Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Entomology

ISSN 1812-5670
DOI: 10.3923/je.2016.141.147

Research Article
Isolation of Multidrug Resistant Pathogenic Bacteria from Common Flies in Dhaka, Bangladesh

[1]Md. Anowar Khasru Parvez, [1]Mahfuza Marzan, [1]Fahima Khatun, [1]Md. Firoz Ahmed, [2]Siraje Arif Mahmud and [3]Sabita Rezwana Rahman

[1]Department of Microbiology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
[2]Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
[3]Department of Microbiology, University of Dhaka, Ramna, Dhaka 1000, Bangladesh

Abstract

Background and Objective: Flies can be mechanical vectors of food borne enteric pathogenic bacteria. In Bangladesh, data on drug resistant fly associated bacteria is limited. Therefore, this study aimed to investigate the presence of drug resistant pathogenic bacteria in common flies found in Bangladesh. **Methodology:** Bacterial and fungal loads on the internal and external parts of common flies: *Musca domestica* and *Lucilia sericata* were enumerated through serial dilution and plating on nutrient agar and selective media. Biochemical tests were performed to identify bacteria, with further confirmation by 16S rRNA sequencing and BLAST search. Antibiogram of the isolated bacteria was performed by disk diffusion method. **Results:** The average count of bacteria from the fly external surface on the nutrient agar was 1.5×10^4 CFU mL^{-1} for *M. domestica* and 1.6×10^6 CFU mL^{-1} for *L. sericata*. On the other hand, the average count of bacteria from the fly internal parts was 6.5×10^5 CFU mL^{-1} for *M. domestica* and 1.1×10^5 CFU mL^{-1} for *L. sericata*. Number of fungal colonies associated with the internal parts of the house fly (*M. domestica*) was 3.3×10^4 CFU mL^{-1} and of the green bottle fly (*L. sericata*) was 4.3×10^4 CFU mL^{-1}. *Salmonella typhimurium*, *Citrobacter*, *E. coli*, *Providencia*, *Shigella*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were isolated from the external parts of both type of flies. From the internal parts of the flies *Serratia marcescens*, *Bacillus*, *Salmonella* and *Enterobacter* were identified. All the isolated organisms from external parts of fly body showed the highest antimicrobial resistance against amoxicillin (95%) followed by cefixime (80%), gentamicin (35%), ciprofloxacin (25%) and chloramphenicol (20%). **Conclusion:** High numbers of bacteria with multiple drug resistance phenotypes are associated with flies. Therefore, further study is needed to determine the range of pathogenic bacteria being spreaded by flies.

Key words: House fly, enteric bacteria, food borne disease, antibiotic resistance

Received: February 09, 2016 Accepted: May 20, 2016 Published: June 15, 2016

Citation: Md. Anowar Khasru Parvez, Mahfuza Marzan, Fahima Khatun, Md. Firoz Ahmed, Siraje Arif Mahmud and Sabita Rezwana Rahman, 2016. Isolation of multidrug resistant pathogenic bacteria from common flies in Dhaka, Bangladesh. J. Entomol., 13: 141-147.

Corresponding Author: Md. Anowar Khasru Parvez, Department of Microbiology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
Tel: +8801737540940

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food Borne Diseases (FBD) have become a public health concern due to the health and economical loss and under-developed countries suffer much than the developed countries[1]. The major cause for food borne diseases is either infection with contaminating pathogens or intoxication with biological or chemical components, where the pathogen or the chemical is ingested with foods. Contamination of foods can occur at any stage of food processing, preparation and delivery due to lack of good hygiene practice. Other environmental sources, such as air and water can deliver pathogenic microbes to foods. Flies can play a role of mechanical vector to deliver the pathogens to foods or food utensils[2] as they are available where organic matters, such as animal carcasses, vegetables wastes or leftover foods are abundant. They are known to transmit some very serious food borne diseases, such as typhoid fever, cholera, protozoan and helminths related diseases[3-5]. In fact several interventional studies showed that the decrease in the density of fly, especially house fly (*M. domestica*) can be accompanied by reduced incidence of diarrhea, dysentery, culture confirmed shigellosis and other food borne diseases[6-9]. Not only bacteria and protozoa, flies have also been reported to carry fungi, such as Mucorales, yeast, *Aspergillus niger* and *Penicillium*, *Fusarium* etc., through their external parts[10,11].

