



## ORIGINAL ARTICLE

# Evaluation of bacterial infection and antibiotic susceptibility of the bacteria isolated from cockroaches in educational hospitals of Mazandaran University of medical sciences

Farzad Motevali Haghi<sup>1</sup>, Hassan Nikookar<sup>2</sup>, Hosna Hajati<sup>3</sup>, Mohamad Reza Harati<sup>4</sup>, Majid Malekzadeh Shafaroudi<sup>5</sup>, Jamshid Yazdani-Charati<sup>6</sup>, Mohammad Ahanjan<sup>7</sup>

<sup>1</sup> Department of Entomology, School of Health, Mazandaran University of Medical Sciences, Sari, Iran

<sup>2</sup> Health Sciences Research Center, Department of Medical Entomology and Vector Control, Mazandaran University of Medical Sciences, Sari, Mazandaran, Iran

<sup>3</sup>Ferdowsi University of Mashhad, Mashhad, Iran

Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

<sup>5</sup> Department of Anatomy and Cell Biology, Sari Medical Faculty, Mazandaran Medical Sciences University, Sari, Iran

<sup>6</sup>Department of Biostatistics, School of Health, Mazandaran University of Medical Sciences, Sari, Iran

<sup>7</sup>Antimicrobial Resistant Nosocomial Infection Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Email: [ahanjan2007@gmail.com](mailto:ahanjan2007@gmail.com)

### ABSTRACT

*This study was done to investigate the bacterial infection and antibiotic susceptibility of the bacteria isolated from cockroaches in educational hospitals of Mazandaran university of medical sciences in 2013. In this descriptive cross-sectional study, cockroaches of 4 hospitals were caught via direct collection in a 45 days period. Medically important bacteria were isolated from their outer surface and digestive tract by standard procedures and antibiogram test was done using different antibiotics. Culturing the supernatant of outer surface wash and the digestive tract of cockroaches resulted in the separation of *Proteus mirabilis*, *Klebsiella pneumonia*, *E. Coli*, *Enterobacter*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Staphylococcus epidermidis*. The main common bacteria were *Proteus mirabilis*, *Klebsiella pneumonia* in three hospitals, and *Pseudomonas aeruginosa*, *Acinetobacter baumannii* in one hospital. *Staphylococcus epidermidis* isolated from different hospitals had the highest susceptibility to various antibiotics, however, *Acinetobacter baumannii* had the lowest susceptibility to different antibiotics. Cockroaches can carrier pathogenic bacteria in hospitals and they can cause hospital-acquired infections. Regarding to their potential in drug-resistant pathogens transmission, periodic fumigation and sanitation programs of the hospitals would be emphasized to combat these insects in the hospitals.*

**Keywords:** Cockroaches, Pathogenic Bacteria, Nosocomial Infection

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### INTRODUCTION

Hospital-acquired infections have emerged as a cause of illness or death in people over recent years [1]. Nosocomial infection or hospital infection is acquired after the patient's admission in the hospital and is manifested during his or her remain there or after furlough in case it is linked to hospitalization. Regardless of region in the world, hospital's environment is a reservoir of pathogenic microorganisms in a higher or lesser extent. It is estimated that in developing countries, Nosocomial infections concern above 25% of hospitalized patients, and in the developed countries from 5 to 10% [2]. It was also reported that the ratio of infections were higher in public hospitals than private hospitals [3]. A great number of such infections are attributed to poor standards of hygiene but it seems likely that peridomestic pests such as the American cockroach (*Periplaneta americana* L., in the family *Blattidae*) and indoor domestic pests such as *Blatte. germanica* are also involved. Cockroaches may not merely carry bacteria capable of causing human illness but may also act as hosts, allowing such bacteria to multiply (4). Cockroaches (*Dictyoptera: Blattidae*) are omnivorous insects and they can potentially carry pathogenic

