



ISOLATION OF *NON-TUBERCULOUS MYCOBACTERIA* FROM *PERIPLANETA AMERICANA* IN BAGHDAD

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Abstract

The aim of this study was designed to investigate the prevalence of non-tuberculous *Mycobacteria* in *Periplaneta americana* in Baghdad governorate. A hundred samples of *Periplaneta americana* were collected from different places in Baghdad governorate include (Al-Hurryyah, AL-khadra, AL-yarmook) from (April to September) in 2018. Samples cultured on Lowenstein-Jenson media, middle brook media and incubated at 37°C for 8 weeks. The diagnosis was based on characteristics of colonies, rate of growth, acid fast stain in direct microscopic examination and the ability of production of chromogenes. In out of (100) samples (26%) collected from AL-huriacity, (20%) from AL-khadra, (16 %) from AL-yarmook. Mycobacterium isolation rate was (10) isolates for *Mycobacterium kansaii*, (7) isolates for *Mycobacterium gordonae*, (5) isolates for *Mycobacterium fortuitum* All isolated bacteria appeared as acid-fast bacilli with acid fast staining and the colonies appeared rapidly within days. In conclusion, this is the first study in Iraq reported the prevalence of non-tuberculous *Mycobacteria* spp. in *Periplaneta americana* in Baghdad governorate. These are atypical mycobacteria important zoonotic disease and these infected *Periplaneta americana* which might play important as source of disease dissemination to humans. The author recommends to take a precautions from these insect especially in house.

Key words : *Periplaneta americana*, non-tuberculous *Mycobacteria*, Baghdad.

Introduction

The American cockroach (*Periplaneta americana*) are highly adaptable, synanthropic, and omnivorous insects (Krame *et al.*, 2009) cockroaches play vital roles in transmitting pathogens like bacteria, virus, helminthes and protozoan that while living close to human dwellings (Pai and Peng 2003). They identified as potential mechanical vector for bacterial pathogen dissemination in health-care facilities, dwelling environments restaurant (Sayyad *et al.*, 2016). The presence of the American cockroach, in human dwellings causes damage and distress, Cockroach body has bad smell secretions, which are one of the sources of allergens responsible for asthma (Arlian, 2002) In particular, cockroaches, such as the peridomestic American cockroach *Periplaneta Americana* (Blattaria, Blattidae) are suspected vectors of food spoilage and pathogenic microbes (Rust, 2008). They move freely from drains, from building to building, gardens, sewers and latrines to human habitations. Because they feed on human faeces as well as human food they can spread

germs that cause disease. They have survived on the earth for more than 300 million years virtually without change The cockroach poses one of the greatest health hazards of all households' pests. Millions of them live in our homes, hotels, shops, supermarkets and bars, spreading diseases which can prove fatal to humans. Many cases of food poisoning are known to be as a result of cockroach contamination. (Zurek and Schal, 2004). Cockroaches frequently feed on human faces, garbage and sewage, therefore they have copious opportunity to disseminate pathogenic agents (Uçkay *et al.*, 2009). They are known as one of the most important agents in transmission and distribution of many different *viruses, bacteria, fungi, protozoa* and to human life, and they are intermediate host for some pathogenic intestinal worms All these bacteria make the food contaminated and eventually it causes the illness in humans which may be fatal. Cockroaches are one of the most appalling sights, especially when they are in our home, bathrooms, and kitchen nearby foods. Any bug indoors will scare someone, but cockroaches are far worse than other household pests. Cockroaches carry many pathogens,

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they pick up from contaminated places such as: drains, sewers, landfills, garbage, bathrooms, and toilets. Many health risks come along with a cockroach infestation. These cockroaches are typical to causes many intestinal diseases and illnesses (Kausar *et al.*, 2013). Cockroaches live in sewage, pipes, latrines, garbage, wall slits, baseboards and filthy places and it carry many germs. There are many species of sanitary importance and one of major importance is *Periplaneta americana* the odorous secretions produced by American cockroaches can alter the flavor of food. Also, if populations of cockroaches are high, a strong concentration of this odorous secretion can be present Cockroaches can pick up disease-causing bacteria, on their legs and later deposit them on foods and cause food infections or poisoning. As cockroach infestation occurs commonly in the hospital environment, they may potentially be implicated as a cause of hospital-acquired infections due to non-tuberculous *Mycobacteria*. House dust containing cockroach faces and body parts can trigger allergic reactions and asthma in certain individuals. (Milca *et al.*, 2006) Non-tuberculous *Mycobacteria* (NTM), also named as environmental Mycobacteria, are isolated from water, soil, dust, and plants. They are divided into two groups: rapidly growing mycobacteria (RGM) where visible colonies appear within seven days, and slowly growing mycobacteria (SGM) which require longer incubation time (Tortoli, 2009). So this study intends to investigate the prevalence of *Mycobacteria* spp. in local in Baghdad governorate and to highlight the importance of epidemiological significance and transmission to human.

