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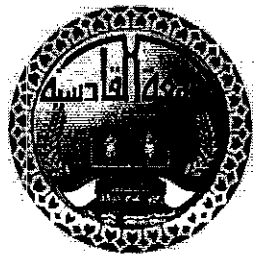
College of Veterinary medicine



Isolation of Staphylococcus aureus from house fly

By

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"بسم الله الرحمن الرحيم"

"وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّن الْعِلْمِ إِلاَّ قَلِيلاً"

"صدق الله العلي العظيم"

أية الإسراء (٨٤)

Certificate of Supervisor

I certify that the research entitled

(Isolation of *Staphylococcus aureus* from house fly) was prepared under my supervision at the college of veterinary medicine / University of Al-Qadissiya .

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Certificate of Department

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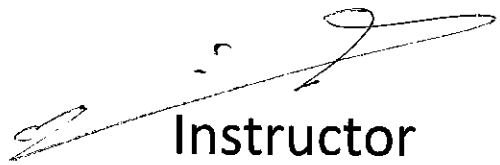

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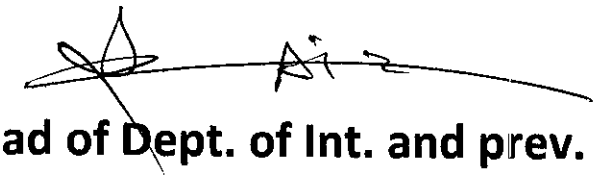

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Roaa

Dedicate

Every challenging word needs self-effort as well as guidance of elders especially who were very close our heart .

My humble effort I dedicated to my sweet and loving

Father and Mother

Whose affection , love , engorgement and prays of day and night make me able to get such success and honor .

Along with all hard working and respect

Dr. Asaad Jassim

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Summary

The study was conducted in teaching station of animal of the college of veterinary medicine of Al-Qadyssiah university, aimed for isolating of *Staphylococcus aurous* from flies near of animals. post hunter of fly by net and cultured on ordinary media which 4 isolates (13.33%) of bacterium was found. All isolates grew on mannitol salts agar, gave positive results of catalase, and negative for oxidase test, Gram positive cocci with formation of clusters. It β-hemolysis of sheep blood on blood agar. And all isolates fermented glucose and lactose.

1-Literature review

1-2:Staphylococci bacteria

1-2-1:Definitions:

Staphylococcus is a genus of family Micrococcaceae , which the spherical Gram - positive cells occur singly , in pairs and in irregular clusters , they are non-motile (Michael, 1998) . Staphylococci (from Greek, Staphyle, and bunch of grapes) owe their name to the fact that they appear to be clustered as a result of division, (Gabriel, 1997). The average size of individual cell is from 0.7 to 1 micrometer, they are Gram positive, non-motile, non-capsulated and do \ not form spores (Bigger,1949), while (Rupp, 1994) stated that Staphylococci are Gram positive one micrometer in diameter , they form clumps.

The cocci sheds by the patients and carriers contaminate hander kerchiefs, bed linen and blankets and may persist on them for days and weeks (Namita, 2003). Staphylococci is the short name for the bacteria which can live harmlessly on many sk in surfaces, especially around the nose, mouth, genitals and anus, but when the skin is punctured or broken for any reason , Staphylococci can enter the wound and cause an infection (Crumpton, 2007).

1-2-2:General characteristics:

They are Gram - positive cocci avoided or spherical, non motile, the hemolytic capacity is available, pat hogenic strains ferments sugars, lactose, Mannitol, with acid production, also produces pus in lesion (Statish,2000). The genus Staphylococcus consist of gram positive cocci that grow in grape like clusters , are aerobic and fucltativly anaerobic produce catalase (Namita, 2003).

1-2-3:History:

Staphylococcus was first seen in human pyogenic lesions by Reckinghausen in 1871, but Sir Alexander Ogeston a surgeon from Scotland established the ro le of the Coccus in an abscess and give the name Staphylococcus , Staph means a bunch of grape and kokks means berry (Namita, 2003). First seen in pus by Koch in 1878 then Pasteur cultivated them for the first time in 1880, named by Alexander Ogeston 1881 (Statish, 2000).

There are 88 species of Staphylococci, researchers and surveillance continue for discovery of other new species (Statish, 2000). Staphylococciare widely distributed in environment; they form part of the normal microbial flora of the skin, upper respiratory tract, and intestinal tract (Monica, 1991).

