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Molecular characterization of multidrug-resistant bacteria isolated from the external and internal parts of the housefly

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ABSTRACT

House flies are mechanical vectors of food borne enteric pathogenic bacteria which may transfer isolates to human and produce diseases. In Bangladesh, there is very limited data on molecular characterization of drug-resistant bacteria from isolated house flies. The research was conducted to determine the pathogenic bacteria isolated from houseflies and their antibiogram. A total of 140 houseflies were randomly collected for microbiological analysis. A group of cultural tests, biochemical tests were used to isolation and identification of isolates and further confirmed through molecular characterization by the presence of 16S r*RNA* gene E1, E2 and *invA*. Additionally, 14 commercially available antibiotics were used by karby-bauer disk diffusion technique for antibiogram study. Results showed that the most isolated bacteria from houseflies' external surfaces were *Escherichia coli* 19.04%, *Salmonella typhimurium* 15.87%, and *Pseudomonas* spp. 7.93% from 63 isolates, while 42 isolates found 35.71%, 28.57%, and 14.28% respectively. PCR amplification bands of *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas* spp. were 584bp, 284bp, and 1497bp, respectively. Almost all of the isolates were highly resistant to erythromycin, gentamycin, bacitracin (100%), followed by kanamycin, methicillin (80%) whereas highly sensitive to ciprofloxacin, chloramphenicol, azithromycin (100%), followed by tetracycline, amoxicillin (85.71%). These pathogenic microorganisms at distinct sampling sites indicate that house flies may transmit vector-borne pathogens to humans. Based on these findings, we recommend vector-borne disease-fighting medications and a sustainable house fly-control approach. We also suggest promoting hygiene and food safety protocol to distance food ingredient from flies in local markets.

INTRODUCTION

The familiar house fly (*Musca domestica*) is a member of the filth fly family, which poses significant public health risks as a possible bearing of pathogens. They live in intimacy with bacteria and other microorganisms. House flies are a very powerful mechanical vector for bacteria, both biologically and etiologically [1]. Due to public health, they have been processed to be probable vectors of more than 100 severe pathogens ranging from virus-bacteria, protozoans, and nematodes [2]. It is currently demonstrated that certain microorganisms can survive inside and on the house flies body surface from 5 - 6 hours up to 35 days [3]. The house flies transmit pathogens through direct contact with surfaces. Due to the viscosity of feces, pathogens or foreign particulates are better able to adhere to the body of a fly [4]. Houseflies can spread infections from broiler fields, hospitals, public parks, garbage/dumping sites, slaughterhouses, and homes [4, 5]. Several dangerous foods borne disease, including typhoid fever, cholera, protozoan and helminthes-related illness believed to be transmitted by houseflies [6]. Food borne diseases have become a global health issue in consequence of health and financial outcomes, with developing countries suffering much more than developed countries. In

reality, many intervention studies have shown that a reduction in fly density, especially of the house flies, is associated with a lower occurrence of dysentery, diarrhea, shigellosis, and supplemental food borne illness [7]. In another study, typical pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus,* and *Vibrio cholerae* were isolated from flies in Dhaka, Bangladesh [8]. Furthermore, only a few experiments on bacterial isolates related with the gut micro biota of fruits flies have been performed in Bangladesh [9].

House flies were also reported to be involved in several disease outbreaks including *E. coli* O157:H7 in Japan and *Vibrio cholerae* in India [4]. The antibiotic resistance of these vector borne diseases poses an additional challenge to the therapeutics of those outbreaks [10, 11]. The contribution of vectors like house flies in the spread of MDR bacteria has not been investigated. However, the widespread prevalence of MDR bacteria in food, livestock, animal-based food products, fresh plants, environmental samples (water, air, soil etc.) as well as farmers [12] suggests an underlying catalyst like arthropod vectors that may actively be spreading these bacteria in a wide range of environments. Our approach was to collect house fly's samples from various locations such as, fish market, chicken market, roadside hotel, and home kitchen. From these flies we isolated *E. coli, Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Bacillus* spp., *Staphylococcus* spp., *Aspergillus* spp., and *Fusarium* spp. from those flies by growing them in selective media. We confirmed those bacteria through molecular characterization by utilizing 16E1 and 16E2 primers for *E. coli*, S139 and S141 for *Salmonella* spp. and Ker P (F) and Ker P(R) primers for *Pseudomonas* spp. Then we evaluated resistance of those bacteria isolated from house flies against commonly used antibiotics. Unfortunately, there is no preceding investigation carried in Bangladesh for isolation and molecular identification of bacteria transmitted by house flies. Therefore, the objective of our research was to isolation and molecular characterization of external and internal isolates of house flies, and the evaluation of their antibiotics resistance profile to unveil the contribution of vectors in the spread of MDR bacterial population.