Flies are very common insects in Bangladesh, as the tropical weather permits, so they can be one of the major contributors of food borne diseases here. In Bangladesh, one study showed the association of house fly (*M. domestica*) with the shigellosis disease, where the number of shigellosis infected patients increased with the rise in the fly number, indicating fly can play role as a mechanical vector of *Shigella*[12]. Another study in the city of Dhaka, Bangladesh, has isolated common pathogenic bacteria from flies, such as *E. coli*, *Staphylococcus aureus* and *Vibrio cholerae* etc.[13]. In addition, only a couple of studies have been conducted in Bangladesh on bacteria associated with gut microbiota of fruit flies[14,15]. Both of these investigators identified several bacterial genera belonging to the family of Enterobacteriaceae, some of which might be potential pathogens. However, till now, there is no data available on the drug resistance pattern of the fly associated microorganisms in Bangladesh. Therefore, it is aimed to provide some baseline data regarding the enteric food borne pathogenic bacteria associated with common flies in Dhaka city, Bangladesh and it is also aimed to investigate whether these flies have the potential to carry drug resistant bacteria with them.

MATERIALS AND METHODS

Collection of flies and specimen preparation: The study was conducted at the Jahangirnagar University campus situated at, Savar, Dhaka, Bangladesh. The total area of the campus is 697.56 acre or 282.51 ha. Thirteen student dormitories accommodate about 15000 students and a major portion of them consumes meals from the canteens of these dorms and adjacent food stalls. It is aimed to collect flies from 3 dormitory canteens and 3 food stalls as they are the most crowded canteens all the year round and are not adjacent to each other. The target sites were kitchen areas after/during the preparation of cooking, when there is the highest availability of the waste product. A total of 45 flies were collected during the month of April-June, 2014 from these 6 sites, when flies are abundant. Sterile insect collection net without any bait was used to collect the flies. They were transferred to sterile vials aseptically and kept in freezing temperature for couple of hours to anaesthetize[16]. The flies were morphologically identified according to Ahmed *et al.*[17].

Isolation of microorganisms from flies: Each fly was suspended in 2 mL sterile saline and vortexed gently for preparing the body wash (external) of the fly[16]. This saline suspension was then diluted upto 5 times and 100 µL of suspension from each dilution tube was inoculated on the different kinds of media, such as nutrient agar (Himedia), MacConkey agar (Sigma) and mannitol salt agar (Oxoid) through spread plate method. For the detection of fungal colonies diluted specimens were spread on Potato Dextrose Agar (PDA) media (Oxoid) supplemented with tetracycline. To isolate microorganisms from the internal parts of the body, external surface of the fly was first washed with diluted alcohol and then with sterile distilled water. After that each fly was crashed in the saline solution with sterile tips. Suspension was then vortexed, diluted and plated as previous. To culture the bacterial and fungal colonies, plates were incubated at 37 and 25°C, respectively for 24 h and then observed. Isolates were preserved in 15% glycerol at -20°C.

Enumeration and identification of isolates: After growth of the microorganisms on different plates, colonies were enumerated and colony forming unit was interpreted according to the dilution factor of the inoculated samples. Isolated colonies in higher dilution were observed on each type of plates. Bacterial colonies with different characteristics were selected from different media for the isolation of representative organisms. The isolates were picked from selective media, such as MacConKey and mannitol-salt agar in

order to identify pathogens. These colonies were then subcultured in nutrient agar plates for pure colony isolation and subsequently Gram stained. From the pure culture, Gram positive and Gram negative bacterial isolates were then presumptively identified through a series of conventional morphological, cultural and biochemical tests according to the criteria described in Bergey's Manual of Determinative Bacteriology[18].

Antimicrobial susceptibility testing of the isolated pathogens: To identify the resistance and susceptibility pattern of the isolated microorganisms, antibiotic sensitivity test was performed with selected antibiotics. Single disc diffusion method[19] was performed for this purpose. Standard antibiotic discs (Oxoid, UK) of amoxicillin (10 µg), cefixime (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg) and gentamycin (10 µg) were used. Each zone size was interpreted with reference to the CLSI standards. The *E. coli* ATCC 25922 and *S. aureus* ATCC 292913 were used as controls to check the quality of the drugs. In literal terms, Multiple Drug Resistance (MDR) means 'Resistant to more than one antimicrobial agent', but the majority define MDR as 'Resistant to three or more antimicrobial classes[20]'. Therefore, an isolate was defined as MDR if it was resistant to three or more antibiotics.