microbes, so they play an important role in the spread of infection in hospitals and environment [4]. It was reported that cockroaches can carrier of 150 bacteria species, 60 yeast species, 90 protozoa species and 45 parasite worms' species (5 & 6). Cockroaches can transport up to fourteen million bacteria on their body surface and seven million in their excremental droppings [7]. Cockroaches' excretion has compounds such as cinamic acid, xanthurenic acid and 8-hydroxyquinaldic acid (tryptophan derivatives) that have mutagenic and carcinogenic properties (6). In hospitals, cockroaches have been detected inside different wards, the intensive therapy zone, surgical section, kitchen, and medicine part (8). These cockroaches can often move between hospital wards, operating theatres, patients' rooms, and food-preparation and storage areas, frequently via gaps in walls such as pipe chases, and their potential to disseminate multidrug-resistant pathogenic agents is a significant concern for many healthcare professionals (4). The hospital provides them with suitable temperature, food, water, and protective cover. Cockroaches can derive nourishment from vomit, spit, phlegm, excrement, human entrails, and on other diverse food sources. They are able to transfer viruses, bacteria, fungi, and other medically significant pathogenic agents in infectious regions (9). They have been proposed as likely transmitters of drug-resistant pathogens. Up to 98% of cockroaches found in a clinical setting can bear pathogenic agents on their teguments or in their intestines (10). So far, numerous pathogenic bacteria, including *Salmonella spp*, *Shigellaspp*, *Campylobacter spp*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiellapneumoniae* have been isolated from cockroaches (11 & 12). It is well documented that the introduction of antimicrobials in the therapeutics of infectious diseases was described over 2,500 years ago. At that time, they were regarded as the solution to all diseases caused by microorganisms. However, their widespread and indiscriminate use has caused the selection of resistant strains, and their empirical use, the lack of institutional standardization policies and pre-established criteria for their acquisition or therapeutic prescription, among other factors, have led to a selective pressure on microorganisms, leading to difficulty in treatment and multi-resistant strains. This has created impasses in the clinical treatment of patients, especially in health-care institutions (13). Thus, the aim of the present study was to investigate the bacterial infection of cockroach vectors and determine the antibiotic susceptibility of bacteria isolated from cockroaches in Sari educational hospitals (such as Imam Khomeini, Fatima Zahra, Bu ali, and Zare hospitals).

## MATERIALS AND MTHODS

Imam Khomeini, Fatima Zahra, Bu ali, and Zare hospitals in Sari, Iran was noticed in this research. The samples were gathered by direct collection (sterile hand gloves and containers) from different parts of the hospitals, laboratory and kitchen. Sampling was done afternoons using sterile tubes and this procedure lasted 45 days. The hunted samples were transferred to the research lab of medical department. Any cockroach's body was washed fully by 1 cc physiological sterile serum in the experimental tubes. Then, the cockroach was brought out of the tubes and the remained liquid contained bacteria was centrifuged at 2000 rpm for 10 minutes. Supernatant was removed and the sediment was used for determination of bacteria. Different selective bacterial media such as eosin methylene blue agar(EMB), blood agar and salmonella shigella agar(SS Agar) were applied for culturing bacteria in 37 ° C for 24 hours. Bacteriological recognition tests were performed by an expert bacteriologist. In order to continue the study, cockroach's guts were picked using a sterile forceps, squashed in a plate, and mixed with 1 ml physiological sterile serum. The suspension was examined for determining the specious and strain of the bacterium. Biochemical tests were performed on colonies from primary cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests such as triple sugar iron (TSI), Simmons citrate (SC), Lysine decarboxylase brothsulfide indolemotility (SIM), urea or MR-VP medium (Methyl Red, Voges-Proskauer). Gram-positive cocci were identified based on their gram reaction, catalase, coagulase and novobiocin susceptibility test results. Isolated bacteria were tested for antimicrobial susceptibility with Kirby-Bauer disk diffusion method using gentamicin (10 µg), ciprofloxacin, ceftizoxime, cephalixin, methicillin,vancomycin, tetracycline, trimethoprim + Sulfamethoxazole, and imipenem on muellerhinton medium.

## RESULTS

Data of the present study showed that the collected cockroaches from different parts of Imam Khomeini, Fatima Zahra, Bu ali, and Zare hospitals were the vector of gram positive and gram negative bacteria. The dominant bacteria isolated from Zare hospital cockroaches were *Proteus mirabilis* (25%), *Klebsiella pneumoniae*(20%), *Pseudomonas aeruginosa* (15%), *E. Coli* (10%), *Enterobacter*(8 %), *Acinetobacterbaumannii* (4 %), *Staphylococcus aureus* (12%), and *staphylococcus epidermidis*(6 %). Most frequent isolated bacteria from Bu Ali and Fatima Zahra hospitals were *Klebsiella pneumoniae*(30 %), *Proteus mirabilis* (22%), *Enterobacter* (15%), *E. Coli* (13%), *Staphylococcus aureus*(12 %), and