MATERIALS AND METHODS

Study design, location, collection of houseflies and processing

The research was conducted for a period of one year from June-2019 to July-2020. The house flies were caught from different places (fish market, chicken market, roadside hotel, and home kitchen) in Dinajpur city and transferred to the Bacteriological laboratory of Hajee Mohammad Danesh Science and Technology University for microbiological analysis. A total of 140 flies were caught from the selected sites (four fish markets, four chicken markets, four roadside hotels and three home kitchens) during the (June-2019- July-2020) with sterile nylon net from 9:00 am to 1:00 pm when the flies were active. About 9-10 flies were collected from each location (total 15 locations). The collected flies were transferred into sterile bottles and then transported to the laboratory for microbiological analysis within very short periods and kept in frozen temperature (0-4°C) for few hours to anaesthetize [13]. Finally, the samples were stored at appropriate conditions (-20°C) for further analysis [13]. According to earlier study of Parvez *et al.,* the houseflies were morphologically identified [13].

Isolation of bacteria and fungus from houseflies

Pathogenic bacteria and fungus from external and internal surface and gut of house flies were isolated by some cultural tests. Each fly was suspended into sterile saline

solution and vortex for external body wash. The suspension was inoculated into nutrient agar and nutrient broth for primary isolation of bacteria [13]. For sub-culturing of the suspected bacteria, we have used different bacteriological agar media like as MacConkey agar, EMB agar, MSA agar, Cetrimide agar and Salmonella–Shigella agar. All bacterial culture petri dishes were incubated at 37°C for overnight for bacterial growth confirmation. Then pure culturing of bacteria was done by following methods described earlier [14]. For fungus isolation PDA media was used. After inoculation sample PDA plates were incubated at 25 °C. All bacteriological and fungal agar media were derived from Hi-Media, India.

Identification of bacteria and fungus

The primary identification of bacteria was done by using gram staining methods which showed morphological and staining characteristics under microscopy [15]. Using standard methods bacteria were identified by different biochemical tests including catalase, oxidase, indole, MR-VP, simon citrate, motility urease [16].

Bacterial genomic DNA extraction and purification

After biochemical test confirmation, for bacterial identification *E. coli*, *Salmonella* spp. and *Pseudomonas* spp. were selected and their 16S rRNA were sequenced. According to [17] laboratory protocol bacterial genomic DNA isolation and purification of *E. coli* was done. *E. coli* was cultured on sodium thioglycolate broth media and by using chloroform isoamyl alcohol genomic DNA of *E. coli* was extracted. For bacterial genomic DNA isolation Tris –EDTA buffer, Tris HCL, Ph 7.4, 10% SDS (fisher scientific), 20% proteinase k (Ambion), Hybridization oven (Biometra OV2, UK), CTAB/NaCl, 24:1 chloroform isoamyl alcohol (Sigma-aldrich) materials used by following protocol [14].

PCR analysis

The PCR reaction was started with the final volume of 25 ml accumulation of 12.5µl of 2x master mixer (Gene Amp Fast PCR Master Mix), DNA temple 2 µl, 0.2µ DNA Tag polymerase, 0.5 µl forward primer, 0.5 µl reverse primer, and 9.3 µl nano grade pure water. The PCR reaction started with primary denaturation at 95°C for 5 min, following by denaturation at 94°C for 30 sec, annealing at 60°C for 30sec, extraction at 72°C for 30sec and terminal extension at 72°C for 5 min with 35 cycles [18]. Afterward, for detection of PCR band agar gel electrophoresis was used with 100 bp DNA ladder. By using thermo scientific Nano Drop 2000 spectrophotometer machine DNA quality and quantity was examined with measuring the DNA concentration (ng/µL) and absorbance ratio (260nm/280nm) of DNA that express the actual purity and concentration of DNA. Finally, we stored DNA at -20°C for further use. The same protocol was applied for *Salmonella* spp. and *Pseudomonas* spp. For detection of PCR band of *E. coli*, 16s rRNA was amplified by PCR using forward primer 16E1 (F): (5' GGGAGTAAAGTTAATCCTTTGCTC 3') and reverse primer 16E2(R): (5' TTCCCGAAGGCCATTCT 3') [19]. For *Salmonella* spp., forward primer S139 (F): (5'GTGAAATTATCGCCA CGTT CGGG CAA 3') reverse primer S141 (R): (5' TCAT CGCA CCGTCAAAGGAACC 3') were used [20] and for *Pseudomonas* spp., forward primer Ker P (F): (5' GAATTCGTGAAGAAGGTTTCT 3') and reverse primer Ker P (R): (5' GGATCCTTACAACGCGCT 3') were applied.

