

Original article

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *ESCHERICHIA COLI* ISOLATES FROM COCKROACHES IN SOUTHWESTERN IRAN

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Summary

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In recent years antibiotic-resistant bacteria including those producing extended-spectrum β lactamases increase, which could affect their pathogencity and lead to many disease in both humans and animals. Enterobacteriaceae commonly express plasmid-encoded β -lactamases family like TEM-1 gene, which encodes resistance to penicillin in Escherichia coli. Insects, especially cockroaches, are important vectors to spread these bacteria in human living environmment. The present study was performed to evaluate the frequency of extended spectrum β -lactamase producing gene TEM-1 and the antimicrobial susceptibility patterns of Escherichia coli strains isolated from cockroaches in Iran using antibiogram and molecular techniques. In this study, 110 cockroaches (Periplaneta americana) were collected from residential environments in Chahrmahal Va Bakhtiari province (Southwest Iran) and after bacterial culture on specific media the antimicrobial susceptibility patterns of isolated Escherichia coli strains from cockroaches were evaluated by disk diffusion test and molecular assay. The amplification of TEM-1 gene by PCR revealed a 296 bp fragment on gel electrophoresis. The results of the present research showed that Escherichia coli infection was detected in 84 samples (76.36%) and 11 samples had the TEM-1 gene (10%). The antibiotic susceptibility pattern using the disk diffusion test indicated that most Escherichia coli isolates from cockroaches were sensitive to imipenem (100%) and amikacin (87.8%), while resistance to aztreonam, ceftazidime, and ceftriaxone was 88.1%, 80.5%, and 78.2%, respectively. These findings showed that cockroaches are important agents for transmission of Enterobacteriaceae and that extended-spectrum B-lactamases producing genes like TEM-1 by Escherichia coli could increase the gastrointestinal infections and antibiotic resistance. So, antibacterial drugs such as aztreonam, ceftazidime, and ceftriaxone must be used with caution, while imipenem and amikacin antibiotics are helpful to reduce Escherichia coli infection caused by cockroaches.

Key words: cockroach, disk diffusion, *Escherichia coli*, extended-spectrum β -lactamases, molecular technique, *TEM-1* gene

INTRODUCTION

Cockroaches have been on earth for more than 270 million years, and are the world's most usual insects. Approximately thirty of over 4,500 identified species of this insect in the world live close to humans (Chamavit et al., 2011). Most commonly, these are the German cockroach (Blattella germanica) and the American cockroach (Periplaneta americana) (Gonzalez-Domenech et al., 2012). Cockroaches generally prefer humid, warm, and dark areas like bathrooms, kitchens, and garages. In hospitals and houses they cause serious health problems by contaminating the food and kitchenware with their saliva and waste (Feizhaddad et al., 2012; Dehghani al., 2014). The presence of cockroaches has health implications, like health facility infection, because the insects move freely from areas around hospitals, which can harbour morbific organisms (Zarchi & Vatani, 2009). In homes and in places with poor standards of hygiene, serious infestations by cockroaches like peridomestic American cockroach (Periplaneta americana) and also the domestic German cockroach (Blattella germanica) (Feizhaddad et al., 2012) can occur. Infectious diseases and their treatment are significant issues in mankind's life and in recent years increasing of microorganism resistance has raised patients' expenses. These pests can serve as carriers, reservoirs and hosts of about 40 different species of pathogenic microorganisms (Chamavit et al., 2011; Heidari Soureshjani & Doosti, 2013; Tetteh-Quarcoo et al., 2013). Their nocturnal lifestyle and dirty habits make them ideal carriers for transmitting numerous pathogenic bacteria, such as Listeria monocytogenes, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella spp., Streptococcus spp., Salmonella spp.,

Campylobacter spp., *Pseudomonas aeruginosa*, and other potential pathogens (Salehzadeh *et al.*, 2007; Kassiri *et al.*, 2014; Doosti *et al.*, 2015). Up to 54% of isolates from hospital environments are found to be human pathogens.

More than 33.3% of cockroach isolates have shown resistance to antimicrobials (Mpuchane et al., 2006). There are also many different virulence factors, which are usually encoded by plasmids, chromosomes, or bacteriophages (Tham, 2012). Escherichia coli (E. coli) is a Gram-negative rod bacillus (Ramberg et al., 2012) and can express plasmid-encoded β -lactamases including TEM-1, TEM-2, and SHV-1. TEM-1 is one of the commonest factors conferring resistance to penicillins but not to extended-spectrum cephalosporins. In the 1980s, a new group of enzymes, the extended-spectrum β-lactamases (ESBLs) are detected (Sharma *et al.*, 2010). ESBLs are β -lactamases that hydrolyse extended-spectrum cephalosporins with an oxyimino side chain. Plasmids responsible for ESBL production frequently carry genes encoding drug resistance. Therefore, antibiotic options with respect to ESBL-producing bacteria are extremely restricted (Rupp & Fey, 2003).

