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**The bacterial infection of German cockroach (*Blatella germanica*)
in hospitals of western Iran**

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Abstract

Cockroaches are considered a serious health problem in some medical centers. They can move freely in hospital wards and transmit pathogenic organisms. This study was carried out in order to detect and identify bacterial infection on outer surface and in digestive organs of *Blatella germanica* as a dominant species of cockroach in hospitals.

In this cross-sectional study 222 *B. germanica* were collected from 14 different wards and units of 5 hospitals in the city of Hamedan in western Iran. Most of them were collected from dining room (34.2%) followed by urology ward (13.5%). After applying anesthesia and washing in 2cc physiology serum, the culture was prepared from this suspension for identification of bacterial infection on outer surface. Next, the outer surface of each cockroach was sterilized and their guts were isolated from mouth to anus. Bacteria extraction and culture were done accordingly.

A total of 12 bacteria species were identified from outer surface and digestive system of cockroaches. On outer surface, *Escherichia coli* was the most dominant species (40%) followed by *Staphylococcus epidermidis* (20%). In digestive system *E. coli* had also the highest percentage (45%) followed by *Enterobacter aeruginosa* (20%). This study can show the importance of cockroaches as potential vectors of medically important microorganisms such as pathogenic bacteria in hospitals. Control programs for removing cockroaches, especially in the hospitals are recommended.

Key word: *Blatella germanica*, Bacterial infection, Hospital, Iran

آلودگی باکتریایی سوسری آلمانی (*Blatella germanica*) در بیمارستان‌ها، غرب ایران

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چکیده

سوسری‌ها به عنوان یک مشکل بهداشتی جدی در مراکز درمانی محسوب می‌شوند. آنها می‌توانند آزادانه در بخش‌های مختلف بیمارستانی حرکت کرده و عوامل بیماری‌زا را جایجا نمایند. این مطالعه با هدف بررسی آلودگی باکتریایی سطح خارج بدن و دستگاه گوارش سوسری آلمانی (*B. germanica*)، به‌عنوان یک گونه غالب سوسری در بیمارستان‌ها، انجام گرفت. در این مطالعه مقطعی تعداد ۲۲۲ عدد سوسری آلمانی از ۱۴ بخش در ۵ بیمارستان شهر همدان صید شدند. سلف سرویس و بخش ارولوزی به ترتیب بیشترین درصد صید را داشتند. به منظور تعیین آلودگی باکتریایی خارجی، سوسری‌ها بعد از

بیهوشی در ۲ سی سی سرم فیزیولوژی شستشو داده شدند و سپس از سوسپانسیون کشت صورت گرفت. تعیین آلودگی باکتریایی دستگاه گوارش بعد از استریل کردن سطح خارجی بدن سوسری و جداسازی لوله گوارشی از دهان تا مخرج همچون مرحله قبل انجام شد. در مجموع ۱۲ گونه باکتری از سطح خارجی و دستگاه گوارش سوسری‌ها جداسازی گردید. باکتری‌های *Escherichia coli* و *Staphylococcus epidermidis* در سطح خارجی بدن و *E.coli* و *Enterobacter aeruginosa* در دستگاه گوارش سوسری‌ها دارای بیشترین درصد فراوانی بودند. این مطالعه اهمیت سوسری‌ها را به‌عنوان ناقلین بالقوه عوامل بیماریزا در بیمارستان‌ها نشان داده و لذا پیشنهاد می‌شود تا برنامه‌های کنترلی برای حذف آنها اجرا شود. واژگان کلیدی: *Blatella germanica* آلودگی باکتریایی، بیمارستان، ایران.

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Introduction

Cockroaches are contaminated with pathogenic bacteria causing leprosy, bubonic plague, dysentery, urinary infections, Hungarian, pimples, and abscesses. They are also infected with almost 150 species of bacteria, 60 species of yeast, 90 species of protozoa and 45 species of parasite worms (WHO/Department of Communicable Disease Prevention, 1999, Basseri *et al.*, 2008, López-Sánchez *et al.*, 2008, Hanafi-Bojd and Sh, 2001)

Cockroaches can be found in various places. They are omnivorous and their habit of vomiting a portion of eaten food wherever they eat it can contaminate humans' food and household surroundings. These nutritional behaviors, their infected surface body, nocturnal activity and the ability to escape and run fast can increase the potential of disease transmission (Service, 1980, Bell *et al.*, 2007, Pechal *et al.*, 2007).

