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Household cockroaches of Quetta city as reservoir for infectious pathogenic bacteria

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Abstract

This study makes an assessment on the role of cockroaches as potential carriers of pathogenic microorganisms. Current research is based on isolation, biochemical conformation and antimicrobial susceptibility tests. Totally, 191 household cockroaches were collected and identified as *Blata orientalis*, *Blattella germanica* and *Periplaneta americana*. A total of 10 Gram positive and negative species were isolated from their external surfaces and in the gastrointestinal tract. Percentage prevalence of contaminations for isolates were found higher in *P. americana* (35.6%) than *B. orientalis* (32%) and least in *B. germanica* (21.6%). The highest percentage of isolates was recorded in female (55.6%) then male (33.6%) cockroaches. The most abundant and common bacterial species belonged to *Escherichia coli* (80%), while *Salmonella* spp. (55%) was recoded as the least isolated specie. The ciprofloxacin and colistin sulphate antibiotics were recorded as most effective drugs against all isolates. The current finding of this study suggests that household cockroaches act as potential vector and reservoir of some important bacterial pathogens.

Keywords: Household cockroaches, isolation, biochemical conformation, antimicrobial susceptibility

1. Introduction

Cockroaches belong to order Blattodea and are considered as an important mechanical vector source to transmit pathogenic organisms including protozoa, bacteria and viruses. The occurrence and distribution of cockroaches are worldwide [1] and so far more than 50 species of cockroaches have been reported around human living structures [2]. Similar kinds of bacterial fauna occurrence in nymphal and adult cockroaches indicate sharing of forage and residential localities [3]. The shedding of nymphal cuticle and damaged body parts during metamorphosis provides rapid dispersal of pathogens [4].

Cockroaches can breed and forage in sewer systems, garbage bins, and latrines [5]. Cockroaches create problematic conditions which include spoilage of food, transfer of pathogens, frequent allergic reactions and psychological distress [6]. The presence of cockroaches is a major cause of acute asthma morbidity [7].

Cockroach's nocturnal habit facilitates the rapid transmission of pathogens during at night time [8]. Household cockroaches carry more than 100 pathogen bacteria on their integuments, in the digestive tracts, and in feces [9]. Cockroaches can transmit various pathogens, both on the external surface and internal medium of human body [10]. It was noted that the presence of bacterial fauna found highest in the stomach than intestine and least on external surfaces [11]. They can provide sufficient space for multiplication of pathogens in gastrointestinal tract as their excretion may contain bacteria for up to 114 days [12].

Most dominant species of cockroaches include American cockroaches (*P. americana*) which are large and black, Oriental cockroaches (*B. orientalis*) are medium and dark brown to black and German cockroaches (*B. germanica*) are in smaller size and brown in colour [13]. American cockroaches carry numerous bacteria like *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., and *Enterococcus* spp [14]. Few researchers conducted on cockroaches deny their direct involvement to cause serious health issues like allergic reactions. However, it is confirmed that they provide shelter to multidrug resistant bacterial pathogens [15].

The disease control management plan includes proper vaccination in infected areas; improved water quality and sanitation recommended by world health organization. These measures help in the prevention of diseases spread by cockroaches. Role of cockroaches as mechanical vectors of source for bacteria needs more research.

The objective of this research was to isolate, identify and check antibiotic resistance in isolated bacterial pathogens from household cockroach species.

2. Material and methods

2.1 Collection of cockroaches

Laboratory work was performed at Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB) University of Baluchistan, Quetta and an ethics approval was taken from the same research institute. The current study was conducted at the above premises during the period March-July, 2016. A total of 191 adult cockroaches were captured with majority at early in the morning and late night hours from houses. These were manually hand caught using sterile entomological forceps and few at and were kept in sterile flask. The collected specimens were examined under laminar flow with the help of the compound microscope to study sexual dimorphism using taxonomic keys as described by Chew [16].