Nucleotide sequencing, and BLAST analysis

For sequencing of *E. coli*, forward primer 16E1 (F): (5' GGGAGTAAAGTTAATCCTTTGCTC 3') and reverse primer 16E2(R): (5' TTCCCGAAGGCCATTCT 3') was used [19]. For *Salmonella* spp., forward primer S139 (F): (5'GTGAAATTATCGCCA CGTT CGGG CAA 3') and reverse primer S141 (R): (5' TCAT CGCA CCGTCAAAGGAACC 3') were used [20]. By using the Genetic Analyzer 3130 (Applied Biosystems) with dideoxy chain termination method (Sanger and Coulson method) PCR products were sequenced in National Institute of Biotechnology (NIB), savar, Dhaka. Same forward and reverse sequences were assembled using SeqMan software and compared with GenBank database of NCBI using BLAST search tool. By using molecular evolutionary analysis software DNA sequences were edited and analyzed as described previously [21]**.** By using the neighbor-joining method phylogenic tree was analyzed and evolutionary distance was measured by computer software [14].

Nucleotide sequence accession number

The nucleotide sequence of *E. coli* and *Salmonella* spp. were subjected to gene bank under accession number MW435870 (Dinajpur/Housefly-8/2019) and (Dinajpur/Housefly-6/2019).

Antibiotic sensitivity test of *E. coli*, *Salmonella* spp. and *Pseudomonas* spp.

An antibiotic sensitivity test was performed on Muller-Hinton agar according to Kirby Bauer disc diffusion tests, as recommended by the Clinical and Laboratory Standards Institutes [6]. A bacterial suspension of 0.5 McFarland standard (equivalent to 1.5 × 108 CFU/ml) was prepared in normal saline solution (HI media, Mombai, India) from a selective culture plate. Then, a 100µl bacterial suspension spread onto Mueller –Hinton agar (Oxoid TM, UK) and antibiotic discs were placed using an automatic disc dispenser. A total of 14 antibiotic discs were used; amoxicillin 11µg, azithromycin 12µg, erythromycin 10µg, gentamycin 35 µg, norofloxacin 20 µg, tetracycline 10 µg, chloramphenicol 15 µg, bacitracin 5 µg, cephalexin 10 µg, colistin 16 µg, nalidixic acid 5 µg, neomycin 15 µg, kanamycin 14 µg, and vancomycin 10 µg, (Hi Media, India). Then the agar plates were incubated at 37°C for 24 hours. After 24 hours incubation, the zone of inhibition was recorded using a millimeter scale. The zone of inhibition was categorized as sensitive and resistant, as according to the disc manufacturer's instruction. MDR means resistant to more than three or more antimicrobial classes against per isolates.

Statistical analysis

Data from different sampling sites (roadside hotel, home kitchen, fish market and chicken) were entered in excel sheets (Microsoft Excel) and analyzed by using R version 3.6.0. The results of all data summarized by descriptive analysis. Chi-square test was also applied to measure the significance difference in several sampling area.

RESULTS

Identification and prevalence of isolated organisms

In this research 63 bacterial and fungus isolates from external surface of houseflies and 42 isolates from internal part of houseflies were identified by different cultural and biochemical tests. *E. coli* was found to be the highest number and give indole, methyl red, motility test positive and oxidase, catalase, voges-proskeur, simmons citrate and urease test negative. *Salmonella* spp. give positive reaction for methyl red, simmon citrate and motility test and remaining tests showed negative reaction. *Klebsiella* spp. showed positive reaction in catalase, urease, indole, and VP test where other tests give negative results. Oxidase, catalase, simmons citrate and motility tests were positive for *Pseudomonas* spp. *Staphylococcus* spp. were positive for catalase, MR-VP, simmons citrate and TSI test and remaining tests showed negative results. Oxidase, catalase, VP and simmon citrate were positive for *Bacillus* spp. and negative for MR, motility, urease tests. TSI test agar slant contains lactose (yellow) color show that *Klebsiella* spp., *Pseudomonas* spp. and glucose (red colour) shows that *Staphylococcus* spp. Butt contains the patterns formation of lactose (yellow) color show that *Klebsiella* spp., *E. coli* and *Staphylococcus* spp. TSI agar butt contains the patterns formation of glucose (red colour) show that *Staphylococcus* spp. This permits detection of the utilization by acid formation as *Staphylococcus* spp. The H_2S formation is present by *Staphylococcus* spp. and absence of substrate *Klebsiella* spp., *E. coli,* and *Pseudomonas* spp. The acid base indicators red is also incorporated to defect carbohydrate formation. Cultural and biochemical test results were presented in Figure 1. Out of 15 sampling sites of houseflies, a total of 63 isolates were isolated from the external surface of housefly whereas total 42 isolates were isolated from internal parts of body of housefly which were represented in Table 1. Out of 63 isolates from external surface *E. coli* were 12 (19.04%), *Salmonella* spp. 10 (15.87%), *Klebsiella* spp. 7(11.11%), *Pseudomonas* spp. 5 (7.93%), *Bacillus* spp. 2(3.17%), *Proteus* spp. 11(17.46%), *Staphylococcus* spp. 11 (17.46%). Out of 42 isolates from internal body part *E. coli* were 15 (35.71%), *Salmonella* spp. 12 (28.57%), *Klebsiella* spp. 6 (14.28%), *Pseudomonas* spp. 6 (14.28%) and *Proteus* spp. 3 (7.14%) found, respectively. 3 (4.76%) *Aspergillus* spp. and 2 (3.17%) *Fusarium* spp. were also identified from external surface of houseflies. *E. coli*, *Salmonella* spp. and *Pseudomonas* spp. were most prevalent in external and internal parts of houseflies.

