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Evaluation of Antibacterial Activity of extract of housefly, *Musca domestica*

KEYWORDS: Maggots, housefly, debridement, antibacterial activity.

SHAGALOLU V.V
D.B.F.Dayanand College of Arts and Science, Solapur, Maharashtra, India

DESPANDE S.N
D.B.F.Dayanand College of Arts and Science, Solapur, Maharashtra, India

BHIKSHAPATI , E.
Centre for Cellular and Molecular Biology, Hyderabad, India

RAO K.R.
Walchand College of Arts and Science, Solapur, Maharashtra India

ABSTRACT *Houseflies are the most common insects and they survive in almost all extreme environmental conditions. It harbors enormous bacterial flora over its body. They successfully survive even in living in very abrasive conditions without any effect on them. They breed in garbage, compost, unhygienic conditions, animal manure etc. They are responsible for transmission of various diseases like dysentery, cholera and other diseases. However, the maggots (larval stages) of houseflies are recently used for treating chronic infected wounds successfully as a debridement therapy. Therefore, it is interesting to know the antibacterial activity of extract of houseflies, hence the present investigation was undertaken to know the native microbial flora from adult and its larvae. And also the study was extended to know the role of the houseflies in prevention of bacterial infections. Antibacterial activity was determined by Agar diffusion method. The bacterial pathogen used for the present study showed inhibitory action for the larval extract of houseflies, Musca domestica.*

Introduction:

Houseflies are the most common insects found in the domestic area. The adult housefly insects are dark molted grey and measures about 7 to 13 mm in length and when stretched with wings from both the sides, it measures 13 to 15 mm across wings. Schiner (1864) has studied extensively the morphological characters of housefly, *Musca domestica*. The body is covered with hair like projections. In the males, eyes are narrowly separated from frontal strips, whereas in females the frontal stripes are widened. The space between eyes in females is about one third of the width of the head. Houseflies act as mechanical vectors of various bacterial pathogens, which may cause diseases like typhoid, cholera, salmonelosis, bacillary dysentery, tuberculosis, anthrax etc. (Majpuria, 1969). Insects live in various habitats which are resided with an extremely large quantity of pathogens. Insects have extremely effective physicochemical barrier as first line defense (Peter *et al*., 2005). They develop host defensive mechanism to counteract microbial infections and they offer powerful resistance against them (Hoffman *et al.,* 1996).It was noticed that various pathogens inhabit in and around various insect (Lynsenko 1985). The most common housefly is the *Musca domestica* which is being extensively studied by various workers especially entomologist. It is considered as a pest because it spreads various diseases. Arora *et al.,* (2001) have studied the effect of antimicrobial activity used for treatment with multidrug resistant *Staphylococcus aureus.* In their study they have demonstrated antibacterial activity in secretions of maggot of *Lucelia cuprina.* Simmons (1935) and Bexfeild *et al.,* (2004) studied the antimicrobial actions in excretions of surgical maggots in various infections. Hubberman *et al.,*(2007) and Daeshcaelien (2007) have studied the secretions of blowfly maggot against gram positive and gram negative bacteria. Jacklic *et al.,* (2008) have studied the antimicrobial activity of maggots against pathogenic bacteria. Hou *et* al., (2007) studied antimicrobial activity of extracts of larvae of housefly *Musca domestica*. Yan et al., (2009) studied expression pattern of *Musca domestica*.

In the present study bacterial surface flora was studied and identified by standard methods. The present study was also focused on evaluation of antibacterial activity of housefly, *Musca domestica* against representative bacteria.

Materials and Methods

Collection of Housefly

Houseflies were collected from poultry manure from local area Bhawani peth, Solapur.

Isolation and identification of bacterial flora from adult housefly body surface and its larvae:

Collected houseflies and its larvae were taken in sterile glass bottle containing sterile saline and well shaken for five minutes. 0.1ml of serially diluted saline suspension was aseptically spread on sterile nutrient agar medium. The plates were incubated at 37°C for 24 hours and bacteria were identified by standard methods.

Preparation of adult housefly its larval extract:

Adult flies were anesthetized with ammonia and individually dissected out the legs, wings and head region with the help of sterile forceps. The flies without legs wings and head were homogenized in saline solution. The house fly larvae were homogenized in sterile saline solution for 3 minutes and the extract was used for antibacterial activity.

Antibacterial activity of housefly and its larval extract:

The antibacterial activity of housefly and its larval extract against *Staphylococcus aureus* and *Eschericia coli* was performed by agar well diffusion method. 0.1 ml of the suspension of test organism was spread on sterile nutrient agar media. The wells were bored by sterile cork borer. 0.1 ml original extract of the adult housefly and the larvae of housefly were taken separately for the antibacterial testing.

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The dilutions of 1:2, 1:4 and 1:8 were added in each well, and allowed to diffuse. Phosphate buffer in one of the wells was used as negative control and Gentamycin was kept as positive control. After diffusion of the extract, the plates were incubated at 37^0 degrees for 24 hours. Zone of inhibition was observed after overnight incubation around the wells.

Results:

Bacterial flora of adult housefly body surface:

Different types of bacterial colonies were observed on nutrient agar after overnight incubation, suggesting the presence of bacteria on housefly body surface of adult housefly and larval body.

Figure 1 (a) indicates bacterial flora of adult housefly

Figure 1 (b) indicates bacterial flora of larval body.

Representative colonies were selected for further purification and identification of bacteria. Both body surfaces of adult housefly and larval body showed the presence of *E.coli, Staphylococcus aureus, Micrococcus species and Bacillus subtilis.* The morphological, cultural and biochemical characteristics of these identified bacteria are depicted in Table 1, Table2, Table3, respectively.

