Isolation of Staphylococcus aureus can be Transmitted by House Fly *Muscadomestica* Collected from Flies Breeding Sites in Wad Medani, Gezira State, Sudan (2016)

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(BSc. Medical laboratory Science, University of Gezira, 2009)

A Dissertation

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Department of Medical Microbiology

Faculty of Medical Laboratory Sciences

University of Gezira

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Isolation of Staphylococcus aureus can be Transmitted by House Fly *Muscadomestica* Collected from Flies Breeding Sites in Wad Medani, Gezira State, Sudan (2016)

Monera Siddig Bakht Jobara

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<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Adam Dawoud Abakar</td>
<td>Main Supervisor</td>
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</tbody>
</table>

Date: 17 / 11 /2016
Isolation of Staphylococcus aureus can be Transmitted by House Fly *Muscadomestica* Collected from Flies Breeding Sites in Wad Medani, Gezira State, Sudan (2016)

Monera Siddig Bakhit Jobara

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<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Adam Dawoud Ababar</td>
<td>Chairman</td>
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</tr>
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<td>External Examiner</td>
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</tr>
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<td>Internal Examiner</td>
<td></td>
</tr>
</tbody>
</table>

Date of Examination 17 / 11 /2016
Declaration

I, hereby declare that, this dissertation is my own work, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgment has been made in the text.

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Acknowledgements

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Isolation of *Staphylococcus aureus* can be Transmitted by House Fly *Muscadomestica* Collected from Flies Breeding Sites in Wad Medani, Gezira State, Sudan (2016)

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Abstract

Houseflies have long been regarded as potential carriers of microorganisms. Since pathogenic microorganisms are widespread in the hospital, restaurants and organic material environment, there is abundant opportunity for flies to become contaminated and, in turn, to contaminate the human and animal environment. The objective of this study was to isolate and identify *staphylococcus aureus* that are picked up by house-fly over the human premises. In this study, totally 25 houseflies were collected from decayed materials of fly breeding sites at some super market wad Madni city to isolate and identify *staphylococcus aureus* on the external surface of house fly *Staphylococcus aureus* from fly samples isolated by using concerture isolation technique. The most common isolated bacteria were *staphylococcus aureus* with 9\25 (45 %). The other species were isolated; were *klebsiella spp* with 2 \25 (10 %), *Escherichia coli* with 1 \25 (5 %), *Proteus spp* with 3\25 (15%), *streptococcus spp* with 5 \25 (25 %), when antimicrobial sensitivity testing was carried some strains of gram positive and negative showed resistance to certain antibiotics. The results of the current study confirm that flies are much more than a nuisance and that they pose potentially serious health risks. Consequently, the population of houseflies has to be controlled, houseflies therefore may act as vectors of potentially bacteria in flies breeding sites.
ملخص الدراسة

الذبابة المنزلية و كم هو معروف من الآفات العالمية، وهي الناقل الميكانيكي لأكثر من مائة من الكائنات الدقيقة. تنتشر المرض في كل من المستشفى والمطاعم والمواد الغذائية في الطبيعة مما يجعل الزباب موث، بالمرض بشكل خاص بسبب الإصابة بكل من الإنسان والحيوان. هدفت هذه الدراسة لعزل ووصف البكتيريا المكورات العنقودية التي تحمل حشرة الذهبية المنزلية والبنكية التي تم جمعها من النفايات العضوية في مدينة ودمدني. تم جمع 25 من الحلقات العنقودية من النفايات العضوية المختلفة في مدينة ودمدني، بغرض عزل ووصف وتعرف نوع البكتيريا من السطح الخارجي الذبابة المنزلية، بعد جمعها في محلول البينون المائي. تم عزل ووصف البكتيريا من هذه الحلقات، كما تم تحديد المضادات الحيوية لها باستخدام تقنية العزل التقليدية. وقد تم توصيف البكتيريا التالية وهي: المكورات العنقودية الذهبية(45%) والتي تمثل أكثر أنواع البكتيريا المعزولة من الزباب، المكورات السحجة(25%)، والمتقلبات (15%)lkisila (10 %)الاتراكية القولونية (5%)، كما تم اجراء اختبارات الحساسية الدوائية لكل أنواع البكتيريا المعزولة. أظهرت الاختبارات ان هناك بعض سلالات البكتيريا من النوعين سالبة لصبغة جرام والموجب لصبغة جرام مقاومة لعدد من المضادات الحيوية. لقد أكدت نتائج الدراسة الحالية أن ذبابة المنزل اضافة لأنها مزاعة، فإنها تشكل خطرًا صحيًا كبيرًا، ولذلك من الضروري محاربة تجمعات واماكن توالد هذه الحشرة والسيطرة عليها.
List of Contents:

