



Household Cockroaches of Quetta city are reservoir for infectious Pathogenic Bacteria

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ABSTRACT

In present study the different pathogenic bacteria were isolated. These bacteria were confirmed through different biochemical tests and the pathogenicity of different bacteria were checked through animal trail. The following Pathogens Citrobacter spp., Clostridium spp., Enterobacter spp., Escherichia coli, Klebseilla spp., Listeria spp., Pseudomonas spp., Salmonella spp., Staphylococcus aureus and Shigella spp., were isolated from Blata orientalis, Blattella germanica and Periplaneta americana of cockroaches. Pseudomonas spp. was the only pathogen was not isolated from B. germanica. P. americana 35.6%, B. orientalis 32% and B. germanica 21.6% were found highly contaminated with pathogens respectively. Percentage of isolated pathogenic bacteria were found higher in female cockroaches (55.6%) then male (33.6%). Biochemical tests were applied to conform the isolated species and in-vitro inoculation of species were also studied in mice body. Significantly statistical analysis of t-test and chi-square were applied for population distribution and rate of contaminations for cockroaches. Antimicrobial susceptibility trails demonstrated that most effective drugs were ciprofloxacin and colistin sulphate against all isolates. Cockroaches are important as potential carriers of pathogenic microorganisms. Effective measures should be taken to remove possible sources of bacteria and control the pests in household environments. Besides, it is difficult to prove direct involvement of cockroaches in transmission of bacterial diseases; the role of cockroaches in the spread of the pathogens such as bacteria is strongly suspected. This prospective study will provide sufficient information about the recent updates about the contaminated cockroaches and health hazard issues in mice.

Key words: House hold cockroaches, isolated, identified, pathogenic bacteria, biochemical test

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INTRODUCTION

Cockroaches belong to order Blattodea and they are considered as an important mechanical vector for a large number of pathogenic organisms including protozoa, bacteria and viruses amongst humans as sanitation and garbage management are serious issues in Quetta city. Occurrence and distribution of cockroaches are worldwide [1] and so far more than 50 species are reported living in or around human living structures [2]. Similar bacterial fauna is observed between adults and nymphs indicated shared foraging and residential locations [3]. Cockroaches have dirty habits with an ability to spoil food, transfer pathogens, cause allergic reactions and psychological distress [4]. Cockroaches breed and forage in sewer systems, garbage bins, and latrines [5]. Dispersal of pathogens is rapid during metamorphosis where nymphal cuticle is shed or loses body parts [6].

Cockroaches transmit various pathogens at a time both externally and internally to humans [7]. Presence of cockroaches is major cause of acute asthma morbidity [8]. Domestic cockroaches contained more than 100 pathogen bacteria carry on their integuments, digestive tracts, cuticles and feces [9]. Presence of bacterial fauna is higher in stomach than in intestine and least on external surfaces of their body respectively [10]. Cockroaches are nocturnal in habit and transmit pathogens rapidly at night time [11]. Large number of microbial fauna multiplies in gut and their excretion of bacteria may last up to 114 days so cockroaches are reservoir for multiplication of pathogens [12].

Large number of bacterial species were isolated from American cockroaches such as *Escherichia coli*, *Klebsiella* spp., *Providencia* spp., *Staphylococcus* spp., and *Enterococcus* spp [13]. Although evidence regarding direct infection are not suggested but serious health issues such allergic reactions and provide shelter to multidrug resistant pathogen of bacteria [14].

Proper vaccination for most infected areas, sanitation, improved water quality and disease control management plans given by WHO may help to overcome on the diseases spread by cockroaches. The role of cockroaches as mechanical vectors of pathogens is unknown but the objective of this research was, hence, to isolate and identify the important pathogenic bacteria from *P. americana* (American cockroaches), *B. orientalis* (Oriental cockroaches) and *B. germanica* (German cockroaches); and these cockroaches were collected from different houses of Quetta city, Baluchistan.