2.2 Isolation from body surface and gut

Each cockroach was frozen at 0 to -5°C for 5-10 minutes to bring under sub-conscious condition. About 2 ml of saline solution (0.9%) was added in the sterile flask, then vortex it and add saline solution (0.01 ml) to the media [17]. The next step was to add 70% alcohol in the flask containing cockroach to decontaminate external surface and transfer the cockroach into sterilize flask to dry at room temperature under sterile conditions. Sterile normal saline (0.9%) was used for 2-3 minutes in order to remove all traces of alcohol. To isolate the internal bacteria, cockroaches were dissected and gastrointestinal tract was macerated aseptically in a sterile pestle and mortar in 2 ml of sterile normal saline. Aliquots (0.01 ml) of both prepared samples, including the external surface and gastrointestinal tract were cultured separately on selective and differential media, including Eosin methylene blue agar (EMBA), Cetrimide agar (CA), Selective agar, Mannitol salt agar (MSA), Reinforced Clostridium medium agar (RCM), Salmonella-Shigella agar (SSA) at 37°C for 24 hours under facultative and anaerobic conditions [18].

3.3 Identification of isolates

General characterization was done following bacteriological procedures of Gram staining. Further, characterization was achieved with biochemical tests which include; Gram staining, IMVIC, sugar fermentation, catalase, oxidase, gelatin liquefaction, Christie Atkins Munch Petersen, CAMP and H₂S production tests [19, 20].

3.4 Antibiotic susceptibility test

A standardized antibiotic sensitivity trail was tested on Mueller Hinton agar followed by the disc diffusion Bauer technique and McFarland turbidity standard method of CLSI Protocol [21]. The diameter of the zone of inhibition around the disc was measured for sensitivity and resistivity responses of specific antimicrobial agent (10-30 µg) for isolates [22].

3.5 Statistical analysis

Population density of collected specimens was calculated on t-test (p (one tailed) < 0.05) assuming independent variable using Microsoft Excel, Analysis tool pack 2010.

4. Results

4.1 Cockroach infestation

During this study three species, the *P. americana*, *B.*

germanica and *B. orientalis* were reported as the most common species in Quetta city. Gram-positive *bacilli*, *cocci* and Gram-negative *bacilli* were isolated from external surface and gastrointestinal tract of the cockroaches. Significant result ($p < 0.02$) for population density was obtained which indicates that male and female cockroaches were similar in population size collected from houses (Table 1). It was found that cockroaches carry 89.2% bacteria on external surfaces (28.9%) and gastrointestinal tract (60.3%) during the dissection of contaminations.

The *P. americana* was the most abundant carry 35.6% isolates of all cockroaches, while least was found isolated from *B. germanica* 21.6% (Figure 1). Percentage frequency of contamination was higher in *P. americana* (male 11.4%, female 24.2%) than *B. orientalis* (male 14%, female 18%) and least in *B. germanica* (male 8.2%, female 13.4%) as mentioned in Figure 2. This result also indicates that female cockroaches exhibit better potential for bacterial species as reservoirs than male cockroaches.

4.2 Isolates from Cockroaches

Total ten colonies were identified as *Citrobacter* spp., *E. coli*, *Enterobacter* spp. and *Klebsiella* spp. grown on (EMBA) media. *Staphylococcus aureus* developed new colonies on (MSA) medium. *S. typhimurium* and *Shigella* spp. colonies formation was confirmed on (SSA) media. *Clostridium* spp. on (RCM), *Listeria* spp. on (SA) and *Pseudomonas* spp. isolates were grown on (CA) media plates respectively (Figure 3).

Escherichia coli 80% was the most dominant isolated specie followed by 69.5% *S. aureus*, 69.5% *Enterobacter* spp., and 69.5% *Citrobacter* spp., placed at second dominant position; *Listeria* spp., 65.6% secured its third position as dominant species. Collectively *Shigella* spp., 61% *Klebsiella* spp., 60% *Pseudomonas* spp., 58% *Clostridium* spp., and 63.6% *Salmonella* spp., 55% were seen as least bacterial isolates (Figure 4). However, *Pseudomonas* spp. presence was not confirmed from *B. germanica* during isolation.

4.3 Biochemical tests

Biochemical tests including IMVIC, sugar fermentation tests, catalase test, oxidase test, gelatin liquefaction test and H₂S production tests were successfully applied and gave results for all isolates. *Listeria* spp was conformed only on CAMP test summarized in Table 2.

