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Isolation of pathogenic bacteria from *Musca domestica* and the effect of antibiotics, captured from Vadodara city, Gujarat

Linta Paulson, Parth Pandya, Bhumi Thakkar and Pragna Parikh

Abstract
House fly, *Musca domestica* has been given more focus as a potential vector in spreading bacterial pathogens. The spread of housefly is season specific and locus bound since it feeds and breeds on dumped organic wastes, open drainages and hospital surroundings are affluent with a copious amount of pathogenic and non-pathogenic microorganisms. Thus, the aim of the study was to isolate and classify different types of bacteria and prove *M. domestica* as a carrier. The bacteria were isolated with the help of media using biochemical estimation and pathogenecity was tested checking its antibiotic susceptibility. The result obtained showed that the pathogenic load of bacteria was more among which, *Pseudomonas* sp was found to be maximum in all the study sites. Thus, the study suggests that the house fly is a dominant carrier for pathogenic bacteria and can be considered to be a vector for several infectious pathogens that are antibiotic resistant.

Keywords: *Musca domestica,* Pathogenecity, *Pseudomonas* sp., vector, antibiotic resistance

1. Introduction
Musca domestica belongs to the group of filth flies which possess tremendous health hazards in public health as potential vector of microorganisms. They live in close association with bacteria and other microorganism. Biologically and ecologically, the house fly behaviour and habitats make them a very effective mechanical vector for microbes [1]. The highly mobile house flies spread bacteria by direct contact with the substrates. The presence of electrostatic charges, the setae and hairs on the body surface cause to have the higher capacity to attach foreign particles. The viscosity of faeces enhances the adhering capacity of pathogens/foreign particles to the fly body [2, 3] .Moreover, external surfaces and alimentary canal of house flies become contaminated by various microbes and can potentially contaminate any substrate by the unique regurgitation type of feeding and faecal excretion.
In continuation, concern for public health and cleanliness are more intensive as population increases in developing countries. The emergence of flies, new pathogens and the growing number of immune-compromised individuals strengthens the need for safer food supplies and good community health [4]. Nevertheless, outbreaks of foodborne and waterborne pathogens are increasingly reported and communicated in India. Due to the indiscriminate form of feeding of houseflies, they have been predicted to be as potential vectors of more than 100 serious pathogens ranging from virus-bacteria (*Vibro cholera*, *Staphylococcus)-protozoans*; (*Entamoeba histolytica*, *Cryptosporidium parvum* and *Entamoeba) to* nematodes (*helminth eggs, Toxocara spp.; Ascaris lumbricoides, Trichiuris trichiura, Enterobius vermicularis, Ancylostoma caninum, Strongyloides stercoralis*, [5].
In addition, these bacteria are more or less susceptible to antibiotic resistance from which they develop multiple-drug resistance (MDR) that has become a serious issue in clinical medicine [6, 7] The heavy use of antibiotics has become a serious public health concern as it has led to the rapid emergence, selection, and spread of resistant, commensally and potentially virulent bacterial strains. The resistant strains have already been reported from the food animals, the animal-based food products, the fresh vegetables, the surrounding environmental samples (water, air, soil etc.) and also from the farmers [8, 9, 10].
Thus, on the basis of literature, the present study lays its hypothesis on isolation and characterisation of bacterial load and to test it pathogenicity using antibiogram on the outer surface and internal surface (midgut) of house fly (*M. domestica*).

To accomplish the hypothesis, four different sites were selected where the housefly was found to be maximum. The rationale behind choosing house fly is due to their abundance in all the sites and its affinity towards the environment present in it.

2. Materials and Methods

2.1 Collection of Houseflies

A total of 100 houseflies were randomly collected from different locations, such as fish market, butcheries, vegetable market and residential area. About 25-30 flies were collected from each location by using a sweep net. Collected flies were transferred into sterile bottles and transported to the laboratory within 30–45 min of collection. After the identification Flies were freeze sacrificed at −20 ^{0}C.

2.2 Isolation of bacteria from external surfaces and alimentary tract of houseflies

One milliliter of sterile saline (0.9%) was added to each test tube containing ten flies, and the tubes were thoroughly shaken for 2 min after that the solutions were transferred in sterile test tubes.

After external washing, houseflies were suspended in 70% alcohol for 5 min to make the external surface of the flies devoid of bacteria and were allowed to dry at room temperature and were given wash with sterile1X PBS for 3 min to remove traces of alcohol. Further, the gut of the houseflies was dissected out and macerated aseptically in a mortar-pestle in 2ml of 1X PBS. Washing samples were collected in test tubes.

