ISOLATION AND CHARACTERISATION OF ANTIBIOTIC RESISTANT BACTERIAL SPP. FROM THE GUT OF HOUSE FLY (MUSCA DOMESTICA)

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Abstract

House Flies (*Musca domestica*) are one of the most abundant and important group of order Diptera serving as vectors for some of the most devastating disease affecting humans. The gut of houseflies is harboured with microbes that act as pathogen in transmitting illness. Antibiotics are used to combat infectious diseases. But gut flora of *Musca domestica* have developed antibiotic resistance property for survival adaptive features. Most commonly found flies were inherited with microbiome showing resistance to antibiotics. Examination of antibiotic resistance bacteria in the gut of *M. domestica* revealed the presence of both Gram positive and Gram negative isolates like Staphylococcus ssp, Escherichia coli, Proteus spp, Bacillus spp. and Klebsiella spp, Analysing the disc potency of antibiotic susceptibility by the isolates recorded a higher range for the antimicrobial agents for Cefotaxime and Amikacin than other antibiotics Ampicillin, Gentamicin and Streptomycin. This was in conformity that houseflies were able to spread diseases showing resistance for antibiotics thereby acting as long term vectors of potential human diseases.

Keywords: *Diptera, House fly, Antibiotics, Antibiotic resistance bacteria*

Introduction

House flies (*Musca domestica*) are common insects of dipteran order in everyday life of human beings. The higher Dipteran order includes the family Muscidae whose well

known members are commonly termed as Flies. House flies reproduce and develop in dead and decaying organic matter. and many common infections are transmitted by them. Some diseases are very serious and even potentially deadly. They are mechanical carriers of microbial organisms spread infectious diseases and simply act as vector in many instances. There are many pathogens that are associated with house flies such as viruses, bacteria, protozoa and fungal organisms. Unlike other insects, such as mosquitoes or ticks, these insects do not specifically require an endogenous environment to live and reproduce. The gut microbiome of housefly serves as the ways and means for disease transmission. Although it can never be eradicated entirely, reducing fly populations and limiting its contact with food in particular is an important part of hygiene.

Systematic Position of Housefly

Kingdom :Animalia
Phylum : Arthropoda
Class : Insecta
Genus : *Musca*
Species : *domestica*

Diarrheal illnesses are some of the more common diseases spread by house flies. The bacterial species such as *E.coli*, *Shigella*, *Campylobacter*, *Enterococcus* which commonly cause diarrheal diseases are found to be associated with gut of houseflies and found in the stool of infected population. More over houseflies have developed antibiotic resistance property in their evolution. Gaining the antibiotic resistance property make them to affect people at any stage of life, as well as the healthcare, veterinary and agriculture industries making it one of the world's most urgent public health problems.

Antimicrobial resistance is the ability of a microbe to resist the effect of medication that once could successfully treat the pathogenic microorganisms. Antibiotics are medicines used to prevent and treat bacterial infections (M. Peteridis et al, 2006). Each year in the India at least 2.8 million people are infected with antibiotic resistant bacterial species and more than 35,000 people are succumbed to death. Antibiotic resistance occurs when bacteria change their response to combat the effects of these medicines.

Therefore analysis of housefly gut flora will give a better understanding to limit the infection.

MATERIALS AND METHODS

SAMPLE COLLECTION

The flies were collected at Dindigul and Vadamadurai including tea shop, kitchens and other public places. The target sites were kitchen area after the preparation of cooking when there is the highest availability of the waste product. A total of 40 flies were collected from these 4 sites during the month of November- December 2021 .Sterile insect collection net without any bait was used to collect the flies. The flies were morphologically identified using the literature. They were transferred to sterile vials aseptically and kept in freezing temperature for couple of hours to anaesthetize.

ISOLATION OF MICROORGANISMS FROM FLIES

Each fly was suspended in 1ml of sterile saline and vortexed gently to remove any adhering debris and microorganisms. Then the flies were surface sterilized in 70 % ethanol for 60 seconds followed by rinse with sterile saline two times. After that each fly was crushed in the saline solution with sterile tips. This suspension was serially diluted from 10^{-1} to $10^{-6}$and 100 µl of sample from each dilution was spread on Nutrient agar (pH 7) media through spread plate method for the identification of bacteria. (**Table. 1)**. To culture the bacterial colonies, plates were incubated at 37ºc for 24 hours and then observed.

IDENTIFICATION OF ISOLATES

After incubation, according to the dilution factor of the inoculated samples organisms were identified based on the colony morphological characteristics. Representative bacterial colonies were selected and isolated using standard pure culture techniques. These colonies were streaked on nutrient agar plates for pure colony isolation. From the pure culture, bacteria were presumptively identified through a series of conventional morphological, cultural and biochemical tests (**Table. 2)** according to the criteria described in Bergey's Manual of Determinative Bacteriology (1923).

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF THE ISOLATED ORGANISMS:

To identify the resistance and susceptibility pattern of the isolated microorganisms, antibiotic sensitivity test was performed with selected antibiotics. Single disc diffusion method was performed for this purpose. Standard antibiotics of Ampicillin (10 mg), Amikacin (30 mg), Gentamycin (10 mg), Streptomycin (10 mg), Ceftriaxone (30 mg) and Cefotaxime (30 mg), was placed in the Mueller Hinton agar plates with bacteria inoculated and incubated at 37^{o}c for 24 hours. The diameters of inhibition zones were measured and compared with the standards given by CLSI (The Clinical & Laboratory Standards Institute). The width zone of diameter indicates the ability of the antibiotic to prevent the bacterial growth.

