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Prevalence and Antimicrobial Resistance of Pathogenic Bacteria Isolated from Cuticles of *Blattella germanica* and *Periplaneta americana* in Gizan City, Saudi Arabia

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ABSTRACT

Cockroaches are major microbial vectors around human dwellings that cause serious public health threats. They harbor a number of pathogenic bacteria on their cuticles with antimicrobial resistance. This study aims at investigating the bacterial carriage of *Blattella germanica,* and *Periplaneta americana* and their antimicrobial resistance in Gizan, Saudi Arabia. 152 cockroaches were trapped in houses in Gizan City during January - July 2018. Standard methods were followed in all the microbiological investigations and antibiotic susceptibility tests using Vitek2 Automated Microbiology System, Biomerieux®. All of the 152 cockroaches were found with bacteria load on their cuticles. Twenty tow species of bacteria belonging to ten genera were identified. However, *Klebsiella pneumoniae* 33 (21.7%), *Serratia marcescens* 26 (17.1%), and *Pantoea agglomerans* 20 (13.1%) were the predominant isolates. Half of the isolates 11 (50. %) were multidrug-resistant strains. High resistance percentages were noted to Ampicillin and Amoxicillin clavulanate (41%), Cefoxitin (36%) and Cefazolin and Fosfomycin (27%). Cockroaches are potential source of pathogenic bacteria with multidrug resistant strains. This fact implies the epidemiological risks, complicating therapeutics, and leads to more medical costs in urban environments. Preventive and control measures are highly needed to minimize cockroach related food-borne diseases and other infections.

KEY WORDS: PATHOGENIC BACTERIA, BLATTELLA GERMANICA, PERIPLANETA AMERICANA, ANTIMICROBIAL RESISTANCE, JAZAN REGION, SAUDI ARABIA.

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INTRODUCTION

Cockroaches are among the most common insects that exist nearly around the world. Some biologists consider them one of the most adaptable and successful animal groups (Bennett et al., 1997). Cockroaches are one of the most serious food and residential pests around the world and invade places where food is stored, prepared or served. They are known to be notoriously resilient and difficult to control (Service 2004). There are about 4,400 species of Cockroaches (Blattaria or Blattodea). They are considered to be the oldest and most primitive of insects, dating to the Permian, about 275 million years ago (termite ancestry) (Brenner and Kramer, 2019).

The filthy behavior of cockroaches along with their nocturnal lifestyle enables them to contribute to food-borne diseases and to transmit many pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp., Shigella dysenteriae, Bacillus cereus,* and *Entamoeba histolytica* (Fotedar et al., 1989; Blazar et al., 2011; Burgess and Chetwyn 1981; Tachbele et al., 2006). In addition, cockroaches not only spoil food, but also cause allergic reactions and psychological disorders (Brenner, 1995). Control strategies should therefore be reoriented to emphasize the biology and ecology of target cockroaches, as well as the use of insecticides, if any, and should be more selective and environmentally friendly (WHO, 1996). Boric acid powder and cockroach gels were proved very effective against cockroaches and thus are highly recommended to be used in households and health care settings to control cockroaches (Noureldin and Farrag, 2008). The use of insecticidal baits had also resulted in a sustained cockroach elimination over a year in New Orleans and led to improve in asthma outcomes (Rabito et al., 2017).

Antimicrobial resistance (AMR) is one of the public health problems that threatens the prevention and effective treatment of a growing group of infections caused by bacteria. The problem of antimicrobial resistance is especially urgent when it comes to antibiotic resistance in bacteria. Bacteria that cause common or severe infections have developed over many decades, and to varying degrees, resistance to each new antibiotic. This necessitates the need for action to avert a growing global crisis in health care facilities (Prestinaci et al., 2015). The multiple resistance of the bacteria isolates from residential areas in Nigeria revealed the importance of surveillance on pattern and origin of antimicrobial drug resistance, as well as, the awareness of people at the resident areas about the danger of cockroaches in residential areas and vicinities (Adeleke et al., 2019).

