالي 13 نوعاً مختلفاً من البكتيريا ، (82٪) عزلة بكتيرية من اجزاء الحشرة الخارجية و (18٪) عزلة من القناة الهضمية. معظم الانواع المعزولة تعود لعائلة Enterobacteriaceae بنسبة 68.18٪. واحتلت Serratia marcescens اعلى نسبة 36.36٪ من بين الأنواع المعزولة ثم تليها Kluyvera intermedia 13.64%. بينما احتلت الانواع المتبقية نسبا متساوية 4.55٪.

الكلمات المفتاحية: *Periplaneta americana*، Enterobacteriaceae، *Serratia marcescens*، *Kluyvera intermedia*، Cockroach ، مدينة الموصل

INTRODUCTION

Insects are regarded as the most important species of animal Kingdom, Insects evolution depended on their various relations with other living organisms especially their useful microorganisms (Engel & Moran, 2013). Recently, the scientific connection between microbiology and entomology increased because of the increased interest by researchers and genetic evolution in sequencing techniques (PCR technique) to facilitate defining microorganisms (Engel & Moran, 2013).

Douglas(2016) pointed out that the natural habitats of microorganisms in the body of an insect are the epidermis and intestine, Although the external structure is immune against microscopic colonization, it can be occupied by certain microorganisms, 1000 bacterial cells was found on surface body of *Drosophila melanogaster*, which is less than the number found inside the fly's body. Mechanical factors determine microbial existence on the external surface (Cuticle) of insects such as ecdysis and Adaptive pattern as well as external secretions of insects which may be antimicrobial. Until now, it is unknown the degree of multiplying of microbes on the external cuticle of insects to form a stable colony number, Many insects evolved their cuticles to encourage the multiplying of microbes during ecdysis, The latter becomes a habitat for some fungi.

Five families of cockroach were classified: Cryptocercidae, Blattellidae, Blaberidae, Blattidae, and Corydiidae, There are 4500 species in tropical and semi-tropical areas, the American cockroach is the largest of all species, Its length is 30mm whereas German cockroach is just 15mm (Hashemi-Aghdam et al.,2017; Kassiri et al.,2018).

The most two important species of cockroaches carrying disease-causing bacteria living in houses and kitchens are the German cockroach (*Blattella germanica)* and American cockroach (*P. Americana*). The existence of American cockroach near houses causes a lot of harm because of its harsh smell secretions as well as some allergy diseases like asthma (Akbari et al., 2015).

The hazardous nature of cockroaches comes from its ability to carry four-strains of poliomyelitis virus, brain fever, yellow fever as well as carrying eggs of seven species of worms causing dangerous diseases (Hashemi-Aghdam et al. ,2017).

Al-galebi et al.,(2011) reported that 14 species of fungi were isolated from the external surface of fifty samples of American cockroach collected from different parts of Al- Diwaniya educational hospital.

In Kassiri et al., study (2018) they identified 23species of fungi from the digestion channel of *P. americana* that belongs to *Rhizopus, Aspergillus, Penicillium, Mucorales, Alternaria, Cladosporium, Chrysosporium, Candida, Rhodotorula, Zygosaccharomyces, and Debaryomyces*.

Some studies showed the isolation of different bacterial species from the outer surface of the American cockroach, the most prominent of which are: Klebsiella, Proteus, Escherichia, Staphylococcus spp, Streptococcus, Pseudomonas, and the isolates of Klebsiella spp. were the most common and the least prevalent isolates of Proteus and Staphylococcus (Kassiri et al, 2014), The species *Enterobacter cloaca, Escherichia coli, Escherichia vulneris, Shigella flexineri, Citrobacter freundii* as well as other disease-causing bacteria are found in the intestine of *P. americana* (Akbari et al., 2015; Memona et al.,2017).

The aim of this study to identify the role of *P. americana* as a carrier of disease-causing bacteria through isolating and determining these microorganisms from parts of the cockroach's body gathered from different areas in city of Mosul.

MATERIALS & METHODS

1-Sampling: Adults of *P. americana* of both sexes were collected (Al-Mayali & Al-Aredhi,2013) from different quarters of the city of Mosul from March till May 2019, The sterile gloves were used to collect (18) lively adult cockroaches, samples were put in separate sterilized, clean tubes (Kassiri et al.,2014). Then, transferred to a researches laboratory at the college of Basic Education / University of Mosul for tests.

2-Diagnose samples: Samples were anesthetized by refrigerating them in 0C° for 5-10 minutes to paralyze them and check them using dissecting microscope, diagnose their species according to standard classification key (Kassiri & Kazemi,2012; Salehzadeh et al.,2007; Teama et al.,2012).