MATERIALS AND METHODS

Study area

Present study was conducted in Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB) University of Baluchistan, Quetta.

Collection of samples

During the period of study, Adult cockroaches were captured (mostly at night time or early morning) from different houses (kitchens, basements, bedrooms, washrooms and store rooms) by hand picking method using sterile gloves and transferred to the sterile test tubes or sterile bottles. These test tubes were directly transferred to laboratory for further process.

Identification of cockroaches

Cockroaches was examined under laminar flow, sex identification was done under a low power microscope. The specimens were confirmed by taxonomic features described by Chew et al [15].

Isolation of pathogen from body surface

External pathogenic bacteria were isolated from cockroaches using 2 mL of sterile normal saline solution (0.9%) and vortexed to remove pathogenic bacteria from external body surface of cockroaches. 0.01 mL saline was inoculated on nutrient, differential and selective media respectively [16]. Plates were incubated at 37 °C for 24 h and kept in facultative and anaerobic conditions.

Isolation of pathogenic bacteria from digestive tract of cockroaches

Cockroaches were placed in flask and washed with 70% alcohol for 5 min to decontaminate external surface, transferred to sterilized flask to dry at room temperature under sterile conditions and then sterile normal saline was used for about 3 min to remove all traces of alcohol. Guts of cockroaches were dissected out, macerated aseptically in a sterile pestle and mortar in 2 mL of sterile normal saline. 0.01 mL saline was inoculated on the nutrient, differential and selective media respectively [17]. Plates were incubated at 37 °C for 24 h and kept in facultative and anaerobic conditions.

Biochemical testing

Morphological identification was performed using microscopic examination of isolated pathogens using Gram staining, IMVIC, sugar fermentation, catalase, oxidase, gelatin liquefaction, Christie Atkins Munch Petersen test (CAMP) and H₂S production biochemical tests [18].

Antibiotic susceptibility test

Standardized antibiotic sensitivity test were performed on Mueller Hinton agar followed by disc diffusion Bauer technique and McFarland turbidity standard method of CLSI protocol, 2008 [19]. Isolates were considered as sensitive and resistant to a particular antimicrobial agent on the basis of inhibitory zones [20].

Pathogenicity of isolated bacteria in mice

Pathogenicity of isolated species was tested using 0.5mL growth suspension having 1×10^9 CFU/mL of each isolated pathogen was injected subcutaneously in to mice. Dead mice were studied for localized lesion on body and disparate organs [21].

RESULTS

Population distribution

Total 191 samples of cockroaches were collected during the months of April to October, 2016 bedrooms, kitchens and store rooms from different houses of Quetta city. Statistical test of t-test was applied at 0.05p for male and female cockroaches assuming the sample size equal. Highly significant ($p > 0.02$) result was obtained that population of both sexes were of same sized (Table- 1). 89.2% cockroaches were carriers of external and internal pathogenic bacteria while 10.8% were found negative of both. *Periplaneta americana* 35.6%, *B. orientalis* 32% and *B. germanica* 21.6% of cockroach species were recorded carriers of pathogens respectively (Figure-1). Statistical analysis of chi-square test (0.05p) was constructed to evaluate contamination rate between male and female cockroaches and highly significant ($p > 0.0241$) results were obtained. *Periplaneta Americana* (11.4% male and 24.2% female), *B. orientalis* (male 14% and female 18%) and *B. germanica* (male 8.2% female 13.4%) were contaminated with pathogens respectively (Figure- 2). *Citrobacter* sp., *Clostridium* sp., *Enterobacter* sp., *E. coli*, *Klebseilla* spp., *Listeria* spp., *Pseudomonas* spp. *Salmonella* spp., *Shigella* spp and *S. aureus* bacterial pathogens were isolated from three different species of cockroaches (Table- 2). *Escherichia coli* 80% was the most dominant species among other isolated bacteria. *Staphylococcus aureus* 69.5%, *Enterobacter* spp. 69.5% and *Citrobacter* spp. 69.5% were the second dominant isolated bacterial species; *Listeria* spp. 65.6% was the third dominant isolated species while *Shigella* spp. 63.6%, *Klebseilla* spp. 61%, *Pseudomonas* spp. 60%, *Clostridium* spp. 58% and *Salmonella* spp. 55% were the least dominant having variable frequencies. *Pseudomonas* spp. was the only specie not isolated from *B. germanica* collected from houses (Figure- 3).

