Original Articles

Frequency of Resistance and Susceptible Bacteria Isolated from Houseflies

B Davari[1], *E Kalantar[2], A Zahirnia[3], SH Moosa-Kazemi[4]

[1]Department of Parasitology, School of Medicine, Medical University of Kurdistan, Sananadaj, Iran
[2]Liver and Digestive Research Center, Tohid Hospital, Medical University of Kurdistan, Sananadaj, Iran
[3]Department of Parasitology, School of Medicine, Medical University of Hamedan, Hamedan, Iran
[4]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Iran

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Abstract

Background: In this study, we determine the vector competence of *Musca domestica* with reference to the transmission of susceptible and resistance bacterial strains in hospitals and slaughter house in Sanandaj City, west Iran.
Methods: Totally 908 houseflies were collected to isolate bacteria from their external body based on standard procedures.Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller Hinton agar based on recommendations of CLSI (formerly the National Committee for Clinical Laboratory Standards).
Results: From collected houseflies, 366 bacteria species were isolated. The most common isolated bacterium at hospitals was *Klebsiella pneumoniae* 43.3% (n= 90) followed by *Pseudomonas aeruginosa* 37% (n= 77), while that of slaughterhouse was *Proteus mirabilis*. 29.1% (n= 46) followed by *Citrobacter freundii* 28.4% (n= 45). Among all the isolates from hospitals, cephalexin, chloramphenicol, ampicillin, and tetracycline, resistance rates were above 32.5% and gentamicin expressed the highest susceptibility among all the isolates from hospitals. It is worth to note that *K. pneumoniae* showed 61% and 44.5% resistance to cephalexin and chloramphenicol respectively. Similarly, all isolates from slaughterhouse were more than 28% and 30% resistant to cephalexin and chloramphenicol respectively. Surprisingly, among all the isolates, *Citrobacter freundii* were highly resistant to gentamicin.
Conclusion: Houseflies collected from hospitals and slaughterhouse may be involved in the spread of drug resistant bacteria and may increase the potential of human exposure to drug resistant bacteria.

Keywords: House fly, bacterium, antibacterial resistance, hospitals, slaughterhouse

Introduction

The common house fly, *Musca domestica* is a medically-important insect worldwide (Fotedar 2001, Graczyk et al. 2001, Kabkaew et al. 2007). Houseflies have been implicated as vectors or transporters of various human pathogens, including *Vibrio cholerae*, Enterobacteriaceae pathogens, *Staphylococcus aureus*, and *Pseudomonas* spp. (Olsen 1998, Fotedar 2001, Rajendran and Pandian 2003). Transmission takes place when the fly makes contact with people or their food. As many as 500000 microorganisms may swarm over its body and legs (Thirumalai Vasan et al. 2008).

"Flies can spread diseases because they feed freely on human food and dirty matter alike. The fly picks up disease-causing organisms while crawling and feeding. The diseases that flies can transmit include enteric infections, eye infections, poliomyelitis and certain skin infections." Thus, houseflies are widely recognized as potential reservoirs and vectors of food borne pathogens (Pandian and Asumtha 2001, Khobdel et al. 2008).

*Corresponding author: Dr Enayat Kalantar,
Email: kalantar_enayat@yahoo.com

It is worth to note that a few studies also indicate that houseflies have been suspected to be reservoirs and vectors for pathogens (Zarin et al. 2007, Barin et al. 2010).

There have been no studies on the carriage of antibiotic-resistant, pathogenic bacteria by *M. domestica* in hospitals and slaughterhouse in Sanandaj. An increasing frequency of antibiotic resistance has been reported from all over of the world. In this regard, an attempt was made to determine the frequency of resistance and susceptible bacteria isolated from houseflies in Sanandaj, west of Iran.

Materials and Methods

Houseflies were captured by a sterile nylon net from the wards and corridors of the Tohid Hospital, Beassat Hospital, and the slaughterhouse of the city. The collected flies were transferred immediately to the Entomology Laboratory, and identified to species level by morphological characters such as thorax, wings and antenna.