Table 1: Morphological characters of bacterial isolates obtained from body surface of house fly [Musca domestica]

Colony code	Shape of bacteria	Gram nature	Motility	Sporulation	Bacterial species identified
C1	Rod	Gram negative	Motile	Non sporulating	E.coli
C2	Spherical	Gram positive	Nonmotile	Non sporulating	S. aureus
C3	Spherical	Gram positive	Nonmotile	Non sporulating	Micrococcus species
C4	Rod	Gram positive	Motile	sporulating	Bacillus subtilis

Table 2: Cultural characters of bacterial isolates obtained from body surface of house fly [Musca domestica]

Colony code	Size [mm]	Shape	Colour	Margin	Opacity	Elevation	Consistency	Bacteria species identified
C1	1	Circular	Cream	Regular	Translucent	low convex	Moist	E.coli
C2	1	Circular	Golden yellow	Regular	Translucent	Low convex	Moist	S. aureus
C3	1	Circular	Light yellow	Regular	Translucent	Low convex	Moist	Micrococcus species
C4	2	Circular	Buff	Irregular	Opaque	Flat	Dry	Bacillus subtilis

Table 3: Biochemical characters of bacterial isolates obtained from body surface of house fly [Musca domestica]

Code of colony	Glu	Lac	Suc	Man	I	MR	VP	CU	H_2S	CAT	UH	GH	NR	SH	COA	Bacteria species identified
C1	+	+	+	+	+	+	-	+	-	+	-	-	+	ND	ND	E.coli
C2	+	+	+	+	-	+	+	-	-	+	-	+	+	ND	+	S. aureus
C3	+	+	+	+	-	-	-	-	--	+	-	-	-	ND	-	Micrococcus species
C4	+	+	+	+	-	-	-	-	-	+	-	-	+	+	ND	Bacillus subtilis

ND= not done, "+" = positive test, "-"= negative test

Glu=Glucose fermentation, Suc=Sucrose fermentation, Man=Mannitol fermentation, Lac=Lactose fermentation, I=indol production test, MR=methyl reduction test, VP=voges proskauer test, CU=citrate utilization test, H2S=H2S production test, CAT=catalase production test, UH=urea hydrolysis test, GH=gelatin hydrolysis test, NR=nitrate reduction test, SH=starch hydrolysis test, COA =coagulase test

Antibacterial activity of adult housefly and its larval extracts:

As shown in figure 2, in the present study the adult housefly extract did not show zone of inhibition against the test organism [*Escherichia coli* and *Staphylococcus aureus*]. This clearly indicates that adult housefly extract did not have antibacterial activity. However, the antibacterial activity was revealed against larval extracts with significant zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*

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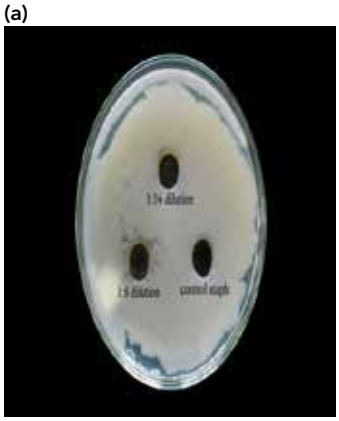
as shown in fig 3 (a) and (b), respectively.

(a)



(b)

Figure 2: Antibacterial activity of adult housefly extracts against (a) *E. coli* **(b)** *S. aureus*.

(a)

(b)

Figure 3: Antibacterial activity of house fly larval extracts against (a) *E. coli* **(b)** *S. aureu*

Discussion:

Houseflies are constant visitors to several areas like refuse dumps, toilets, human and animal wounds, domestic waste and other areas of poor sanitary conditions. Wide range of microorganisms is associated in these environments. It is therefore quite evident that there is occurrence of bacteria, fungi and parasites on the body surface of houseflies. In the present study, when the bacterial flora of housefly body surface was studied, it revealed the presence of *Staphylococcus aureus,Bacillus subtilis, Escherichia coli and Micrococcus species*.Mawak and Olukose (2006) analyzed the bacterial pathogens and parasites by using standard microscopic and cultural methods and reported the presence of eight parasites and bacterial pathogens including *Staphylococcus aureus, Escherichia coli,Sal monella,Klebsiella,Shigella, Streptococcus,Pseudomonas* and *Proteus* species. Banjo *et al.,* (2005) studied bacteria and fungi associated from housefly *Musca domestica* larvae and reported the presence of *Staphylococcus aureus, Streptococcus,Pseudomonas* and *Bacillus* species. Though different bacterial species were found to be associated with body surface of housefly, the occurrence of bacteria with body surface of housefly in the present study is consistent finding with Banjo *et al.,* (2005) and Mawak and Olukose (2006).

Jaklic *et al.*, (2008) studied the antimicrobial activity if maggots against pathogenic bacteria and noted that the larval therapy of *Lobelia seriata* can be used to treat bacterial infections. Hou *et al*., (2007) also reported antibacterial activity of extracts of larva of *Musca domestica.* The result of present study is consistent with the findings of Jaklic *et al*., (2008) and Hou *et al.,* (2007)

Conclusion

The present study concludes that the adult housefly and its larvae on body surface carries different bacterial species like *E.coli, Staphylococcus aureus, Micrococcus species and Bacillus subtilis.* The present study also concludes that the housefly larval extract shows significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* as compared to adult housefly extract suggesting the use of larval extracts of housefly for the treatment of chronic ulcers, injuries and wound infections caused by bacteria which is known as maggot debridement therapy. However, further investigation is necessary for practical applications of extracts for the said therapy.

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