4.4 Antimicrobial susceptibility analysis

The antimicrobial susceptibility analysis of all antibiotics showed commonest and multidrug resistant pattern. Polypeptides (colistin sulphate), quinolones (ciprofloxacin) and tetracyclines (tetracycline) were found as effective common antibiotics. Multidrug resistant pattern was restricted to penicillin (carbenicillin), glycopeptides (vancomycin), lincosamides (lincomycin) and amino-glycoside (kanamycin) for isolated pathogen (Table- 3).

Clostridium spp. isolates were sensitive to all antibiotics except Lincomycin, while *S. aureus* showed sensitivity to all antibiotics. *Listeria* spp. was found to have multi-resistant to three antibiotics and showed sensitivity of maximum 45 mm zone of inhibition on ciprofloxacin. Overall, over two third of isolates were found resistant to the drugs. For individual strains of bacteria, *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Pseudomonas* spp., *Salmonella* spp. and *Shigella* spp. were found to have multi-drug resistance to four antibiotics, whereas *Klebsiella* spp. resistance to three antibiotics.

Table 1: Mean and variance of cockroaches for estimation of population density in Quetta city.

Cockroaches	Male	Female	t-test	p-value*
<i>P. americana</i>	32	29	2.68	0.02
<i>B. orientalis</i>	34	31		
<i>B. germanica</i>	38	27		
Mean	34.66	29		
Variance	9.33	4		

* Significant difference ($p < 0.05$) obtained for male and female distribution of cockroaches.

Table 2: Biochemical tests applied for classical bacterial species identification isolated from cockroaches.

Bacterial isolates, shape and biochemical tests											
Biochemical tests		a**	b	c	d	e	f	g	h	i	j
		Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Cocci	Rods
IMVIC	Indole	-	+	-	-	+	v	-	-	-	-
	MR	-	+	-	-	-	+	+	-	+	+
	VP	+	-	+	-	-	-	-	-	+	+
	Simmon Citrate	+	-	+	-	+	-	-	+	+	-
Sugar fermentation	Dulcitol	-	v	v	-	d	-	-	-	-	-
	Glucose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	-	+	v	+	+	+	+
	Trehlose	+	+	+	-	+	v	+	v	+	+
	Dextrose	+	+	+	-	+	+	+	+	+	+
	Xylose	+	+	+	v	+	+	+	+	-	-
	Sorbitol	+	+	+	-	v	v	+	v	-	v
	Ionsitol	+	-	+	-	-	-	-	-	-	-
	Mannitol	+	+	+	+	+	v	+	-	+	-
Other	Urease test	-	-	+	-	+	-	-	+	+	-
	Gelatin test	-	-	-	-	+	-	-	+	+	-
	Catalase test	+	+	+	+	+	+	+	-	+	+
	Oxidase test	-	-	-	-	-	-	-	-	-	-
	CAMP test	*	*	*	*	*	*	*	*	*	+
	Motility test	+	+	-	+	+	-	+	-	-	t
H ₂ S gas test	-	+	-	-	+	-	+	+	-	-	

* +/- trends indicate positive and negative, v= variable mostly positive; d= 26-75% positive; * not applicable, t= tumbling.

** Abbreviations: a. *Enterobacter* spp, b. *E. coli*, c. *Klebsiella* spp., d. *Pseudomonas* spp., e. *Citrobacter* spp., f. *Shigella* spp., g. *Salmonella* spp., h. *Clostridium* spp., i. *S. aureus*, j. *Listeria* spp.

Table 3: Antimicrobial resistance and sensitivity evaluated for Gram positive and negative bacterial species on selective antibiotics.

Gram	Pathogens	Antibiotics** with zone of inhibition (mm)						
		CAR	CT	CIP	TET	VAN	LC	KAN
Positive	<i>Clostridium</i> spp	9	1	27	15	20	0	20
	<i>S. aureus</i>	5	8	23	20	15	14	15
	<i>Listeria</i> spp	0***	15	45	10	13	0	0
Negative	<i>Salmonella</i> spp	0	13	38	0	8	0	0
	<i>E. coli</i>	0	18	26	26	0	0	0
	<i>Klebesilla</i> spp	8	18	20	0	0	0	15
	<i>Enterobacter</i> spp	0	17	36	10	0	0	0
	<i>Citrobacter</i> spp	0	15	26	10	0	0	0
	<i>Shigella</i> spp	0	12	24	0	0	0	14
<i>Pseudomonas</i> spp	0	10	31	0	0	0	11	

**Antibiotics include; (CAR) Carbenicillin, (CT) Colistin sulphate, (CIP) Ciprofloxacin, (TET) Tetracyclines, (VAN) Vancomycin, (LC) Lincomycin, (KAN) Kanamycin.

