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CARRIAGE OF MULTI-DRUG RESISTANT GRAM-NEGATIVE PATHOGENIC BACTERIA BY THE HOUSE FLY *MUSCA DOMESTICA*

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Abstract

Fifteen house flies were used to isolate bacteria from external body surface and alimentary tract. A total of 50 isolates were obtained from the house flies, of which 25 (50%) were collected from the external body surface and 25 (50%) from alimentary gut. Fifteen isolates (30%) were obtained from Shamsunnahar Hall (SN) dining room, 22 (44%) from Dhaka Medical College Hospital (DMCH) and 13 (26%) from Rokeya Hall (RH) canteen. Six Gram-negative bacteria were isolated from the house flies namely, *Escherichia coli* (36%), *Shigella* spp. (22%), *Salmonella* spp. (18%), *Pseudomonas* spp. (10%), *Klebsiella* spp. (8%) and *Enterobacter* spp. (6%). *E. coli* was the highest in number in all three study areas which was 33% in SN Hall dining, 36% in DMCH, and 39% in RH canteen. *E. coli* was present in 32 and 40% of external body surface and gut samples, respectively. Bacterial susceptibility to antimicrobial agents showed that *E. coli* isolates were highly resistant (66-77.7%) to ampicillin, ciprofloxacilin and penicillin antibiotics. *Salmonella* isolates were sensitive to chloramphenicol but it was (55.5%) resistant to ampicillin, penicillin, tetracycline, gentamycin and imipenem antibiotics. In case of *Shigella* and *Pseudomonas*, 72.72 and 80% isolates were resistant to tetracycline and chloramphenicol, respectively. Among the *Enterobacter* spp. 66.66% were resistant to chloramphenicol, imipenem, vancomycin and tetracycline, while *Klebsiella* showed 100% resistant pattern to tetracycline in the study. It was observed that house flies carry several multi-drug resistant Gram-negative bacteria in their body surface and alimentary tract and played a role in the transmission of serious diseases to human.

Introduction

The house fly (*Musca domestica*) belongs to the order Diptera. It is, by far, the most common of all domestic flies, accounting for about 91% of all flies in human habitat, and one of the most widely distributed insects, found in all over the world(1). This is not only a nuisance pest, but also is considered as one of the major insect vectors which transmits and disseminates different human pathogens, including viruses, bacteria, protozoans, worms and fungi particularly in temperate and tropical countries(2-3). It has

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been reported to be responsible for spreading diseases from animal to animal and from animal to man[4]. House flies have been implicated in the transmission of serious diseases such as anthrax, ophthalmia, typhoid fever, tuberculosis, cholera, and infantile diarrhoea[5-7] and have been demonstrated to harbor or transmit other pathogenic bacteria including *Salmonella* spp.[8], *Chlamydia* spp., *Campylobacter jejuni*[9], *Klebsiella* spp.[10], *Escherichia coli* O157:H7[11-12], *Yersinia pseudotuberculosis*[13], and *Helicobacter pylori*, the causative agent of gastric ulcer[14]. Recently, these insects were reported to be involved in disease outbreaks including *E. coli* O157 : H7[15] in Japan and *Vibrio cholerae* in India[16]. In addition to their role in disease transmission, flies are usually regarded as indicator organisms, symptomatic of disposal problems and reflecting the sanitary level of the community. House flies trapped in hospitals may also participate more in the dispersion of antibiotic resistance into the environment[17]. Recent studies focused on house flies in agricultural and food service settings have suggested that Antiretroviral Therapy (ART) opportunistic pathogens can also be transmitted from animal feedlots to food and humans through house fly vectors[18].

Despite the abundance of house flies in localities of Dhaka city, there are very few information about their role as mechanical transmitters of antibiotic resistant pathogenic bacteria. The present study therefore aims to observe the prevalence of Gram-negative bacteria frequently carried by the house flies and to study their resistance pattern to commonly used antibiotics.