Plasmid profiling: Plasmids of the identified bacteria from flies were extracted by conventional alkaline lysis method[21]. In short, cells from an overnight grown liquid culture were collected by spinning at 6000 rpm for 5 min and then suspended into 100 µL of solution I. Two hundred microlitre of solution II was added for alkaline denaturation and kept in ice for 10 min. Then 150 µL of neutralizing solution III was added and again kept in ice for 5 min. After centrifugation at 13000 rpm for 7 min, the supernatant was collected and the plasmid was precipitated by double volume of ice cold ethanol followed by centrifugation at 13000 rpm for 10 min. The pellet was washed with 70% cold ethanol. After drying, the plasmid was dissolved in TE buffer. Plasmid DNA was separated by horizontal agarose gel electrophoresis in 0.8% agarose slab gel in tris-acetate EDTA (TAE) buffer at room temperature using 50 V for 3 h and then visualized with UV-transilluminator for detection of plasmid bands.

Sequencing of 16S rRNA gene: In order to confirm the biochemical test results for bacterial identification, 4 bacterial isolates were selected and their 16S rRNA were sequenced. The genomic DNA was extracted and purified using DNA extraction kit (Promega, USA). The 16S rDNA was amplified by PCR using universal primer pair for prokaryotes: 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GAAAGGAGGTGATCCAGCC-3')[22]. The PCR products were purified with the PCR Clean-Up System kit (Promega, USA) and sequenced on a sequencer (ABI 3700 PE sequencer, Applied Biosystem, USA). Forward and reverse sequences were assembled using DNASTAR Lasergene SeqMan software and compared to the GenBank database of the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST).

RESULTS

Collection and identification of flies and heterotrophic count of microorganisms: The microbial counts obtained from body wash and body crash are listed in Table 1. The average count of bacteria from the fly external surface on the nutrient agar was 1.5×10^4 CFU mL^{-1} for house fly and 1.6×10^6 CFU mL^{-1} for green bottle fly. On the other hand, the average count of bacteria from the fly internal parts was 6.5×10^5 CFU mL^{-1} for house fly and 1.1×10^5 CFU mL^{-1} for green bottle fly. Number of fungal colonies associated with the external parts of the house fly was 1.0×10^4 CFU mL^{-1} on an average and of green bottle fly was 2.6×10^5 CFU mL^{-1} on an average. In MacConkey agar plates, the bacterial counts from internal parts of both flies were higher than those from the external surfaces. Some colonies on the MacConkey agar media showed lactose fermentation and the others were non lactose fermenter. In the MSA, the growth of staphylococci was observed in both fly samples. Number of fungal colonies associated with the internal parts of the house fly (*M. domestica*) was 3.3×10^4 CFU mL^{-1} and of the green bottle fly (*L. sericata*) was 4.3×10^4 CFU mL^{-1}. Three different colonies on Potato Dextrose Agar (PDA) media, were yeasts as identified by microscopy. Three colony characteristics were found C1-orange-red colored, C2-white colored, rough colony surface and C3-cream colored smooth colony surface, all were round and convex. Type C1 and C2 were isolated from *M. domestica* and type C2 and C3 were isolated from *L. sericata*. The C1 was found to be *Rhodotorula*. Other 2 yeast types are yet to be determined.

Biochemical characterization and presumptive identification of the isolated bacterial pathogens: After performing various morphological, cultural and biochemical tests, 20 bacteria were identified from the external parts of the flies and 11 bacteria from the internal parts of the flies. The results of identification tests for isolates from fly body wash are summarized in Table 2. From the external body surface of

Table 1: Average count of microorganisms on *Musca dosmestica* (house fly) and *Lucilia sericata* (green bottle fly) external surface and internal body parts on different types of media

	Average count				
	External surface		Internal parts		
Types of media	*Musca domestica*	*Lucilia sericata*	*Musca domestica*	*Lucilia sericata*	Colony characteristics
Nutrient agar	1.5×10^4	1.6×10^6	6.5×10^5	1.1×10^5	Whitish colony, moist, glistening, red colony, round and convex
MacConkey agar	2.0×10^3	8.0×10^3	4.0×10^4	4.5×10^4	Pink to whitish, round and slightly raised to flat
Mannitol salt agar	1.5×10^5	1.0×10^3	4.0×10^5	2.0×10^3	Small, off white to transparent, round and convex
Potato dextrose agar	1.0×10^4	2.6×10^5	3.3×10^4	4.3×10^4	Orange, white and round to convex