<table>
<thead>
<tr>
<th>Subject</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supervision Signatures</td>
<td>I</td>
</tr>
<tr>
<td>examination Signatures</td>
<td>ii</td>
</tr>
<tr>
<td>Declaration</td>
<td>iii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>V</td>
</tr>
<tr>
<td>Abstract (English)</td>
<td>Vi</td>
</tr>
<tr>
<td>Abstract (Arabic)</td>
<td>Vii</td>
</tr>
<tr>
<td>Table of content</td>
<td>Viii</td>
</tr>
<tr>
<td>List of tables</td>
<td>xi</td>
</tr>
<tr>
<td>List of figures</td>
<td>xii</td>
</tr>
<tr>
<td>List of abbreviations</td>
<td>xiii</td>
</tr>
</tbody>
</table>

Chapter one

Introduction

1.0 Introduction 1
1.1. Problem Identification 2
1.2. Problem Justification 2
1.3. General Objective 3
1.4. Specific Objectives: 3

Chapter Two

Literature Review

2.1 Life cycle of house fly 4
2.1.1 Housefly as a vector of diseases 4
2.1.2 Role of insects in disease transmission 5
2.1.3 Food borne infections and houseflies 5
2.1.4 Disease transmission in health care facilities 6
2.2. Staphylococcus 7
2.2.1. Staphylococcus aureus 7
2.2.2. Pathogenicity of Staph aureus 7
2.2.3. Transmission of disease 8
2.2.4. Virulence factors 8
2.2.5. Identification of Staphylococci 9
2.3. Other Study 9

Chapter Three

3. Methodology 14
3.1 Study area 14
3.2. Study design 14
3.3. Study population 14
3.4. Sample size 14
3.5 Ethical considerations 14
3.6 Inclusion criteria 14
3.7 Exclusion criteria 14
3.8. Material 14
3.8.1 Culture media 14
3.8.2 Nutrient agar 14
3.8.3 Blood agar 14
3.8.4 MacConkey agar 14
3.8.5 Hydrogen peroxide for catalase test 15
3.8.6 Plasma for coagulase test 15
3.8.7 Disc for antimicrobial sensitivity test 15
3.8.8 Urease medium 15
3.8.9 Citrate medium 15
3.8.10 Peptone water 15
3.8.11 Oxidase 15
3.9 Method 15
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.9.1. Sample collection (houseflies)</td>
<td>15</td>
</tr>
<tr>
<td>3.9.2. Bacteria Isolation</td>
<td>15</td>
</tr>
<tr>
<td>3.9.3 Gram staining techniques</td>
<td>16</td>
</tr>
<tr>
<td>3.9.4 Biochemical reactions</td>
<td>16</td>
</tr>
<tr>
<td>Catalase test</td>
<td>16</td>
</tr>
<tr>
<td>Coagulase test</td>
<td>16</td>
</tr>
<tr>
<td>3.10. Antimicrobial Sensitivity test</td>
<td>16</td>
</tr>
</tbody>
</table>

**Chapter Four**

**Results**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. Colonial morphology and biochemical</td>
<td>18</td>
</tr>
<tr>
<td>4.2. Gram-positive bacteria</td>
<td>19</td>
</tr>
<tr>
<td>4.3. Gram-Negative Bacteria</td>
<td>19</td>
</tr>
<tr>
<td>4.4. Antibiotic Susceptibility Test</td>
<td>20</td>
</tr>
</tbody>
</table>

**Chapter Five**

**Discussion and Conclusion & Recommendations**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Discussion</td>
<td>24</td>
</tr>
<tr>
<td>5.2 Conclusion</td>
<td>25</td>
</tr>
<tr>
<td>5.3 Recommendations</td>
<td>25</td>
</tr>
<tr>
<td>References</td>
<td>27</td>
</tr>
</tbody>
</table>
## List of tables