* Indicates formation of highest inhibitory zone.

** Indicates sensitivity of isolates on antibiotics.

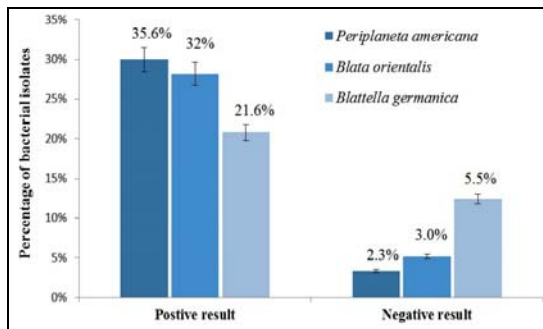


Fig 1: Prevalence of isolates of isolates in each collected household cockroaches.

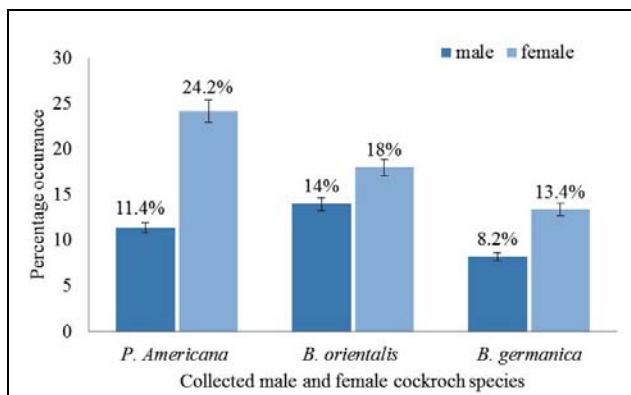


Fig 2: Relative abundance of bacterial species isolates from male and female cockroaches.

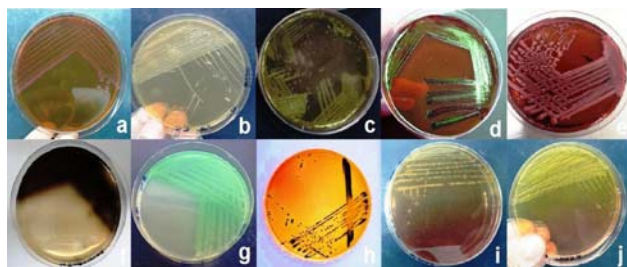


Fig 3: Media plates; a. *Citrobacter* spp., b. *Clostridium* spp., c. *E. coli*, d. *Enterobacter* spp., e. *Klebsiella* spp., f. *Listeria* spp., g. *Pseudomonas* spp., h. *S. typhimurium*, i. *Shigella* spp., j. *S. aureus*.

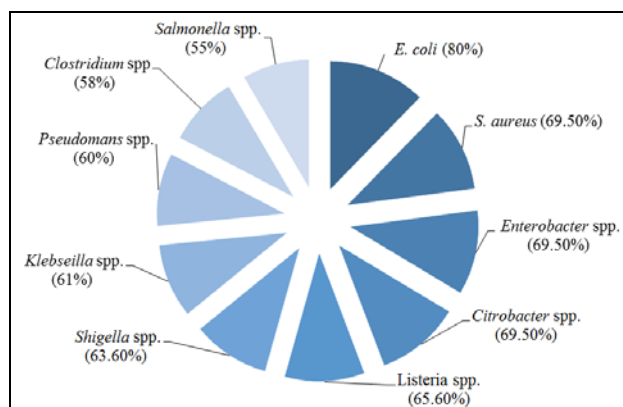


Fig 4: Indicates bacterial diversity occurrence in percentages isolated from cockroaches.

5. Discussion

A total of 10 bacterial isolates were identified and isolated from the external surface and the gastrointestinal tract of household cockroaches. This was the first attempt on bacterial isolation from *B. orientalis* in this region.