Table 2: Morphological and biochemical tests for presumptive identification of 20 bacterial isolates from fly body wash

			Biochemical tests								
Isolates	Growth media	Gram reaction	Indole	Methyl Red (MR)	Voges-proskauer (VP)	Citrate	Urease	Catalase	Oxidase	KIA	Presumptive identification
A	MacConkey	- rod	+	+	-	-	-	+	-	Acid butt, Alk slant, gas^+	*Escherichia coli*
B	MacConkey	- rod	-	-	-	+	-	+	-	Alk or no change butt, Alk slant, gas^+	*Providencia* sp.
C	MacConkey	- rod	-	-	-	-	-	+	-	Alk or no change butt, Alk slant, gas^+	*Alkaligenes faecalis*
D	MacConkey	- rod	-	+	-	-	-	+	-	Acid butt, Alk slant, H_2S^+	*Citrobacter* sp.
E	MacConkey	- rod	-	-	-	+	-	+	-	Acid and raised butt, Alk slant, H_2S^+	*Citrobacter* sp.
F	MacConkey	- rod	-	-	-	+	-	+	-	Acid and creak in butt, Alk slant, gas^+	*Providencia* sp.
G	MacConkey	- rod	+	+	-	-	-	+	-	Acid butt, Alk slant, gas^+	*Escherichia coli*
H	MacConkey	+ rod	-	-	-	-	-	+	-	Acid butt, acid slant, gas^+	*Bacillus cereus*
I	MacConkey	- rod	+	-	-	+	-	+	-	Acid butt, Alk slant, gas^+	*Salmonella typhimurium*
J	MSA	- rod	-	-	-	+	-	+	-	Alk or no change butt, Alk slant, gas^+	*Citrobacter* sp.
K	MSA	- rod	-	-	-	+	-	+	-	Acid butt, Alk slant, H_2S^+	*Citrobacter* sp.
L	MSA	+ cocci	-	+	-	-	-	+	-	Alk slant, Alk butt, H_2S^-, gas^+	*Staphylococcus aureus*
M	SS	+ cocci	-	+	-	+	-	+	-	Alk slant, Alk butt, H_2S^-, gas^+	*Staphylococcus aureus*
N	SS	- rod	-	+	-	-	-	+	-	Acid butt, Alk slant, H_2S^+	*Shigella* sp.
O	SS	- rod	-	+	-	+	-	+	-	Acid butt, Alk slant, H_2S^+	*Salmonella typhimurium*
P	SS	- rod	+	+	-	-	-	+	-	Acid butt, Alk slant, H_2S^+, gas^+	*Klebsiella pneumoniae*
Q	SS	- rod	-	+	-	-	-	+	-	Acid butt, Alk slant, H_2S^+	*Shigella* sp.
R	SS	- rod	-	-	-	+	-	+	-	Acid butt, Alk slant, H_2S^+	*Salmonella typhimurium*
S	SS	- rod	-	+	-	+	-	+	-	Acid butt, Alk slant, gas^+	*Salmonella typhimurium*
T	SS	- rod	-	+	-	+	-	+	-	Acid butt, Alk slant, H_2S^+	*Salmonella typhimurium*

MSA: Mannitol salt agar, SS: *Salmonella shigella* agar

Table 3: 16S rRNA sequence based identification of selected isolates using BLAST

Isolates	Matching strain description	Maximum identity (%)	Accession No.	Match with biochemical test result
B	*Providencia rettgeri* strain ALK310 16S rRNA gene, partial sequence	99	KC456526.1	Yes
F	*Providencia rettgeri* strain NRC109 16S rRNA gene, partial sequence	100	KP244262.1	Yes
P	*Klebsiella pneumoniae* strain XH209, complete genome	99	CP009114.1	Yes
K	*Citrobacter freundii* strain AAKNVR10 16S rRNA gene, partial sequence	100	HQ324431.1	Yes

the flies we identified: *Salmonella typhimurium* (n = 5), *Citrobacter* (n = 4), *E. coli* (n = 2), *Providencia* (n = 2), *Shigella* (n = 2), *S. aureus* (n = 2), *Bacillus cereus* (n = 1), *Klebsiella pneumoniae* (n = 1) and *Alkaligenes faecalis* (n = 1). From the internal parts of the flies, *Serratia marcescens* (n = 4), *Bacillus* (n = 4), *Salmonella* (n = 2) and *Enterobacter* (n = 1) were identified.