*staphylococcus epidermidis*(8%). The dominant bacteria isolated from Imam Khomeini hospital were *Pseudomonas aeruginosa* (23%), *Acinetobacter baumannii* (8%), *Staphylococcus aureus*(18 %), *Klebsiella pneumoniae*(16 %), *E. Coli* (10%), *Staphylococcus epidermidis* (7%), *Proteus mirabilis* (10%), and *Enterobacter*(8%). It was interesting that about 80% of cockroaches' outer surface bacteria was also found in their digestive tract. The results of the antibiogram tests using gentamicin (10 µg), ciprofloxacin, ceftizoxime, cephalixin, methicillin, vancomycin, tetracycline, trimethoprim + Sulfamethoxazole, and imipenem antibiotics are shown in tables 1, 2, 3, and 4.

Table 1. Drug resistance of isolated bacteria from 41 cockroaches in Zare Hospital.

	<i>Proteus mirabilis</i>	<i>Klebsiella pneumoniae</i>	<i>E. Coli</i>	<i>Enterobacter</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
gentamicin	(40%)5	(75%)8	(33%)3	(33%)3	(75%)8	(100%)7	(40%)5	(0%)2
ciprofloxacin	(20%)5	(34%)8	(33%)3	(33%)3	(62%)8	(87%)7	(20%)5	(0%)2
ceftizoxime	(0%)5	(45%)8	(0%)3	(0%)3	(50%)8	(87%)7	(0%)5	(0%)2
cephalexin	(%20)5	(35%)8	(0%)3	(33%)3	(50%)8	(100%)7	(60%)5	(0%)2
methicillin	NT	NT	NT	NT	NT	NT	(100%)5	(0%)2
vancomycin	NT	NT	NT	NT	NT	NT	(0%)5	(0%)2
tetracycline	(80%)5	(55%)8	(33%)3	(33%)3	(100%)8	(100%)7	(100%)5	(0%)2
trimethoprim + Sulfamethoxazole	(40%)5	(65%)8	(33%)3	(33%)3	(100%)8	(100%)7	(80%)5	(0%)2
imipenem	(20%)5	(25%)8	(0%)3	(0%)3	(37%)8	(%87)7	NT	NT

NT: Not test

Table 2. Drug resistance of isolated bacteria from 28 cockroaches in Fatmia Zahra Hospital.

	<i>Proteus mirabilis</i>	<i>Klebsiella pneumoniae</i>	<i>E. Coli</i>	<i>Enterobacter</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
gentamicin	(20%)4	(40%)5	(0%)3	(33%)3	(80%)5	(100%)2	(25%)4	(50%)2
ciprofloxacin	(0%)4	(20%)5	(33%)3	(33%)3	(60%)5	(100%)2	(25%)4	(0%)2
ceftizoxime	(0%)4	(40%)5	(0%)3	(0%)3	(60%)5	(100%)2	(25%)4	(0%)2
cephalexin	(50%)4	(40%)5	(33%)3	(33%)3	(60%)5	(100%)2	(50%)4	(0%)2
methicillin	NT	NT	NT	NT	NT	NT	(100%)4	(0%)2
vancomycin	NT	NT	NT	NT	NT	NT	(0%)4	(0%)2
tetracycline	(80%)4	(40%)5	(66%)3	100%)3 (	(100%)5	(100%)2	(100%)4	(0%)2
trimethoprim + Sulfamethoxazole	(75%)4	(100%)5	(66%)3	100%)3 (	(100%)5	(100%)2	(100%)4	(0%)2
imipenem	(25%)4	(20%)5	(0%)3	(0%)3	(60%)5	(50%)2	NT	NT

NT: Not test

Table 3. Drug resistance of isolated bacteria from 26 cockroaches Bu Ali Hospital.

	<i>P. mirabilis</i>	<i>K. pneumoniae</i>	<i>E. Coli</i>	<i>Enterobacter</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
gentamicin	(20%)5	(80%)5	(33%)3	(50%)2	(100%)4	(100%)2	(33%)3	(0%)2
ciprofloxacin	(20%)5	(25%)5	(33%)3	(0%)2	(50%)4	(100%)2	(33%)3	(0%)2
ceftizoxime	(20%)5	(50%)5	(0%)3	(0%)2	(50%)4	(50%)2	(33%)3	(0%)2
cephalexin	(20%)5	(60%)5	(33%)3	(66%)2	(75%)4	(100%)2	(33%)3	(0%)2
methicillin	NT	NT	NT	NT	NT	NT	(100%)3	(0%)2
vancomycin	NT	NT	NT	NT	NT	NT	(0%)3	(0%)2
tetracycline	(80%)5	(100%)5	100%)3 (	(100%)2	(100%)4	(100%)2	(66%)3	(0%)2
trimethoprim + Sulfamethoxazole	(50%)5	(100%)5	(33%)3	(50%)2	(100%)4	(100%)2	(66%)3	(0%)2
imipenem	(0%)5	(0%)5	(0%)3	(0%)2	(0%)4	(50%)2	NT	NT