They are found mainly in or on the bodies of mammals (Michael, 1996).Staphylococciare widely distributed in the environment, they form part of the normal microbial flora of the skin , upper respiratory tract and intestinal tract (Monica, 1991). Species of Staphylococciare associated with skin, skin glands and mucous membrane of warm blooded animals (Roy, 2000). They can also be found in the tips of the fingers, most commonly on the index finger as well as the thumb (Ryan, 2004) Staphylococci are found in a variety of animal products such as meat and dairy products (Roy, 2000).

In 20 to 30 percent of healthy persons, however *Staphylococcus aureus* occurs as a harmless communal in the anterior nares and on moist areas of the skin , such individuals who carry the bacteria and do not suffer from the disease are called carriers others carry it in other parts of the body without becoming ill, include the nostrils, mouth and throat, the

breasts, and the genital, urinary and upperrespiratory tracts (Monica, 1991).

1-2-4:Pathogenicity:

Many species of Staphylococcican be pathogenic causing a variety of conditions (Michael, 1998). Staphylococcus is a group of bacteria that can cause a multitude of diseases as a result of infection of various tissues of the body (Melissa, 2009).Three species are pathogenic in human and animals; *Staphylococcus aureus,Staphylococcus epidermidis* and *Staphylococcussaprophyticus* (Gabriel, 1997). Staphylococci are generally harmless unless they become very heavily concentrated on skin surface or they are able to enter the body through a break in the skin (Crumpton, 2007). *Staphylococcus aureus* is commonly carried on the skin or nose of healthy people , some cause an infection (Namita, 2003). It is the main species of medical important and associated with many diseases in human and animals (Monica, 1985). *Staphylococcus aureus* is a gram positive, which mean that the cell wall of the bacteria consist of a very thick peptidoglycan layer, they are spherical, form clusters in 2 planes and have no flagella (Crumton, 2007). Pathogenic Staphylococci are ubiquitous, they are carried usually in the anterior nacres of about 20% of healthy adults and on the skin of about 20% , of population are long-term carriers (Kluytman, *et al.*, 1997). Staphylococci are divided on pigment formation in to : *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*, Staphylococcus is lemon – yellow (William, *et al.*, 2009). Many strains of Staphylococci have developed resistant to penicillin and other antibiotics (Vinay, 1997). Many strains of *Staphylococcus aureus* are multi-resistant to antibiotics and are of increasing important, MRSA strains have caused major out breaks world – wide (Kuroda, *et al.*, 2001). Route of infection: Infection

by Staphylococcus occur through the skins and the mucous membrane by air – drop and air – dust way, so to be infectedit is possible through the open wounds, the burns, the eyes, the skin, the blood, also it is possible the transfer of infection with the tods, the catheters, the surgical dressing, the object of withdrawal and also the food (William, et al., 2009). Most infection are minor and causes sores such as pimples and boils , these infections can become worse in some patients if untreated , causing redness , swelling and pus (William, et al., 2009). Staphylococci can cause a wide variety of diseases in human and other animals either through toxin production or invasion (Ryan,etal.2004). Staphylococci infection is a well known infections, which is caused by one of the Staphylococcal bacteria, the majority of these infections are due to Staphylococcus aureus (Melissa, 2009). *Staphylococcusaureus* produce a large number several extracellular toxins (i.e., alpha, beta, gamma, and delta hemolytic toxins, and toxic shock toxin) and enzymes (i.e., lipase, nuclease, protease and hyaluronatelyase) (Abdelnour, 1993). The role of each individual factor and to overcome in, entero in virulence is not fully under stood (Jawest, et al, 1980), it is likely that they are responsible for establishment of infection, enabling of potential virulence factors, among these are the organism to bind to connective tissue, to resist killing by the bacteria activities of hormonal factors such as complement and to overcome uptake and intracellular killing by phagocytes.

1-2-5:Diseases caused by Staphylococcus*aureus*

In humen,*Staphylococcus aureus* most frequently causes diseases in humans in various suppurative (pus-forming) infections. It causes superficial skin lesions such as boils, styes and furunculosis; more serious infections such as pneumonia, mastitis, and urinary tract infections; and deep- seated infections such as osteomyelitis and endocarditis (Jarvis and Martone 1992; Ellis *et al*; 2003). *Staphylococcus aureus* is also a serious bacterial cause of food- borne infections. Staphylococcal food poisoning is one of the economically most serious food-borne diseases worldwide (Evensonet *al*., 1988; Bennett, 2005).