16S rRNA sequencing for confirmation of genus identification: Representative isolates were selected for 16S rRNA sequencing in order to confirm the biochemical identification results. For this, bacterial 16S rDNA was partially sequenced, each representing around 1.4 kb. The selected isolates were B, F, P and K as shown in Table 2. The results of sequencing and BLAST are shown in Table 3. After BLAST search, isolates B and F showed maximum identity of 99 and 100%, respectively, with *Providencia rettgeri*, isolate P, with *Klebsiella pneumoniae* (99%) and isolate K with *Citrobacter freundii* (100%). This result was consistent with the biochemical test results. Therefore, biochemical identification of the isolates could be validated as correct.

Antimicrobial susceptibility and multidrug resistant pattern of bacterial isolates: The antimicrobial susceptibility tests of the 20 isolates from fly surface were performed against 5 antibiotics. The sensitivity, resistance and intermediate

Table 4: Multidrug resistant isolates and their resistant patterns

Bacteria	Resistant pattern
Providencia spp., *Citrobacter* spp. and *Staphylococcus aureus*	Amoxicillin, cefixime and gentamicin
E. coli and *Shigella* spp.	Amoxicillin, cefixime and ciprofloxacine
Salmonella typhimurium and *Klebsiella pneumoniae*	Amoxicillin, cefixime, ciprofloxacine and chloramphenicol
Salmonella typhimurium	Amoxicillin, cefixime, gentamicin and chloramphenicol
Shigella spp.	Amoxicillin, ciprofloxacine and chloramphenicol

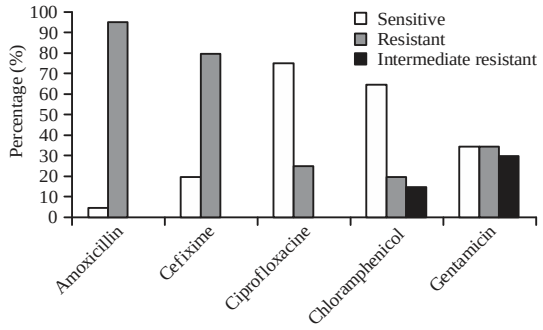

Fig. 1: Sensitivity pattern of antibiotic resistant bacteria isolated from flies

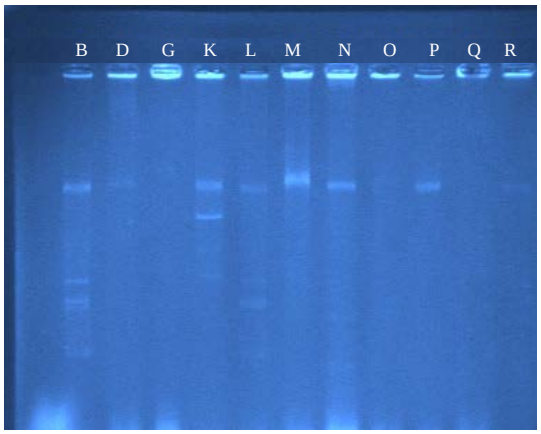

Fig. 2: Plasmid profiling of the resistant bacteria. Lane B: Providencia sp., lane K: *Citrobacter* sp., lane L: *Staphylococcus aureus* and lane N: *Shigella* sp. Lane B shows 4 plasmid bands, lane K and L shows 3 plasmid bands and lane N shows 2 plasmid bands

resistance pattern of the isolates against test antibiotics are shown in the Fig. 1. Among the 20 isolates, amoxillin was least effective, as 95% (n = 19) of the isolates were resistant to it. This was followed by cefixime containing 80% (n = 16) resistant strain. The most effective antibiotic was found to be ciprofloxacin as more isolates (80%, n = 16) were sensitive to this antimicrobial as compared to others. Intermediate resistance strains were found only against chloramphenicol and gentamicin. Total 11 of 20 isolates (55%) were resistant to three different groups of antimicrobial classes and may be called as MDR strain (Table 4). *Salmonella typhimurium* isolates were the most dangerous MDR strains showing resistance to 5 antibiotics. This was followed by *K. pnuemoniae* and *Shigella* isolates showing resistance to four antibiotics. Other MDR isolates were *Providencia*, *Citrobacter*, *S. aureus* and *E. coli*.