In case of external surface out of 63 bacterial isolates 17 (26.98%) were found in rode side hotel followed by 8 (12.70%) in home kitchen, fish market 25 (39.68%) and chicken market 13 (20.63%) respectively (Table 2), while in case of internal surface, out of 42 bacteria 14(33.33%) was found in fish market followed by 12(28.57%) in chicken market, 9 (21.42%) in road side hotel and 7(16.67%) in home kitchen (Table 3). The microbial load on houseflies (both external surface and internal parts of body) is much higher in fish market than other places which were presented in Table 2 and 3. There is significant different in the frequency of positive samples for *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Bacillus* spp., *Aspergillus* spp. and *Fusarium* spp. among the treatment of roadside hotels, home kitchens, fish markets and chicken markets (Table 2).

Table 1. Frequency of isolated bacteria and fungus in houseflies from different samples.

Isolates	Number of isolates /Number of samples (63/80) external surface	Percentage of positive isolates	Number of isolates /Number of samples (42/60) internal body	Percentage of positive isolates
E. coli	12	19.04%	15	35.71%
Salmonella spp.	10	15.87%	12	28.57%
Klebsiella spp.	7	11.11%	06	14.28%
Pseudomonas spp.	5	7.93%	06	14.28%
Bacillus spp.	2	3.17%	-	-
Proteus spp.	11	17.46%	03	7.14%
Staphylococcus spp.	11	17.46%	-	-
Aspergillus spp.	3	4.76%	-	-
Fusarium spp.	2	3.17%	-	-
Total isolates	63		42	105

Not detected isolates, - ; 63= Total number of isolates from external surface; 80= Total number of samples; 42= Total number of isolates from internal body; 60= Total number of samples; Total number of samples= 80+60=140; and Total number of isolates= 63+42=105.

Table 2. Distribution of bacteria and fungus from external surface of housefly according to sampling sites.

Isolates	Roadside Hotel	Home Kitchen	Fish market	Chicken market	X^2	P-Value
E. coli	4	0	4	4	4.200	0.241
Salmonella spp.	2	1	4	3	2.083	0.555
Klebsiella spp.	2	2	3	0	2.792	0.425
Pseudomonas spp.	1	0	2	2	2.245	0.523
Proteus spp.	0	0	2	0	6.048	0.109
Bacillus spp.	4	2	4	1	2.567	0.463
Staphylococcus spp.	4	3	2	2	1.046	0.790
Aspergillus spp.	0	0	2	1	3.711	0.294
Fusarium spp.	0	0	2	0	6.048	1.109
Total	17(26.98%)	8(12.70%)	25(39.68%)	13(20.63%)	Total=63	

Chi-square, $\chi 2$

Table 3. Distribution of bacteria and fungus from internal part of housefly according to locations.

Isolates	Roadside Hotel (4)	Home Kitchen (3)	Fish market (4)	Chicken market (4)	Total
E. coli	04	03	04	04	15
Salmonella spp.	02	03	04	03	12
Klebsiella spp.	01	01	02	02	6
Pseudomonas spp.	01	-	03	02	6
Proteus spp.	01	-	01	01	3
Bacillus spp.	-	-	-	-	
Staphylococcus spp.	-	-	-	-	-
Aspergillus spp.	-	-	-	-	-
Fusarium spp.	-	-	-	-	-
Total	9 (21.42%)	7 (16.67%)	14(33.33%)	12(28.57%)	42