Multidrug resistance of many bacterial species is considered a serious threat for patients in a sense that it restricts and limits therapeutic options. Cockroaches are becoming medically important as external vectors for many infectious diseases. Nonetheless, the public health significance of this vector so far has not been well documented, and to the best of our knowledge, no data has been published on this issue in Saudi Arabia and Jazan region in particular. Therefore, the aim of this study was to isolate pathogenic bacteria from the external surfaces of the cockroaches and to determine their antimicrobial resistance statuses.

MATERIAL AND METHODS

Study area: Jazan region (Fig. 1) is situated in the subtropical zone, Southwest Saudi Arabia, lies between 16°-12, and 18°-25, latitude north. It is surrounded by the Red Sea (260 km) from the west and by Arabic Republic of Yemen (120km) from the south and east and Asir region from the north, with total area of about 22,000 km2 (Al-Sheikh, 2011) and 1.6 million populations (GASTAT 2017: https://www.stats.gov.sa/en/5655).

Sampling and identification of cockroaches: Cockroaches were randomly trapped from 37 different households of Gizan City. Cockroaches were trapped in food-baited pitfall traps. Traps were placed at night in kitchens, toilets, and bathrooms and collected the next morning and transported to the laboratory of the Saudi Center for Disease Prevention and Control (SCDC) in Jazan for identification and further processing.

Figure 1: Map of Saudi Arabia showing Jazan region.

Cockroaches were identified to the species level using CDC pictorial key: (https://www.cdc.gov/nceh/ehs/docs/pictorial_keys/cockroaches.pdf). and (Harwood and James, 1979).

Bacterial isolation: Trapped cockroaches were frozen at 0 ^{0}C for 10 minutes. Then, each cockroach was placed in a sterile test tube and 5 ml. of sterile 0.9% normal saline was added to each test tube and thoroughly shaken for vigorously washing in the centrifuge for 2 minutes at 3000 rpm. A loop full of each suspension was cultured on MacConkey agar (MAC) and blood agar plate and incubated overnight at 37 ^{0}C.

Identification of bacterial isolates and antimicrobial susceptibility testing (AST): Following the manufacturer's instructions of Vitek 2 Automated Microbiology System, Biomerieux®; two plastic tubes (the first fills with 3 ml of 0.45% NaCl and the second is empty) were prepared.

Then a suitable number of every pure colony from the culture media was taken by sterile loop and placed in the 3 ml saline tube. The suspension was then centrifuged at 15,000g for 3 minute and measured for McFarland turbidity by DensiChek (McFarland range for GNB and GNB is 0.5 - 0.63). The former two steps were repeated to get the needed McFarland turbidity.ID card was placed in the suspension tube and AST card placed in the empty tube, and both tubes were put in the cassette, then the cassette was loaded in a smart carrier to enter needed data. The cassette then was loaded to the Vitek 2 to start the run for bacteria identification and Susceptibility testing. The cards were read by kinetic fluorescence measurement and the results reported within11 to 24 hrs.

RESULTS AND DISCUSSION

One hundred fifty two cockroaches were collected from 37 different households of Gizan City. Out of these, 98 (64.5%) were German cockroaches (*B. germanica*), and 54 (35.5%) were American cockroaches (*P. americana*) (Table 1). In this study, both *Blattella germanica* and *Periplaneta americana* were identified in the households of Gizan City.

Table 1. Bacteria species isolated from *Periplaneta americana* and *Blattella germanica* in Gizan City, Saudi Arabia