Plasmid profiling of MDR bacterial isolates: Plasmids were isolated from bacteria by alkaline lysis method and visualized by agarose gel electrophoresis. Out of 11 MDR bacterial isolates, multiple plasmid bands were found in *Providencia* (Lane B), *Citrobacter* (Lane K), *S. aureus* (Lane L) and *Shigella* (Lane N) (Fig. 2). *Providencia* (Lane B) shows to contain 4 plasmid bands, *Citrobacter* and *S. aureus* (Lane K and L) both have 3 plasmid bands and *Shigella* (Lane N) shows two plasmid bands. *Providencia* and *S. aureus* were isolated from housefly (*M. domestica*), while *Citrobacter* and *Shigella* were isolated from green bottle fly (*L. sericata*) and this result indicates that the MDR isolates could be carrying resistance genes in their mobile genetic elements, such as plasmids and are capable of horizontal gene transfer into non-resistance strains.

DISCUSSION

It is found that high numbers of Gram negative enteric pathogenic bacteria are carried with the external parts of house fly (*M. domestica*) and green bottle fly (*L. sericata*) as well as with their internal body parts. Gram positive bacteria and fungus were also isolated from both external and internal parts. High bacterial load was found on both types of flies in differential media also. However, we could not find any noteworthy difference between the average count of fly's external and internal body specimens, but the range of microbial load on external parts of the flies were wider than the internal parts.

Thirty one bacterial isolates were identified from external and internal parts of the flies of, which some were potential enteric pathogenic bacteria such as *S. typhimurium*, *Shigella* and *K. pneumoniae*. Other enteric bacteria which can play role as opportunistic pathogens were also identified, such as *S. marcescens*, *Providencia* and *Enterobacter*. *Salmonella*

were isolated from both internal parts and external surface specimen of flies, whereas *S. marcescens* was isolated only from the internal parts and may be a normal flora of fly gut. In different studies, flies were found to carry mostly Gram negative enteric pathogenic bacteria, including *E. coli*, *Salmonella*, *S. typhi*, *Shigella*, *Pseudomonas aeruginosa*, *Proteus*, *Acinetobacter*, *Serratia* and *Enterococcus*[23-25]. Similar types of Gram negative bacteria were also isolated in the present study, whereas flies were also found to carry, both internally and externally, *S. aureus*, a Gram-positive bacterium capable of both infection and intoxication. This finding also supports previous report indicating that fly can carry infectious dose of *S. aureus* in their gut[26].

The bacteria associated with fly surface could be more dangerous in spreading disease than those present inside. Therefore, the antibiotic sensitivity test was done only for the isolates from external body parts, not for the bacteria isolates from internal body parts. The antibiotic resistant bacteria was detected from the flies, showed highest resistance (95%) against amoxicillin and highest sensitivity (75%) against ciprofloxacin. About 55% of the bacterial isolates were multidrug resistant, which is a matter of concern. Intermediate resistance against chloramphenicol and gentamicin is an indicator that these pathogens are on the way of acquiring resistance against these drugs. Currently there is no data on the antimicrobial resistance bacteria associated with flies in Bangladesh. However, a study conducted in Iran, found antimicrobial resistant bacteria associated with flies with above 32.5% resistance against cephalexin, chloramphenicol, ampicillin and tetracycline[27]. In another study conducted in China, multidrug resistant *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Aeromonas hydrophila* were identified[28] and drug resistant enterococci and staphylococci were identified in flies collected near confined poultry feeding operations[29]. Wei *et al.*[30] demonstrated that both antibiotic resistant and sensitive bacteria can persist in the intestinal gut of house fly and green bottle flies. Animal carcasses, garbage, hospital waste or even sewage samples are potential source of resistant bacteria. Therefore, flies that are exposed to these sources, can easily pick up antibiotic resistant bacteria and disseminate them. Recently, Ahmed *et al.*[31] have reported presence of MDR bacteria in adjacent poultry firms and veterinary hospital area. Therefore, the flies have the potential to carry drug resistant bacteria through their external surface. Though we have found plasmids in *Providencia*, *Citrobacter*, *S. aureus* and *Shigella*, this could not tell us about the putative genes associated with plasmids, nor showed, which genes are responsible for their drug resistance mechanism. Currently this is a limitation of this study. Therefore, further study is required to investigate whether those plasmids carry the resistance genes. The 16S rDNA sequencing was also used to confirm the identification of MDR isolates in order to study them further.