Above 100 variations and point mutations in the *TEM* gene have been defined. These variations are the major cause of resistance to β -lactams in these isolates (Jain & Mondal, 2008). Over 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1. TEM-1 is capable to hydrolyse penicillins and early cephalosporins like cephaloridine and cephalothin (Patricia, 2001).

ESBLs-producing *E. coli* are now a serious problem of public health worldwide. However, there are still few reports on the prevalence of ESBL producing E. coli isolates from cockroaches and their related genes in Iran (Zaniani et al., 2012: Goudarzi et al., 2014). In recent decades, the antibiotic-resistant bacteria like those producing TEM-type enzymes and the clinical use of β-lactams and other penicillins are followed by the researchers and the medical community (Robin et al., 2007). Therefore, the objective of the present study was to investigate the prevalence of extended spectrum β -lactamase producing genes including TEM-1 among isolated E. coli from cockroaches in Iran using antibiogram test and molecular assay.

MATERIALS AND METHODS

Sampling

This study was carried out in Chaharmahal Va Bakhtiari province (southwest Iran). A total of 110 P. americana cockroaches were collected randomly from residential environments over a period of 2 months (June 2013-August 2013). The sample collection was done from kitchens, bathrooms, water carets, parking, wardrobe departments, cellar, basement, and pets and livestock whereabouts using sticky trap methods and sterile hand gloves. Then, the samples were transported immediately to the Biotechnology Research Center in separate sterile tube to avoid any contamination. The number of collected specimens from Shahrekord, Farsan, Boroujen, and Kiar were 44, 30, 21, and 15, respectively.

Samples preparation and antibiotic susceptibility testing

Each cockroach was frozen at 0 $^{\circ}$ C for 5–10 min, 2 mL of sterile physiological saline (0.9%) was added to each test tube

and the cockroaches were thoroughly shaken for 2 min. One hundred µL of washing solution from the body of each cockroach were cultured on MacConkey agar (Merck, Germany) plates and incubated at 37 °C for 24 hours. Then, each colony was cultured by dilution method on Mueller-Hinton agar plates (BD, Franklin Lakes, NJ). E. coli (ATCC 25922) was used as positive control strain. The antimicrobial susceptibility profiling using the disk diffusion test was done according to the 2011 guidelines of the European Committee of Antimicrobial Susceptibility Testing (EUCAST) (Kahlmeter et al., 2006). Standard antibiotic disks (i2a, Perols Cedex, France) were used for determination of antibiotic susceptibility patterns: imipenem (10 µg), amikacin (30 µg), cefepime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), and ceftriaxone (30 µg). The isolated bacteria after 24 h of incubation at 37 °C were categorised into susceptible, resistant, or intermediate by determination of inhibitor zone around disks according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) published in January 2013 and CLSI breakpoints were used for minimum inhibitory concentrations (MICs) interpretation.

DNA extraction

Total DNA was extracted from each colony of cultured bacteria using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendation. The quality of extracted DNA was quantified by NanoDrop ND-1000 (PeqLab) at 260/280 nm wavelength of according to the method described by Sambrook & Russell (2001).

Gene amplification

In order to confirm the presence of *TEM-1* gene in *E. coli* isolates from cockroaches

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the polymerase chain reaction (PCR) using specific oligonucleotide primers including TEM-1-F: 5'-TCCGCTCATGAG ACAATAACC-3' and TEM-1-R: 5'-GAC GATACACCACGCCATAATA-3' were obtained from the study of Khalaji et al. (2013). Briefly, each amplification reaction was carried out in a total volume of 25 µL (final concentration between 1 and 2 mM) consisting of 200 µM dNTP mix, 2.5 μ L of 10× PCR buffer, 1 unit of Taq DNA polymerase (all Fermentas, Germany), 1 µM of each forward and reverse primer, and 1 µg of template DNA. Two µL of sterile ultrapure deionised water instead of template DNA were used as a negative control. The micro-tubes were placed in Gradient Palm Cycler (Corbett Research, Australia) for gene amplification. The thermo-cycling conditions included initial denaturation at 95 °C for 5 min and followed by 32 cycles consisting of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min at the end of the amplification program. PCR products were separated on 1.5% agarose gel electrophoresis. The electrode buffer was TBE (10.8 g of Trisbase 89 mM, 5.5 g of 2 mM boric acid, 4 mL of 0.5 M EDTA (pH=8.0), combined in sufficient H₂O and stirred to dissolve). PCR products (10 μ L) were applied to the gel. Constant voltage of 80 V for 30 min was used for products separation. The 100 bp DNA ladder (Fermentas, Germany)

was used as a molecular weight marker to determine the length of the amplified fragments.