NT: Not test

Table 4. Drug resistance of isolated bacteria from 21 cockroaches Imam Khomeini Hospital.

	<i>Proteus mirabilis</i>	<i>Klebsiella pneumoniae</i>	<i>E. Coli</i>	<i>Enterobacter</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
gentamicin	(33%)3	(60%)4	(33%)3	(0%)2	(66%)3	(100%)2	(33%)3	(0%)1
ciprofloxacin	(33%)3	(25%)4	(33%)3	(0%)2	(33%)3	(100%)2	(33%)3	(0%)1
ceftizoxime	(0%)3	(50%)4	(0%)3	(0%)2	(0%)3	(50%)2	(0%)3	(0%)1
cephalexin	(66%)3	(33%)4	(33%)3	(33%)2	(66%)3	(100%)2	(33%)3	(0%)1
methicillin	NT	NT	NT	NT	NT	NT	(100%)3	(0%)1
vancomycin	NT	NT	NT	NT	NT	NT	(0%)3	(0%)1
tetracycline	(100%)3	(0%)4	(66%)3	(0%)2	(100%)3	(100%)2	(66%)3	(0%)1
trimethoprim + Sulfamethoxazole	(66%)3	(0%)4	(33%)3	(50%)2	(100%)3	(100%)2	(66%)3	(0%)1
imipenem	(0%)3	(0%)4	(0%)3	(0%)2	(0%)3	(0%)2	NT	NT

NT: Not test

## DISCUSSION

Hospitals patients suffering from different ailments and cockroaches would have been contaminated during their nocturnal movements from one ward to another and to other areas including toilets [5]. This emphasizes the significant role of cockroaches in the transmission of nosocomial infections [14]. Our study revealed that the two identified species including *Proteus mirabilis* (25%) and *Klebsiella pneumoniae* (20%) were the most frequent bacteria isolated from cockroaches in the Zare, Bu Ali, and Fatima Zahra hospitals. However, *Pseudomonas aeruginosa* (23%), *Staphylococcus aureus* (18%), and *Staphylococcus epidermidis* (10%) were the most frequent bacteria isolated from cockroaches in the Imam Khomeini hospital. In agreement with these results, Fakoorzibaet al. (2010) reported that the most frequent extracted bacterium was *Klebsiella*. Also, the results of an investigation in a Brazilian health care institution showed that among the enterobacteria, *Klebsiella pneumoniae* was the most prevalent, and it was isolated from 18% of American cockroaches (15). Isolation of nine pathogenic bacteria from the external surface of American cockroaches caught in health and medical services centers in Khorramshar County has been reported. *Klebsiella* (47.9%) and *Pseudomonas* (37%) were the most common recognized bacteria (16). In contrast to our result, some researchers reported that the most frequent extracted bacterium from cockroaches' external body surface was *Escherichia coli* and the second was *Proteus*, which were potential pathogens (17). Furthermore, an investigation done in three hospitals of Ahwaz County, Southwestern Iran, nine species of pathogenic bacteria were extracted. The most frequently bacteria separated in the mentioned study were *E. coli* (86.7%) and *P. vulgaris* (73.3%) [18].

