

## COCKROACHES (*Periplaneta americana*): RESERVOIRS OF METALLO $\beta$ LACTAMASE AND EXTENDED SPECTRUM $\beta$ -LACTAMASE PRODUCING BACTERIA IN MEDICAL CITY HOSPITAL IN BAGHDAD, IRAQ

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### ABSTRACT

Cockroaches are usually major carriers of pathogenic bacteria, prompting economic and serious health risk problem for humans by carrying bacteria and allergens. This study focuses on isolation of bacteria from *Periplaneta americana* in Medical City Hospital in Baghdad. In total, thirty adult cockroaches were collected randomly in February 2017. Antibiotic susceptibility test was done for each bacterial species. In total, 21 bacterial isolates were identified. The most common diagnostic bacterium isolated from the cockroaches was found to be *Klebsiella pneumoniae* (33.33%). Coagulase -ve Staphylococci were found in 19% of the isolates. About 42.8% of cockroach bacterial isolates were found to express ES $\beta$ Ls trait while 19% of isolates expressed M $\beta$ Ls trait. Two isolates harbored the *bla*TEM ES $\beta$ Ls gene. None of the isolates harbored *bla*IMP, *bla*CTX or *bla*VIM. Current study indicates that *Periplaneta americana* is reservoirs for bacterial isolates which produce ES $\beta$ Ls and M $\beta$ Ls.

**Key words:** *Periplaneta americana*, ES $\beta$ Ls, M $\beta$ Ls, Antibiotic resistance.

### INTRODUCTION

*Periplaneta americana* is also known as the ship cockroach, Bombay canary and kakerlac (Bell and Adiyodi, 1981). Cockroaches may harbor and disseminate bacteria to the environment; these insects important in the spread of microorganisms (Fotedar et al., 1991, Prado et al., 2006).

Cockroaches carry bacteria, destructive and offensive, live in pipes of sewage, latrines, wall slits, garb-age and baseboards (Prado et al., 2006; Prado et al., 2002), from *Periplaneta americana* and other insects isolated bacteria which are often pathogenic isolates in hospital (Mikulak et al., 2013; Moreira et al., 2005). The epidemiology of hospital infections, especially the transmission of antibiotic -resistant *Pseudomonas aeruginosa*, *E. coli* and *Klebsiellae spp.* (Kassiri et al., 2014). Cockroaches can transfer a large number of microbe on the surface of body (Akinjogunlaa et al., 2012).

One of the mechanisms of antimicrobial resistance in the microorganisms is the production of extended spectrum beta lactamases (ES $\beta$ Ls) (Mesa et al., 2006). The genes encoding metallo  $\beta$ - lactamases (M $\beta$ Ls) are carried by transposable elements that transfer rapidly (Mendes et al., 2004). Increased rates of mortality have been authenticated for patients infected with bacteria which produce M $\beta$ Ls (Lucenaa et al., 2014).

The aim of this study was to detection of the ES $\beta$ Ls and M $\beta$ Ls-producing bacteria in *Periplaneta americana* from Medical City Hospital in Baghdad.

### Methods

**Collection of bacteria:** Thirty *Periplaneta americana* were captured, transferred to sterilized flasks. Each cockroach was immersed and homogenized in 0.8% normal saline. After that the resulting solution was cultured on the nutrient agar, Mac-Conkey,s agar and Blood agar, incubated for 48 hours at 37°C.

**Antimicrobial susceptibility testing:** The susceptibility of the bacterial isolates was determined against the antibiotics like Ciprofloxacin (CIP); Imipenem (IMP), Amoxicillin/clavulanic acid (AMC), Cephalothin (KF), Cephalexin (CL), Azithromycin (AZM), Ceftriaxone (CRO), Ampicillin (AM), Cefotaxime (CTX), Ceftazidime (CAZ), Tetracycline (TE) and Aztreoname (ATM), On Mueller-Hinton agar Plate.

**Phenotypic Detection of ES $\beta$ Ls production:** Each isolate tested to production of ES $\beta$ Ls by disk approximation test, the results were interpreted according to Collee et al., (1996) and Drieux et al., (2008): Tested bacterial isolates were cultured onto Muller-Hinton agar. Augmentin disk was placed in the center of plate; Ceftazidime and Cefotaxime disks were placed at 3cm from center disk. The zone of inhibition of the Cefotaxime, Ceftazidime and Clavulanic acid disks were determined after incubation at 35°C.

**Detection for Metallo- $\beta$  lactamase(M $\beta$ Ls):** The test was done according to Bashir et al., (2011) as follows: Tested bacterial isolates were cultured onto Mueller Hinton agar media according to Kirby-Bauer method. The inhibition zones of the imipenem and imipenem EDTA discs were determined after incubation at 35°C.

**Detection of beta lactamase enzymes genes:** bacterial isolates were submitted to detection for some  $\beta$ -lactamase genes by using PCR technique, *bla*IMP, *bla*VIM, *bla*TEM and *bla*CTX-M, ampli-

fication of DNA was carried with PCR System. The primers used in current study are listed in Table 1.