The samples were serially diluted in 0.85% saline and 9ml distilled water (1:9 dilutions). Dilutions of 10^{-2} to 10^{-6} were prepared and 0.1ml of each fraction was plated on the nutrient agar medium, from which, bacteria were cultured and isolated on respective medium to study its colony morphology and biochemical responses.

A loopful of the washing was inoculated onto the surface of agar plates (MacConkey's medium, blood agar). The plates containing MacConkey's medium and blood agar were incubated aerobically and anaerobically at 37°C for 24 h. The Identification of bacteria identified by colonial morphology, Gram staining, and biochemical phenotype was carried out according to the Bergey's manual of systematic bacteriology. Typical colonies were sub cultured and were subsequently identified as pathogenic bacteria by Gram staining, and urease, oxidase, and catalyse activities. Moreover, for further characterization, bacteria were identified by various biochemical tests to understand the biochemical character of gram positive bacteria and gram negative bacteria.

2.3 Quantitative Estimation of Bacterial Isolates [11]

2.3.1 Colony count and viable count

The percentage of pathogen isolated from the external and internal body/surface of *M. domestica* were determined and recorded. For each case (0.05 ml), different dilution of 0.05ml of washings cultured on blood agar and Mac Conkey agar plates in duplicate. The isolated bacteria were kept at 37^{o}C and colony count as performed (c.f.u). Supplementary, we took the mean count of the plates and calculated the viable count of particular bacteria for 2ml of the solution.

Viable count = No. of colonies x dilution factor / Vol. of sample

2.4 Antibiograms

Antimicrobial sensitivity test was carried out by using stimulus combi-disc diffusion method. 1ml of cultured sample was introduced in 20ml of sterile molten Mueller Hinton Agar (MHA). After the solidification of agar, the combi disk was placed onto the media with the help of sterile forceps. Incubated the plate at 35-37^{o}C for 16-18 hrs. The disc diffusion test is based on the size of the zone of inhibition. Antimicrobial susceptibility testing with the disc is a simple and rapid method and provides a reproducible means of testing bacterial sensitivity and resistance to the various antibiotics and chemotherapeutic agents.

2.5 Data analysis

The statistical analysis were evaluated by Chi – Square test keeping (Having degree of freedom= 2) $\alpha = 0.05$ using Graph-pad Prism 6 software using the following formula.

$$\chi^2 = \frac{(\text{Observed Value} - \text{Expected Value})}{\text{Expected Value}} + \frac{(\text{Observed Value} - \text{Expected Value})}{\text{Expected Value}} + \ldots$$

The significance was noted at $p<0.05$.

3. Result

3.1 Identification of Bacteria from *M. domestica*

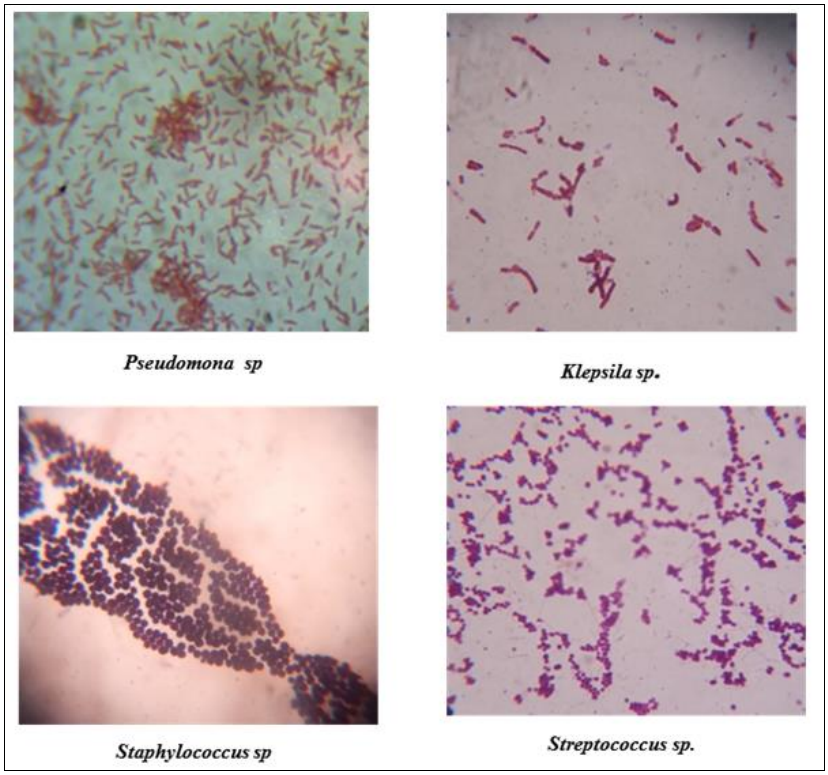
A total of 8 species of bacteria were identified from the isolated colony from internal and external surface of *M. domestica.* (Table 1). Of the total % species were Gram–ve and 3 were Gram+ve. (Fig 1) The collected samples from the different sites were washed with saline and were cultured on nutrient agar medium. The colony thus formed was then identified based on their morphology and biochemical test (Table 1). As far as butchery site is concerned *E. coli* and *Streptococcus sp*. were common on external and internal surface, however, *Klebsiella sp* and *Pseudomonas sp* were exclusively found on the external surface and *Staphylococcus sp* was solely found on the internal surface. From fish market the E. coli was cofound to be present on external as well as internal surface of *M. domestica*, However the species found on the external surface includes *Pseudomonas, Klebsiella, Staphyllococcus* whereas that found from the internal surface include *Strreptococccus, Enterobacter* and *salmonella.* From vegetable market *pseudomonas was* common for internal and external surface of *M. domestica.* The identified species from external surface was *Enterobacter* and *Bacillus* where as from internal surface *E. coil* was present. From residential area *E. coil* was common for internal and external surface. However, *Enterobacter* was found on external surface and pseudomonas was found on internal surface (Table 1)