Table 1: Distribution frequency of fully matured cockroaches in houses of 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		

* Difference recorded between adults was found highly-significant ($p < 0.05$).

Table 2: Comparative studies on bacterial isolates of external and internal sources from *P. americana*, *B. orientalis* and *B. germanica* species of cockroaches

Gram	Isolates	<i>P. Americana</i>				<i>B. orientalis</i>				<i>B. germanica</i>			
		Whole body*		Digestive tract		whole body		Digestive tract		whole body		Digestive tract	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Positive	<i>Enterobacter</i> spp.	+	+	+	+	+	+	+	+	+	+	+	+
	<i>E. coli</i> .	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Klebsiella</i> spp.	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Pseudomonas</i> spp.	+	+	+	+	+	+	+	+	-	-	-	-
	<i>Citrobacter</i> spp.	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Shigella</i> spp.	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Salmonella</i> spp.	+	+	+	+	+	+	+	+	+	+	+	+
Negative	<i>Clostridium</i> spp.	+	+	+	+	+	+	+	+	+	+	+	+
	<i>S aureus</i>	+	+	+	+	+	+	+	+	+	+	+	+
	<i>Listeria</i> spp.	+	+	+	+	+	+	+	+	+	+	+	+

* Whole body represents the external surface of cockroaches

+ Present, - Absent

Biochemical tests

Biochemical tests included IMVIC, sugar fermentation tests, catalase test, oxidase test, gelatin liquefaction test and H₂S production tests were carried out to confirm isolated species of bacteria included *Citrobacter* spp., *Clostridium* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., *S. aureus* and *Shigella* spp. CAMP was used for conformation for *Listeria* spp (Table- 3).

Table 3: Biochemical tests used for confirmation of bacterial pathogens presence

		<i>Pathogens with shapes</i>									
Gram staining		G. negative bacteria					G. Positive bacteria				
Biochemical tests		<i>Enterobacter</i> spp	<i>E.coli</i>	<i>Klebsiella</i> spp	<i>Pseudomonas</i> spp	<i>Citrobacter</i> spp	<i>Shigella</i> spp	<i>Salmonella</i> spp	<i>Clostridium</i> spp	<i>Staph aureus</i>	<i>Listeria</i> spp
		Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Cocci	Rods
IMVIC tests	Indole	-	+	-	-	+	V	-	-	-	-
	MR	-	+	-	-	-	+	+	-	+	+
	VP	+	-	+	-	-	-	-	-	+	+
	Simmon Citrate	+	-	+	-	+	-	-	+	+	-
Sugar fermentation tests	Dulcitol	-	v	v	-	d	-	-	-	-	-
	Glucose	+	+	+	+	+	+	+	+	+	+
	Maltose	+	+	+	-	+	V	+	+	+	+
	Trehlose	+	+	+	-	+	V	+	v	+	+
	Dextrose	+	+	+	-	+	+	+	+	+	+
	Xylose	+	+	+	v	+	+	+	+	-	-
	Sorbitol	+	+	+	-	v	V	+	v	-	v
	Inositol	+	-	+	-	-	-	-	-	-	-
	Mannitol	+	+	+	+	+	V	+	-	+	-
Others biochemical tests	Urease test	-	-	+	-	+	-	-	+	v	-
	Gelatin test	-	-	-	-	+	-	-	+	+	-
	Catalase test	+	+	+	+	+	+	+	-	+	+
	Oxidase test	-	-	-	-	-	-	-	-	-	-
	CAMP test	*	*	*	*	*	*	*	*	*	+
	Motility test	+	+	-	+	+	-	+	-	-	Tumbling
	H ₂ S gas test	-	+	-	-	+	-	+	+	-	-

* +/- trends indicate positive and negative, v= variable mostly positive; d= 26-75% positive;

* not applicable.