Bacteria species	Gram (-ve /+ve)	Cockroaches (N = 152)		Total bacteria species No (%)
		B. germanica No (%)	*P. americana* No (%)	
Klebsiella *Klebsiella pneumoniae*	-ve	15	18	33 (21.7%)
Klebsiella oxytoca	-ve	8	-	8 (5.3%)
Klebsiella pneumoniae spp. ozaenae	-	5	5 (3.3%)	
Klebsiella pneumoniae *spp. pneumoniae*	-ve -ve	-	3	3 (1.9%)
spp. pneumoniae *Citrobacter*				
Citrobacter gillenii	-ve	5	-	5 (3.3%)
Citrobacter werkmanii	-ve	4	-	4 (2.6%)
Citrobacter braaakii	-ve	2	-	2 (1.3%)
Citrobacter freundii *Serratia*	-ve	-	1	1 (0.66%)
Serratia marcescens	-ve	26	-	26 (17.1%)
Serratia plymuthica	-ve	7	-	7 (4.6%)
Serratia fonticola	-ve	-	8	8 (5.3%)
Enerobacter				
Enerobacter aerogenes	-ve	3	-	3 (1.9%)
Enerobacter cloacae complex	-ve	-	2	2 (1.3%)
Pseudomonas				
Pseudomonas aeruginosa	-ve	4	-	4 (2.6%)
Pseudomonas luteola	-ve	1	-	1 (0.66%)
Aeromonas				
Aeromonas samonicida	-ve	-	1	1 (0.66%)
Aeromonas hydrophila complex	-ve	-	5	5 (3.3%)
Pantoea				
Pantoea agglomerans	-ve	20	-	20 (13.2%)
Pantoea spp.	-ve	-	6	6 (3.95%)
Enterococcus				
Enterococcus durans	+ve	-	4	4 (2.61%)
Kluyvera				
Kluyvera ascorbata	-ve	3	-	3 (1.9%)
Hafnia				
Hafnia alveii	-ve	-	1	1 (0.66%)
Total	-	98 (64.5%)	54 (35.5%)	152 (100%)

Cockroaches are widely distributed in Saudi Arabia. For example, in Jeddah province, four species had been identified; German cockroach (*Blattella germanica*), American cockroach (*Periplaneta americana*), brown-banded cockroach (*Supella longipalpa*), and Oriental cockroach (*Blatta orientalis*) (Noureldin and Farrag, 2010). *Blattella germanica* was the most dominant species in both households and other properties. Cockroaches possess nocturnal as well as omnivorous features; thus considered the ideal vectors of pathogenic microorganisms including protozoa, bacteria, helminthes, fungus, and virus (Tatfeng et al., 2005: Salehzadeh et al., 2007). They are also of public health significance due to their ability to produce potent allergens (Özdemir, 2014). Among others, including house dust mites (HDMs), molds, pets, and rodents, cockroaches are considered one of the common indoor allergens (Al-Ghamdi et al., 2019).

Twenty two species of bacteria belonging to ten genera were identified from all cockroaches (Table 1). However, *Klebsiella pneumoniae* 33 (21.7%), *Serratia marcescens* 26 (17.1%), and *Pantoea agglomerans* 20 (13.1%) were the predominant isolates, followed by *Klebsiella oxytoca* and *Serratia fonticola* 8 (5.3%), and *Serratia plymuthica* 7 (4.6%).Cockroaches are common in many human dwellings, particularly in places where food is handled or stored. They are also found in wards and laboratory rooms of hospitals (Donkor, 2019).

Table 2. Resistance status of bacterial isolates identified from cockroaches collected from households in Gizan City, Saudi Arabia

Bacteria isolates	Antibiotics*																			
	AMC	AMP	CEZ	CEX	CER	CRA	CEL	MER	IMP	AZT	FOS	CLN	ERY	NIT	TE	TRS	CEF	CED	PIP	TIG
Klebsiella pneumoniae	R	R	R	R	S	S	S	S	S	R	R	S	S	S	S	R	R	I	R	I
Klebsiella oxytoca	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S
Klebsiella pneumoniae spp. ozaenae	S	R	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S
Klebsiella pneumoniae spp. pneumoniae	S	R	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S
Citrobacter gillenii	S	S	S	I	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
Citrobacter werkmanii	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
Citrobacter braaakii	S	I	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Citrobacter freundii	R	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
Serratia marcescens	R	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
Serratia plymuthica	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Serratia fonticola	R	R	R	I	I	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S
Enerobacter aerogenes	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Enerobacter cloacae complex	R	S	S	R	S	S	R	S	S	S	S	S	S	I	R	S	S	S	S	S
Pseudomonas aeruginosa	R	R	R	R	S	S	S	S	S	S	R	S	S	R	S	S	S	S	S	S
Pseudomonas luteola	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R	I	S	S	S
Aeromonas samonicida	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Aeromonas hydrophila complex	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S
Pantoea agglomerans	S	I	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	I	S
Pantoea spp.	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Enterococcus durans	S	S	S	S	R	R	S	S	S	S	S	I	R	S	S	S	S	S	S	S
Hafina alvei	R	R	S	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
Kluyvera ascorbata	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Resistance Percentage	41%	41%	27 %	36 %	9 %	5 %	9 %	5 %	5 %	9 %	27 %	5 %	5 %	9 %	14 %	14 %	5 %	0 %	5	0