Finally the gel were stained with ethidium bromide. The gel images were obtained using UVIdoc gel documentation system (Uvitec, UK).

Statistical analysis

The frequency of *TEM-1* gene and the results of antibiotic susceptibility test of *E. coli* isolated from cockroaches were collected and analysed by SPSS software version 20 (SPSS Inc. Chicago, IL, USA).

RESULTS

The antibiotic susceptibility pattern using disk diffusion test showed that 0%, 4.9%, 69.5%, 80.5%, 88.1%, and 78.2% of isolates were resistant to imipenem, amikacin, cefepime, ceftazidime, aztreonam, and ceftriaxone, respectively. The percentages of susceptible isolates were as follows: 100% to imipenem, 87.8% to amikacin, 21.8% to ceftriaxone, 19.5% to ceftazidime, 14.2% to cefepime, and 5% to aztreonam (Table 1).

The amplified fragments of *TEM-1* gene of *E. coli* isolated from cockroaches by PCR on 1.5% agarose gel electrophoresis revealed bands with the length size of 296 bp (Fig. 1).

In this study, after bacterial culture and molecular investigation 84 samples (76.36%) were infected with *E. coli* and

Table 1. The antibiotic susceptibility patterns of E. coli isolates from cockroaches

Antibiotic disks	MIC (µg/mL)	Sensitive (%)	Intermediate (%)	Resistant (%)
Imipenem	≤1≥4	100.0	-	_
Amikacin	≤16≥64	87.8	7.3	4.9
Cefepime	≤8≥32	14.2	16.3	69.5
Ceftazidime	≤8≥32	19.5	-	80.5
Aztreonam	≤8≥32	5.0	6.9	88.1
Ceftriaxone	≤ 2	21.8	—	78.2

11 (10%) of them were positive for *TEM-1* gene (Table 2).



Fig. 1. The 1.5% agarose gel electrophoresis of PCR products using specific oligonucleotide primers for detection of *TEM-1* gene. Lane M: 100 bp DNA ladder (Fermentas, Germany); lane 1: *E. coli* ATCC 25922 (EUCAST quality control strain); lane 2: negative control (no DNA); lanes 3 and 4: amplified fragments of *TEM-1* gene (296 bp).

DISCUSSION

ESBLs production rate by *Enterobacteriaceae* has increased perceptibly during the two recent decades (Nejati *et al.*, 2012). The prevalence of ESBLs among *Enterobacteriaceae* is variable in each country. In the present study, the frequency of ESBLs producing gene included *TEM-1* among *E. coli* isolated from cockroaches

was investigated by the antibiogram test and molecular technique. A total of 110 *P*. *americana* cockroaches were collected from residential environments in Chahrmahal Va Bakhtiari province (southwestern Iran). After bacterial culture and molecular investigation 84 samples (76.36%) were infected with *E. coli* and 11 samples (10%) were positive for *TEM-1* gene.

There are different reports worldwide for outbreaks caused by ESBL bacteria. In a study on the prevalence of ESBL-producing E. coli and Klebsiella pneumoniae in Chicago, it was revealed that a common plasmid expressing TEM-1 was found in isolates from numerous patients in some hospitals (Patricia, 2001). Bell et al. (2002) indicated that the frequency of ESBL-producing E. coli in the medical centres located in Australia varied from 0-1% and within 13-35% for mainland Chinese centres. Moreover, the higher prevalence rates (>20%) of ESBL K. pneumoniae phenotypes were observed in clinical centres. In a recent study in Iran on detection of TEM and SHV genes in K. pneumoniae isolated from cockroaches, 32 samples (17.87%) were positive for TEM and 15 samples (8.37%) – positive for SHV (Doosti et al., 2015). In the present research, the frequency of TEM-1 gene in isolated E. coli from cockroaches was 10%. Investigation of β -lactamase

Table 2. Frequency of isolated *E. coli* and *TEM-1* gene positive organisms in each region of the Chahrmahal Va Bakhtiari province (Southwestern Iran) using bacterial culture and molecular assay