After identification, 1 ml of sterile physiological saline solution was added to each vial, which was shaken vigorously for 1 min with the fly remaining inside. The fly was then removed from the saline, and was checked for bacteria dislodged from the external surfaces of the fly.

Bacterial Counts

Serial dilutions of a subsample of each bacterial suspension were prepared in sterile saline. Each dilution was then inoculated onto two plates of plate-count agar and incubated overnight at 37 °C. Colony forming units (CFU/ ml) were then counted so that the total numbers of bacteria recovered from the external surface of each fly could be estimated. Briefly, houseflies individually were shaken thoroughly in sterile saline solution (2 ml) for 2 min. The suspension was then serially diluted and inoculated on MacConkey agar, and Blood agar. Plates were incubated for 24 h at 37 °C. The resulting isolates were characterized morphologically and further identifications were carried out following the methods of Koneman et al. 1992.

Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method on Mueller Hinton agar based on recommendations of CLSI (formerly the National Committee for Clinical Laboratory Standards) (NCCLS, 2003). The following antibiotics were used in this study: erythromycin, streptomycin, ampicillin, tetracycline, kanamycin ,chloramphenicol, co-trimoxazole, gentamicin, ciprofloxacin, nitrofurantoin, ceftriaxone, and cephalexin which were purchased from Patan Teb Company.

Results

From the 908 houseflies collected from the hospitals and slaughterhouse at Sanandaj, 366 (40.3%) bacterial species were isolated (Table 1).

The most common bacterium isolated from *M. domestica* at hospitals was *Klebsiella pneumoniae* 43.3% (n= 90) followed by *Pseudomonas aeruginosa* 37% (n= 77), while that of slaughterhouse was *Proteus mirabilis* 29.1 (n= 46) followed by *Citrobacter freundii* 28.4% (n= 45) (Table 2).

Among all the isolates from hospitals, cephalexin, chloramphenicol, ampicillin, and tetracycline, resistance rates were above 32.5% and gentamicin expressed the highest susceptibility among all the isolates from the hospitals. It is worth to note that *K. pneumoniae* showed 61% and 44.5% resistance to cephalexin and chloramphenicol, respectively (Table 3).

Similarly, all the isolates from slaughter house were more than 28%, 30% resistance to cephalexin and chloramphenicol respectively (Table 4). Surprisingly, among all isolates, *Citrobacter freundii* were highly resistant to cephalexin.

Table 1. Bacterial carrying rates for *Musca domestica* collected from hospitals and slaughter house at Sanandaj

No. of bacterial species isolated from each fly	Number isolates for habitats						
	Hospitals			Slaughter House			Total
	Male	Female	Total	Male	Female	Total	
Total positive flies	61	147	208	56	102	158	
Total negative flies	78	132	210	127	205	332	
Total flies examined	139	279	418	183	307	490	908

Table 2. Details of bacteria isolated from House Fly collected from hospitals and Slaughter house at Sanandaj

Bacteria	Number isolates for habitats			
	Hospitals		Slaughter house	
	Number	Percent	Number	Percent
K. pneumoniae	90	43.3	23	14.5
P. aeruginosa	77	37.0	0.0	0.0
***Citrobacter** freundii*	12	05.7	45	28.4
E. coli	19	09.1	26	16.4
Bacillus cereus	10	04.8	18	11.4
Proteus mirabilis	0.0	0.0	46	29.1
Total	208	100	158	100

Table 3. Antibiotic susceptibility pattern (%) of identified bacteria in hospitals at Sanadaj, Iran

