BACTERIAL CONTAMINATION OF EXTERNAL SURFACE OF COCKROACHES AND THEIR ANTIBIOTIC RESISTANCE IN HOSPITALS OF HAMADAN, IRAN

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ABSTRACT

Objective: To determine the type of cockroaches contaminated by bacterial species and their antibiotic resistance in different hospitals of Hamadan city.

Methodology: This cross sectional study was carried out on 179 samples of cockroaches collected from hospitals in Hamadan city, Iran. The body surface was washed with physiological sterile serum and the the solution was centrifuged at 2000 rpm for five minutes. bacteria were identified using the phenotypic method. Antibiotic resistance of bacteria against various antibiotics was checked with disk diffusion method. Data were analyzed using SPSS version 20 software. chi-square test was applied for significance. P value <0.05 was regarded as significant.

Results: Out of 179 cockroaches, 117 (65.36%) and 62 (34. 64%) were American and German cockroaches respectively. In total, 173 (96. 64%) of them were contaminated with bacteria. Three hundred and fourteen (76%) and 99 (24%) strains of bacteria were isolated from both types of cockroaches. E. coli was the most common (119 cases) and Morganella was the lowest species found. Gram positive bacteria showed the highest antibiotic resistance to Clindamycin with 106 (79.7%) and Tetracycline with 43 (32.33%) highest susceptibility. Gram-negative bacteria had the highest resistance to Amoxicillin and the highest sensitivity to Norfloxacin.

Conclusion: Bacterial contamination of cockroaches in hospital environment is an important concern in health care systems. Moreover, antibiotic resistance observed in separated bacteria can enhance the pathogenicity of bacteria.

Key Words: Bacterial contamination, Antibiotic resistance, Cockroaches, Iran

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INTRODUCTION

Insects are the most populous arthropods that are important in economy, health and medical terms. The relationship between human health and some insects has been proven in the nineteenth century[1]. Cockroaches have been known as Blattaria, and so far about 4,000 cockroaches have been identified, of which less than 1% are pests. Pest cages are residential areas, warehouses, bakeries, baths, hospitals and even ships and harbors. They live in the dark and narrow gaps and come out at night for feeding[1]. The secretions and shells caused by replacement of the skin contain sensitive substances that cause dermatitis, itching and many acute respiratory diseases[2]. Laboratory tests have been used to isolate and detect multiple allergens using serological methods on the secretions and fecal particles of cockroaches. Cockroaches are considered as the second most important cause of asthma after dust-induced sensitivities. The ability to run and swiftly move makes them difficult to control and transmit pathogens[3]. In fecal cockroaches, there are compounds such as Zantoric acid, Zinc citric acid and 8-hydroxycinnedic acid, all of which are derivatives of tryptophan and have a mutagenic and carcinogenic effect. The role of cockroaches in the development and spread of nosocomial infections is very important[1, 4]. Cockroaches are naturally infected with pathogenic bacteria causing leprosy, pygmy plague, bloody diarrhea, urinary tract infections, pimples, abscesses and food poisoning. German, American and Oriental cockroaches are the dominant cockroaches of the bacterial vector[5]. Cockroaches cause the spread of diseases due to feeding of a variety of food and even human extinction, with the transmission of a large number of harmful microor-

ganisms[6].

This study was aimed to determine the type of cockroaches, their contamination with bacterial species and their antibiotic resistance.

METHODOLOGY

This cross sectional study was conducted from March 2015 to February 2016, in Hamadan, Iran. It was approved by the Ethics Committee of Hamadan University of Medical Sciences, Hamadan. The registration code of project was 9503111014. The study settings were two hospitals; Sina and Fatemiyeh teaching hospitals. In this study, 179 cockroaches were sampled. They were transported to laboratory after observation using sterile gloves. To avoid contamination, separate containers were used for transfer. Then each of the samples was placed at 4 degrees below zero for 5 minutes to be anesthetized. Species of cockroaches were determined according to the detection key. Then, each cockroach was placed in sterile glasses containing 5 cc of physiological saline and washed and shaken vigorously to release probable microorganisms from its external surface[2].

