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Study Of ß-lactamases Producing *Enterobacteria* isolated from German cockroach (*Blatella germanica*) in hospitals

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Abstract

38 German cockroach (*Blatella germanica*) were captured to determine the potential role of the cockroaches in carrying ß-lactamases producing pathogens from hospital. Only cockroaches captured whole and live were utilized for the study. After that each cockroach was placed in a test tube with 5 ml sterile saline solution , and then homogenized. The resulting solution was cultured on the culture media. 30 isolates of Enterobacteria were identified , the most frequent were *Klebsiella pneumoniae* (40%), *Pseudomonas aeruginosa* (16.6%), *E. coli* and *Proteus mirabilis*(each 13.3%) , *Serratia marcescens* (10 %) and *Enterobacter aerogenes* (6.66%) .

Bacterial isolates were tested against (10) antimicrobial agents: Amoxicillin, Erythromycin ,Cefoperazone , Cefazolin , Aztreoname , Ofloxacin , Cephalothin , Trimethoprim , Carbenicillin , and Gentamicin . Results showed that all the isolates were resistant to Erythromycin, Cefazolin and Amoxycillin , and all the isolates have showen multiple resistance for antibiotics. All isolates (100%) were susceptible to Aztreoname and Ofloxacin. The majority of isolates remained susceptible to Cefoperazone (70 %) and Trimethoprim (75 %) .

The results showed also that 14 isolates (46.66 %) had the ability to produce ß -lactamase enzymes . 2 isolates (6.66 %) were able to produce Extended-Spectrum ß -lactamases (ESBLs).

دراسة انتاج انزيمات البيتالاكتاميز من البكتريا المعوية المعزولة من الصرصر الألماني في بعض مستشفيات مدينة بغداد

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الخلاصة

تم جمع 38 حشرة من الصرصر الألماني (*Blatella germanica*) من مستشفى مدينة الطب/بغداد لدراسة دورها في نقل البكتريا المرضية المقاومة لمضادات الحياة في المستشفيات والمنتجة لأنزيمات البيتالاكتاميز. جمعت الصراصر بأستخدام قناني زجاجية ، بعدها وضع كل صرصر في أنبوبة أختبار تحوي 5 مل من الملح الفسيولوجي المعقم ومزجت محتويات الأنبوبة بالمازج(vortex) لعمل معلق بكتيري ، بعد زرع المعلق البكتيري على الأوساط الزرعية المناسبة شخصت 30 عزلة بكتيرية مختلفة تعود للعائلة المعوية وكانت اعلى نسبة عزل هي لبكتريا *Klebsiella pneumoniae* أذ تم عزلها بنسبة 40% ثم بكتريا *Pseudomonas aeruginosa* بنسبة عزل 16.6 % ، ثم بكتريا *E.coli* وبكتريا *Proteus mirabilis* اذ عزلت بنسبة 13.3% لكل منها ، ثم تأتي بعدها بكتريا *Serratia marcescens* بنسبة عزل 10 % وبكتريا *Enterobacter aerogenes* بنسبة 6.66 % .

أختبرت حساسية هذه العزلات لعشرة من مضادات الحياة : الآموكسسلين و الأرثرومايسين و الجنتامايسين و الترايميثوبريم و الأوفلوكساسين و السيفازولين و الأزتريونام و السيفوبيرازون و السفالوثين و الكاربنسلين . أظهرت النتائج ان جميع العزلات كانت مقاومة لمضادات الأرثرومايسين و ألأموكسسلين والسيفازولين، وان جميع العزلات تحمل مقاومة متعددة لمضادات الحياة . بالمقابل كانت جميع العزلات حساسة لمضادي الأزتريونام و الاوفلوكساسين ، كما بقيت معظم العزلات حساسة لمضاد السيفوبيرازون (70%) والترايميثوبريم (75%) .

من جانب أخر أظهرت 14 عزلة (46.66 %) من هذه العزلات قابليتها على انتاج انزيمات البيتالاكتاميز. وكانت عزلتان (6.66%) منتجه لأنزيمات البيتالاكتاميز واسعة الطيف من مجموع 30 عزلة تحت الدراسة.

Introduction

Insects play a role as alternate vectors for bacteria (1).Cockroaches are capable of carrying many human pathogens and they may have a role in transmitting some serious nosocomial infections to man and his domestic animals (2) .

How much diversity exists in the antibiotic resistance patterns within natural populations of enteric bacteria, and how have the levels of resistance changed since the use of antibiotics became widespread? Increased introduction of antimicrobial agents into the environment via medical therapy, agriculture, and animal husbandry , this has exacerbated the problem of controlling microbes in a disease setting and has caused a resurgence of bacterial diseases worldwide due to the acquisition and transfer of virulence factors and antibiotic resistance genes (3) .These problems are further compounded by the persistence of resistance determinants in bacterial genomes over hundreds of generations, even in the absence of antibiotics as selective agents (4).