Antibiotic	Bacteria														
	***K.* spp. (90)**			***P. aeruginosa* (77)**			***E. coli* (19)**			***B. cereus* (10)**			***Proteus* mirabilisa (12)**		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ery	46.6	31.1	11.1	39.0	36.3	24.6	26.0	42.1	31.5	20.0	60.0	20	16.0	41.6	41.6
Strep	38.8	34.4	26.6	32.5	41.5	25.9	31.0	26.3	42.1	30.0	50.0	20.0	33.0	66.6	0.0
Amp	43.3	38.8	17.7	35.0	45.4	19.4	31.0	26.3	42.1	30.0	40.0	30.0	25.0	33.3	41.6
Tetra	43.3	41.1	15.5	29.0	53.2	16.8	21.0	42.1	36.8	20.0	50.0	30.0	16.0	50.0	33.3
Kana	27.8	23.3	48.8	26.0	41.5	32.4	15.0	57.8	26.3	20.0	60.0	20.0	16.0	41.6	41.6
Chlo	44.5	46.6	08.8	32.5	23.3	44.1	31.0	42.1	26.3	20.0	70.0	10.0	16.0	58.3	25.0
Co-tri	35.5	31.1	33.3	31.0	32.4	36.3	26.0	63.1	10.5	10.0	50.0	40.0	08.3	33.3	58.3
Gen	11.0	16.6	72.2	09.0	46.7	44.1	15.0	42.1	42.1	20.0	50.0	30.0	16.0	50.0	33.3
Cipro	15.5	22.2	62.2	14.0	49.3	36.3	15.0	63.1	21.0	10.0	60.0	30.0	08.3	58.3	33.3
Nitrof	17.7	15.5	66.6	16.8	23.3	59.7	21.0	47.3	31.5	20.0	50.0	30.0	16.0	50.0	33.3
Ceftri	24.4	26.6	48.8	22.0	54.5	23.3	26.0	42.1	31.5	30.0	60.0	10.0	25.0	41.6	33.3
Cepha	61.0	31.1	07.7	44.0	28.5	27.2	42.0	47.3	10.5	40.0	50.0	10.0	33.0	50.0	16.6

Erythromycin, Streptomycin, Ampicillin, Tetracycline, Kanamycin ,Chloramphenicol, Co-trimoxazole, Gentamicin, Ciprofloxacin, Nitrofurantoin, Ceftriaxone , Cephalexin
(S) = Sensitive, (I) = Intermediate, (R) = Resistant

Table 4. Antibiotic susceptibility pattern (%) of identified bacteria at slaughter house in Sanandaj, Iran

Antibiotic	*Klebsiella* spp. (23)			*Proteus* mirabilis (46)			*E. coli* (26)			*Citrobacter freundii* (45)			*Bacillus cereus* (18)		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ery	21.7	43.4	34.7	15.0	43.4	41.3	19.0	61.5	19.2	15.4	17.7	66.0	11.1	33.3	55.0
Strep	26.0	30.4	43.4	15.0	52.1	32.6	23.0	69.2	07.6	24.4	22.2	53.0	11.1	22.2	66.0
Amp	21.7	26.0	43.4	17.0	60.8	21.7	27.0	46.1	23.0	20.3	17.7	62.0	22.2	27.7	50.0
Tetra	13.0	34.7	52.1	13.0	43.4	43.4	19.0	69.2	11.5	22.2	08.8	68.0	16.6	22.2	61.0
Kana	17.0	26.0	56.5	15.0	41.3	43.4	15.0	65.3	19.2	24.4	24.4	51.0	05.5	22.2	72.0
Chlo	30.0	34.7	34.7	14.0	60.8	26.0	23.0	57.6	19.2	15.5	15.5	68.0	11.1	33.3	55.0
Co-tri	30.0	21.7	47.8	06.5	52.1	41.3	27.0	53.8	15.3	06.6	11.1	82.0	16.6	38.8	44.0
Gen	08.6	56.5	34.7	13.0	43.4	43.4	15.0	46.1	38.4	08.8	13.3	77.0	0.0	22.2	77.0
Cipro	13.0	39.1	47.8	06.5	54.3	39.1	11.5	65.3	23.0	04.4	11.1	84.0	0.0	38.8	61.0
Nitrof	13.0	52.1	34.7	15.0	43.4	41.3	19.0	53.8	26.9	20.0	26.6	53.0	0.0	27.7	72.0
Ceftri	21.7	34.7	43.4	19.0	39.1	41.3	23.0	57.6	19.2	17.7	22.2	60.0	0.0	44.4	55.0
Cepha	47.0	30.4	21.7	28.0	45.6	26.0	30.0	65.3	03.8	66.0	20.0	13.3	05.5	33.3	61.0