The solutions obtained were centrifuged at 2000 rpm for five minutes. The sediments were cultured on various media; Eosin Methylene Blue (EMB) and blood agar, incubated at 37° C for 24 hours. For each colony, differentiation tests were conducted to specify the type of each bacterium. For gram-negative bacteria gram stain, fermentation of sugars, motility test, Triple Sugar Iron (TSI), Sulfide Indole Motility (SIM), and Simmon citrate (Merck) were employed. For identification of gram-positive bacteria, gram stain, catalase test, oxidase test, fermentation of sugars (mannitol) were employed and sensitivity to antibiotics was checked[2,7]. The resistance of bacterial strains was determined using the Kirby-Bauer disk diffusion method. A suspension with turbidity equal to 0.5 McFarland standards was provided for each bacterial strain[8]. Suspensions were placed in Mueller-Hinton agar medium and exposed to the different disks of antibiotics and incubated for 24 hours at 37° C. All antibiotic disks were purchased from Mast Group Ltd. (England) and included various antibiotic disks such as erythromycin (15 µg), ampicillin (30 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (75/23 -25/1 µg), norfloxacin (10 µg), ceftriaxon (30 µg), ceftizoxime (30 µg), Amoxicillin (25 µg), penicillin (10 µg) nalidixinacid (30 µg), clindamycin (2 µg), carbenicillin (100 µg), vancomycin (30 µg), ceftazidime (30 µg), gentamicin (30 µg), cefalotin (30 µg) and cefotaxim (30 µg). The diameter of growth inhibition zone of all samples were measured and categorized in three groups of sensitive to resistant, as per guidelines recommended by The Clinical & Laboratory Standards Institute (CLSI)[9].

Data were analyzed using SPSS version 20 software package developed by IBM cooperation, using chi-square tests. P value <0.05 was regarded as significant.

RESULTS

Out of 179 cockroaches, 117 (65. 4%) were American and 62 (34.6%) were German cockroaches. Frequency of cockroach species in Sina and Fatemieh hospitals is shown in table 1.

Bacterial contamination in American cockroaches was found to be 314 (76%) and 99 (24%) in German cockroaches as shown in table 2.

A total of 413 bacteria were isolated. The abundance and relative frequency of bacterial strains is shown in Table 3. Antibiogram results showed that gram-positive bacteria generally had high resistance to antibiotics and more than 70% of the antibiotics were resistant in this study (Table 4).

In gram-negative bacteria, the highest resistance observed to antibiotic was that to Amoxicillin with 94.77% and the least resistance to Nitrofurantion; 35.82% (Table 5).

DISCUSSION

In developing countries, nosocomial infections are a

Table 1: Frequency of cockroach species

Hospital	Cockroach species		
	P. Aamericana N(%)	B. Germanica N(%)	Total N(%)
Sina	63(66.3)	32(33.7)	95(100.0)
Fatemieh	54(64.3)	30(35.7)	84(100.0)
Total	117(65.4)	62(34.6)	179(100.0)

Table 2: Frequency of type of bacterial strains in each cockroach species

Bacteria	P. Aamericana N(%)	B. Germanica N(%)	Total N (%) N(%)	
Esherichia.coli	104(25.2)	15(3.6)	119(28.8)	X2 =12.499 P=0.00
Klebsiella	71(17.2)	20(4.8)	91(22.0)	
Coagulase Negative Staphylococci	78(18.9)	12(2.94)	90(21.8)	
Proteus	25(6.1)	6(1.5)	31(7.5)	
Enterococcus	14(3.4)	4(0.1)	18(4.4)	
Micrococcus	6(1.5)	9(2.2)	15(3.6)	
Pseudomonas	10(2.4)	1(0.2)	11(2.7)	
Serratia	0(0.0)	13(3.1)	13(3.1)	
Streptococcus B.	5(1.2)	1(0.2)	6(1.5)	
Streptococcus A.	0(0.0)	6(1.5)	6(1.5)	
Staphylococcus Aureus	4(0.1)	4(0.1)	4(0.1)	
Enterobacter	0(0.0)	2(0.5)	2(0.5)	
Morganela	1(0.2)	0(0.0)	1(0.2)	
No Bacteria	0(0.0)	6(1.5)	6(1.5)	
Total N (%)	314(76.0)	99(24.0)	413(100.0)	

Table 3: Frequency of bacterial strains in Sina and Fatemiyeh Hospital (n=413)