Table 1: Colony characteristics of isolates from external and internal surface

				Biochemical Test													
Sample	**Colour**	**Shape**	**Gram's Staining**	**Indole test**	**MR test**	**VP test**	**Glucose**	**Manitol**	**Catalase test**	**Oxidase**	**Nitrate red.test**	**Starch hydrolysis**	**Simmon's citrate**	**Motility test**	**Lactose ferm. test**	**Hemolysis test**	**EMB plate**	**Bacteria in species level**
B	White	Circular	-ve	+	+	+	AG	AG	+	-	+	-	+	-	-	-	-	*Klebsiella* sp.
	Transparent	Circular	-ve	+	+	-	AG	A	+	-	+	-	-	+	+	-	+	*E.coli*
	Milky white	Circular	+ve	-	+	-	-	A	-	-	-	-	-	-	-	+	-	*Streptococcus* sp.
	White	Irregular	-ve	-	-	-	-	-	+	+	+	-	+	-	-	-	-	*Pseudomonas* sp.
BI	Milky white	Circular	+ve	-	+	+	A	AG	+	-	+	-	-	-	-	+	-	*Staphylococcus* sp.
	Transparent	Circular	-ve	+	+	-	AG	A	+	-	+	-	-	+	+	-	+	*E.coli*
	Milky white	Circular	+ve	-	+	-	-	A	-	-	-	-	-	-	-	+	-	*Streptococcus* sp.
F	Greenish	Circular	-ve	-	-	-	A	-	+	+	+	-	+	+	-	-	-	*Pseudomonas* sp.
	White	Irregular	-ve	+	+	+	AG	AG	+	-	+	-	+	-	-	-	-	*Klepsila* sp.
	Yellow	Circular	+ve	-	+	+	A	AG	+	-	+	-	-	-	-	+	-	*Staphylococcus* sp.
	Transparent	Circular	-ve	+	+	-	AG	A	+	-	+	-	-	+	+	-	+	*E.coli.*
FI	Transparent	Circular	-ve	+	+	-	AG	A	+	-	+	-	-	+	+	-	+	*E.coli.*
	White	Circular	+ve	-	+	-	-	A	-	-	-	-	-	-	-	+	-	*Streptococcus* sp.
	Transparent	Circular	-ve	-	-	+	AG	A	+	-	+	-	+	+	+	-	-	*Enterobacter* sp.
	Translucent	Circular	-ve	-	+	-	AG	AG	+	-	-	-	+	-	-	-	-	*Salmonella* sp.
V	Transparent	Circular	-ve	-	-	+	AG	A	+	-	+	-	+	+	+	-	-	*Enterobacter*
	White	Circular	+ve	-	+	-	-	-	+	-	+	+	-	-	-	-	-	*Bacillus* sp.
	White	Irregular	-ve	-	-	-	-	-	+	+	+	-	+	-	-	-	-	*Pseudomonas* sp.
VI	White	Irregular	-ve	-	-	-	-	-	+	+	+	-	+	-	-	-	-	*Pseudomonas* sp.
	Transparent	Circular	-ve	+	+	-	AG	A	+	-	+	-	-	+	+	-	+	*E.coli*
R	Transparent	Circular	-ve	+	+	-	AG	A	+	-	+	-	-	+	+	-	+	*E.coli*
	Transparent	Circular	-ve	-	-	+	AG	A	+	-	+	-	+	+	+	-	-	*Enterobacter* sp.
RI	Transparent	Circular	-ve	+	+	-	AG	A	+	-	+	-	-	+	+	-	+	*E.coli*
	White	Irregular	-ve	-	-	-	-	-	+	+	+	-	+	-	-	-	-	*Pseudomonas* sp.