Antibiotics trails and Pathogenicity of mice

Drug sensitivity test were performed using selective antibiotics classes for all isolated bacterial pathogens (Table- 4). Effective antibiotics were polypeptides (colistin sulphate), quinolones (ciprofloxacin) and tetracyclines (tetracycline). Multidrug resistant pattern was observed using other classes of antibiotics included pencilline (carbenicillin), glycopeptides (vancomycin), lincosamides (lincomycin) and amino-glycoside (kanamycin) for isolated pathogen.

Subcutaneous injections of purified bacterial species were inculcated in to mice and studied clinical symptoms after every 12 h. *Klebsiella* spp. showed nasal discharge, laziness, and reluctant after inoculation in mice after 24 h. Autopsy after 48 h show symptoms included swollen body lesion on nose, lesions on body, necrosis of lung tissues and swollen kidneys. *Staphylococcus aureus* inoculation developed laziness, reluctant fever and pneumonia like symptoms. Autopsy was performed after 24 h show autopsy swollen body, body hairs losing, hemorrhages on body, hemorrhages on lung, abscess in muscles, enteritis and endocarditis were observed.

In case of *Clostridium* spp. laziness, reluctant, kicking on belly and diarrhea were observed. Death of mice was seen after 48 h and autopsy show reduction in body weight, red patches on ventral side of the body, internal organs like pulpy kidney, liver shrunk and enteritis. *Escherichia coli* showed laziness, dizziness, abdominal cramping, sudden watery diarrhea,

loss of appetite, fever, bruising and dehydration; while on autopsy red patches on ventral side of the body lungs and heart shrunk, liver abscess and enteritis were observed. After inoculation of *Shigella* spp. laziness, and dizziness, abdominal cramps, loss of appetite were observed; while on autopsy, lesions on intestine were observed. In case of *Listeria* spp fever, abdominal cramping, diarrhea, loss of balance and convulsions were observed but during autopsy intestine were affected while after inoculation of *Salmonella* spp.in mice clinical signs can occur after 12 to 72 h after infection but the typical signs of *Salmonella* spp. abdominal cramping, diarrhea, fever and vomiting was observed but during autopsy affected gastrointestinal tract were observed.

Inoculation of *Pseudomonas* spp. in mice developed conditions like fever, dizziness and vomiting and during autopsy overall red patches on the body, hemorrhages on lung, brain abscess and eye infected with *Keratitis* (inflammation of the cornea) were seen. Inoculation of *Citrobacter* spp. and *Enterobacter* spp. in mice laziness and dizziness were observed but during autopsy brain abscess, lungs were affected and kidneys became enlarged. All these symptoms of affected organs were compared to controlled mice.

Table 4: Antimicrobial resistance of isolates bacterial species from cockroaches.

Gram	Pathogens	Antibiotics* with zone of inhibitions (mm)						
		CAR	CT	CIP	TET	VAN	MY	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: (CAR) Carbenicillin; (CT) Colistinsulphate; (CIP) Ciprofloxacin; (TET) Tetracyclines; (VAN) Vancomycin; (MY) Lincomycin; (KAN) Kanamycin.