Bacteria	Sina Hospital N (%)	Fatemiyeh Hospital	Total N (%)	
Esherichia.Coli	66(16.0)	53(12.8)	119(28.8)	X2 =82.584 P=0.00
Klebsiella	69(16.7)	22(5.3)	91(22.0)	
Coagulase Negative Staphylococci	67(16.2)	23(5.6)	90(8.21)	
Proteus	11(2.7)	20(4.8)	31(7.5)	
Enterococcus	7(1.7)	11(2.7)	18(4.4)	
Micrococcus	4(1.0)	11(2.7)	15(3.6)	
Serratia	13(3.1)	0(0.0)	13(3.1)	
Pseudomonas	1(0.2)	10(2.4)	11(2.7)	
Streptococcus B.	6(1.5)	0(0.0)	6(1.5)	
Streptococcus A.	6(1.5)	0(0.0)	6(1.5)	
Staphylococcus aureus	0(0.0)	4(1.0)	4(1.0)	
Enterobacter	2(0.5)	0(0.0)	2(0.5)	
Morganela	1(0.2)	0(0.0)	1(0.2)	
No Bacteria	0(0.0)	6(1.5)	6(1.5)	
Total N (%)	253(61/3)	160(38/7)	413(100)	

big threat to all patients in general and indoor patients in particular. The sources can be diverse. In recent years, emphasis has been made on these sources and finding of ways to eradicate these potential threats. Hospitals are inhabited by different types of arthropods. Cockroaches are one of the abundant creature occupying different parts of health care facilities[10]. In the study by Mahjoob et al.,[11] it was reported that in the Hormozgan city, cockroaches isolated from hospitals, German cockroaches had frequency of 88.8%, and Oriental cockroaches were 0.8%, the lowest incidence. Australian cockroaches were reported as 2.4%[11]. In the study conducted in Kashan hospitals, 88 cockroaches were counted as 73 (82.9%) German cockroaches and 13 (17.1%) American cockroaches[12]. In another study in the city of Cushion, Taiwan, 558 cockroaches were caught, out of these 21.32 % and 78.67% were American and German cockroaches, respectively[13]. In a study by Karimi Zarchi in three se-

Table 4: Antibiotic resistance results in gram-positive bacterial strains

Bacteria	Antibiotics N(%)					
	Vancomycin	Carbenicillin	Clindamycin	Tetracycline	Erythromycin	Penicillin
Coagulase Negative Staphylococci	79(87.8)	72(80.0)	77(85.6)	72(80.0)	79(87.8)	79(87.8)
Staphylococcus Aureus	0(0.0)	0(0.0)	4(100.0)	0(0.0)	4(100.0)	4(100.0)
Micrococci	6(40)	0(0.0)	9(60.0)	0(0.0)	2(13.3)	1(6.7)
Enterococcus	13(72.2)	10(55.6)	16(88.9)	7(38.9)	14(77.8)	18(100.0)
Streptococcus B.	0(0.0)	5(83.3)	0(0.0)	3(50)	6(100.0)	0(0.0)
Streptococcus A.	0(0.0)	ND	ND	ND	ND	0(0.0)
Total	98(73.7)	87(65.4)	106(79.7)	82(61.6)	105(78.9)	102(73.4)
	P=0.00					

ND: Not Done

Table 5: Antibiotic resistance results in gram negative bacterial strains

Bacteria	Antibiotics N(%)									
	Ceftrizoxime	Cefotaxime	Ceftazidime	Norfloxacin	Amoxicillin	Sulfamethoxazole	Nitrofurantion	Nalidixinacid	Tetracycline	Gentamaicin
Klebsiella	56(61.5)	41(45.1)	54(59.3)	90(98.9)	91(100.0)	41(45.1)	42(46.1)	41(45.1)	26(28.6)	28(30.7)
Pseudomonas	11(100.0)	10(90.9)	8(72.7)	5(45.4)	11(100.0)	9(81.81)	10(90.9)	10(90.9)	11(100.0)	7(63.6)
Esherichia.Coli	68(57.1)	50(42.1)	71(59.7)	46(38.6)	107(89.9)	56(47.1)	26(21.8)	34(28.6)	75(63.0)	43(36.1)
Protous	10(32.2)	11(35.4)	11(35.4)	11(35.4)	29(93.5)	11(35.4)	11(35.4)	21(67.7)	28(90.3)	15(48.4)
Morganela	0(0.0)	0(0.0)	0(0.0)	1(100.0)	1(100.0)	0(0.00)	0(0.0)	1(100.0)	0(0.0)	0(0.0)
Serratia	0(0.0)	0(0.0)	0(0.0)	0(0.0)	13(100.0)	0(0.00)	6(46.1)	7(53.8)	8(61.5)	13(100.0)
Enterobacter	1(50.0)	1(50.0)	1(50.0)	1(50.0)	2(100.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)
Total	146(55.3)	113(42.2)	145(54.1)	154(57.5)	254(94.8)	118(44.0)	96(35.8)	115(42.9)	170(63.4)	107(39.9)