External surface: B (butcheries), F (Fish market), V (Vegetable market), R (Residential area),
Internal Surface: BI-1 (butcheries), FI (Fish market), VI (Vegetable market), RI(Residential area) ; AG- Acid and Gas, + Presence, - Absence

3.1.1 Classification of bacteria using Gram Staining



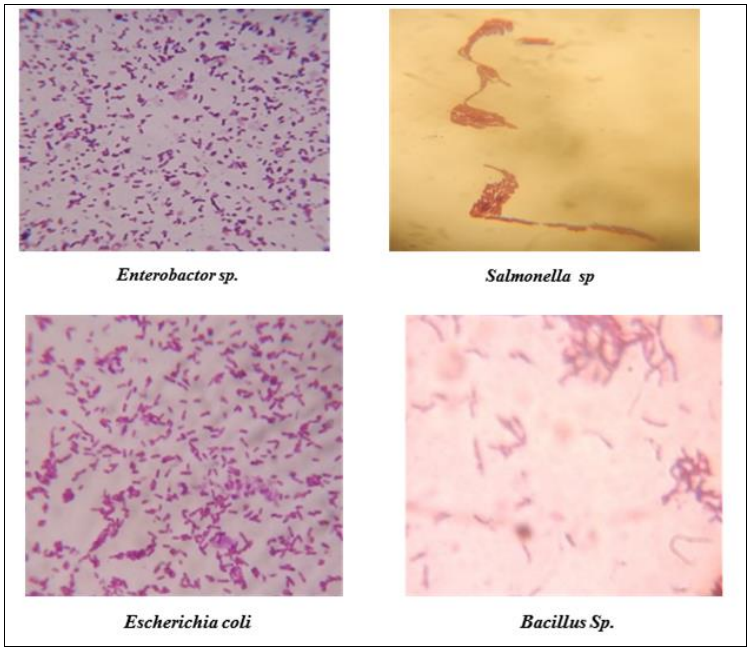


Fig 1: Gram staining of Isolated Bacteria from the external and internal surface of the *M. domestica*

Table 2: Isolated Colonies from the External and the Internal surface of *M. domestica*

Sr.no	Bacteria	B	F	V	R	BI	FI	VI	RI
1	*Pseudomonas* sp.	+	+	+	-	-	-	+	+
2	*Klebsiella* sp.	+	+	-	-	-	-	-	-
3	*Staphylococcus* sp.	-	+	-	-	+	-	-	-
4	*E. coli*	+	+	-	+	+	+	+	+
5	*Streptococcus* sp.	+	-	-	-	+	+	-	-
6	*Enterobacter* sp.	-	-	+	+	-	+	+	-
7	*Salmonella* sp.	-	-	-	-	-	+	-	-
8	*Bacillus* sp.	-	-	+	-	-	-	-	-

External surface: B (butcheries), F (Fish market), V (Vegetable market), R (Residential area),
Internal surface: BI-1 (butcheries), FI (Fish market), VI (Vegetable market), RI (Residential area)

3.2 Quantitative Estimation of Bacterial Isolates

Table 3 and Fig 2 represents Quantitative estimation of total bacterial load /fly from isolated colony from *M. domestica*. Among all the three sites explored the highest load was found to be in the Fish market followed by butchery and vegetable market. The p-value obtained from Chi-Square statistical analysis was less than 0.05 (p<0.05). Hence, the data is statistically highly significant.