3-Dissecting the samples: Samples were put in a sterile Petri dish. Antenna, Dermal wings, Membrane wings Front legs and Back legs cut from the insect body using sterilized anatomy tools, Then isolated parts were put in sterilized small tubes containing 2 ml of sterilized physiological solution

with a concentration of 0.9% NaCl, three replications for each part, tubes were shaken for 5 minutes to obtain liquid containing bacteria (Algalebi et al.,2011). To isolate bacteria from the digestive tract of cockroach, the insect was sterilized by washing it in ethyl alcohol 70% for 2 minutes then by a sterilized physiological solution 0.9% for 2-3 minutes to remove alcohol away and let to dry (Menasria et al., 2014). Then, the head, legs were separated, abdomen opened using thin pointy anatomical tweezers with a small scissor, digestive tract removed using a tiny needle (Atiokeng Tatang et al.,2017). The digestive tract was transferred to tubes(three replications) containing 2ml of sterilizing physiological solution. The digestive tract is squashed inside tubes by sterilized glass pole to get a suspension in order to isolate bacteria from the digestive tract (Akbari et al., 2015; Kassiri et al., 2018; Teama et al., 2012).

4-Sample Culturing: using a sterilized swab, for each prepared bacterial suspension, using a streaking plate method over MacConkey agar, Brain and heart infusion agar poured into sterilized Petri dishes(Prado et al.,2006). MacConkey agar was used due to its selectivity especially for gram-negative bacteria where Lactose fermentation can be recognized. Then Plates were incubated under 37Cº for 24- 48hours. obtain colonies pure using the streaking method on nutrient agar media for several times. Pure cultures are saved in slant nutrient agar media under 4Cº (Maleki-Ravasan et al., 2014).

5-Diagnosis of bacteria: Bacteria was diagnosed by examining the colony morphology on culture media (shape, size, color, the edge of the colony is regular or irregular), After staining the bacteria with a Gram stain, a microscopic examination was done (Brooks et al.,2013). Then Vitek2 device provided by the American BioMerieux company was used to classify bacteria according to their species.

RESULT & DISCUSSION

This study focused on the American cockroach because it is one of the most important types of medical insects, It lives in filthy environments such as sewers, The easiness of its movement and transference from a region to another carrying its disease-causing germs from contaminated areas to human food (Akbari et al., 2015). These insects are also found in hospital environments such as patient's rooms and intensive care units due to an abundance of good circumstances for their reproduction and growth (Naher et al.,2018).

The results of isolation and identification of bacteria in the studied samples of American cockroach parts showed the diagnosis of twenty-two different bacterial isolations. As shown in table 1 most of these isolates (82%) were from external insect parts involving Antenna, front and back legs, dermal, and membrane wings while the smallest percentage about (18%) were isolated from the digestive tract. Moges and his team (2016) showed that out of 181 bacterial isolates, from different cockroach parts, 110 (61%) isolated from external parts while 71(39%) were isolated from internal parts. The high ratio of bacterial species isolated from external parts may belong to high variation in the types of contaminated insects habitats moving around.

Table(1): cockroach parts and species isolated from

Insectal Parts	Bacterial species	Number of isolates
Antenna	*Kluyvera intermedia*	1
	Leuconostoc mesenteroides	1
	Kocuria rosea	1
Dermal wings	*Kluyvera intermedia*	1
	Serratia marcescens	4
	Pseudomonas aerugenosa	1
Membrane wings	*Citrobacter freundii*	1
	Staphylococcus warneri	1
	Serratia marcescens	1
Front legs	*Kluyvera intermedia*	1
	Serratia marcescens	1
	Alcaligenes faecalis	1
	Lactococcus lactis spp.lactis	1
Back legs	*Enterobacter gergovia*	1
	Serratia marcescens	1
Digestive tract	*Streptococcus sanguinis*	1
	Serratia marcescens	1
	Proteus mirabilis	1
	Escherichia coli	1
Total number		22

Returning to table 2 note that most isolated species, which made up about 68.18%, belonged to the family of Enterobacteriaceae. Species of this family

are considered as widely distributed Microorganisms in natural environments and many of their species are common in the intestine of humans and animals, including insects, and sometimes cause enteric diseases (Morales-López *et al*.,2019). Members of Enterobacteriaceae are very medically important organisms that it made up about 50% of all laboratory pathogens isolated from many bacterial infections as sepsis, urinary tract infections, intestinal tract infections, nosocomial infections, meningitis, pneumonia, wound, abscess (Dos Santos *et al*.,2015).