In animals, S. aureus can cause pustular inflammation of the skin and other organs, mastitis being the most serious (Nagase *et al*., 2002). *Staphylococcus aureus* is an important cause of mastitis in cattle, sheep and goats (Yazdankhah et al., 2001; Rodrigues daSilva*et al*., 2005). Staphylococcus intermedius causes pyoderma, staphylococcal pustular dermatitis, and otitis externa in dogs and cats. Staphylococcus hyicus;

1-2-6: Role of flies for transmission of bacteria

Flies are one of the largest and most divers order of insect andwith the medical and veterinary significant, so some of the true fly speciesaffect human and livestock health indirectly through disease transmission andtransmission of disease agent occur when diptera physically carry pathogenfrom one place or host to another host often via body parts that collectcontaminates as the insect feed on dead animals or excrement, and the mainpoint about the mechanical transmission in that the pathogen undergoes nodevelopment and no multiplication (Goddard*et al*., 2008).

Vazirianzadeh*et al.*(2008) mentioned that flies play important role mechanical vector for lots of pathogenic microorganism agents, including: bacteria, protozoa, worms, fungi and viruses amongst humans and animals.

Keeping in mind all of the above mentioned studies, the capability of the flies as a factor for spreading infections as compared to other ways of transmission, must be seen as a quantitative problem and depending on many factors. The intimate association between *Staphylococcus sp.* and the house fly was also observed by Labib (1990), where 136 isolates of this species were isolated from the house fly samples collected from four Egyptian hospitals. The following strains of Streptococci were isolated by Merdan and Allam (1974), *Streptococcus agalactiae, Strept. salivaius, Strept. lactis, Strept. equines, Strept. pyogenes*and *Strept. faecalis*. Also Umeche and Mandah (1989), arrived to the same observations. Cholera, the causative organism of which is Vibrio comma, was among the first disease in which house fly was incriminated as a vector. Though, flies have the mechanism and habits for the transmission of the tubercle bacillus, no conclusive work has established the relationships of the flies to such transmission (Fotedar, 2001; Nazni, 2005) . The importance of the house fly wings in mechanical transmission of vibrio cholera was discussed by Yab et, al (2008) because of the low transfer rate of the bacteria to wings.

More than one hundred pathogen associated fly species especially house fly disease to human and animal doso mechanically, The most common bacteria isolated were *E. coli*, Staphytococus,Streptococcus , Enterobacter, there bacteria were cause disease conditions inhuman and animals. typhoid fever Salmonella is a well knownentric disease andeffects human world, cholera is another entric disease of great

importanceShiyella, causing dysentery and diarrhea, and Escherichia coli causinguregental and intestinal infection are wide spread entric disease (Lane andCrosskey, 1993).

Chapter one: literature review

Chapter two: material and methods

The Material and Method

2-1:Materials:

2-1-1:Equipment and apparatuses:

NO.	Equipment and apparatuses	Company
1	Light microscope	Olympus (Japan)
2	Incubator	Memmert (Germany)
3	Autoclave	Trilp (Italy)
4	Sensitive electrical balance	Sartorius (Germany)
5	Oven	Yagami (Japan)
6	Hood	LabTech (UK)
7	Refrigerator	Kiriazi (Egypt)
8	Benzene flame	Sartorius (Germany)
9	Loop	Townson (Japan)
10	Disposable petri dish	China
11	Filter papers	GMBH (Germany)
12	Sterile cotton swap	Memmert (Germany)
13	Slide and cover slide	Beroslide (Germany)
14	Magnetic stirrer	HYSC (Korea)
15	Plastic test tubes	AFCO (Jordan)
16	Screw capped tubes	Hirschmann (Germany)
17	Distillator	LabTech (UK)
18	Glass beakers	Hysil (UK)
19	Wooden sticks	Supreme (China)
20	Tube racks	China
21	Latex gloves	Unimed (K.S.A)

2-1-2:Chemicals:

NO.	Chemicals and Solutions	Company
1	Ethanol 70%	B.D.H (UK)
2	Gram's stain set which include : Crystal Violet , Iodine , Ethanol , Safranin	England UK
3	NaCl	Thomas Baker India
4	Hydrogen peroxide	Fluka (Switzerland)

2-1-3: Culture media:

2-1-3-1:Blood Agar base:
it was prepared according to the company

2-1-3-2:Nutrient Agar
it was prepared according to the company

2-1-3-3:Mannitol salt Agar:
it was prepared according to the company

2-1-3-4:Nutrient Broth:

it was prepared according to the company

2-1-4:. Reagents:

2-1-4-1:Catalase reagent:

it was prepared according to the company.