Table 3: Quantitative Estimation of Bacterial Isolates from *M. domestica*

Sample	Bacteria	Viable Count	(%)	Total Bacterial Load/ fly
B	*Klebsiella* sp.	22.40 x 10^2	21.13	106 x 10^2 (31%)
	E. coli	10.40 x 10^2	9.81	
	Streptococcus sp.	10.60 x 10^2	10	
	Pseudomonas sp.	14.00 x 10^2	13.21	
BI	*Staphylococcus* sp.	16.00 x 10^2	15.09	
	E. coli	22.40 x 10^2	21.13	
	Streptococcus sp.	10.40 x 10^2	9.81	
F	*Pseudomonas* sp.	8.00 x 10^2	6.14	130.2 x 10^2 (39%)
	Klebsiella sp.	12.00 x 10^2	9.22	
	Staphylococcus sp.	15.00 x 10^2	11.52	
	E. coli	16.00 x 10^2	12.23	
FI	*E. coli*	18.20 x 10^2	13.98	
	Streptococcus sp.	24.00x 10^2	18.43	
	Enterobacter sp.	22.00 x 10^2	16.90	
	Salmonella sp.	15.00 x 10^2	11.52	
V	*Enterobacter* sp.	11.00 x 10^2	14.86	74 x 10^2 (22%)
	Bacillus sp.	14.00 x 10^2	18.92	
	Pseudomonas sp.	16.00 x 10^2	21.62	
VI	*Pseudomonas* sp.	13.00 x 10^2	17.57	
	E. coli	20.00 x 10^2	27.03	
R	*E. coli*	8.90 x 10^2	33.84	26.30 x 10^2 (8%)
	Enterobacter sp.	6.65 x 10^2	25.29	
RI	*E. coli*	10.40 x 10^2	39.54	
	Pseudomonas sp.	0.55 x 10^2	2.09	

External surface: B (butcheries), F (Fish market), V (Vegetable market), R (Residential area),
Internal surface: BI-1 (butcheries), FI (Fish market), VI (Vegetable market), RI (Residential area

3.2.1 Colony count and viable count

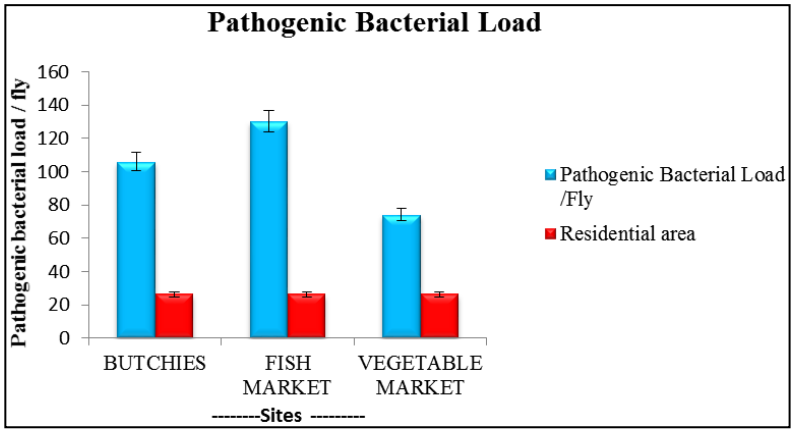


Fig 2: A Comparative assessment of bacterial load in three different sites carried by *M. Domestica.* Residential area was taken as control area as due to hygienic prevailing hygienic condition. (**) denotes the significance at $p<0.01$.