<table>
<thead>
<tr>
<th>Serial NO</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>colony morphology and biochemical reactions of various bacterial species isolated from decaying material by the house fly</td>
<td>15</td>
</tr>
<tr>
<td>4.2</td>
<td>Percentage of Antibiotic susceptibility tests of Staph aureus identified from 25 samples of house fly picked up from decaying organic material in supermarkets of wadmadeni</td>
<td>21</td>
</tr>
<tr>
<td>4.3</td>
<td>Percentage of Antibiotic susceptibility tests of Strepto species identified from 25 samples of house fly picked up from decaying organic material in supermarkets of wadmadeni</td>
<td>22</td>
</tr>
<tr>
<td>4.4</td>
<td>Antibiotic susceptibility tests of Proteus identified bacteria from house fly of decaying organic material in supermarkets of wadmadeni</td>
<td>23</td>
</tr>
<tr>
<td>4.5</td>
<td>Antibiotic susceptibility tests of E.coli identified bacteria from house fly of decaying organic material in supermarkets of wadmadeni</td>
<td>24</td>
</tr>
<tr>
<td>1.6</td>
<td>Antibiotic susceptibility tests of Klebsiela identified bacteria from house fly of decaying organic material in supermarkets of wadmadeni</td>
<td>24</td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>Serial NO</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Gram-positive bacteria isolated</td>
<td>17</td>
</tr>
<tr>
<td>4.2</td>
<td>Gram-Negative bacteria isolated</td>
<td>18</td>
</tr>
<tr>
<td>4.3</td>
<td>Frequency of all bacteria isolated</td>
<td>19</td>
</tr>
</tbody>
</table>
## Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AK</td>
<td>Amikacin</td>
</tr>
<tr>
<td>AMC</td>
<td>Augmentin</td>
</tr>
<tr>
<td>TE</td>
<td>Aztracyclin</td>
</tr>
<tr>
<td>AT</td>
<td>Ceftazidime</td>
</tr>
<tr>
<td>C</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>CIP</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>E</td>
<td>Erythomycin</td>
</tr>
<tr>
<td>E. Col</td>
<td>Escherchia coli</td>
</tr>
<tr>
<td>FC</td>
<td>Fusidicaad</td>
</tr>
<tr>
<td>GEN</td>
<td>Gentamycin</td>
</tr>
<tr>
<td>IPM</td>
<td>Imipenem</td>
</tr>
<tr>
<td>I</td>
<td>Intermediate</td>
</tr>
<tr>
<td>KIA</td>
<td>Kliger iron agar</td>
</tr>
<tr>
<td>No</td>
<td>Number</td>
</tr>
<tr>
<td>P</td>
<td>Pericillin</td>
</tr>
<tr>
<td>PI</td>
<td>Piperacillin</td>
</tr>
<tr>
<td>R</td>
<td>Resistant</td>
</tr>
<tr>
<td>S</td>
<td>Sensitive</td>
</tr>
<tr>
<td>VA</td>
<td>Vancomycin</td>
</tr>
</tbody>
</table>
Chapter one

Introduction

1.0 Introduction:

The house fly, *Musca domestica* is well-known cosmopolitan pest. It has a world-wide distribution and is found throughout the country in close association with human activities. It receives the common name of house fly by virtue of being the most common fly found in and around houses in addition to being a nuisance pest (Moon, 2002). It is a vector of many pathogenesis carrier of over 100 different pathogenic organisms including organisms for diseases, typhoid, cholera, bacillary dysentery, ophthalmic neonatorum and infantile diarrhea as well as parasitic worms [Sassaki 2000, Fotedar 1992].

House flies *Musca domestica* are of medical and veterinary importance due to their capacity to act as mechanical vectors of microorganisms originating in animal manure and other decaying organic substrates [Chakrabarti 2010]. How-ever, its classification as a "disease causing fly" as follows (1) its confirmed association with food-borne pathogens *Escherichia coli*, *Salmonella*, and *Shigella*.(2)the fact that it is ecologically associated with humans (synanthropic ),(3) its association with domestic vironments (pedophilic) ,(4) its equal attraction to excrements and human food sources, and (5) its municative behavior that allows the house fly to easily move from heavily contaminated to human populated areas [Olsen,A.R1998].

Houseflies *Musca domestica* a have been shown to be carriers of several species of bacteria; this is because of their close association with decaying organic matters, garbage and feces [Holt,Ps,2007] *Insects*, such as houseflies, that develop in decaying organic material may *transmit antibiotic –resistance bacteria* from the manure of animals and other decaying organic substrates to residential setting. The hairy proboscis and feet with glandular hairs and pads that secrete sticky material enable the flies to pick up the pathogens on to their bodies. In addition the regurgitation of vomits and deposit of fecal droplets during feeding process contribute to the flies' ability to spread the pathogens [Rosfe, oandKapperud 1983].The common house fly ,is a medically-important insect
worldwide [Fotedar, 2001; Graczyk, 2001; Kabkaew, 2007]. House flies have been implicated as vectors or transporters of various human pathogens, including *Vibrio cholerae*, *Enterobacteriaceae* pathogens, *Staphylococcus aureus*, and *Pseudomonas* spp. [Rajjendran, 2003]. Transmission takes place when the fly makes contact with people or their food. As many as 500,000 microorganisms may swarm over its body and legs [Thirumalaj, 2008]. A number of researchers have studied and isolated pathogens, such as *Vibrio cholerae* [Fotedar, 2001], *Escherichia coli* 0157:H7 [Sassaki, 2002], *Salmonella* and *Campylobacter* [Olsen, 2000; Nichols, 2005; Wales, 2010] from house flies and reported them as a potential source for transmission and spread of these pathogens. While feeding or resting, house flies often defecate, leaving fly specks and organisms passing through their digestive system [Sassaki, 2000]. This a simple mechanical transfer of microbes by vector whose behavior places the contaminants from decayed and diseased source they visit [Holt, 2007]. According to flies can contaminate clean surfaces with approximately 0.1 mg of food per landing. The organisms associated with house flies number in the hundreds and commonly include dysentery-causing and tissue-infecting agents such as *Bacillus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Spp.*, *Escherichia*, *Shigella* coli, *Bacillus anthracis*.