Materials and Methods

The present study was conducted in Entomology Laboratory, Department of Zoology and Microbiology Laboratory, Department of Microbiology, University of Dhaka.

Sample collection: For the collection of the house fly three selective locations were chosen. The locations were dining room of Shamsunnahar Hall, canteen of Rokeya Hall and Dhaka Medical College Hospital in Dhaka, Bangladesh. These locations were selected because the infestation of house flies in these areas was very high which made the environment unhygienic.

House flies were collected several times from August, 2015 to March, 2016 from these study areas. The fly was collected by using sterile insect net and hand picking. For microbial investigation, house flies were collected using sterile screw capped jars and sterile hand gloves. Collected house flies were placed into the sterile tube individually inflicting minimal injury to them. The tubes were transferred to lab immediately after capturing and anesthetized by keeping them at 0ºC for 5 min. Identification was made by examining the fly inside test tube under a dissecting microscope and following standard taxonomic keys[2].

Bacteriological analysis of the house flies: Each house fly was suspended in 2 ml sterile normal saline (0.85%) and was thoroughly shaken for two min in the vortex machine. This wash was taken as external body homogenate sample[10]. After external body washing, the flies were soaked in 70% ethanol for five min to decontaminate their external surface and dried, followed by washing with sterile saline to remove traces of ethanol. The alimentary tract of flies was aseptically dissected out using autoclaved sterilized entomological dissecting needles under a dissecting microscope. The instrument was dipped in ethanol and flamed between dissections. The excised gut was homogenized in 5 ml of sterile normal saline water.

A total of 30 samples consisting of 15 external body surfaces and 15 gut homogenates were analyzed. All collected homogenates were cultured by using spread plate technique into Xylose Lysine Deoxycholate agar (XLD), Eosin Methylene Blue (EMB) and MacConkeyagar media, followed by incubation at 37ºC for 24 hrs. Growths on all plates were observed and the characteristics of the isolate colonies were noted. To obtain pure culture, isolated colonies were sub-cultured on nutrient agar (NA) media. The isolates were identified based on morphological features observed in Gram staining, cultural characteristics and biochemical tests (Kligler's iron agar test, indole production test, citrate utilization test, catalase test, oxidase test, motility assay and urease production test as recommended in Bergey's Manual of Bacteriology[19].

Analysis of antibiotic resistance pattern: Bacterial susceptibility to anti-microbial agent was determined *in vitro* by standardized agar-disc diffusion method known as the Kirby Bauer method using commercial antibiotic discs (Oxoid, UK). Penicillin G (PG 10), chloramphenicol (CRO 30), vancomycin (VA 30), amoxicillin (AML 10), antibiotics and disc potencies used were ampicillin (AMP 10), tetracycline 10 μg (TET 10), gentamicin 10 μg (CN 10), imipenem 10 μg (IPM 10) and ciprofloxacin 5 μg (CIP 5).

A suspension of each test organism was prepared and was diluted with normal saline to match the equivalent turbidity of 0.5 MacFarland standards. A sterile cotton swab was dipped into suspension and excess fluid was removed by pushing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then streaked evenly over the entire surface of a Muller-Hinton Agar (pH 7.3) plate to obtain uniform inoculum. Antibiotic discs were then applied aseptically to the surface of the inoculated plates with appropriate spatial arrangement by means of sterile forceps. The plates were then inverted and incubated at 37°C for 24 hrs. After incubation, the plates were examined and the diameters of the zones of complete inhibition were measured in millimeter. The zone diameter for individual anti-microbial and agents were translated into susceptible, intermediate and resistant categories by referring to an interpreting table.

Results and Discussion

A total of 15 house flies (*Musca domestica)* were collected and were recognized by their morphological characteristics. A total number of 50 bacterial isolates were obtained from 30 samples. The highest number of isolates 22 (44%) were collected from DMCH, 15 (30%) isolates were collected from Shamsunnahar Hall dining room and 13 (26%) isolates were from Rokeya Hall canteen.