**Table 1:** Primers selection that used in this study

Genes	Primer name	Sequence	Product Size(bp)	Reference
<i>bla</i> TEM	TEM -F TEM -R	5'ATGAGTATTCAACATTTCCG-3' 5'-TTAATCAGTGAGGCACCTAT-3'	861	(Grimm et al., 2004)
<i>bla</i> CTX	CTX - F CTX - R	5'-CGCTTTGCGATGTGCAG-3' 5'-ACCGCGATATCGTTGGT-3'	550	(Bhattacharjee et al., 2007)
<i>bla</i> IMP	IMP -F IMP -R	5'-CATGGTTTGGTGGTCTTGT-3' 5'-ATAATTTGGCGGACTTTGGC-3'	488	(Sung et al., 2008)
<i>bla</i> VIM	VIM -F VIM -R	5'-ATTGGTCTATTTGACCGCGTC-3' 5'-TGCTACTCAACGACTGAGCG-3'	780	(Sung et al., 2008)

## RESULTS

**Isolation of bacteria from Cockroaches:** Thirty *Periplaneta americana* were collected from Medical City Hospital in Baghdad. The most bacterium isolated from cockroaches was found to be *Klebsiella pneumoniae* (33.33%), 19% were *E. coli*, 14.28% were *Serratia marcescens*. Coagulase -ve Staphylococci were found in 19% of the isolates (Table 2).

**Table-2:** Bacterial isolates from *Periplaneta americana*.

Bacterial isolates	Isolates no.	%
<i>S.marcescens</i>	3	14.28

<i>K.pneumoniae</i>	7	33.33
Coagulase-ve Staphylococci (CNS)	4	19
<i>Pseudomonas aeruginosa</i>	3	14.28
<i>E.coli</i>	4	19
Total	21	100

**Antibiotic susceptibility testing:** All bacterial isolates were multidrug resistant, the resistance was 100% to ampicillin, Amoxycillin/clavulanic acid, cephalixin and ceftriaxone, while the most isolates were susceptible to imipenem (Table 3).

**Table 3:** Resistance Percentages of Bacterial isolates in the current study.

Antimicrobial agents	Resistance no. ( % )	No. (species) of resistance isolates
Ciprofloxacin	6( 82.5)	1( <i>S.marcescens</i> ) ; 3( <i>K.pneumoniae</i> ) ; 2( <i>Coagulase-ve Staphylococci</i> )
Imipenem	4( 19)	1( <i>S.marcescens</i> ) ; 2( <i>K.pneumoniae</i> ), 1( <i>P. aeruginosa</i> )
Cephalothin	18( 85.7)	2( <i>S.marcescens</i> ) ; 7( <i>K.pneumoniae</i> ) ; 4( <i>Coagulase-ve Staphylococci</i> , 2( <i>P. aeruginosa</i> ) , 3 ( <i>E.coli</i> )
Aztreoname	13( 61.9)	6( <i>K.pneumoniae</i> ), 4( <i>Coagulase-ve Staphylococci</i> , 1( <i>P. aeruginosa</i> ) (2 <i>E.coli</i> )
Ampicillin	21(100)	3 ( <i>S.marcescens</i> ), 7( <i>K.pneumoniae</i> ), 4 ( <i>Coagulase-ve Staphylococci</i> , 3 ( <i>P. aeruginosa</i> ), 4 ( <i>E.coli</i> )
Amoxycillin/ clavulanic acid	21 (100)	3 ( <i>S.marcescens</i> ), 7 ( <i>K.pneumoniae</i> ), 4 ( <i>Coagulase-ive Staphylococci</i> , 3 ( <i>P. aeruginosa</i> ), 4 ( <i>E.coli</i> )
Ceftazidime	9( 42.8)	3 ( <i>K.pneumoniae</i> ), 4( <i>Coagulase-ve Staphylococci</i> , 2 ( <i>E.coli</i> )
Cefotaxime	13( 61.9)	1 ( <i>S.marcescens</i> ) ; 6 ( <i>K.pneumoniae</i> ) ; 4 ( <i>Coagulase-ive Staphylococci</i> , 2 ( <i>E.coli</i> )
Cephalexin	21(100)	3 ( <i>S.marcescens</i> ), 7 ( <i>K.pneumoniae</i> ), 4 ( <i>Coagulase-ve Staphylococci</i> , 3( <i>P. aeruginosa</i> ), 4 ( <i>E.coli</i> )
Tetracycline	20 (95.2)	3 ( <i>S.marcescens</i> ), 6 ( <i>K.pneumoniae</i> ), 4 ( <i>Coagulase-negative Staphylococci</i> (CNS), 3 ( <i>P. aeruginosa</i> ), 4 ( <i>E.coli</i> )
Azithromycin	13( 61.9)	2( <i>S.marcescens</i> ), 5( <i>K.pneumoniae</i> ), 3( <i>Coagulase-negative Staphylococci</i> (CNS), 1 ( <i>P. aeruginosa</i> ), 2 ( <i>E.coli</i> )
Ceftriaxone	21(100)	3( <i>S.marcescens</i> ), 7 ( <i>K.pneumoniae</i> ), 4 ( <i>Coagulase-negative Staphylococci</i> (CNS), 3( <i>P. aeruginosa</i> ), 4 ( <i>E.coli</i> )