2-1-4-2: Oxidase reagent:

it was prepared according to the company.

2-1-4-3: Gram stain:

it was prepared according to the company.

2-1-4-4: Peptone water:

it was prepared according to the company.

2-2:The methods

Sampling 30 housefly samples were collected in the field of Vet. Med. Col. Of Al-Qadiyssia. University. At the period extend from (Dec. 2015 – Feb 2016).

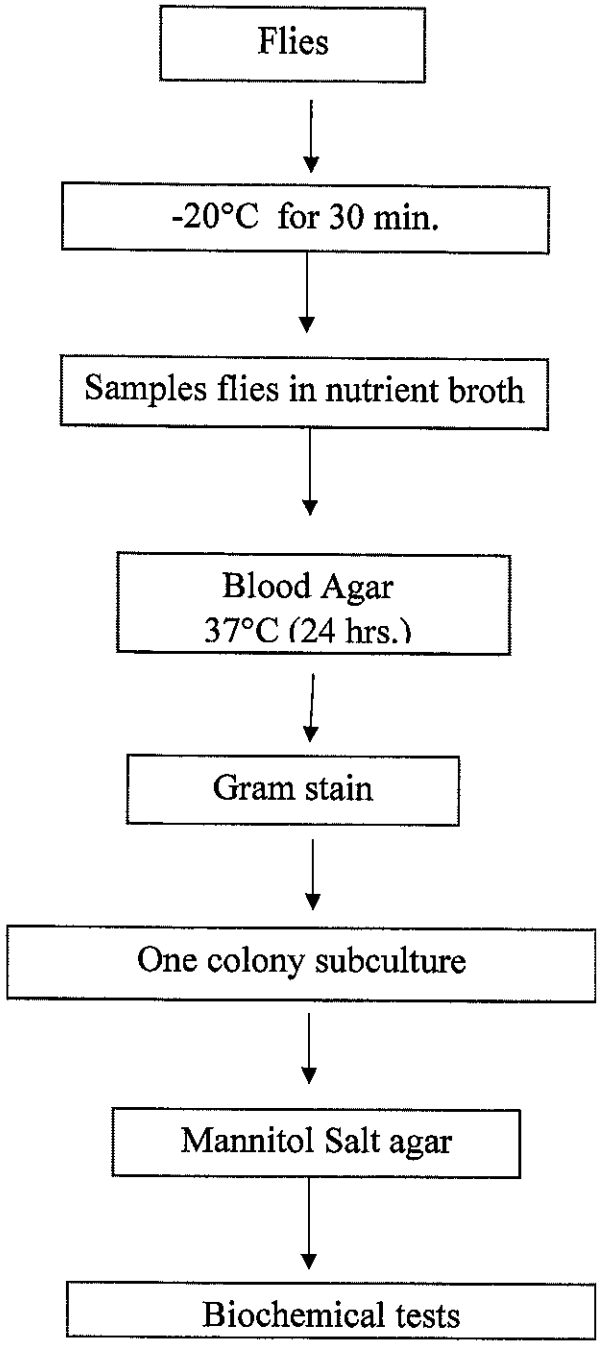
The Samples were captured from the field (flies that are near to the farm animal) by a sterile net then kept in a sterile plastic tubethen into a refrigerator at (-4° C) for half hour.

Bacterialculture according toSingh and Prakash(2008).all samples were cultured on nutrient broth incubated at (37° C) for 24 hrs. Loopfulsof super facial layers of turbid bacterial broth were streaked on blood agar overnight incubation at (37° c).The diagnosis depend on morphological characteristics (shape, color and size)of colony then examined via Gram stain.

Gramstain , a single colony was taken form blood agar by loop and spreaded on clean slide and fixed with heat and staining with Gram stain then examined the bacteria under microscope used oil immersion (Jawetz*et al.*,2007).

Biochemical testsof Staphylococcus bacterium were conducted according to Singh and Prakash(2008).