Not detected isolates, -

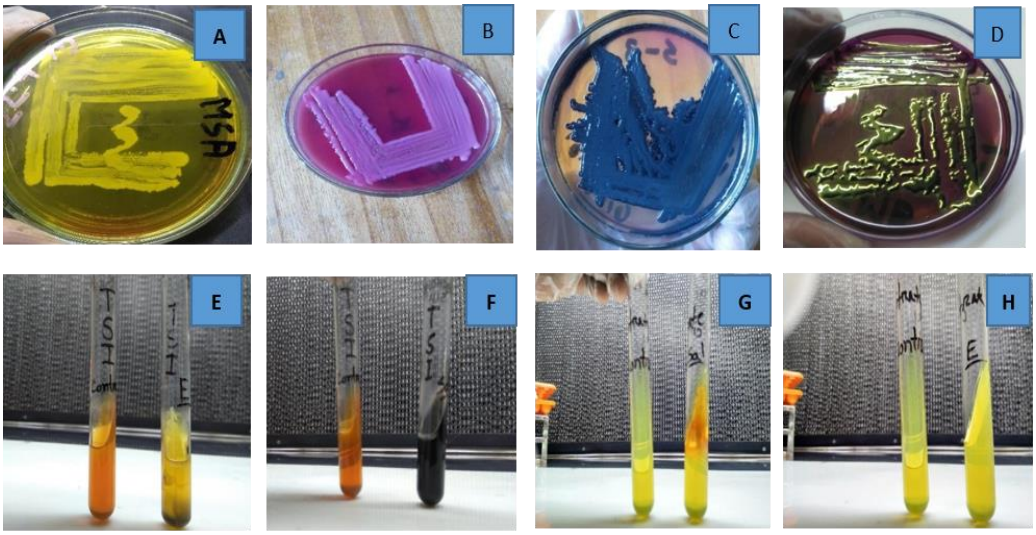

Figure 1. Cultural characteristics of *Staphylococcus* spp. on MSA (A), *Klebsiella* spp. on MacConkey agar (B), *Salmonella* spp. on SS agar (C) and *E. coli* on EMB agar (D), Biochemical test result of *E. coli* positive in TSI (E), *Salmonella* spp. positive in TSI (F), *Salmonella* spp. positive in Citrate test (G), and *E. coli* negative in Citrate test (H).

PCR, gene sequencing and phylogenic tree analysis

To confirm biochemical test results the three isolates were selected for 16S rRNA sequencing. *E. coli* DNA was amplified by using the Forward primer E1-: (5'GGGAGTAAAGTTAATCCTTTGCTC3') and Reverse primer E2-: (5'TTCCCGAAGGCCATTCT3') and found 584 bp band on gel electrophoresis which was presented in Figure 2 whereas, phylogenic tree analysis was mentioned in Figure 3. For *Salmonella* spp. DNA was amplified with specific S139- F and S141- R primers and 284 bp band was found which is shown in Figure 4 and phylogenic tree in Figure 5. In Figure 6 showed that the PCR band of *Pseudomona* spp. was 1497 bp. Sequencing was experimented by using BLAST tool and results found with 99.99% homology in *E. coli* with accession number MW435870 Dinajpur/Housefly-8/2019, *Salmonella typhimurium* found 98.96% homology with accession number Dinajpur/Housefly-6/2019 from GenBank analysis. The result was consistent with the biochemical results. In this research, our accession number was compared with different bacterial accession number which was found from other sources.

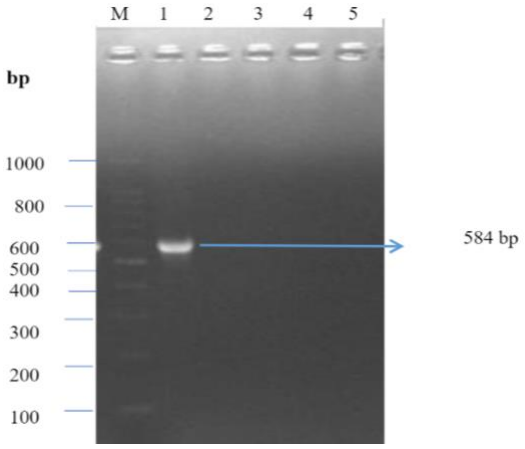

Figure 2. Amplification of E. coli by E1 and E2 Primer, Ladder 1: test sample, Lane M: Marker- 1000 bp *E. coli* showed 584 bp band.