lected hospitals in Tehran city, 305 cockroaches, 55.6% , 12.1% and 22.3% were American, Germans and Oriental cockroaches, respectively[14]. In another study in the city of Cushion in Taiwan, 226 cockroaches were caught, of which 123(54.42%) were American cockroaches and 103(45.57%) were German cockroaches[10]. Above-mentioned studies are consistent with this study. It seems that these two cockroache forms are the dominant and active species of Diktyo Ptera in the hospitals of Iran. Because of many differences in cockroaches, their size, habitat, pesticide use, environmental conditions and health status can be affected.

In particular, it was revealed that cockroaches from the Sina Hospital had more bacterial species in their body, which had a significant difference with the cockroaches from Fatemiyeh Hospital. In the study of Mahjub et al.,[20] bacterial species were identified in educational hospitals of Hormozgan. Among these bacteria, the highest frequency was related to E. Coli with 26.4% and the lowest was related to Advarcilla with 0.4%; as well as Pseudomonas, Enterococcus bacteria and Escherichia isolated from the body of cockroaches, and the presence of Salmonella and Shigella as Less abundant and other important bacteria such as Klebsiella pneumoniae, Streptococcus pneumoniae and Staphylococcus aureus, which is sufficient reason to explain the high contamination level of this insect[1]. In the study of Fakoorziba at home (as a control) and three hospitals in Tehran, the most frequent was the Klebsiella and Enterobacter[14]. In a study conducted by Karimi Zarchi in three selected hospitals in Tehran,[19] bacterial strains

were isolated. Among them, E. Coli had the highest percentage of infection, followed by Klebsiella pneumoniae, Bacillus, Streptococcus group D and Perteus vulgaris, respectively[15]. A study by lin in Hainan, China, showed that most cockroaches were contaminated with E Coli, Pseudomonas irvinezas, Salmonella, Staphylococcus aureus, Shigella and Peretheus[16].

Another study by Chaichanawongsaroj et al., in Bangkok, Thailand, reported that 99 cockroaches were hunted from hospitals, residential areas and restaurants, and high percentages of Klebsiella, E.Coli, Entero Bacteriace and Citro bacteria was found. It was showed that the insects play a role in the transmission of these bacteria[17]. The review of the above studies showed that the results of this study were consistent with the frequency of E. coli.

In research conducted by Soltani et al. in Iran, 100% of the isolates of the S. aureus were susceptible to Vancomycin[18]. In another study in Chita Gong, Bangladesh, significant resistance to penicillin (68.0%), erythromycin (60.0%) and Clindamycin (31.0%) was reported[19] which is consistent with the present study. In a study done in Varna, India, 8 types of Gram-negative bacteria were isolated. Klebsiella pneumoniae and Pseudomonas aeroginosa had the highest drug resistance. Resistance to Ampicillin and Trimetoprim/Sulfamethoxazole was reported as 100.0%[20], which is consistent with the present study. The main cause of antibiotic resistance is the unjustified use of antibiotics[21].

High levels of resistance in hospital bacteria can also be justified because antibiotic use varies from hospital to hospital. The bacteria in the hospitals have more virulence and resistance genes that cause the disease, and on the other hand, these bacteria can transfer these genes evenly to other sexes through genetic transmissions. However, the role of cockroaches in creating hospital infections is much more important than it seems.

CONCLUSION

Bacterial contamination of cockroaches in hospital environment is an important concern in health care systems. Moreover, antibiotic resistance observed in separated bacteria can enhance the pathogenicity of bacteria.