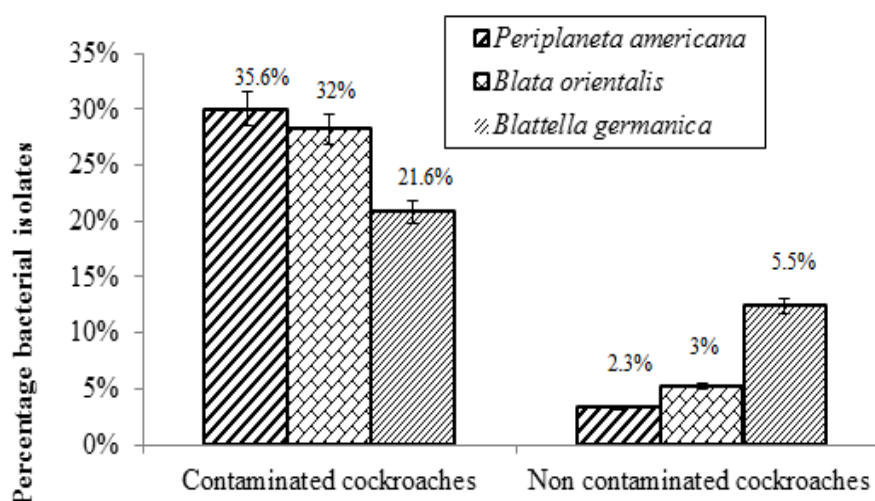


Figure 1: Comprehensive studies of non-contaminated and contaminated pathogen carrier cockroaches.

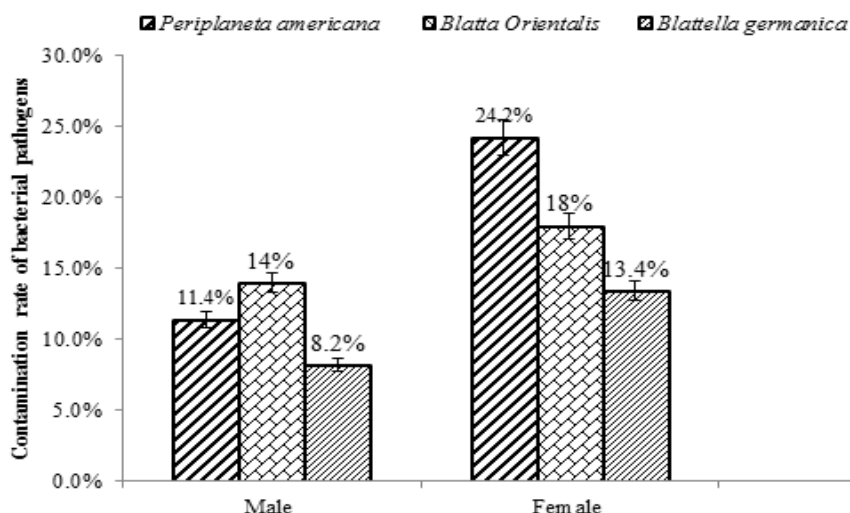


Figure 2: Sex based contamination rate of bacterial pathogen among male and female cockroach species.