Cockroaches are associated with nosocomial infections, which issue has attracted less attention so far (Stypułkowska-Misiurewicz *et al.*, 2005). It has been reported that 96.1% of collected cockroaches from hospitals and 98.7% from houses are able to carry bacteria pathogens and play an important role in the spread of bacterial pathogens in hospitals and the environment (Elgderi *et al.*, 2006). The species *Enterobacter*, *Proteus*, *Citrobacter*, *Aerobacter*, *Klebsiella*, *Bacillus*, *Serratia*, and *Edwardsiella* were isolated from external surface of cockroaches collected from Tehran hospitals (Fakoorziba *et al.*, 1998).

Blatella germanica has been reported as one of dominant species in hospitals by some researchers (Lee *et al.*, 2003). With regard to the existence of this species in hospitals and its importance in mechanical transmission of pathogenic agents, the current study was conducted in order to identify species of bacteria on the outer surface and in the digestive system of *B. germanica* in the hospitals.

Materials and Methods

This cross-sectional study was conducted in five hospitals in Hamedan city located in the west of Iran. Fourteen units and wards of the hospitals including infectious disease ward, operating room, general surgery, orthopedics, emergency room, urology, dialysis,

ophthalmology, cardiology, radiotherapy, burns unit, psychiatry, neonatal and dining room (self-service) were examined.

Considering the prevalence of *E. coli* as the most abundant bacterial species in the exterior of cockroaches, and having $P=25.7\%$, $d= 0.06$ and $\alpha = 0.05$; the minimum sample size required for this study was estimated to be 222 cockroaches according to the following formula.

$$n = \frac{z^2 p(1 - p)}{d^2}$$

Cockroaches were collected by hand, sticky traps and glass traps, which was done based on previous studies (Wang and Bennett, 2006, Smith and Appel, 2008). Using sterile gloves, all cockroaches were taken into sterile glass or plastic containers and transferred to the laboratory. To prevent the spread of contamination, each container was used only for one cockroach.

Glass traps contained a glass or plastic jar with 20 cm height and 15 cm in diameter, constricted by funnel paper. To prevent cockroaches from escaping, the upper interior parts of the jars were lubricated with oil. Malt juice and bread were placed inside the jars as the bait to attract the cockroaches. To lead cockroaches into the trap, two methods were used; digging the ground and putting the glass trap in it at the same level with the ground. This method was not feasible due to stone or ceramic floors of hospitals. In the second method, which was used in this study, paper strips were put between the upper edge of the jar and the ground surfaces (WHO/Department of Communicable Disease Prevention, 1999).

A tile with a size of 15×15 was used for the sticky trap. A piece of bread or cake as a bait was placed at the center of a circle prepared from a non-dry adhesive. The attracted cockroaches to the bait were caught in the sticky trap. These traps were set before sunset in the appropriate places such as sick rooms; in the corners, under dressers and refrigerators to be collected the following night.

Having been taken to the laboratory, each of cockroaches was anesthetized by the solution of chloroform, observed by stereo-microscope and identified by a specific key. In this way, the genus and species of each sample were recorded. To identify bacterial infection of outer surface, they were washed in a sterile glass containing 2 ml sterile saline. The prepared suspension was used for culture of bacteria. To identify bacterial infection of digestive system, each of samples was sterilized by specific solution and rinsed in serum. Then, their guts were isolated from mouth to anus and bacteria culture was done similarly. Blood Agar (BA), Eosin methylen-blue lactose sucrose agar (EMB), selenite F (SF), salmonella shigella agar (SS), chocolate agar, Macconkey agar, Mannitol Salt Agar (MS), Laysin, Simmons Citrate, Orthonitro Phenul – -D-galacto Pyramside (ONPG), Methyl Red(MR), Sulfide-Indol-Motility(SIM), Triple Sugar Iron Agar (TSI) Coagulase Test, Catalase Test and Oxidase Test were used as culture media in this study.

As for microbial culture, at first, the swab was cultured on an EMB amplifying environment such as the McKinney or SS environment and subsequently on enriched environments like selenite F. The samples were tested after 24 hours at 37° C in order to detect bacterial strains using differential, biochemical and serological tests.

Results

In the current study, 222 samples of *B. germanica* were collected and examined. Most of them were collected from dining room (34.2%), followed by urology ward (13.5%) (Table 1).