**Detection of ES $\beta$ Ls and M $\beta$ Ls production:** Detection of ES $\beta$ Ls and M $\beta$ Ls enzymes were illustrated that 9 (42.8%) of cockroach bacterial isolates were produced ES $\beta$ Ls, Four (19%) of isolates were positive for metallo- $\beta$ -lactamases (Table 4).

The results in current study showed the presence of *bla*TEM in Two cockroach bacterial isolates (*P. aeruginosa* and *K. pneumoniae*). None of the isolates harbored *bla*IMP, *bla*CTX or *bla*VIM genes.

**Table 4:** Production of ESβLs and MβLs enzymes by bacterial isolates from *Periplaneta americana*.

Bacterial Isolates	No. (%) of isolates that produced ESβLs	No. (%) of isolates that produced MβLs
<i>S.marcescens</i>	3( 14.28 )	1( 4.76)
<i>K.pneumoniae</i>	1( 4.76 )	2(14.28)
<i>P. aeruginosa</i>	2( 9.52 )	1( 4.76 )
<i>E.coli</i>	3(14.28)	-
Total	9( 42.8%)	4(19)

## DISCUSSION

The most common isolated bacterium from cockroaches was found to be *Klebsiella pneumoniae* (33.33%). Most Gram-ve bacteria isolated from arthropods: *Escherichia*, *Klebsiella*, *Proteus*, *Serratia*, *Citrobacter* and *Acinetobacter* (Tachbele et al., 2006, Gliniewicz et al., 2003) and the most Gram +ve bacteria: *Streptococcus*, *Enterococcus*, *Staphylococcus* and *Micrococcus* (Mikulak et al., 2013).

*Klebsiella pneumoniae* was the most frequent bacterial isolates in the current study. *K. pneumoniae* was collected from insects by other studies in hospitals (Hsiu-Hua et al., 2004; Salehzadeh et al., 2007). Al-Dulaimi (2016) found the multi-antibiotics resistance among *E. coli* from nosocomial infections.

In hospitals, *Periplaneta americana* play a role as important vectors in the hospitals infections, especially the transfer of antibiotics-resistant *Klebsiella* spp *E. coli* and other potential pathogenic micro-organisms (Akinjogunlaa et al., 2012).

Most bacterial isolates in current study were multi-antibiotic resistant. Prado et al., (2006), recorded that 13% of *S. marcescens* isolated from *Periplaneta americana* were resistance to gentamicin. *S. liquefaciens* collected from the *Periplaneta americana* showed resistance to most antimicrobial agents; they were carried ESBL and AmpC enzymes (Czajka et al., 2003). The isolates of *Citrobacter freundii* with ESBL and AmpC enzymes were also collected from cockroaches (Mikulak et al., 2013). A study done by Akinjogunlaa et al., (2012) showed that bacterial isolates from cockroaches were resistance to Streptomycin (36.1%), (36.0%) to Tetracycline, (40.3%) to Amoxicillin, (35.5%) to Erythromycin, (34.6%) to Penicillin, (27.8%) to Cephalothin, (23.4%) to Sulfamethoxazole, (18.4%) to Ciprofloxacin and (17.7%) to Levofloxacin. Al Marjani (2008) reported that all isolates from cockroaches were resistant to Cefuroxime, Amoxycillin and all the isolates have showed multiple antibiotics resistance for antibiotics. Al Marjani et al., (2013)

revealed that *P. aeruginosa* isolated from clinical sources were resistance 100% for Carbencillin; 80 % for Cefixime, 84% for Amoxicillin/clavulanic acid. About 42.8% of cockroach bacterial isolates in current study were found to express ESβLs while 19% of isolates expressed MBLs. The isolates of *S. liquefaciens* from the cockroaches in Poland hospital were contain AmpC and ESBL mechanisms of resistance (Czajka et al., 2003). Loucif et al., (2016) reported the ESBLs and *Enterobacteriaceae* which produce carbapenemase from German cockroaches caught in Hospital in Algeria.

The wide spread of bacteria which produce CTX-M-15 ESBL enzymes and the emergence of *Enterobacteriaceae* which produce OXA-48-type carbapenemase in Algerian hospitals related with the carriage of such antibiotics-resistant bacteria by cockroaches in hospital through their particular feeding habits (Cuzon et al., 2015, Ahmed-Kazi et al., 2014).

**Conclusion** Our study documented a high percentage of antimicrobial resistance of the bacteria especially for the commonly used Beta lactams. *Periplaneta americana* can act a vector for ESβLs and MβLs-producing bacteria.

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