Based on the biochemical tests and growth on selective agar medium, it was found that out of 50 isolates, 18 (36%) were *E. coli*, 9 (18%) were *Salmonella* spp., 11 (22%) were *Shigella* spp., 5 (10%) were *Pseudomonas* spp., 4 (8%) were *Klebsiella* spp. and 3 (6%) were *Enterobacter* spp. (Figs 1, 2).

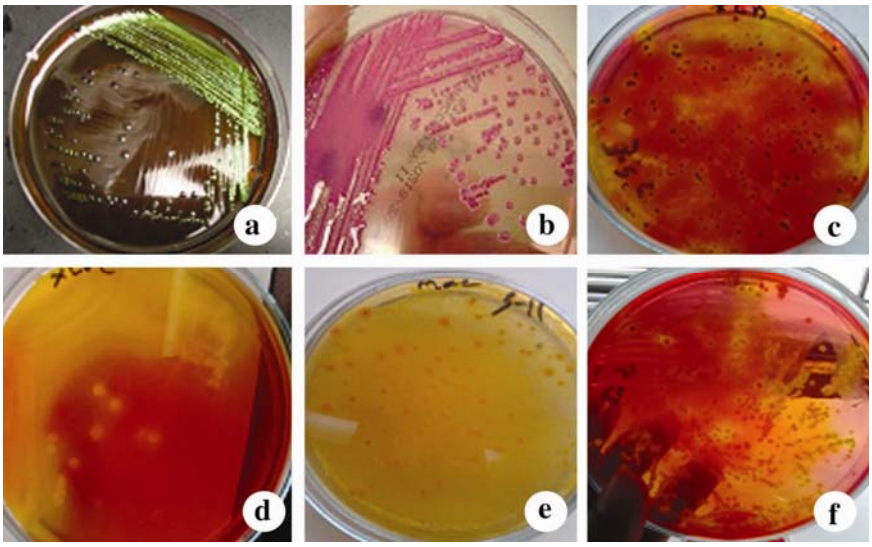

Fig. 1. Growth of different bacterial isolates on different selective growth medium. (a) *Escherichia coli* on EMB agar medium, (b) *Klebsiella* spp. on MacConkey agar medium, (c) *Salmoenlla* spp. on XLD agar medium, (d) *Shigella* spp. on XLD agar medium, (e) *Pseudomonas* spp. on MacConkey agar medium and (f) *Enterobacter* spp. on XLD agar medium.

Among the 15 isolates obtained from fly samples of Shamsunnahar hall dining room, the number of *E. coli* and *Shigella* spp. was 5 (33%) each. Distribution of *Salmonella* spp., *Enterobacter* spp. and *Klebsiella* spp. were 3 (20%), 1 (7%) and 1 (7%), respectively. Among the 22 isolates collected from DMCH, *E. coli* 8 (36%) was the most dominant bacteria which was followed by *Salmonella* spp. 4 (18%), *Pseudomonas* spp. 3 (14%), *Shigella* spp. 3 (14%). *Enterobacter* spp. 2 (9%) and *Klebsiella* spp. 2 (9%). In Rokeya Hall canteen also *E. coli* 5 (39%) was predominant. Other bacteria were collected from this area included *Shigella* spp. (23%), *Pseudomonas* spp. (15%), *Salmonella* spp. (15%) and *Klebsiella* spp. No *Enterobacter* spp. was found in this study area (Table 1).

The isolates from the external body surface were 32% *E. coli*, 24% *Salmonella* spp., 20% *Shigella* spp., 12% *Pseudomonas* spp., 4% *Enterobacter* spp. and 8% *Klebsiella* spp. From the isolates of gut there were 40% *E. coli*, 12% *Salmonella* spp., 24% *Shigella* spp., 8% *Pseudomonas* spp., 8% *Enterobacter* spp. and 8% *Klebsiella* spp. (Fig. 3).