Table (2): the total numbers and percentages of studied bacterial species

Total	percentage	Number	Bacterial species	Family
68.18	36.36	8	*Serratia marcescens*	*Enterobacteriaceae*
	13.64	3	*Kluyvera intermedia*	
	4.55	1	*Citrobacter freundi*	
	4.55	1	*Proteus mirabilis*	
	4.55	1	*Escherichia coli*	
	4.55	1	*Enterobacter gergovia*	
9.09	4.55	1	*Lactococcus lactis spp.lactis*	*Streptococcaceae*
	4.55	1	*Streptococcus sanguinis*	
4.55	4.55	1	*Kocuria rosea*	*Micrococcaceae*
4.55	4.55	1	*Staphylococcus warneri*	*Staphylococcaceae*
4.55	4.55	1	*Pseudomonas aeruginosa*	*Peudomonadaceae*
4.55	4.55	1	*Leuconostoc mesenteroides*	*Leuconostocaceae*
4.5	4.55	1	*Alcaligenes faecalis*	*Alcaligenaceae*

In current study, as shown in figure 1, the species *Serratia marcescens* constituted the highest isolated ones. It represents about 36.36% of all isolates followed by *Kluyvera intermedia* about 13.64% then *Citrobacter freundii*, *Proteus mirabilis*, *Escherichia coli*, *Kocuria rosea*, *Staphylococcus warneri*, *Pseudomonas aeruginosa*, *Leuconostoc mesenteroides*, *Alcaligenes faecalis*, *Lactococcus lactis spp. lactis*, *Enterobacter gergovia*, and *Streptococcus sanguinis* with about (4.55%). Despite the different percentages of the bacterial species that were obtained, the current study agreed with Kassiri and Kazemi, 2012 study in terms of many isolated bacteria as *Klebsiella*, *Pseudomonas*, *Escherichia coli*, *Staphylococcus*, *Streptococcus*, *Serratia*, and *Proteus* and differed from it in some of them. Also, our study agreed with the study of Maleki-Ravasan *et al*.,2014 in terms of isolation of many popular Enterobacteriaceae species as *Serratia*, *Escherichia*, *Kluyvera*, *Klebsiella*, and non Enterobacteriaceae species as *Pseudomonas* and *Staphylococcus*. Through Figure 1, may note the high isolation percentage obtained for *Serratia marcescens* species, about 36.36%, compared to the other isolated species. Raymann et al,2018 also isolate this species from more than 60% of studied honey bee workers. This high percentage may be related to the widely distribution of this species in many environments as soil, water and plant materials(Kwak et al.,2014).

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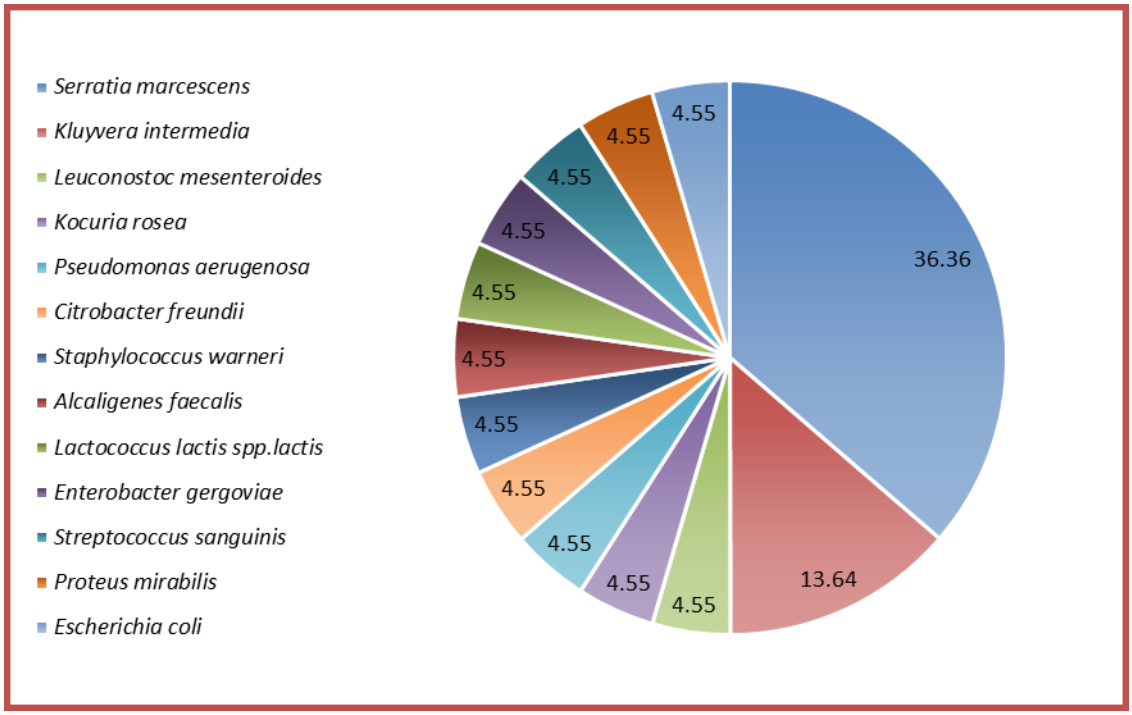

Figure(1): The percentage of isolated studied species

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