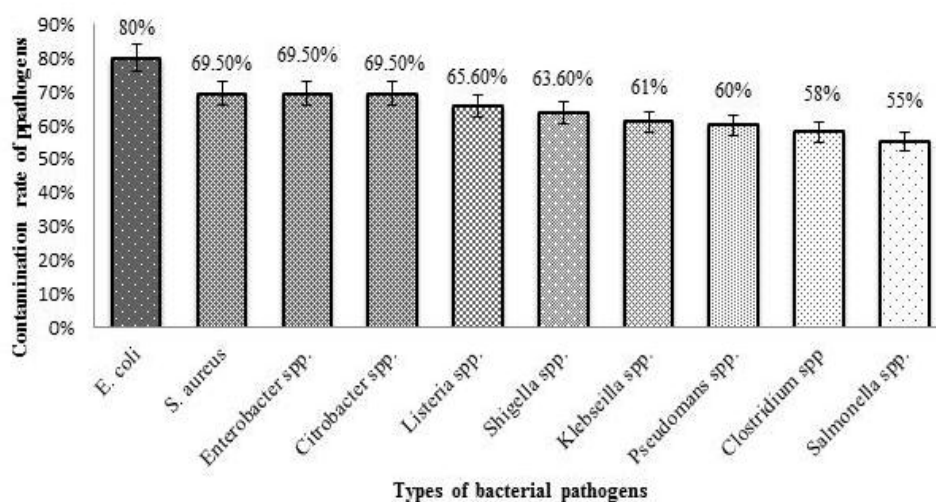


Figure 3: Isolated bacterial pathogens from selective household cockroaches.

DISCUSSION

Cockroach species include *P. americana* and *B. germanica* are potential carriers of pathogenic bacteria in Pakistan Malik et al [22]; Masood et al [23] and Memona et al [24]. Collection of *B. orientalis* was made first time collected from houses along with other cockroach species. For this research work, total 191 cockroaches were collected from the houses, among which cockroaches were identified as 35.6% *P. americana*, 32% *B. germanica* and 21.6% *B. orientalis*. Eighty-nine point two percent cockroaches were found positive for carriers of pathogenic bacteria and 10.6% results were recorded negative.

We have isolated large number of pathogenic bacteria from exterior (body surfaces) and anterior (digestive tract) of the house cockroaches. For example, among Gram negative bacterial strains, we have found *Citrobacter spp.*, *E. coli*, *Enterobacter spp.*, *Klebsella spp.*, *Salmonella spp.*, *Shigella spp.* and *Pseudomonas spp.* Similar bacterial species were isolated from house cockroaches by Elgderi [10]; Pai et al [25] and Lamiaa et al [26]. We have also isolated gram positive bacteria include *Clostridium spp.*, *Listeria spp.* and *S. aureus*. Chitsazi et al [27] had also reported these species incidence in house cockroaches. *Pseudomonas spp.* was not identified from *B. germanica* of residential areas; similar observation was also given by Fotedar et al [28].

Most frequently found bacterial pathogen among house cockroaches was *E. coli*. This result is in agreement with Bouamamaa et al [13]. In our research; the biochemical tests

performed for identification of bacterial species are the same as used by Holt *et al* [18] and Sherman [29] except CAMP confirmatory test that can be used only for the identification of *Listeria* spp.

During this course of study pathogenic bacteria include; *Citrobacter* spp., *E. coli*, *Enterobacter* spp., *Salmonella* spp., *Klebsiellas* spp. and *Pseudomonas* spp. Pathogens have shown multidrug resistance to carbenicillin, lincomycin and vancomycin. Most effective antibiotics were ciprofloxacin and colistin sulphate showed highest mortality against all isolates. Existence of antibiotic resistant pathogenic bacteria in/on household cockroaches is potentially danger signal toward the possible future spread of these bacteria through cockroaches.

Pathogenic bacteria, isolated from household cockroaches, when inoculated in mice have caused development of disease symptoms like diarrhea, gastroenteritis, abdominal cramping, respiratory infections; pneumonia, dizziness, typhoid and vomiting.

The collected specimens of *P. americana* and *B. orientalis* showed three to four times larger body length than *B. germanica*. They also showed more bacterial flora compared to that shown by *B. germanica*. This coincidence may strengthen the assumption that the capability of harboring microorganisms by cockroaches is also size dependent along with other factors e.g. sanitation conditions of the environment.

CONCLUSION

Cockroaches including *P. americana*, *B. orientalis* and *B. germanica* possess high potential capabilities to carry pathogenic microorganisms. Evidences are not fully supported direct involvement in the transmission of bacterial diseases but it is strongly suspected in the spread of pathogenic bacteria by cockroaches. Public awareness regarding potential of cockroaches in the carrying pathogens can help people at the household level to keep their houses, kitchens and toilets clean. It can prevent them from infestation of cockroaches.

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