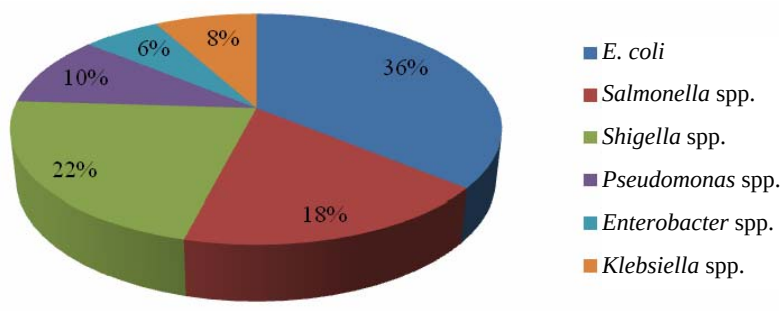

Fig. 2. Percentage of different bacterial isolates in the house fly samples.

Table 1. Distribution of bacterial isolates among the study area.

Isolates	SN Hall dining	Rokeya Hall	DMCH	Total
E. coli	5(33%)	5(39%)	8(36%)	18
Salmonella spp.	3(20%)	2(15%)	4(18%)	9
Shigella spp.	5(33%)	3(23%)	3(14%)	11
Pseudomonas spp.	0	2(15%)	3(14%)	5
Enterobacter spp.	1(7%)	0	2(9%)	3
Klebsiella spp.	1(7%)	1(8%)	2(9%)	4
Total	15(30%)	13(26%)	22(44%)	50

It was observed that house flies carry a variety of pathogenic bacteria and may play an important role in the transmission of diseases to humans and animals. Six Gram-negative bacteria *E. coli*, *Pseudomonas* spp., *Enterobacter* spp., *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. have been isolated from the alimentary tract and external body surface of the house flies. This result is consistent with other studies where the external organs (legs, wings and mouthparts) as well as intestinal tract of *M. domestica* have been reported to constitute a large source of bacteria[20,11]. These findings are also in agreement with the results in Ahwaz[21], which showed presence of *E. coli, P. aeruginosa* and *K. pneumonia* on the house fly collected from slaughterhouse and zoo.

All the isolates were resistant to all antibiotics tested, in a varying degree (Fig. 4). Among the 18 *E. coli* isolates 66-77% were resistant to chloramphenicol, ciprofloxacin and penicillin antibiotics. While 50% was found resistant to vancomycin, amoxocilin and tetracyclin.

Salmonella spp. isolates were 100% sensitive to chloramphenicol but it was highly (55.5%) resistant to penicillin, tetracycline, gentamycin and imipenem. Among 11 *Shigella* isolates, 72.72% were resistant to tetracycline and penicillin which are followed by 63.63% resistance to chloramphenicol and vancomycin. The isolates also showed resistance

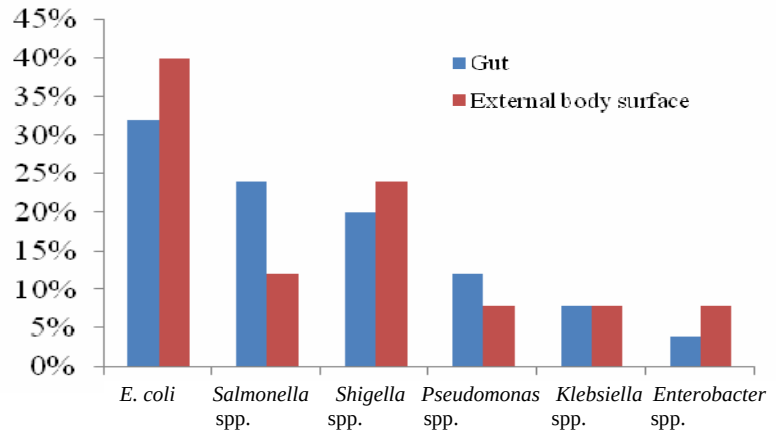

Fig. 3. Distribution of different isolates in external body surface and gut samples.