RESULTS AND DISCUSSION

House fly 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 with 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.

In the present study, it is observed that the bacteria isolates recovered from *M. domestica* were both Gram positive and Gram negative species like Staphylococcus spp. Escherichia coli, Proteus spp. Bacillus spp. and Klebsiella spp. (**Table. 1).** Our observations goes in accordance with Thomas et al., (2005) where bacterial genera such as *Bacillus* spp. and *Staphylococcus* spp. were found to occur in gut and external parts of house fly. De Jesus AJ et al. (2004) who conducted a study on house flies reported that the most prevalent type of bacteria reported from the gut of the house flies was *E.coli.* However, this finding is not in line with what we observed in the present study. This contradiction can be attributed to the different sampling places from where the flies were captured for analysis. It can be concluded that the type and number of bacteria harboured by house flies differs to a high degree with the sampling areas from where these flies were captured. It was explained by Akhtar M, (2009) that house flies were important vector in carrying of various types of bacteria particularly *Staphylococcus* spp. and *Bacillus* spp. which is in agreement with the results of the present study.

The term antibiotic resistance is a subset of antimicrobial resistance, as it applies to microorganisms becoming resistance to antibiotics. Analyzing the diameter of inhibition

zone in Antibiotics Sensitivity Test by the bacteria isolates from the gut of Housefly revealed that the Ecoli showed susceptible reaction to the exposed antibiotics (Liu Y etal, 2013). Likewise, the staphylococcus spp. recorded the resistance mode of response to the experimented antibiotics (**Table. 3).** Then the disc potency exhibited by the bacteria isolates from gut environment of flies were found to be higher for the antimicrobial agent Cefotaxime and Amikacin than other antibiotics Ampicillin, Gentamicin and Streptomycin **(Fig. 1).** The higher disc potency was due to the broad-spectrum antibiotics showing a counter effect to maximum gut isolates.

Houseflies containing bacteria have developed resistance to antibiotics that were once commonly used to treat them. For example, *Staphlococcus* sp, and *Neisseria* sp, are almost resistant to benzyl penicillin. In the past, these infections were usually controlled by penicillin. Antibiotic resistance happens when bacteria develop the ability to defeat the drugs designed to kill them (Burrus V and Waldor MK, 2004).The antibiotic resistance property of flies leads to higher medical costs; prolonged hospital stays and increased mortality. However, the important point is that all these studies have emphasized that house flies are important carriers of pathogenic organisms and we should take precautionary and safety measures from housefly infection.

CONCLUSION

Houseflies are perennial organism in transmitting diseases, affecting our health. Analyzing the gut bacteria revealed the presence of cocci bacteria and the bacterial population were able to survive and even multiply in the presence of broad spectrum antibiotics. On conclusion houseflies were found to inherit resistant property for antibiotics thereby they proliferate in large numbers serving as vectors in most of the environment.

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Table: 1. IDENTIFIED BACTERIAL ISOLATES FROM THE GUT OF HOUSEFLY

Sl.No	Spotted pure isolates	Identified organism
1	G1	Staphylococcus spp.
2	G2	Escherichia coli
3	G3	Proteus spp.
4	G4	Bacillus spp.
5	G5	Klebsiella spp.

G- Different bacterial Isolates

Table: 2. BIOCHEMICAL CHARACTERIZATION OF BACTERIAL ISOLATES FROM THE GUT OF HOUSEFLY

S.No	Testes	G1	G2	G3	G4	G5
1	Simple staining	Cocci	Cocci	Cocci	Cocci	Cocci
2	Gram's staining	-ve	-ve	-ve	-ve	-ve
3	Indole test	+ve	+ve	-ve	-ve	-ve
4	Methyle Red	+ve	+ve	-ve	+ve	-ve
5	Catalase test	+ve	+ve	+Ve	-ve	-ve
6	Simmons citrate agar test	+ve	+ve	-ve	+ve	-ve
7	Urease test	+ve	+ve	-ve	+ve	-ve
8	Starch hydrolysis test	+ve	+ve	-ve	+ve	-ve
9	Nitrate test	-ve	+ve	+ve	-ve	+Ve

G- Different bacterial Isolates , + Positive , - Negative

Table 2. ANTIBIOTICS SENSITIVITY TESTING BY DISC DIFFUSION OF BACTERIA ISOLATES FROM THE GUT OF HOUSEFLY.

S.No	Antimicrobial Agent	Disc potency (µg/piece)	Staphylococcus spp.	Escherichia coli	Proteus spp.	Bacillus spp.	Klebsiella spp.
1	Ampicillin	10	I	S	S	I	R
2	Gentamicin	10	R	S	I	S	NIL
3	Streptomycin	10	I	I	S	R	NIL
4	Ceftriaxone	30	I	R	R	I	I
5	Cefotaxime	30	R	S	I	R	I
6	Amikacin	30	R	I	R	NIL	S

R= Resistance S= Sensitive , I= intermediate. R,S and I were determined after in vitro susceptibility test

Fig. 1. ANTIBIOTIC SENSITIVITY TEST OF GUT MICROBES OF HOUSE FLY