Erythromycin, Streptomycin, Ampicillin, Tetracycline, Kanamycin, Chloramphenicol, Co-trimoxazole, Gentamicin, Ciprofloxacin, Nitrofurantoin, Ceftriaxone , Cephalexin

Discussion

"The biology and ecology of *M. domestica* make it an ideal mechanical vector of human and animal pathogens. Cattle barns, poultry houses, slaughter houses, and hospitals are sites where house flies can reproduce" (Peter et al. 2007).

Many scientists indicated that the external organs of *M. domestica* (legs, wings and mouthparts) constituted a large source of bacteria they isolated (Graczyk 1999, Mutsuo et al. 1999). The results of this study indicated that *M. domestica* could play a great role as a mechanical carrier of bacteria. In this study, most of the bacteria isolated were medically important, including *K. pnenumonae, P. aeruginosa, Proteus mirabilis.* These findings agree with the results of Vazirianzadeh et al. (2008) in Ahvaz, which showed presence *Escherichia coli, P. aeruginosa,* and *K. pneumonia* on the housefly collected from slaughterhouse and zoo.

Our results are in accordance with other reports which highlight the importance of houseflies in carrying various pathogenic bacteria particularly *K. pneumoniae* being the most important at USA and Iran (Thaddeus et al. 2001, Khalil et al. 1994). The house flies caught in hospitals carried pathogenic bacteria more often than those caught at slaughter house which is in contrast with Sulaiman et al. (2000) study.

One of the most important problems facing global public health today is antimicrobial resistance. The problem is most horrible in developing countries, where the bacterial infections causing human disease are also those in which emerging antibiotic resistance is most evident (Shears 2000, Kalantar et al. 2008).

The resistance patterns of the *K. pneumoniae* isolated in the present study are shown in Table 3 and 4. The *K. pneumoniae* isolated from the hospitals houseflies were more resistant to cephalexin as compared to that of isolated from the slaughterhouse. Fotedar et al. (1992) and Sramova et al. (1992) reported similar multiple-resistance to antibiotics, in *Klebsiella* spp. from houseflies in hospitals environments.

Similarly, the resistance patterns of the *P. aeroginosa* from hospitals environment houseflies were frequently multiple-resistant, with more than 32% of the isolates each being resistant to erythromycin, streptomycin, ampicillin, and cephalexin. Therefore, houseflies trapped in hospitals may also participate more in the dispersion of antibiotic resistance into the environment. Multiple resistances to antibiotics are common among *P. aeruginosa* isolated from different clinical sources in Iran (Kalantar et al. 2009).

The present study indicates that housefly *M. domestica* poses a possible health risk to communities proved that the isolated strains of bacteria were resistant to various antibiotics. It is well-established fact that the resistance to various antimicrobials may be due to presence of some virulence gene, involvement of secretion machinery of multi drug efflux proteins, through mutations in bacterial genome or by gaining additional genes through horizontal gene transfer or by physiology dependent resistance (Mitchell et al. 2004, Rangrez et al. 2006).

In conclusion, we report that houseflies collected in hospitals and slaughterhouse may be involved in the spread of drug resistant bacteria and may increase the potential for human exposure to drug resistant bacteria. It is recommended that suitable steps must be taken to control the flies and monitor the sensitivity pattern of the pathogens transmitted by the houseflies.

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