Extended-spectrum ß-lactamases are a rapidly evolving group of ß-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam , typically, they derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these ß-lactamases(5) . An increasing number of ESBLs not of TEM or SHV lineage have recently been described , the presence of ESBLs carries tremendous clinical significance(6) . The ESBLs are frequently plasmid encoded, Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited (7).

Host ecology and environment also factor into the patterns of antibiotic resistance in natural populations. For example, strains of *Salmonella* are likely to have experienced different selective pressures for resistance than *Escherichia coli* strains have experienced because they reside primarily in nonmammalian hosts and are far less likely to have encountered most commercial antibiotics in their natural environments. However, such organisms have been exposed to naturally occurring antimicrobial agents, such as the small-polypeptide defensins, that are distributed broadly in mammalian and nonmammalian hosts (4).

The aims of this study were to determine the potential role of the *Blatella germanica* in carrying pathogens in hospital , and to test the antimicrobial susceptibility of these microorganisms , and study the ability of Cockroaches isolates for ß-lactamases production.

Material and Methods

1) **Isolation of bacteria from Cockroaches** : 38 Cockroaches (*Blatella germanica*) were captured in the morning , they were placed in flasks , rinsed with 70% alcohol, transferred to sterilized flasks, and then taken to the laboratory. Only cockroaches captured whole and live were utilized for the study. After that each cockroach was placed in a test tube with 5 ml sterile saline solution (0.8%) and then homogenized. The resulting solution was cultured on the following three medium: MacConkey's agar, Blood agar and nutrient agar. Bacterial isolates were identified according to (8) by using the cultural and biochemical examinations and API 20-E system.

2) **Antimicrobial susceptibility:** The disks diffusion test was used to determine antimicrobial susceptibility of Bacterial isolates on Mueller-Hinton agar by use of the antibiotics : Amoxicillin (AX) , Erythromycin(E) ,Cefoperazone (CEP) , Cefazolin (CZ) , Aztreoname (ATM) , Ofloxacin(OFX) , Cephalothin (KF) , Trimethoprim(TMP) , Carbenicillin (PY), and Gentamicin (CN)**.**

3) **Detection of beta-lactamases** : The ability of Bacterial isolates for beta-lactamases production were tested according to (9), by rapid iodometric method as followes: Test Bacterial isolates were removed with a loop from an overnight culture on solid medium and suspended with Penicillin solution , at 1 h. two drops of starch indicator were added to the suspension ,followed by one drop of iodine reagent and were mixed thoroughly . A blue colour developed immediately , persistence of the blue colour for longer than 10 min. constitutes a negative result.

4)) **Detection of Extended-Spectrum beta-lactamases (ESBLs)**: The ability of Bacterial isolates for ESBL**s** production were tested according to (5), by using a clavulanate double –disk diffusion method: synergy between cefotaxime and Clavulanate was detected by placing a disk of amoxicillin / clavulanate (20 µg/10 µg . respectively) and a disk of

cefotaxime(30 µg), 30 mm a part (center to center) on an inoculate agar plate . A clear extension of the edge of the cefotaxime inhibition zone toward the disk containing Clavulanate was interpreted as synergy , indicating the presence of an ESBLs

Results and Disscussion

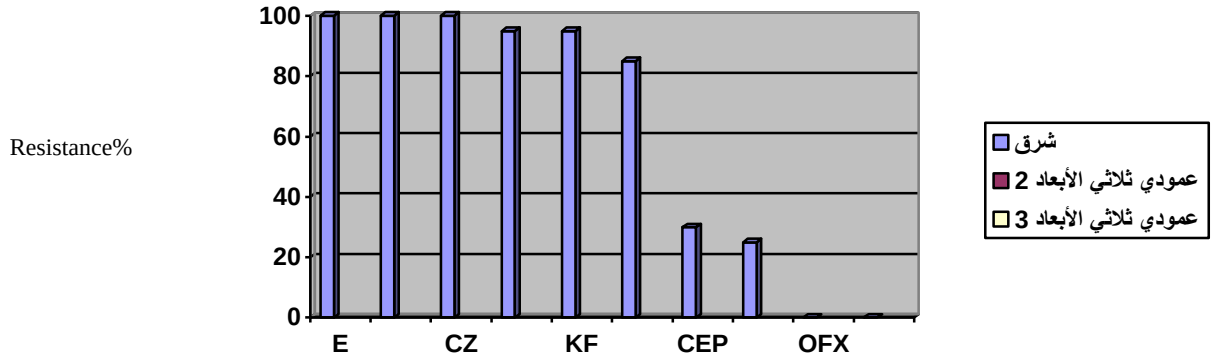
In the present study , 30 isolates of Enterobacteria were identified , the most frequent were *Klebsiella pneumoniae* (40%), *Pseudomonas aeruginosa* (16.6%), *E. coli* and *Proteus mirabili*s(each 13.3%) , *Serratia marcescens* (10 %) and *Enterobacter aerogenes* (6.66%) . Table (1).