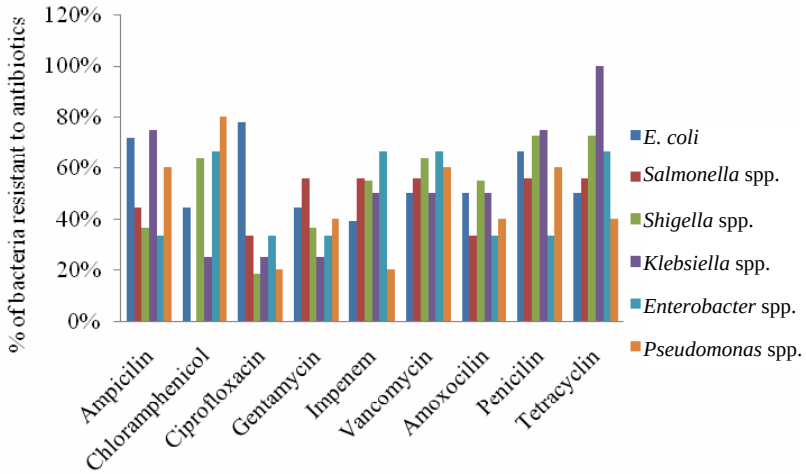

Fig. 4. Antibiotic resistance pattern of the bacterial isolates obtained from house flies.

pattern to other antibiotics. About 66% *Enterobacter* spp. isolates were resistant to chloramphenicol, imipenem, vancomycin and tetracycline. In case of *Pseudomonas*, 4 (80%) isolates were resistant to chloramphenicol and resistant to other antibiotics varied between 20 and 60%. Multi-drug resistance was also observed in the *Enterobacter* spp. isolates of which 3 (66.66%) were resistant to chloramphenicol, imipenem, vancomycin and tetracyclin. However, 100% *Klebsiella* isolates were resistant to tetracycline and resistance to other antibiotics was also observed in varying degrees.

All the isolates obtained from the house flies in this study are commonly associated with various diseases of human. To confirm the pathogenicity of these isolates, further investigation is necessary to detect presence of specific virulence genes. However, presence of the potential pathogenic bacteria from house flies in present study reinforces what has been known for a long time, that house fly posses a possible health risk to communities in proximity to population of flies. Good environmental sanitation practices and measures must be adopted to control house flies.

In the present study, the antibiotic resistance patterns of the bacteria isolated from house flies were also examined. It has been observed that all 50 isolates were resistant to commonly used antibiotics, including the third generation antibiotic imipenem, which is of particular concern. Multi-drug resistant *Escherichia coli*, *Salmonella*, *Klebsiella* spp. and *Pseudomonas* spp. were also isolated from house flies collected in hospitals and urban environments in Libya[22]. This study indicates that house flies collected from kitchen area of houses and the hospital area may be involved in the spread of drug resistant bacteria and may increase the potential for human exposure to drug resistant bacteria. This is really alarming as antibiotic resistance has become a serious public health problem in recent days, resulting in reduced effectiveness of antibiotics with greater mortality rates, prolonged hospitalization and increased health care costs.

In a country like Bangladesh where lack of sanitation and personal hygiene prevails among majority of the population, presence of multi-drug resistant pathogens like *E. coli*, *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Klebsiella* spp. and *Pseudomonas* spp. in house fly should be considered as a serious public health concern. This further emphasizes on regular monitoring of the sensitivity pattern of the pathogens transmitted by the house flies. Study at molecular level to identify the specific genes responsible for antibiotic resistance would provide an insight into the epidemiological profile of the drug-resistance as well as would also allow designing effective therapy and control measures against different diseases.

Conclusion

This study concludes that presence of house flies in human dwelling could be very dangerous, as they carry pathogenic, multi-drug resistant bacteria. The potential of house flies to transmit disease should not be ignored. Generating awareness among people about maintaining general sanitation and hygienic standards to control house fly is necessary. In sensitive places like hospitals and food processing establishments use of even pest control on regular basis might be implicated.

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