Table 4: Antibiogram test result of gram-negative bacteria

Sample	Bacteria	Tricarcillin	Meropenem	Levofloxacin	Moxifloxacin	Cefprozil	Cefpirome	Ceftizoxime	Cefpodoxime	Cefoparazone	Piperacillin	Tobramycin	Sparfloxacin	Gatifloxacin	Imipenem
B	*Klebsiella sp*	S	I	S	S	I	S	S	S	S	I	S	S	S	S
	E. coli	I	S	S	S	R	S	S	S	S	R	S	S	S	S
	Pseudomonas sp.	R	S	S	S	S	I	S	-	S	R	S	S	S	S
BI	*E. coli*	R	R	S	I	S	R	S	S	I	S	S	-	S	S
F	*Pseudomonas sp.*	R	S	S	I	S	S	R	I	I	R	S	S	S	S
	Klebsiella sp	S	I	S	S	S	S	I	I	S	S	S	-	R	-
	E. coli.	R	S	-	-	I	I	I	S	S	S		I	S	-
FI	*E. coli.*	S	R	S	S	S	R	S	R	I	S	S	R	-	-
	Enterobacter sp.	S	R	-	S	S	R	S	S	S	S	R	S	S	S
	Salmonella sp.	S	R	S	S	S	I	S	S	S	S	I	S	S	S
V	*Enterobacter*	S	-	I	S	S	I	S	S	-	S	S	-	S	S
	Pseudomonas sp.	R	S	I	S	I	S	S	S	S	S	S	I	S	I
VI	*Pseudomonas sp.*	S	I	S	S	R	S	S	S	-	S	S	S	I	R
	E. coli	S	I	R	I	S	R	S	I	-	I	S	S	S	I
R	*E. coli*	S	I	S	S	S	S	I	I	-	S	S	I	S	S
	Enterobacter sp.	I	S	S	S	S	I	S	S	S	-	S	S	S	S
RI	*E. coli*	S	-	I	S	-	S	S	S	I	S	S	S	S	S
	Pseudomonas sp.	I	S	-	I	S	S	S	S	S	S	S	I	S	S
B	*Klebsiella sp*	S	I	S	S	I	S	S	S	S	I	S	S	S	S
	E. coli	I	S	S	S	R	S	S	S	S	R	S	S	S	S
	Pseudomonas sp.	R	S	S	S	S	I	S	-	S	R	S	S	S	S
BI	*E. coli*	R	R	S	I	S	R	S	S	I	S	S	-	S	S
F	*Pseudomonas sp.*	R	S	S	I	S	S	R	I	I	R	S	S	S	S
	Klebsiella sp	S	I	S	S	S	S	I	I	S	S	S	-	R	-
	E. coli.	R	S	-	-	I	I	I	S	S	S		I	S	-
FI	*E. coli.*	S	R	S	S	S	R	S	R	I	S	S	R	-	-
	Enterobacter sp.	S	R	-	S	S	R	S	S	S	S	R	S	S	S
	Salmonella sp.	S	R	S	S	S	I	S	S	S	S	I	S	S	S
V	*Enterobacter*	S	-	I	S	S	I	S	S	-	S	S	-	S	S
	Pseudomonas sp.	R	S	I	S	I	S	S	S	S	S	S	I	S	I
VI	*Pseudomonas sp.*	S	I	S	S	R	S	S	S	-	S	S	S	I	R
	E. coli	S	I	R	I	S	R	S	I	-	I	S	S	S	I
R	*E. coli*	S	I	S	S	S	S	I	I	-	S	S	I	S	S
	Enterobacter sp.	I	S	S	S	S	I	S	S	S	-	S	S	S	S
RI	*E. coli*	S	-	I	S	-	S	S	S	I	S	S	S	S	S
	Pseudomonas sp.	I	S	-	I	S	S	S	S	S	S	S	I	S	S

R- Resistant S – Sensitive I – Intermediate

3.2 Quantitative Estimation of Bacterial Isolates

Table 3 and Fig 2 represents Quantitative estimation of total bacterial load /fly from isolated colony from *M. domestica*. Among all the three sites explored the highest load was found

to be in the Fishmarket followed by butchery and vegetable market. The p-value obtained from Chi-Square statistical analysis was less than 0.05 (p<0.05). Hence, the data is statistically highly significant.

Table 5: Antibiogram test result of gram-positive bacteria

	Sample	Bacteria	Clindamycin	Teicoplanin	Lomefloxacin	Moxifloxacin	Ampicillin	Cefaclor	Roxithromycin	Clarithromycin	Lincomycin	Sparfloxacin	Gatifloxacin	Linezolid	Vancomycin
1	B	*Streptococcus sp.*	*R*	*I*	*I*	*S*	*R*	*R*	*S*	*I*	*S*	*S*	*R*	*I*	*I*
2	BI	*Staphylococcus sp.*	*I*	*S*	*R*	*S*	*I*	-	*S*	*I*	*S*	*S*	*R*	*I*	*I*
3		*Streptococcus sp.*	*R*	*R*	*I*	*S*	*S*	*R*	*I*	*S*	*S*	*I*	*I*	*I*	*R*
4	F	*Staphylococcus sp.*	I	*S*	*S*	*S*	*S*	*I*	*S*	*I*	-	*I*	*S*	*S*	*I*
5	FI	*Streptococcus sp.*	*S*	*S*	-	*S*	-	*I*	*S*	*S*	*I*	*S*	*R*	*S*	*I*
6	V	*Bacillus sp.*	*S*	*I*	*R*	*R*	*S*	*S*	*I*	*I*	*S*	*S*	*R*	*I*	-