Regions	Number (%) of samples	Number (%) of samples positive for <i>E. coli</i>	Number (%) of <i>TEM-1</i> positive samples
Shahrekord	44 (40.00%)	37 (84.10%)	7 (18.92%)
Borujen	30 (27.27%)	21 (70.00%)	2 (9.52%)
Farsan	21 (19.10%)	20 (95.24%)	2 (9.52%)
Kiar	15 (13.63%)	6 (40.00%)	-
Total	110 (100.00%)	84 (76.36%)	11 (10.00%)

producing Enterobacteria isolated from German cockroaches (Blatella germanica) in hospitals of Iraq showed that the most frequent were K. pneumoniae (40%), Pseudomonas aeruginosa (16.6%), E. coli and Proteus mirabilis (each 13.3%), Serratia marcescens (10%) and Enterobacter aerogenes (6.66%). In addition, all isolates were susceptible to aztreonam and ofloxacin, while in the present work 100% of E. coli isolates from cockroaches were susceptible to imipenem. Moreover in another study (Al-Marjani et al., 2008), 14 isolates (46.66%) had the ability to produce β -lactamase enzymes and two isolates (6.66%) were able to produce ESBLs. Elgderi et al. (2006) isolated 25 latent pathogen species from hospital and home cockroaches in Libya including Klebsiella, Enterobacter, Serratia and Streptococcus species. The study revealed that the place of collecting the cockroaches can affect on results according to transmitted microorganisms: Klebsiella and Enterobacter were more prevalent among the home cockroaches than in those from hospital environment (Elgderi et al., 2006). In a study done in Berkeley, USA the prevalence of ESBL bacteria was 44% (Hawkey, 2008). In a study in Japan (Yagi et al., 2000) ESBL producers prevalence was 40%, and in another study in Japan a high presence of bla_{CTX}. M-positive (CTX-M-9-group) E. coli by clonal spread was observed (Suzuki et al., 2009). In France, the incidence of this phenotype has been reported to be 30-40% in hospitalised patients and 6% in ambulatory patients (Sirot, 1992). In some studies in southern east of Asia the outbreak of ESBL has been reported to be 20%, but in some areas it has been more than 60% (Khorshidi et al., 2011). Another research revealed that the incidence of ESBL bacteraemias was 40%, 18.4%

in Europe and 3% in Sweden (Coque et al., 2008).

Enterobacteria family bacteria isolates from cockroaches in Brazilian health care institutions were found in 74.6% of the samples and 96% of them were resistant to gentamicin, 84% to ampicillin, 75.3% to cephalothin, 66.7% to ampicillin-sulbactam, 50% to aztreonam and 30% to chloramphenicol (Prado *et al.*, 2006). In the present study, 88.1%, 80.5%, 78.2%, and 69.5% of *E. coli* isolates from cockroaches were resistant to aztreonam, ceftazidime, ceftriaxone, and cefepime, as well as 100% and 87.8% were sensitive to imipenem and amikacin, respectively.

The isolated microorganisms in the study of Vazirianzadeh et al. (2008) were E. coli, K. pneumoniae, Neisseria species, Bacillus cereus, Bacillus subtilis, and S. aureus based on sampling of external body and faeces pellets of Egyptian cockroaches in different regions of Ahvaz city (Southwest Iran). In a study carried out at the medical centres of Khorramshahr County in Iran, pathogenic microorganisms were isolated from the external surfaces of all American cockroaches: Klebsiella (47.9%), Pseudomonas (37%), E. coli (30.1%), Staphylococcus (24.6%), Enterobacter (19.2%), Streptococcus (15.1%), Serratia (8.2%), Bacillus (4.1%), and Proteus (2.7%) (Keseler et al., 2009). In another study, E. coli strains isolated from cockroaches (Periplaneta americana) were reported as important medical infection and it was pointed out that cockroaches are potential vectors in the epidemiology of nosocomial infections. It is apparent that multiple drug-resistant bacteria of medical importance were isolated from cockroaches. These insects can act as mobile supply of such bacteria and can start a fresh prevalence of infections in neonates, life-threatening septicaemia, immunocompromised patients and intensive care units (Wannigama *et al.*, 2014).

ESBLs producing bacteria are a major global concern in the clinical and community setting. According to the findings of the present study, the increasing antibiotic resistance of bacteria like ESBLs in E. coli pathogenicity is important and resistance genes such as *TEM-1* are risk factors for transmission of gastrointestinal infections to humans. This study revealed that imipenem and amikacin are effective antibiotics against E. coli isolated from cockroaches. The established antimicrobial patterns to aztreonam, ceftazidime, and ceftriaxone suggested that cockroaches can spread resistance against these antibacterial drugs, therefore they should be used with caution.

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