*AMC: Amoxicillin-clavulanic acid, AMP: Ampicillin, CEZ: Cefazolin, CEX: Cefoxitin, CER: Cefuroxime, CRA: Cefuroxime Axetil, CEL: Cefalotin, MER: Meropenem, IMP: Imipenem, AZT: Azteronam, FOS: Fosfomycin, CLN: Clindamycin, ERY: Erythromycin, NIT: Nitrofurantoin, TE: Tetracycline, TRS: Trimeth/Sulfa, CEF: Cefotaxime, CED: Ceftazidime, PIP: Piperacillin, TIG: Tigecycine. R: Resistant, S: Susceptible, I: Intermediate, NR: Not Reported.

Similar results were obtained in Ethiopia where *Klebsiella pneumoniae* 32 (17.7%), *Escherichia coli* 29 (16%), and *Citrobacter spp.* 27 (15%) were the predominant isolates (Moges et al., 2016). It is also reported that *Klebsiella pneumonia* was the most prevalent nosocomial bacteria in a tertiary hospital in Ghana (Patience et al. 2013). In

Iraq, *Klebsiella* (42.56%), *Pseudomonas* (38.61%), and *Proteus* (35.34%) were found to be the primary bacterial isolates in households and hospitals (Hams et al., 2014). *Blattella germanica* was found to carry 12 species of bacteria isolates (54.5%) (11Gram-negative and 1 Gram-positive), while *Periplaneta americana* harboured 10 spescies (45.5%) (all Gram-negative).Only one Gram-positive bacteria speices (*Enterococcus durans*) was found on the cuticle of *B. germanica*. Interestingly, *Klebsiella pneumonia* was the only bacteria species found on both *B. germanica* and *P. americana*.

Both *Blattella germanica* and *Periplaneta americana* in this study were found to harbor bacterial pathogens associated with food spoilage organisms and foodborne illness through their bodies. With this in mind, cockroaches could be potential vectors and reservoirs for foodborne bacteria and nosocomial infections in Gizan City.Cockroaches are reported to harbor variety of pathogens that potentially cause serious diseases such as typhoid, diarrheal syndromes and gastroenteritis (Graczyk et al., 2005). *Klebsiella pneumonia* is well known as a gram-negative pneumonia that can cause both nosocomial and community-acquired pneumonia. It is responsible for a range of serious infections involving the lungs, abdominal cavity, soft tissues surgical sites, intra-vascular devices, urinary tract and causing bacteraemia (Shon et al., 2013).

Serratia marcescens on other hand, causes variety of infections including meningitis, urinary tract infection, septicaemia, wound infection and respiratory tract infection (Gouin et al., 1993; Cox, 1985; Komer et al., 1994). It has also been reported to infect the left side of the heart causing endocarditis in the community and in hospitals (Cohen et al., 1980). This species has been implicated in ICU [21%] followed by male medical [18.5%] and emergency department [12.3%] in a hospital in Mekkah, Saudi Arabia (Faidah et al, 2015).*Pantoea agglomerans*, formerly known as *Enterobacter agglomerans* may cause a wide variety of nosocomial infections, including meningitis, urinary tract infections, wound and burn infections, pneumonia, infections of intravascular and other prosthetic devices (Donnenberg, 2015).

Worryingly, the present study demonstrated high resistance percentages to Ampicillin and Amoxicillin clavulanate (41%) followed by Cefoxitin (36%) and each of Cefazolin and Fosfomycin (27%). While least resistance rate was observed to Imipenem, Meropenem, Piperacillin, Eryhromycin and others (5%) (Table 2).In Mekkah city of Saudi Arabia, the resistance of *Serratia* strains to the tested antibiotics was high, except for Imipenem and Meropenem. The resistance was higher with Ampicillin (97.5%), Cefoxitin (90%) and Tetracycline (86%) (Faidha et al, 2015).