R- Resistant S – Sensitive I – Intermediate

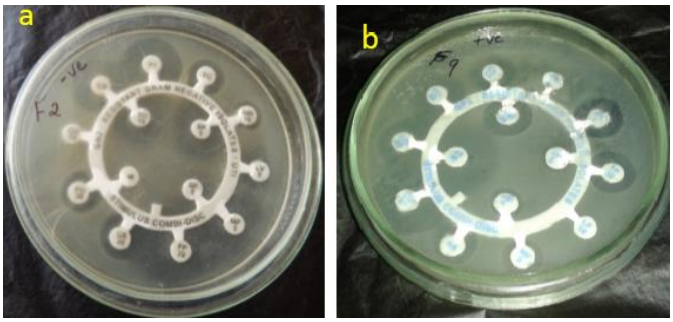
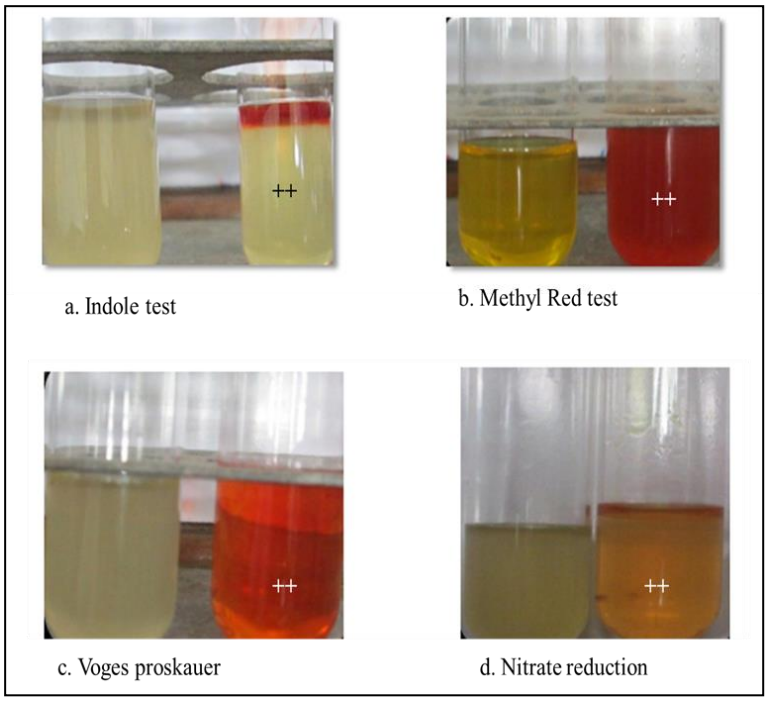


Fig 3: Colonies of Gram negative Bacteria (a) and Gram positive bacteria (b) isolated from house fly.



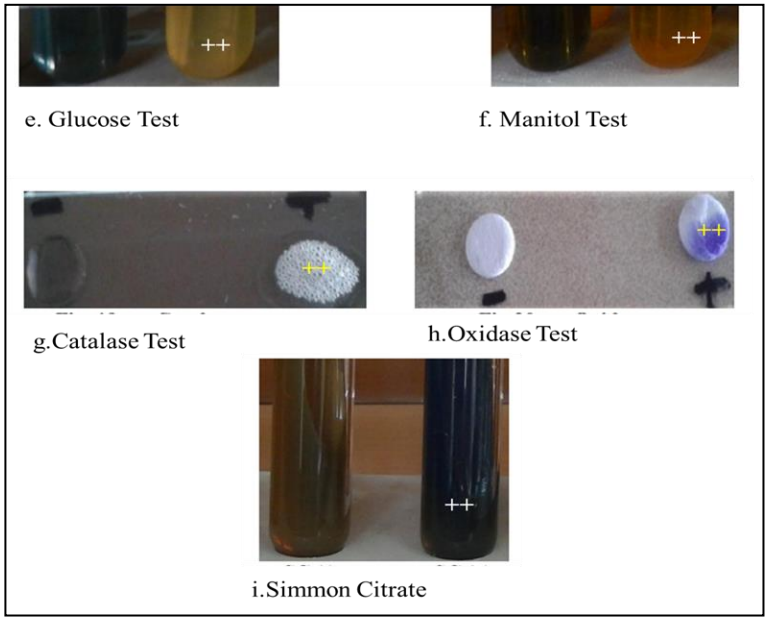


Fig 4 (a-i): Biochemical characterization of isolated bacteria using various digestive test. ++ depicts the presence of bacteria for that particular test.

Antibiogram test results for Gram –ve and Gram +ve are presented in Table 3 and 4. The antibiogram test was carried out to have a view on resistivity and sensitivity of pathogenic bacteria. The analysis of multiple drug resistance test (Table 4, 5 and Fig 3) shows Genus specific differences. All the class of antibiotics like which affects the cell wall synthesis, protein synthesis and nuceleic acid synthesis were selected for to screen the antibiotic resistance of gram negative strain. The bacteria were classified according to the sensitivity of it towards antibiotic as Sensitive, Intermediate and resistant. The isolated bacteria were showing resistance to a certain class of antibiotics, but the majority of them were sensitive to the most of the antibiotic groups and others were intermediate. Intermediates can become resistant by the continuous exposure to the same antibiotic. However, the results obtained also suggested that pathogenic load like of *E. coli*, *Pseudomonas* sp, *Enterobacter* sp and *Streptococcus* sp were more resistant to different class of antibiotics compared to other gram negative bacteria.