CONCLUSION

It can be concluded that high number of bacteria and some of clinical interest are associated with flies. Hence, regular surveillance is necessary to determine the range of pathogenic bacteria that can be carried out with flies. To limit the access of fly in the kitchen, net should be used in the window. Proper disposal of the animal carcasses and other kitchen garbage are highly recommended. Good hygiene practice should be implemented in the food processing zone to keep the contact of flies with the foods as minimal as possible.

SIGNIFICANT STATEMENTS

Flies are one of the major contributors of food borne diseases in Bangladesh. Due to indiscriminate use of antibiotics, pathogens carried by these vectors are also becoming resistant to commonly used antibiotics. Although, few studies have been conducted locally on the pathogenic bacteria associated with house fly, there is no data available on the drug resistance pattern of the fly associated microorganisms in Bangladesh. Therefore, it is aimed to investigate whether fly associated pathogen have the potential to carry drug resistant bacteria. For the 1st time, it is reported that the occurrence of drug resistant pathogenic bacteria associated with flies in Dhaka, Bangladesh. It is concluded that, this high number of drug resistant pathogenic bacteria could possess a major threat to public health.

REFERENCES

1. Fratamico, P.M., A.K. Bhunia and J.L. Smith, 2005. Foodborne Pathogens: Microbiology and Molecular Biology. Caister Academic Press, Wymondham, Norfolk, UK., Pages: 273.
2. Zurek, L. and J.R. Gorham, 2008. Insects as Vectors of Foodborne Pathogens. In: Wiley Handbook of Science and Technology for Homeland Security, Voeller, J.G. (Eds.). Wiley Inc., Hoboken, NJ., ISBN: 9780471761303, pp: 1683-1695.
3. Anderson, J.F., 1909. The differentiation of outbreaks of typhoid fever due to water, milk, flies and contact. Am. J. Public Hygiene, 19: 251-259.