2-4:Design of experimental study.



1-4: Diagram showed the design of study

Chapter three: results

3-Results

A total of 30flies samples were tested and *S. aureus*was isolated from 4 (13.33%) samples based on cultural and biochemical properties. After subculture of bacteria, the bacterium give cluster gram positive cultured on mannitol salt agar medium, if it changes the medium which selective and biochemical were done, All the 4 isolates showed β-hemolysis on blood agar media enriched with 5% sheep blood. Gram-stained smears of the pure cultures exhibited clusters of Gram-positive cocci (Figure 1). The isolates also fermented mannitol with the color change of MSA (Mannitol Salt Agar) and production of small yellow colonies (Figure 2). These isolates were positive for catalase. In catalase test; Hydrogen peroxide was broken-down into water and oxygen. Production of oxygen was indicated by bubble formation, whereas the negative control did not produce any bubble .The biochemical characteristics were documented in table (3-1), all isolates gave positive results for fermentation of glucose , lactose and mannitol.

Table (3-1):show the biochemical characteristic of *Staphylococcus aureus*.

Sample	Grams stain	catalase	oxidase	Glucose	lactose	mannitol
1	+cocci	+	-	+	+	+
٢	+cocci	+	-	+	+	+
٣	+cocci	+	-	+	+	+
٤	+cocci	+	-	+	+	+
Other	G^+ and G^- bacilli	-				

Figure 1: Gram's staining of *S. aureus*(100X). Grapes like (black arrow) Gram positive cocci.

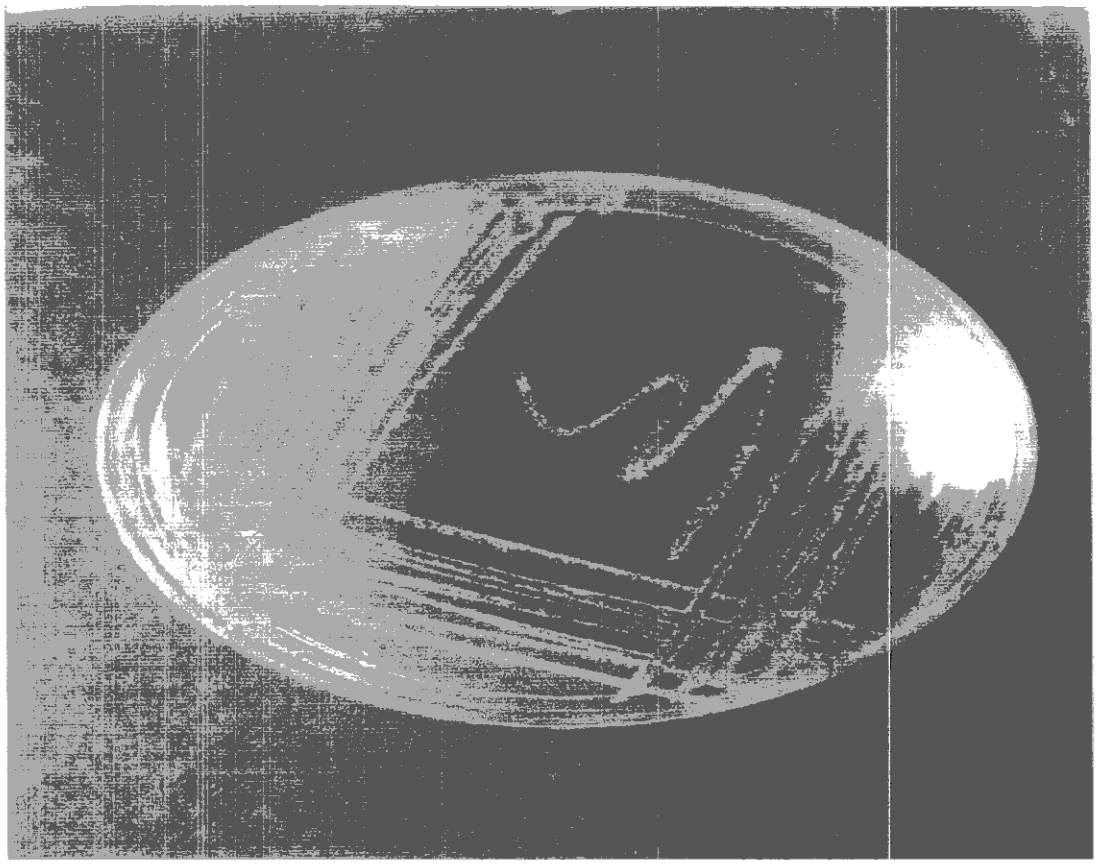


Figure 2: Fermentation of mannitol salt agar by *S. aureus.* Formation of yellow color colony (black arrow).