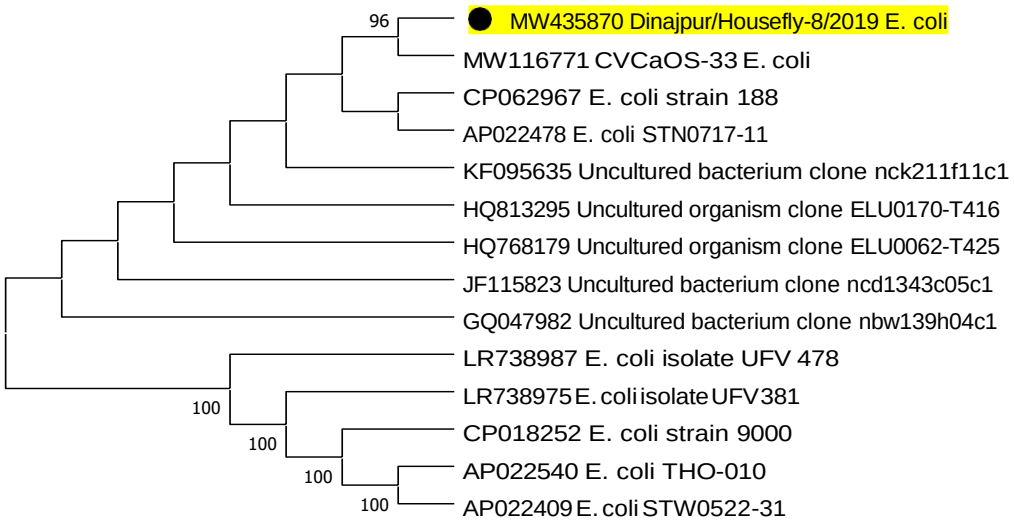

Figure 3. The phylogenetic tree made from 16S ribosomal RNA of *E. coli* from housefly which differ from others.

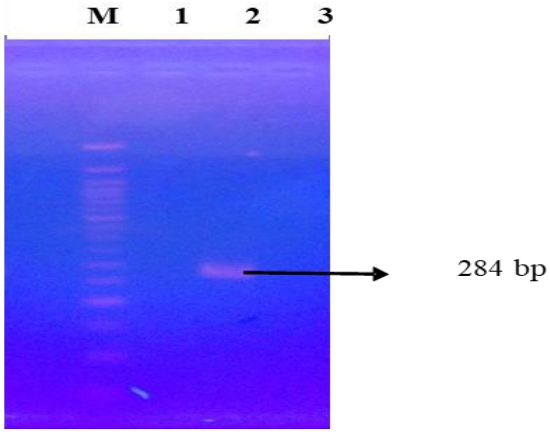

Figure 4. Amplification of *Salmonella typhimurium* by s139 and s141 primer. Test sample of *Salmonella typhimurium* showed band at 284 bp and Lane M: Marker (50 bp).

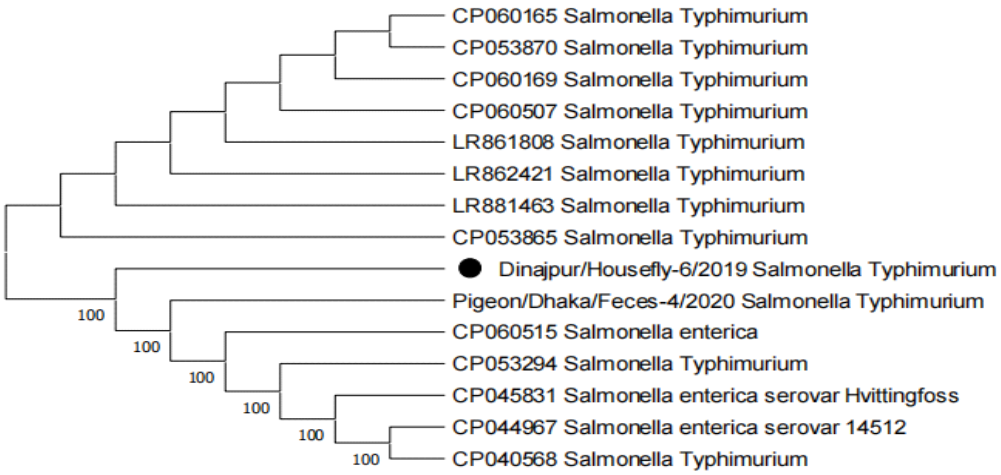

Figure 5. The phylogenic tree made from 16S ribosomal RNA of *Salmonella typhimurium* from housefly which also differ from others.

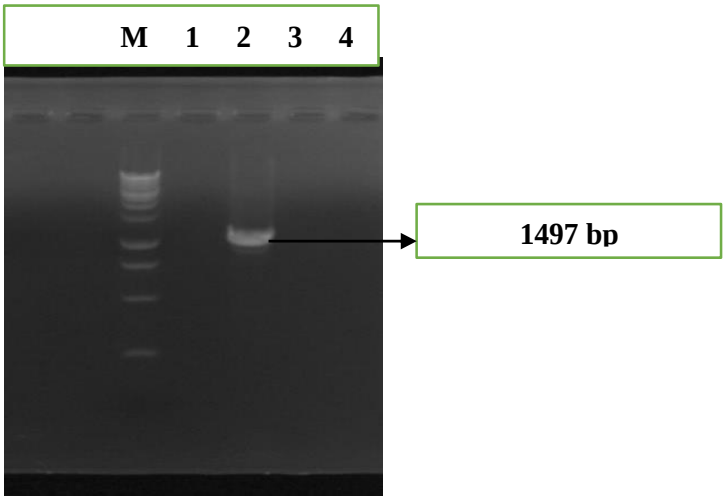

Figure 6. Amplification of *Pseudomonas* spp. by ker P (F) and ker P (R). Test sample of *Pseudomonas* spp. showed band at 1497bp and Lane M: Marker (1kb).