The present result shows that *B. orientalis* and *P. americana* measured three to four times larger; indicates the presence of rich bacterial flora than *B. germanica*. This coincidence may strengthen the assumption that the capability of harboring microorganisms by cockroaches is size dependent along with other factors including sanitation condition of the environment. There was no significant difference between the population size of male and female cockroaches and shows the uniformly occurrence for both sexes of cockroaches in houses. In this study a high percentage of contamination of bacteria was found in female cockroaches which were due its larger body size than male. Cockroaches harboured important bacterial pathogens including Gram positive stains;

Clostridium spp., *S. aureus* and *Listeria* spp., whereas majority of isolates of were Gram negative stains; *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp. [11, 23, 24, 25, 26], *Pseudomonas* spp. was the only specie not identified from *B. germanica* [27].

Bacterial species richness varies among cockroach species such that *E. coli* secured its top position and least was obtained by *Salmonella* specie. This result was in agreement with observation of Bouamamaa [14]. Most of the bacterial species were isolated from the gastrointestinal tract than external surface of the cockroaches. Our findings show marginal difference between contaminated and non-contaminated dissected cockroaches.

Few antibiotics including carbenicillin, lincomycin and vancomycin showed resistance to Gram positive and negative bacteria. Community medicines such as ciprofloxacin and colistin sulphate were susceptible to bacterial colonies and our results were in concordance with those reported by Moges [28] that these antibiotics have minimal impact on bacterial isolates of cockroaches.

6. Conclusion

Cockroaches are fast running arthropod and their rapid movement helps in the transmission of pathogens. Body parts such as mouth and legs have a sufficient reservoir ability to carry bacterial fauna when in contact with contaminations. Overall, our research suggests the possible involvement of cockroaches in the spread and transmission of pathogenic bacteria. Antibiotics including ciprofloxacin and colistin sulphate show susceptibility on all isolates of household cockroaches. Besides, cockroaches must be eradicated from houses as they have developed enough resistance potential against other antibiotics.

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6. References

1. Beccaloni G, Eggleton P. Order Blattodea. In: Zhang, Z.-Q. (Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa. 2013; 3703(1):46-48.
2. Kinfu A, Erko B. Cockroaches as carriers of human intestinal parasites in two localities in Ethiopia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008; 102(11):1143-1147.
3. Le Guyader A, Rivault C, Chaperon J. Microbial organisms carried by brown-banded cockroaches in relation to their spatial distribution in a hospital. Epidemiology and Infection. 1989; 102(03):485-492.
4. Mpuchane S, Allotey J, Matsheka I, Simpanya M, Coetzee S, Jordaan A *et al.* Carriage of micro-organisms by domestic cockroaches and implications on food safety. International Journal of Tropical Insect Science. 2006a; 26(03):166-75.
5. Mpuchane S, Matsheka IM, Gashe BA, Allotey J, Murindamombe G, Mrema N. Microbiological studies of cockroaches from three localities in Gaborone, Botswana. African Journal of Food, Agriculture, Nutrition and Development. 2006b; 6(2).
6. Brenner RJ. Economics and medical importance of German cockroaches. In: Rust MK, Owens JM, Reiersen