Chapter four: discussion

4-Discussion:

Many studies interested in pathogenic and non-pathogenic bacteria and trueflies, those non-biting flies have been found to carry a variety of pathogenicbacteria with potential for transmitted to human and animals, five species ofnon-biting muscidae, Calliphoridae and Sacrophagidae from the public marketand isolated microorganism associated with these flies, *Muscadomestica*maysettle and feed on decaying organic matters which contain many bacteria, if latter the fly a light on food for human composition and harmful bacteria are deposited on it (Butler*et al.*, 2010), they may adhene to the hair on the house fly leg or body and they may remain in the pseudotrachea or oesophagus, to be flushed out on the food which has come from a source of infection(Iwasa*etal.*, 1999).

The objective of the study is to isolate and identify the *staphylococcus aurous*that can be found on the housefly.The biology and ecology of Muscadomestica make it an ideal mechanical vector ofhuman and animal pathogens. Garbage, cattle barns, poultry houses, slaughter houses, and hospitals are sites where house flies can reproduce (Peter*et al.*,2007). This study showed that fourisolates of *Staphylococcus aurous* were isolated from flies, Our results are in accordance with other reports which highlight the importance of house flies in caring various pathogens (Ahmed *et al.*,2013) .

Many scientists indicated that the external organs of Muscadomestica legs, wings,and mouth parts) constituted a large source of bacteria they isolated (Graczyk*et al.*, 1999; Ahmed *et al.*,2013).

Nazni*et al.* (2005) have isolated *Bacillus sp.,Staphylococcus sp.* and *Micrococcus sp.* from faeces and, vomits of houseflies too other than from the external body of houseflies.

Lamiaa*et al.* (2007) have compared thetransmitted bacteria between houseflies and American cockroach, *Periplanetaamericana*. They have isolated *Staphylococcus aureus*, *Staphylococcusepidermidis*, *Streptococcus spp.*, *Salmonella spp.*, *Shigella spp.*, *Proteusvulgaris*, *Proteus spp, Serratia spp.*, *Klebsiella spp.*, *Enterobacter spp.* And *Escherichia coli* from the external surface of both insects. This is an evident forfurther study using different insects and different parts or secretions of these insects as dispersing bacterial diseases.

Sukantason, *et al.* (2007), studied the density of pathogenic bacteria from these diptera families the density on *Chrysomamegacephala*which found carry a high density of bacteria (Forty two species) as expected carried a greater number of bacteria than the housefly, the most common bacteria isolated from flies was *E. coli* that the dominal bacteria to conjoin these *insects,* that noted different species of true fly carried some of the same bacteria too, *Klebsiella, Enterobacter, Proteus, Propionibacterium*as Gram negative and *Staphylococcus* dominate Gram positive bacteria to conjoin these flies as well as different species of flies carried some of the same bacteria , *Streptopococcus, Actinomyces , Enterococcus,* flies feed and oviposit on sources of bacteria such as faeces and during these activities become contaminated with or ingest microbes, a comprehensive list of the numerous species of bacteria associated with flies was compiled by Greenberg(1971).

The biochemical characters of staphylococcus aurous were combatable with showed by Ishii *et al.*(2006) andThaker*et al.*(2013) Jahan*et al.* (2015) whose reported that Staphylococcus aurous produced of catalase and hemolysed of sheep blood and fermented of glucose , lactose as well as mannitol.

Chapter five: conclusion and recommendation

5-Conclusions and recommendations:

5-1:Conclusions:

The study was concluded :

1-The flies is a main vector for transported of *Staphylococcus aurous.*

2-The proportion of bacteria were isolate is high.

3- The outer surface of flies is main site for transported of many bacteria.

4-The flies near of animals considered the main vector for transmission of many pathogens.

5-2: Recommendation:

1-Recommended a new study to determine any other pathogenic bacteria for animals healthy transmitted by flies and others ecto-parasites.

2- Study relation between strains of bacteria in flies and it isolates from clinical cases.

3- Diagnosed the bacteria by newly technique.

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