Overall antibiotic sensitivity and resistance patterns

In our study, all isolates were used under commercially available antibiotics and determined the resistant patterns. Out of 10 antibiotics *E. coli* were resistant against erythromycin, gentamicin, and bacitracin 100% followed by methicillin and kanamicin (80%) respectively, whereas sensitive to chloramphenicol, ciprofloxacin, and azithromycin (100%) (Table 4). Out of 8 antibiotics *Salmonella* spp. were highly resistant to erythromycin, gentamicin and bacitracin (100%) followed by tetracycline 80%. Whereas, sensitive to chloramphenicol and ciprofloxacin (100%, 80%) (Table 4). Among 6 antibiotic *Pseudomonas* spp. were highly resistant to ciprofloxacin and gentamicin (100%) whereas, sensitive to tetracycline, amoxicillin (85.71%) and vancomycin (71.43%) (Table 4).

Table 4. Antibiotic sensitivity test results.

Name of antibiotics with disc concentration (µg)	*E. coli* (n=10)		*Salmonella* spp. (*n=10*)		*Pseudomonas* spp. (7)	
	(%S)	(%R)	(%S)	(%R)	(%S)	(%R)
Chloramphenicol (30µg)	80	20	100	0	NT	NT
Amoxicillin (11 µg)	50	50	NT	NT	85.71	14.29
Methicillin (5 µg)	20	80	NT	NT	NT	NT
Tetracycline (30 µg)	NT	NT	20	80	85.71	14.29
Ciprofloxacin (5 µg)	100	0	80	20	14.29	85.71
Vancomycin (10 µg)	30	70	70	30	71.43	28.57
Gentamicin (10 µg)	0	100	0	100	0	100
Kanamycin (14 µg)	20	80	30	70	28.57	71.43
Erythromycin (10 µg)	0	100	0	100	NT	NT
Azithromycin (15µg)	100	0	NT	NT	NT	NT
Bacitracin (5 µg)	0	100	0	100	NT	NT

Note: S= Sensitive, R= Resistance, NT= Not tested

Multidrug-resistant bacteria

In general, about 40% (4/10) of *E. coli*, 50% (5/10) of *Salmonella* spp. and 57.14% (4/7) of *Pseudomonas* spp. were found to be resistant more than three antibiotics in our research. Bacteria that represented resistant three or more than three antibiotics from different class indicates MDR. Overall, in our study 57.14%, 50% and 40% of *E. coli*, *Salmonella* spp. and *Pseudomonas* spp. were found to transmit MDR traits, respectively. MDR isolates are shown in Table 5.

Table 5. MDR *E. coli*, *Salmonella* spp. and *Pseudomonas* spp. isolated from houseflies.

Antimicrobial compound	Number of MDR isolates (%)
E. coli (n=10)	
E, B, VA	1(10%)
VA, Met, AMX	1(10%)
B, VA, AMX	1(10%)
AMX, B, VAN, E	1(10%)
Total	4(40%)
Salmonella spp. (n=10)	
TE, K, E	1(10%)
TE, AMX, B	1(10%)
TE, E, B, GEN	1(10%)
V, K, B, AMX	1(10%)
TE, B, K, E, GEN	1(10%)
Total	5(50%)
Pseudomonas spp. (7)	
GEN, CIP, K	1(14.28%)
GEN, V, TE	1(14.28%)
TE, CIP, V	1(14.28%)
CIP, K, TE, GEN	1(14.28%)
Total	4(57.14%)

MDR: Multidrug-resistant; *E. coli*: *Escherichia coli*; E: Erythromycin; B: Bacitracin; VA: Vancomycin; Met: Methicillin; AMX: Amoxicillin; TE: Tetracycline; K: Kanamycin; GEN: Gentamycin; CIP: Ciprofloxacin.

DISCUSSION

The present research was guided for the characterization of bacteria such as *E. coli* and *Salmonella typhimurium* and *Pseudomonas* spp. which isolated from internal body part of houseflies in Dinajpur district. The isolates were identified by cultural and molecular approaches. The finding of this research indicated that pathogenic microorganisms are transmitted mechanically by housefly which is similar earlier findings [1]. In this study, bacterial load on the external body parts of the fly was wider than that on the internal body parts of the fly, which is close to previous findings [13]. Our research revealed many pathogenic isolates that are significant for human health. Pathogenic bacterial species were also identified from house flies from other parts of the world [22, 23]. In addition, salmonellosis is one of the important zoonotic diseases in the world which is spread out by *Salmonella* spp. Nowadays salmonellosis increasing not only industrialized country but also developing countries and infecting with diarrheal disease in human and animal [24]. *E. coli* is another of the most predominant pathogens causing urinary and gastrointestinal infections and septicemia in human and animal. In addition, *E. coli* has the ability to preserve resistant genes and transfer these genes to other bacteria and environment [24].