4. Graczyk, T.K., R. Knight and L. Tamang, 2005. Mechanical transmission of human protozoan parasites by insects. Clin. Microbiol. Rev., 18: 128-132.
5. Fotedar, R., 2001. Vector potential of houseflies (*Musca domestica*) in the transmission of *Vibrio cholerae* in India. Acta Tropica, 78: 31-34.
6. Watt, J. and D.R. Lindsay, 1948. Diarrheal disease control studies; effect of fly control in a high morbidity area. Public Health Rep., 63: 1319-1333.
7. Lindsay, D.R., W.H. Stewart and J. Watt, 1953. Effect of fly control on diarrheal disease in an area of moderate morbidity. Public Health Rep., 68: 361-367.
8. Cohen, D., M. Green, C. Block, R. Slepon, R. Ambar, S.S. Wasserman and M.M. Levine, 1991. Reduction of transmission of shigellosis by control of houseflies (*Musca domestica*). Lancent, 337: 993-997.
9. Chavasse, D.C., R.P. Shier, O.A. Murphy, S.R. Huttly, S.N. Cousens and T. Akhtar, 1999. Impact of fly control on childhood diarrhoea in Pakistan: Community-randomised trial. Lancet, 353: 22-25.
10. Srivoramas, T., T. Chaiwong and M.R. Sanford, 2012. Isolation of fungi from adult house fly; *Musca domestica* and the blow fly *Chrysomya megacephala* in Ubon Ratchathani province, Northeastern Thailand. Int. J. Parasitol. Res., 4: 53-56.
11. Zarrin, M., B. Vazirianzadeh, S.S. Solary, A.Z. Mahmoudabadi and M. Rahdar, 2007. Isolation of fungi from housefly (*Musca domestica*) in Ahwaz, Iran. Pak. J. Med. Sci., 23: 917-919.
12. Farag, T.H., A.S. Faruque, Y. Wu, S.K. Das and A. Hossain *et al*., 2013. Housefly population density correlates with shigellosis among children in Mirzapur, Bangladesh: A time series analysis. PLoS Negl. Trop. Dis., Vol. 7. 10.1371/journal.pntd.0002280
13. Khan, A.R. and F. Huq, 1978. Disease agents carried by flies in Dacca city. Bangladesh Med. Res. Council Bull., 4: 86-93.
14. Khan, M., A.A. Mahin, M.K. Pramanik and H. Akter, 2014. Identification of gut bacterial community and their effect on the fecundity of pumpkin fly, *Bactrocera tau* (Walker). J. Entomol., 11: 68-77.
15. Pramanik, M.K., A. Al-Mahin, M. Khan and A.B. Miah, 2014. Isolation and identification of mid-gut bacterial community of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Res. J. Microbiol., 9: 278-286.
16. Vazirianzadeh, B., S.S. Solary, M. Rahdar, R. Hajhossien and M. Mehdinejad, 2008. Identification of bacteria which possible transmitted by *Musca domestica* (Diptera: Muscidae) in the region of Ahvaz, SW Iran. Jundishapur J. Microbiol., 1: 28-31.
17. Ahmed, Z.U., Z.N.T. Begum, M.A. Hassan, M. Khondeker and S.M.H. Kabir, 2008. Encyclopedia of Flora and Fauna of Bangladesh, Volume 21: Arthropoda: Insecta III (*Neuroptera*, *Mecoptera*, *Lepidoptera*, *Trichoptera*, *Deptera*, *Siphonoptera*). Asiatic Society of Bangladesh, Dhaka.
18. Hensyl, W.R., 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, Baltimore, pp: 179-209.
19. CLSI., 2014. Performance standards for antimicrobial susceptibility testing: Twenty-fourth informational supplement. Document No. M100-S24, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA., USA., January 2014.
20. Magiorakos, A.P., A. Srinivasan, R.B. Carey, Y. Carmeli and M.E. Falagas *et al*., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect., 18: 268-281.
21. Birnboim, H.C. and J. Doly, 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res., 7: 1513-1523.
22. Lane, D.J., 1991. 16S/23S rRNA Sequencing. In: Nucleic Acid Techniques in Bacterial Systematic, Stackebrandt, E. and M. Goodfellow (Eds.). John Wiley and Sons, New York, USA., ISBN-13: 9780471929062, pp: 115-175.
23. Chaiwong, T., T. Srivoramas, P. Sueabsamran, K. Sukontason, M.R. Sanford and K.L. Sukontason, 2014. The blow fly, *Chrysomya megacephala* and the house fly, *Musca domestica*, as mechanical vectors of pathogenic bacteria in Northeast Thailand. Trop. Biomed, 31: 336-346.
24. Nazni, W.A., B. Seleena, H.L. Lee, J. Jeffery, T.T.A. Rogayah and M.A. Sofian, 2005. Bacteria fauna from the house fly, *Musca domestica* (L.). Trop. Biomed., 22: 225-231.
25. Sulaiman, S., M.Z. Othman and A.H. Aziz, 2000. Isolations of enteric pathogens from synanthropic flies trapped in downtown Kuala Lumpur. J. Vector Ecol., 25: 90-93.
26. Nayduch, D., H. Cho and C. Joyner, 2013. *Staphylococcus aureus* in the house fly: Temporospatial fate of bacteria and expression of the antimicrobial peptide defensin. J. Med. Entomol., 50: 171-178.
27. Davari, B., E. Kalantar, A. Zahirnia and S.H. Moosa-Kazemi, 2010. Frequency of resistance and susceptible bacteria isolated from houseflies. Iran. J. Arthropod-Borne Dis., 4: 50-55.
28. Liu, Y., Y. Yang, F. Zhao, X. Fan, W. Zhong, D. Qiao and Y. Cao, 2013. Multi-drug resistant gram-negative enteric bacteria isolated from flies at Chengdu airport, China. Southeast Asian J. Trop. Med. Public Health, 44: 988-996.
29. Graham, J.P., L.B. Price, S.L. Evans, T.K. Graczyk and E.K. Silbergeld, 2009. Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. Sci. Total Environ., 407: 2701-2710.
30. Wei, T., K. Miyanaga and Y. Tanji, 2014. Persistence of antibiotic-resistant and -sensitive *Proteus mirabilis* strains in the digestive tract of the housefly (*Musca domestica*) and green bottle flies (*Calliphoridae*). Applied Microbiol. Biotechnol., 98: 8357-8366.
31. Ahmed, M.Y., S. Islam, M.R. Amin, F. Shamma, S.A. Mahmud and N. Adnan, 2013. Dissemination of MDR bacteria from poultry litters and veterinary wastes in Savar, Bangladesh. Jahangirnagar Univ. J. Biol. Sci., 2: 93-101.