- DA, Ed. Understanding and controlling the German cockroach. Oxford University Press, New York. 1995, 77-92.
7. Gore JC, Schal C. Cockroach allergen biology and mitigation in the indoor environment. Annual Review of Entomology. 2007; 52:439-63.
 8. Fischer OA, Matlova L, Dvorska L, Svastova P, Pavlik I. Nymphs of the Oriental cockroach (*Blattella orientalis*) as passive vectors of causal agents of avian tuberculosis and paratuberculosis. Medical and Veterinary Entomology. 2003; 17(2):145-150.
 9. Allotey J, Mpuchane S, Gashe BA, Simpanya M, Matsheka I. Trapping of *Blattella germanica* (L) populations in human dwellings in Gaborone. Botswana. Journal Applied Zoology Research. 2009; 20(2):175-88.
 10. Graczyk TK, Knight R, Tamang L. Mechanical transmission of human protozoan parasites by insects. Clinical Microbiology Reviews. 2005; 18(1):128-32.
 11. Elgderi RM, Ghenghesh KS, Berbash N. Carriage by the German cockroach (*Blattella germanica*) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospitals and households in Tripoli, Libya. Annals of Tropical Medicine & Parasitology. 2006; 100(1):55-62.
 12. Fotedar R, Banerjee U. Vector potential of the German cockroach in dissemination of *Pseudomonas aeruginosa*. Journal of Hospital Infection. 1993; 23(1):55-59.
 13. Tatteng YM, Usuanlele MU, Orukpe A, Digban AK, Okodua M, Oviasogie F *et al.* Mechanical transmission of pathogenic organisms: the role of cockroaches. Journal of Vector Borne Diseases. 2005; 42(4):129.
 14. Bouamamaa L, Sorlozano A, Laglaoui A, Lebbadi M, Aarab A, Gutierrez J. Antibiotic resistance patterns of bacterial strains isolated from *Periplaneta americana* and *Musca domestica* in Tangier, Morocco. The Journal of Infection in Developing Countries. 2010; 4(04):194-201.
 15. Tungtrongchitr A, Sookrung N, Munkong N, Mahakittikun V, Chinabut P, Chaicumpa W *et al.* The levels of cockroach allergen in relation to cockroach species and allergic diseases in Thai patients. Asian Pacific Journal of Allergy and Immunology. 2004; 22(2-3):115-121.
 16. Chew GL, Carlton EJ, Kass D, Hernandez M, Clarke B, Tiven J *et al.* Determinants of cockroach and mouse exposure and associations with asthma in families and elderly individuals living in New York City public housing. Annals of Allergy, Asthma & Immunology. 2006; 97(4):502-513.
 17. Fotedar R. Vector potential of houseflies (*Musca domestica*) in the transmission of *Vibrio cholerae* in India. Acta Tropica. 2001; 78(1):31-38.
 18. Al-bayati NY, Al-Ubaidi AS, Al-Ubaidi IK. Risks associated with cockroach *Periplaneta americana* as a transmitter of pathogen agents. Diyala Journal of Medicine. 2011; 1(1):91-97.
 19. Holt JG, Sneath NR, Staley PH, Williams JT, Stanley T. Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore, 1994, 787-789.
 20. Sharma KK, Soni SS, Meharchandani S. Congo red dye agar test as an indicator test for detection of invasive bovine *Escherichia coli*. Veterinarski Arhiv. 2006; 76(4):363-366.
 21. Wayne PA. CLSI (Clinical and laboratory standards institute). Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement. M100-S18. Clinical and Laboratory Standards Institute. 2008, 17.
 22. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45(4):493.
 23. Pai HH, Ko YC, Chen ER. Cockroaches (*Periplaneta americana* and *Blattella germanica*) as potential mechanical disseminators of *Entamoeba histolytica*. Acta Tropica. 2003; 87(3):355-359.
 24. Lamiaa B, Lebbadi M, Ahmed A. Bacteriological analysis of *Periplaneta americana* L.(Dictyoptera; Blattellidae) and *Musca domestica* L.(Diptera; Muscidae) in ten districts of Tangier, Morocco. African Journal of Biotechnology. 2007; 6(17).
 25. Kassiri H, Kazemi S. Cockroaches [*Periplaneta americana* (L.), Dictyoptera; Blattellidae] as carriers of bacterial pathogens, Khorramshahr County, Iran. Jundishapur Journal of Microbiology. 2011; 5(1):320-2.
 26. Nejati J, Keyhani A, Moosa-Kazemi SH, Mohammadi M, Mahjoob M, Boostanbakhsh A. Cockroaches' bacterial infections in wards of hospitals, Hamedan city, west of Iran. Asian Pacific Journal of Tropical Disease. 2012; 2(5):381-4.
 27. Fotedar R, Nayar E, Samantray JC, Banerjee U, Dogra V, Kumar A. Cockroaches as vectors of pathogenic bacteria. The Journal of Communicable Diseases. 1989; 21(4):318-322.
 28. Moges F, Eshetie S, Endris M, Huruy K, Muluye D, Feleke T, Ayalew G, Nagappan R. Cockroaches as a source of high bacterial pathogens with multidrug resistant strains in Gondar town, Ethiopia. BioMed Research International, 2016.