4. Discussion

M. domestica frequently comes into contact with human food and excrement and has been reported to be involved in the dissemination of numerous diseases. The close association of the housefly and bacteria, and its role in transmission of pathogens, makes it an ideal model organism to study the importance and variation of the microbiota of vector species [12]. Meat and vegetables are the major source of food on which the human beings commonly rely. There are different studies which assert houseflies as the potential vector to carry the microorganism externally and internally. There are varied reasons that make *M. domestica* a potential carrier of microorganisms[13]. House flies have microscopic hairs on every part of the body excluding the eye and these bristles make them the perfect carrier for bacteria and also pollen. It is an evolutionarily optimised vehicle for the dispersal of microorganisms in the environment [14]. The presence of hairy structures on observed is the major causes for the microorganisms getting attached externally. The previous study results also confirm that *M. domestica* carries the microbes internally through the salivary flow system and the digestive system [15].

In the present study, it is observed that the bacteria isolates recovered from *M. domestica* were both Gram positive and Gram negative bacteria, our observations goes on accordace with the previous study done in the poultry farms of Malasiya in which the researchers have observed bacterial genera such as *Bacillus* sp., *Escherichia* sp. and *Klepsilla* sp. [16, 17], *Staphylococcus* sp., *Bacillus sp*. and *Escherichia coli* cause diarrhoea which has been found from external and internal parts of house fly. *Klepsiella sp*. is causative agent of pneumonias and some hospital acquired infections [16, 18]. Surface infection of housefly by *Klepsiella* with inoculating rate up to 38% was the most common bacterial inoculation on housefly collected in food stalls, the wet market and rubbish dumping site in Malaysia [17, 19].The results of the current study confirm that flies are much more than a nuisance and that they pose potentially serious health risks as mechanical vectors [20, 21].

The data of Mile and Misra's [11] method for the viable count (table 3) unveils the enormousness of the bacterial colonies in the *M. domestica*. The present study reveals that the more bacterial load was found from house fly present at the fish market followed by butchery and vegetable market. This is due to the fact that housefly is attracted more to fish because of moist/slimy secretion present on the fish. The collected samples of housefly were loaded with both pathogenic and non-pathogenic microorganisms. The possible reason may be due to the structural compatibility of housefly which makes itself a facilitative carrier of pathogenic and non-pathogenic microorganisms.

Antibiograms of different bacterial isolates depicts the signal of multiple drug resistance (MDR) due to the varied reasons. The research study reports have affirmed that the widespread use of drugs for pest management and preservation are one of the major reason behind the MDR of bacteria [22]. The heavy use of antibiotics in livestock production contributes to resistance development and a growing reservoir of the resistant enterococcal population [8, 23]. Majority of bacteria that cause vegetable decay are a possible agent for the human infections [24]. The present result implies a indirect use of antibiotics for disease residential area and growth

enhancement cause drug resistance in bacteria. According to Wegener, *et al.*, [25]. Most of the drugs that exist in fodder are analogous to the drug used for the human medication. However, the development of antibiotic resistance among clinical isolates as well as commensally bacteria causes a great concern because of the potential dissemination and horizontal transfer of antibiotic resistance genes in the environment, primarily from the agricultural to urban environments [26, 27].

Hence, the study unveils the presence of MDR bacteria in the area where the dumping of waste and open drainage outlets occur. In addition, the housefly samples collected in this study were from the locations adjacent to the municipal hospital, therefore *M. domestica* is prone to spread the nosocomial as well as multiple drug resistant infectious bacteria. In fact, the breeding and spread of housefly normally occur in these unhygienic ecological conditions and is a potential carrier for vector borne disease.

5. Conclusion

The present study concludes that the ubiquitous presence of different bacteria are associated with possible breeding sites of flies *M. domestica* in unhygienic sites and is considered to be potential carrier as vector for infectious pathogens. Both the surfaces of house fly were carrying the pathogenic load of which bacterial load was accounted more on external surface. Among all the gram negative bacteria *E. coli*, *Pseudomonas* sp, *Enterobacter* sp and *Streptococcus* sp were found to be more prevalent and more resistant to different class of antibiotics. With such emphasis given to flies as a mechanical vector in spread of disease and the alarming fact in this study reports that some of the isolated colonies exhibited multiple drug resistance in the combi-disc analysis. Moreover, the presence of flies indicates the sanitary deficiency and unhygienic condition which may leads to human health problems. Hence, it is advisable to eliminate possible breeding sites for flies for which suitable and applicable control methods such as environmental sanitations should be implemented and flies should be prevented from gaining access to contaminate human material.

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