It has been reported that the resistance of bacterial isolates from cockroaches to Ampicillin in Taiwan is ranged between 13.7% to 100%, Chloramphenicol (14.3% to 71.4%), Tetracycline (14.3% to 73.3%), and Trimethoprim-sulfamethoxazole (14.3% to 57.1%). This was found in two gram-positive and five gram-negative bacteria including *Klebsiella pneumonia* and *Serratia marcescens* (Pai et al., 2004).In Indonesia, resistance of bacterial isolates, including *Klebsiella ozaenae*, to Amoxicillin, Vancomycin, and Chloramphenicol were detected but still sensitive to Ciprofloxacin and Ofloxacin (Astiti et al., 2018).

This study demonstrated that for individual bacterial strains, *Kl. Pneumoniae* had multi-resistance to 9 of the tested antibiotics, *Se. marcescens*, and *Pseudomonas aeruginosa* to 6, and *Hafnia alvei*, *Se. fonticola* to 5 (Table 2). In Libya, multiple resistance to at least 6 different antibiotics was observed among the bacteria isolated from the hospital and household cockroaches including *Klebsiella*, *Enterobacter* and *Serratia* species (Elgedri et al., 2006). Moreover, most bacterial isolates of *Klebsiella* from households and hospital cockroaches in India revealed drug resistance to at least 4 antimicrobial agents (Fotedar et al., 1991).

All isolates in this study showed 100% susceptibility to Ceftazidime and Tigecycine. This would help when tailoring effective reserve therapies against resistant bacterial isolates indicated in the present results. Results of this study also showed that half of the isolates 11 (50. %) were multidrug-resistant strains. Association of nosocomial infections with multidrug resistance organisms including bacteria are regarded as a major cause of morbidity and mortality (Faidah et al., 2015). It is well known that multi drug resistance strain could arise due to expression of genes that code for multidrug efflux pumps or due to accumulation of resistant genes in a single bacterial cell (Nikaido, 2009). Antibiotic resistance is often associated with selection and its subsequent proliferation of multi drug resistance strains or the horizontal transfer of genetic elements such as plasmids that encoding resistance (Chmelnitsky et al., 2013).

There are several mechanisms contribute towards virulence and antimicrobial resistance in Gram-negative bacteria. This include inactivating of the antimicrobial agent by resistance determinants, decreasing antimicrobial drug concentrations within the cell and modifying the antibiotic or its target sites (Nordmann and Poirel, 2008; Fernández et al., 2011; Kumar et al., 2011). In combating antimicrobial resistance, the World Health Organization (WHO) have identified several key shortcomings. These issues include: (i) irrational use and unconfirmed drug quality (ii) infections poor prevention and control (iii) lack of commitment and data, and (iv) languishing research into new antimicrobial agents and tools, including diagnostic tests and antimicrobials (Leung et al., 2011). This study indicates the importance of identifying and detecting of multi-drug-resistant bacterial strains from cockroaches for the first time in Jazan region and Saudi Arabia, which in turn cause therapeutic difficulties or failures. Therefore, therapies for such bacterial infections must be tailored to individual bacterial isolates and should be based on antimicrobial susceptibility testing (Donnenberg, 2015).

CONCLUSION

Cockroaches in Gizan City are potential source of pathogenic bacteria with multidrug resistant strains. Twenty tow species of bacteria belonging to ten genera were identified from cockroaches with *Klebsiella pneumoniae*, *Serratia marcescens* and *Pantoea agglomerans* being the predominant isolates. Half of the isolates were multidrug-resistant strains. High resistance percentages were noted to Ampicillin and Amoxicillin clavulanate, followed by Cefoxitin, Cefazolin and Fosfomycin.This fact implies epidemiological risks and complicating therapeutics and leads to more medical costs in urban environments. Preventive and control measures in households and other facilities focusing on hygiene measures within an integrated vector management approach are highly needed to minimize cockroach related food-borne diseases and other nosocomial infections that may arise in Gizan City.

Conflict of interest:
Authors declared that they have no conflict of interest.

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