In this study we isolated *E. coli* 19.04%, *Klebsiella* spp. 11.11% and *Staphylococcus* spp. 17.46% from gut of house flies and the results were more or less similar to as well as author [3].

The internal parts of the houseflies *Salmonella typhimurium* 28.57%, *E. coli* 35.71%, *Pseudomonas* spp. 14.28%, *Proteus* spp. 7.14% and *Klebsiella* spp. 14.28% were identified (42 isolates) and these findings is similar to previous investigators [25]. Cultural and biochemical tests of *E. coli, Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp., *Aspergillus* spp., and *Fusarium* spp. were comparable to previous investigators [25-28]. *E. coli* (the most common), *Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Salmonella* spp. were isolated from housefly's outer part, which are close to the decisions of authors [29]. Microbial load on houseflies (both external surface and internal parts of body) is very high in fish market 39.68%, 33.33%)

than other places and the research was similar to earlier researcher [20]. In these present study, 584 bp band of *E. coli*, 284 bp band of *Salmonella typhimurium* was confirmed by PCR and similar results were investigated from previous research [14, 19, 20].

The results of antibiotic sensitivity tests revealed that most of the pathogens were multidrug resistant. In addition to, all isolates were resistant to erythromycin, gentamycin, kanamycin, and methicillin while being susceptible to ciprofloxacin, chloramphenicol tetracycline, azithromycin, amoxicillin which is close to the results of author [13]. In addition, gentamicin is highly resistant to *E. coli*, *Salmonella* spp. and *Pseudomonas* spp. whereas, other findings showed that *E. coli* was resistant [29]. Ciprofloxacin showed resistant against *Pseudomonas* spp. [25]. However, research was conducted in Iran, they found 32.5% MDR antibiotics including chloramphenicol, tetracycline and ampicillin against antibiotic resistant bacteria associated with gut of houseflies [30]. In another research was conducted in China, found multidrug resistant *E. coli* [31]. Additionally other researchers found amoxicillin, ciprofloxacin, gentamicin was resistant to *E. coli* and *Salmonella* spp. which is related to our findings [32]. In these current study, Tetracycline resistant *Salmonella* spp. were detected, and the results were agreed with previous research in fish market in Bangladesh [20]. No available research data on antibiotic resistant isolates in home kitchen, roadsides hotel in Bangladesh.

It is mentioned in this study that pathogenic bacteria were identified from local market, and this is related to fish, meat, vegetables, and other food ingredients. The resistance genes are easily transferred from flies to human and animal and create infectious diseases. These resistance genes transfer from one infected individual to other persons and affects. Houseflies carry antibiotic resistance gene from various environmental settling and spread infection to human and animals. The prevalence of these pathogenic microorganisms in fish markets, chicken markets, roadside hotels and home kitchens indicated a potential risk of pathogen transmission from houseflies to human, resulting in illness, necessitating increased control steps. Food borne illness in human beings would occur if pathogenic bacteria transferred by houseflies. Therefore, the personnel who are involved in this business should be conscious about proper sanitation and also conscious about the use of antibiotics.

CONCLUSIONS

Houseflies may carry antibiotic resistant gene from isolates from different sources and transmit disease causes bacteria to animal and human. This research has a great significance for human and animal health. *Staphylococcus* spp., *Bacillus* spp., *E. coli*, *Salmonella typhimurium*, *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp., *Aspergillus* spp. *and Fusarium* spp. in houseflies (external surface and internal parts) found from fish market, chicken market, roadside hotel, and home kitchen. These isolated pathogens were found to be more frequent and more resistant to different classes of antibiotics. A net can be used in the window to keep flies out of the kitchen. It is strongly advised that animal carcasses and other kitchen waste be properly disposed of. To keep the interaction of flies with the foods to a minimum, appropriate hygiene practices should be adhered to in the food processing sectors. Government should take proper steps about controlling antibiotic resistance against human, animal, and environmental determinants. Further study is needed to determine the pathogenic isolates spreading by houseflies, and also detect the resistant gene that carries pathogenic bacteria.

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AUTHORS CONTRIBUTION

FJA, NAR designed, conceptualization, writing the manuscript. MAH checked plagiarism and writing review and correction. MHH and MSR contributed to data analysis. JA finally checked the manuscript and editing. Finally, all authors who are involved in this research read and approved the manuscript for publication.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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