Klebsiella pneumoniae is a common cause of serious nosocomial gram-negative infections, including ventilator-associated pneumonia, urinary tract infection, and bloodstream infection (BSI) . Infections due to ***K. pneumoniae*** occur in both outbreak settings and settings of endemicity ,Unfortunately, isolates of ***K. pneumoniae*** are becoming increasingly resistant to antibiotics(10).

Table 1: Enterobacteria isolates from *Blatella germanica*

Microorganism	Isolates no.	%
Klebsiella pneumoniae	12	40
Pseudomonas aeruginosa	5	16.6
E. coli	4	13.3
*Proteus mirabili*s	4	13.3
Serratia marcescens	3	10
Enterobacter aerogenes	2	6.66
Total	30	100

A total of 30 Cockroaches isolates were tested against **(** 10 **)** antibiotics. Results showed that all the isolates were resistant to Erythromycin, Cefazolin and Amoxycillin , and all the isolates have showen multiple resistance for antibiotics. All isolates (100%) were susceptible to Aztreoname and Ofloxacin. The majority of isolates remained susceptible to Cefoperazone (70 %) and Trimethoprim (75 %) (Fig 1) .



-- Antibiotics –

Amoxicillin (AX) , Erythromycin(E) ,Cefoperazone (CEP) , Cefazolin (CZ) , Aztreoname (ATM) , Ofloxacillin(OFX) , Cephalothin (KF) , Trimethoprim(TMP) , Carbenicillin (PY), and Gentamicin (CN).

The results showed also that 14 Cockroaches isolates (46.66 %) had the ability to produce ß- lactamase enzyme , 2 isolates (6.66 %) were able to produce Extended-Spectrum ß -lactamases (Table 2).

The predominant mechanism of cephalosporin resistance in isolates from both hospital and community settings was the production of CTX-M-type ESBLs, with CTX-M-producing *Escherichia coli* as the most numerous resistant organism overall. Other major mechanisms of cephalosporin resistance included production of non-CTX-M ESBLs and AmpC ß-lactamases. Most ESBL (both CTX-M and non-CTX-M) producers were multiply resistant to non-ß-lactam antibiotics, including trimethoprim, ciprofloxacin and gentamicin (11) . Cotton *etal.* (12) recently experienced an outbreak of nosocomial disease due to extended spectrum beta-lactamase-producing ***Klebsiella pneumoniae*** in a neonatal unit infested with cockroaches .

Over the last 20 years, there has been an increased resistance to ß-lactams because of the secretion of extended-spectrum ß-lactamases (ESBLs) mediated by plasmids (13). This type of resistance is now observed in all species of ***Enterobacteriaceae*** and is currently disseminated throughout the world (14 **).** A common environmental source of ESBL-producing organisms has occasionally been discovered. Examples have included contamination of ultrasonography coupling gel, bronchoscopes , blood pressure cuffs (15), and Cockroaches (12).

Since around 2000—earlier in Poland and Spain and later in France and the UK—dramatic shifts have occurred in the prevalence and types of extended-spectrum ß-lactamases (ESBLs) in Europe. Before this watershed, most producers were nosocomial isolates, often ***Klebsiella*** spp. or ***Enterobacter*** spp. from specialist care units, and had mutant TEM or SHV ESBLs. Subsequently, CTX-M ESBLs have become dominant, with much greater penetration into ***Escherichia coli***, and with many infections in 'complicated community' patients, usually with underlying disease, recent antibiotic usage, or healthcare contact **(** 16 **,** 17).

Table 2 : Production of ß - Lactamase by Enterobacteria isolates from *Blatella germanica*

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Microorganism	**No. of isolates that produced ß -lactamase**	**% from all Enterobacteria isolates**
Klebsiella pneumoniae	4	**13.33**
Pseudomonas aeruginosa	3	**10**
E. coli	3	**10**
Proteus mirabilis	2	**6.66**
Serratia marcescens	1	**3.33**
Enterobacter aerogenes	1	**3.33**
Total	**14**	**46.66**

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