Table 1- Distribution of *B. germanica* in hospital wards

Hospital Wards	No	%
Orthopedics	6	2.7
Emergency	9	4.1
Operating room	6	2.7
ICU	4	1.8
General surgery	2	0.9
Dining room	76	34.2
Dialysis	1	0.5
Urology	30	13.5
Eye	29	13.1
Burn	20	9
Infectious	5	2.3
Psychiatry	9	4.1
Elective surgery	20	9
Neonatal	5	2.3
Total	222	100

All in all, 12 bacteria species were identified from outer surface and digestive system of cockroaches. *Escherichia coli* was the most dominant species (40%) on the outer surface, followed by *Staphylococcus epidermidis* (20%). In the digestive system *E. coli* had also the highest percentage (45%) followed by *Enterobacter aeruginosa* (20%).

Proteus vulgaris was detected with the lowest percentage on surface as well as in the digestive organs. Similarly, *Citrobacter diversus* and *Enterobacter agglomerans* had the lowest frequency in digestive system of the cockroaches (Table 2).

Table 2- The frequency of bacteria isolated from *B.germanica*

Bacteria spp.	Digestive organ		Outer surface	
	No	%	No	%
<i>Escherichia coli</i>	65	29.3	57	25.7
<i>Shigella Sonnei</i>	4	1.8	1	5
<i>Shigella disenteria</i>	9	4.1	0	0
<i>Staphylococcus aureus</i>	4	1.8	9	4.1
<i>Staphylococcus epidermidis</i>	8	3.6	29	13.1
<i>Citrobacter freundii</i>	16	7.2	18	8.1
<i>diversus Citrobacter</i>	3	1.4	8	3.6
<i>aeruginosa Pseudomonas</i>	10	4.5	29	13.1
<i>Proteus mirabilis</i>	6	2.7	1	5
<i>Proteus vulgaris</i>	3	1.4	4	1.8
<i>Salmonella typhi</i>	9	4.1	5	2.3
<i>Salmonella paratyphi A</i>	2	9	0	0
<i>Morganella morganii</i>	1	5	0	0
<i>Entrobacter aeruginosa</i>	33	14.9	12	5.4
<i>Entrobacter cloacae</i>	7	3.2	8	3.6
<i>Klebsiella Oxytoca</i>	10	4.5	0	0
<i>Klebsiella pneumoniae</i>	8	3.6	6	2.7
<i>Streptococcus pneumoniae</i>	4	1.8	6	2.7
<i>Providencia Spp</i>	5	2.3	2	9
<i>Serratia marcescens</i>	30	13.5	25	11.3
<i>Enterococcus</i>	41	18.5	26	11.7
<i>Edwardsiella</i>	0	0	1	5
<i>Entrobater agglomerans</i>	3	1.4	0	0

Discussion

Several studies carried out in other parts of Iran have shown two species of American and German cockroaches to be the predominant ones (Mohammadi, 1996, Doroodgar *et al.*, 2005).

The results of this study showed that the dining room of the hospital has the highest rate of *B. germanica*, which can be considered a suitable place for the presence of this species. In a similar study done in Poland, *B. germanica* was detected in 70% of hospitals in which the dining room, laundry storage, and the elevator had the highest frequencies of the presence of this cockroach. In this investigation it was found that the outer surface body of

roaches was infected with several bacteria that can cause nosocomial infection (Gliniewicz *et al.*, 2002). A similar research demonstrated that there is an insignificant difference among the collected species. *B. germanica* has been observed more frequently in clinics, whereas *Periplaneta americana* has been reported from non-clinical environments (Pai *et al.*, 2004).

Similar to the results of the current study, various studies have isolated different bacteria species on cockroaches' bodies. In a similar study, *E. coli* was the most isolated bacterium from cockroaches followed by *Streptococcus* and *Bacillus* (Zarchi and Vatani, 2009). In another research conducted in China most of cockroaches were found in sewerage in which they were infected with *E. coli*, *Pseudomonas arinosa*, *Salmonella* and *Staphylococcus aureus* (Lin *et al.*, 2008). Likewise, in a study in Hamedan city, 98% of *B. germanica* showed contamination with high bacterial load. In this study bacteriological examinations demonstrated that almost all of tested cockroaches had at least one of some microorganisms such as bacteria, fungi and worms. The researchers have emphasized the important role of cockroaches as potential vectors of medically important microorganisms in hospitals (Salehzadeh *et al.*, 2007).

Along the same lines, the results of the current study show that *B. germanica* is considered as a mechanically important vector for spread of pathogenic bacteria. Therefore, the presence and abundance of infected cockroaches in the hospitals is very dangerous and threatens public health. It is, therefore, recommended that serious programs be implemented in order to control cockroaches as well as evaluate the insecticides in the hospitals.

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