Materials and Methods

Collection and identification of cockroaches

A (100) samples of Cockroaches were collected in period from (April -Septemper) in 2018 from different places in Baghdad governorate include (Al-Hurryiah-, AL-khadra, ALyarmook). Cockroaches were collected using sterile hand-gloves and sterile screw-capped. Then transport to laboratory and examine under dissecting microscope and diagnosis as *Periplaneta americana* The collected cockroaches were killed by using chloroform in a sterile jar and sterile physiological saline was used for wash The external body surface vortexing in (2min). After external body washing, the cockroaches were soaked in 90% ethanol for (5min) to decontaminate their external surfaces and were dried. They were then re-washed with sterile saline to remove traces of ethanol, The excised gut was then homogenized in sterile normal saline and were decontaminated by equal amount of sterile 4% NaOH was added to it. Then incubated at 37°C for

15 minutes with vortex shaking every 5 minutes. The mixture was centrifuged at 3,000 rpm for 15 minutes and a part supernatant poured off. The deposit was neutralized by 2N HCL using a drop of phenol red as indicator was inoculated by 0.1ml. of deposit and incubated at -37°C (Cruickshank, 1975). Media were checked for growth twice a week for eight weeks. All grown colonies were Ziehl-Neelsen (ZN) stained to confirm that they comprised acid-fact bacilli. Diagnosis of *Mycobacteria* was based on rate of growth, characteristics features of bacterial colonies, direct microscopic examination by acid fast stain and the ability of production of chromogens (Quinn., *et al.*, 2006; Tachbele, 2006).

Results

The result of this study revealed that the Non-Tubercolus *Mycobacteria* (NTM) were detected in out of (100) samples (26%) collected from AL-huriacity, (20%) from AL-khadra, (16%) from AL-yarmook. Mycobacterium isolation rate was (10) isolates for *Mycobacterium kansaii*, (7) isolates for *Mycobacterium gordonae*, (5) isolates for *Mycobacterium fortuitum* At the same time the reality of isolates. The colonies of the isolates were appeared within (days). Moreover, smears from colonies stained with Ziehl-Neelsen (ZN) technique revealed the presence of acid-fast rods (AFR) as in (Table 1).

Table 1: Percentage of Non tuberculous Mycobacteria isolates in Baghdad city.

Places	No. of sample	No. of isolates	Percentage
AL-huria	50	13	26%
AL-khadra	25	5	20%
AL-yarmook	25	4	16%

Discussion

Nontuberculous Mycobacteria (NTM) are ubiquitous organisms found in the environment as saprophytes and are emerging as main cause of infectious diseases worldwide. The NTM were first recognized as human pathogens in the year 1950 and since then more than 170 NTM species have been identified and spectated. The disease caused due to NTM has also gained increasing attention in countries with high incidence of tuberculosis due to risk of misdiagnosis with multidrug (MDR-TB) (Anandh and Jayanna, 2017) See comment in PubMed Commons below some species of cockroaches have been known to carry mycobacteria that cause tuberculosis, leprosy, and a whole host of other diseases. This is why many people believe cockroaches are dirty and potentially

dangerous. In reality, though, most of the thousands of species of cockroaches live in areas far away from humans. Scientists are often found that American cockroaches in close association with humans and can contact and obtain pathogenic agents pathogen can then be mechanically transmitted to human population in is and the presence of cockroach human dwelling is never favorable, and an issue the also a risk factor for human sanitation (Sayyad *et al.*, 2016). There is a little study on isolation of mycobacteria from cockroach, See comment in PubMed Commons below Previous research approved that non- tuberculous mycobacteria excreted through the faces of infected animals and this may be a source of infection total samples *Mycobacteria* colonies appeared in 23% faces samples within days In study of fisher the by nymphs of oriental cockroach the investigated by oral infection with mycobacterial suspensions of *M. avium*, *M. paratuberculosis* were isolated from dropping at 3 days post infection (Fischer *et al.*, 2003) Other study of pai and Peng (2003) this study to isolate mycobacteria from hospital household cockroaches from 90 hospitals and 40 households in south Taiwan among 203 cockroaches collected six *Mycobacterium* spp. were isolated and identified by polymerase chain reaction–restriction fragment length polymorphism analysis. In 12 cockroaches (*P. americana*): four *Mycobacterium kansaii*, three *Mycobacterium xenopi*, two *Mycobacterium gordonae*, one *Mycobacterium hemophilium*, one *Mycobacterium fortuitum*, and one *Mycobacterium avium* in another study that cockroach are possible mechanical carrier of tuberculosis and the bacilli were found in all smears of the intestinal tract of cockroaches (Read, 1933). Cockroaches identified as potential mechanical vector for bacterial pathogen dissemination in health-care facilities and this study was the first study in Iraq on isolation of non-tuberculous mycobacteria infection in cockroach and People should be aware of the potential pathogenic transmission by cockroach and should keep bathroom and kitchens, and toilets clean and prevent infestation of cockroach in their houses..

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