Original Articles

Frequency of Resistance and Susceptible Bacteria Isolated from Houseflies

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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, cephalixin, 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 cephalixin and chloramphenicol respectively. Similarly, all isolates from slaughterhouse were more than 28% and 30% resistant to cephalixin 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).

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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 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 slaughterhouse 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 | *K. pneumoniae* (90) | *P. aeruginosa* (77) | *E. coli* (19) | *B. cereus* (10) | *Proteus mirabilis* (12) |
|------------|----------------------|----------------------|----------------|------------------|------------------------|
|            | R | I | S | R | I | S | R | I | S | 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 14.1 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 52.1  | 32.6 23.0 69.2 07.6    | 24.4 22.2   | 53.0 11.1 22.2           | 66.0 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 19.0 69.2 11.5    | 22.2 08.8   | 68.0 16.6 22.2           | 61.0 61.0           |
| Kana       | 17.0 26.0 56.5 15.0  | 41.3 43.4 15.0 65.3    | 19.2 24.4   | 51.0 05.5 22.2           | 72.0 72.0           |
| Chlo       | 30.0 34.7 34.7 14.0  | 60.8 26.0 23.0 57.6    | 19.2 15.5   | 68.0 11.1 33.3           | 55.0 55.0           |
| Co-tri     | 30.0 21.7 47.8 06.5  | 52.1 41.3 27.0 53.8    | 15.0 65.3   | 82.0 16.6 38.8           | 44.0 44.0           |
| Gen        | 08.6 56.5 34.7 13.0  | 43.4 43.4 15.0 46.1    | 38.4 08.8   | 77.0 0.0 22.2            | 77.0 77.0           |
| Cipro      | 13.0 39.1 47.8 06.5  | 54.3 39.1 11.5 65.3    | 23.0 04.4   | 84.0 0.0 38.8            | 61.0 61.0           |
| Nitrof     | 13.0 52.1 34.7 15.0  | 43.4 41.3 19.0 53.8    | 26.9 20.0   | 53.0 0.0 27.7            | 72.0 72.0           |
| Ceftri     | 21.7 34.7 43.4 19.0  | 39.1 41.3 23.0 57.6    | 19.2 17.7   | 62.0 30.0 44.4           | 61.0 61.0           |
| 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, Cephalxin

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, Mutso 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. pneumoniae*, *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. pneumoniae* 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 cephalxin 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. aeruginosa* from hospitals environment houseflies were frequently multiple-resistant,
with more than 32% of the isolates each being resistant to erythromycin, streptomycin, ampicillin, and cephalixin. 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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