REFERENCES

1. Mullen G, Durden L. Medical and veterinary entomology: 3rd Ed. Academic press; 2018.
2. Nazari M, Mehrabi T, Hosseini SM, Alikhani MY. Bacterial contamination of adult house flies (musca domestica) and sensitivity of these bacteria to various antibiotics, captured from Hamadan City, Iran. J Clin Diagn Res 2017; 11:DC04-7.
3. Mikó I, Copeland RS, Balhoff JP, Yoder MJ, Deans AR. Folding wings like a cockroach: a review of transverse wing folding ensign wasps (Hymenoptera: Evaniidae: Afrevania and Trissevania). PLoS One 2014; 9:e94056.
4. Cloarec A, Rivault C, Fontaine F, Le Guyader A. Cockroaches as carriers of bacteria in multi-family dwellings. Epidemiol Infect 1992; 109:483-90.
5. Gaugler R. Medical Entomology for students. 5th Ed. Am Entomol 2016; 62:130.
6. Ash N, Greenberg B. Vector potential of the German cockroach (Dictyoptera: Blattellidae) in dissemination of Salmonella enteritidis serotype Typhimurium. J Med Entomol 1980; 17:417-23.
7. Hosseini SM, Zeyni B, Rastyani S, Jafari R, Shamloo F, Tabar ZK et al. Arabestani MR. Presence of virulence factors and antibiotic resistances in enterococcus sp collected from dairy products and meat. Der Pharm Lettre 2016; 8:138-45.
8. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol 2009.
9. Patel JB. Performance standards for antimicrobial susceptibility testing: 29th Ed. Clin Lab Stand Inst; 2017. Available at: https://clsi.org/media/2663/m100ed29_sample.pdf
10. Pai HH, Chen WC, Peng CF. Cockroaches as potential vectors of nosocomial infections. Infect Control Hosp Epidemiol 2004; 25:979-84.
11. Mahjoob M, Nejate G, Keihane A. Bacterial contamination of external surface and gut of cockroaches in teaching hospital, Hamadan. Hormozgan Med J 2010; 14:80-6.
12. Doroodgar A, Khorshidi A, Shajari GR, Tashakkor Z. Bacterial infection of cockroaches in Kashan hospitals, 2001. J Kashan Uni Med Sci 2005; 8:30-8.
13. Pai HH. Multidrug resistant bacteria isolated from cockroaches in long-term care facilities and nursing homes. Acta trop 2013; 125:18-22.
14. Fakoorziba MR, Eghbal F, Hassanzadeh J, Moemenbellah-Fard MD. Cockroaches (Periplaneta americana and Blattella germanica) as potential vectors of the pathogenic bacteria found in nosocomial infections. Ann Trop Med Parasitol 2010; 104:521-8.
15. Zarchi AAK, Vatani H. A survey on species and prevalence rate of bacterial agents isolated from cockroaches in three hospitals. Vector-Borne Zoonotic Dis 2009; 9:197-200.
16. Lin YZ, Cui YB, Yang W, Rao LY, Pan W, Chen JL. Investigation on species composition of cockroaches and bacteria-carrying on their bodies in five cities of Hainan. Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi 2008; 26:77-8.
17. Chaichanawongsaroj N, Vanichayatanarak K, Pipatkul-

lachat T, Polrojpanya M, Somkiatcharoen S. Isolation of gram-negative bacteria from cockroaches trapped from urban environment. Southeast Asian J Trop Med Public Health 2004; 35:681-4.

18. HAGHI Ashteiani M, Sadeghi far N, Abedini M, Soroush S, Taheri-Kalani M. Etiology and antibacterial resistance of bacterial urinary tract infections in Children Medical Center, Tehran, Iran. Acta Med Iran 2007; 45:153-7.

19. Islam A, Nath AD, Islam K, Islam S, Chakma S, Hossain MB et al. Isolation, identification and antimicrobial resistance profile of Staphylococcus aureus in Cockroaches (Periplaneta americana). J Adv Veter Anim Res 2016; 3:221-8.

20. Wannigama DL, Dwivedi R, Zahraei-Ramazani A. Prevalence and antibiotic resistance of Gram-negative pathogenic bacteria species isolated from Periplaneta americana and Blattella germanica in Varanasi, India. J Arthropod-borne dis 2014; 8:10-20.

21. Mehrgan H, Rahbar M. Prevalence of extended-spectrum β-lactamase-producing Escherichia coli in a tertiary care hospital in Tehran, Iran. Int J Antimicrob Agents 2008; 31:147-51.

CONTRIBUTORS

SN conceived the idea, planned the study and drafted the manuscript. FH, SN helped acquisition of data, did statistical analysis, editing and final writing of the manuscript. SMH and MN did literature search, helped in acquisition of data and refining the text after going through initial manuscript. All authors contributed significantly to the submitted manuscript.