It is well known that cockroaches can transfer pathogenic organisms from infected sources to uncontaminated material in the hospitals (19 & 20). Therefore, their existence in hospitals can be a serious public health problem. On the other hand, many bacterial strains isolated from arthropods caught in the hospitals or nearby areas, were resistant to antibiotics and chemotherapeutics. They could have the following mechanisms of resistance: 1- ESBL (extended spectrum of beta lactamases) and AmpC (AmpC beta lactamases) – (Gram – negative rods), 2- methicillin resistance (MRSA – methicillin resistant *Staphylococcus aureus*, 3- MRSE – methicillin resistant *Staphylococcus epidermidis*, 4- MRCNS – methicillin resistant coagulase – negative *Staphylococci*), 5- MLSb (resistance to macrolides, lincosamides, streptogramin B) – (*Staphylococci*) (2). In agreement with our findings, the strains of *Klebsiella spp* were obtained from cockroaches caught in the hospitals (21). It was reported that more than 85% of these isolates were resistant to more than four types of antibiotics, whereas all strains of *Klebsiella spp* isolated from the control cockroaches were sensitive to those antibiotics (21). Generally, *Klebsiella spp* strains are characterized typically by presence of chromosomally encoded beta-lactamases (class A), which give them resistance to penicillins, and what is more, many of them have beta-lactamases with a wide range of activity. The latter gives them the resistance to carbenicillin, ampicillin and quinolones. It is also documented that the strains of *Klebsiella pneumoniae* and *E. coli*, (especially resistant - for example those which produce ESBL), have a high ability to spreading in the hospital environment (22). Our study showed that *E. coli* isolated from hospital cockroaches was susceptible to Ceftizoxime (100%), while *Klebsiella pneumoniae* was susceptible to Imipenem. Presence of MRSE – methicillin resistant *Staphylococcus epidermidis* in the hospital environment is undesirable, because there is the probability of the transmission of mecA DNA genes, which are responsible for resistance to methicillin, from one species of *Staphylococci* to the other (e.g. on *Staphylococcus aureus*) (23 & 24). Strains of *Staphylococci* (*S. epidermidis*, *S. xylosus* and *S. equorum*) which were isolated from the cockroaches caught in the hospitals in Warsaw, were resistant to methicillin, and what is more, those *S. epidermidis* and *S. equorum* were

resistant to erythromycin and clindamycin (MLSb mechanism - induced mechanism of resistance to macrolides, lincosamides and streptogramin B). It was reported that extracted *Staphylococcus hominis* strain was resistant to at least three commonly used antibiotics, including gentamicin (22). In present study, *Staphylococcus aureus* and *Staphylococcus epidermidis* were sensitive to Vancomycin (100%). German cockroaches from hospitals were also source of *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus casseliflavus*, *Enterococcus durans* and *Enterococcus avium* strains (Czajka *et al.*, 2003). All those bacteria were sensitive to antibiotics. Isolated *E. casseliflavus* strain was medium-sensitive to vancomycin, (it is connected with vanC phenotype of this species, which have resistance to low concentrations of vancomycin) but medium sensitivity to teicoplanin (22). Previous study showed that strains of *Enterococci* carried by Diptera were resistant to some aminoglycosid chemotherapeutics, but sensitive to vancomycin (22). It is known, that *Enterococci* acquire and transmit genes determining resistance to many antibiotics which are used in hospitals against various types of bacteria, e.g. *Streptococcus spp*, *Staphylococcus spp*. An example is transmission of resistance gene vancomycinvanA to *S. aureus* (25). Strains of *Pseudomonas spp.* were often isolated from insects caught in different environments, including also hospital areas. From hospitals' cockroaches strains of *P. aeruginosa* and *P. putida*, - resistant to penicillins, cotrimoxazole and trimethoprim were obtained. They didn't produced beta - lactamase of extended ESBL substrate spectrum (-) nor induced beta-lactamase IBL (-) as well (24). In present study, *Staphylococcus epidermidis* isolated from different hospitalshad the highest susceptibility to various antibiotics, however, *Acinetobacter baumannii* had the lowest susceptibility to various antibiotics.

Evaluation of the bacterial strains isolated from cockroaches of the hospital ' environment is helpful for determination of their resistance to the antibacterial drugs, agents using for the disinfection in the hospital, and the assessment of the insecticides effectiveness and arthropods' resistance. It is reported that some bacteria species such as the *Pseudomonas* species can form biofilms, so they can become resistant to various bactericidal disinfectants (4 & 26). So, the use of slow-acting insecticide gels (each a combination of insecticide, food, attractant and water) is helpful in baiting programs. Also, it was reported that the application in sticky traps of a synthetic analogue of the volatile sex pheromone, blattellaquinone, could improve control of German cockroaches (27).

In conclusion, the periodic fumigation and provide a national or international cockroaches' control programs, especially in public places such as hospitals, is recommended to reduce the incidence of nosocomial infections through mechanical insect disseminators such as cockroaches. Certainly, these findings and other similar studies will increase the awareness and susceptibility of health systems in removing this insect from hospitals' environment.

#### COMPETING INTERESTS

The authors declare no conflict of interest.

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