**1.1. Problem Identification:**

Insects, such as houseflies, that develop in decaying organic material may transmit *antibiotic-resistance bacteria* from the manure of animals and other decaying organic substrates to residential setting. The habitats in which it develops (e.g., manure), its dependence on a live microbial community, its feeding mechanism (regurgitation), its attraction to human food, and its ability to fly long distance make this insect a very good candidate for dissemination of fecal bacteria, including human and animal pathogens [Alam, 2004].

**1.2. Problem Justification:**

There is note that there is increased in food boring disease carried by *Musca domestica*, or *as nascomial infection*. The isolation and identification of *Staphylococcus aureus* of microorganism carries by *Musca domestica* due to increase of incidence of
food poisoning and illness of *S. aureus*. *Musca domestica* transmitted the main important antibiotic resistant bacteria species. *Musca domestica* has a high health risks.

1.3. **General Objective:**

To isolation of *Staphylococcus aurous* can Transmitted by *Musca domestica* collected from Wad Medina flies breeding sites.

1.4. **Specific Objectives:**

1. To isolate and *identify Staphylococcus aurous* in collected samples.
2. To isolate and identify bacteria found in natural association with of *Musca domestica*.
3. To determine the rate of each species of bacteria in Musca domestic.
4. To detect antibiotic resistance species from isolated species.
5. To determine the health risks of *Musca domestica*.
2.1 Life cycle of house fly:

House fly has four distinct life stages: egg, larva, pupa and adult (John, 2006). As a mechanical vector this species of fly may be responsible for carrying pathogens which cause diseases such as infantile diarrhoea, shigellosis diarrhoea, dysentery, typhoid, and also intestinal worm eggs. It has been proved in Pakistan (Chavasse, 1998) and in the Gambia (Emerson, 1999) that fly control reduced incidence of diarrhoea by about 24%. In many parts of the Sudan the majority of human diseases are associated with the decline in the environmental sanitation measures due to deterioration in the public health services and accumulation of untreated garbage such as domestic waste, waste associated with sale of cooked food, fruits, fish and meat in different marketing places (Rabaab, et al 2007).

2.1.1 Housefly as a vector of diseases:

The housefly *Musca domestica* is known to be a vector of diseases. These flies are prevalent in items that are exposed. Contamination of drinking water, food and other dairy products with faecal remains are common features. Hence the likelihood of human excrement being transmitted by flies is great. Housefly are the most important insect pest associated with poultry, where the accumulated organic waste and favorable environmental conditions often promote rapid development of large populations. Large populations of *M. domestica* may reduce yields and contribute to substantial public health problems when they enter nearby human habitations. The housefly is a carrier of germs which transmit diseases that cause havoc to man. Such disease include typhoid fever (caused by Salmonella typhi), cholera (caused by Vibrio cholera), *staphylococcal* food poisoning (caused by *Staphylococcus aureus*) and Shigelllosis that caused by *Shigella* spp. (Banjo, et al, 2005)
2.1.2 Role of insects in disease transmission:

Though insects comprise one of the most diverse taxa of life, little is known about the implication of insect associated bacteria on human health (Allen et al., 2009). The arthropod borne infections have an extensive distribution over the face of the globe and such diseases have produced much suffering, economic loss and death in human population. Majority of the arthropod borne bacterial diseases are transmitted by housefly mosquitoes, lice, ticks, mites or fleas. Arthropods associated with human infection often serve as vectors for the pathogenic microorganisms. A vector is an organism capable of mechanically or biologically transferring a pathogen from one organism to another. Two groups of non-biting insects most frequently screened for food borne pathogens are houseflies and cockroaches (Ahmad et al., 2011). These insects have been implicated as mechanical or biological vectors for bacterial pathogens including Salmonella spp., Shigella spp., klebsiella spp., proteus spp, E. coli O157: H7 that can cause diseases in humans and animals (Zurek and Gorham, 2008).

2.1.3 Food borne infections and houseflies:

All the food borne infections are associated with poor hygienic practices where the oral - faecal route is maintained with the food providing the vital link between hosts. Factors contributing to foodborne illness include improper cleaning of raw foods, cross contamination with microbes, inadequate heating and insufficient cooking of foods (Centers for Disease Control and Prevention, 2004). Insects such as houseflies or cockroaches feeding on faecal waste also have a role in transferring microbes to food either mechanically through their body parts or through faecal pellets. Fomites too play a role in the maintenance of the faecal-oral route of transmission. Characteristics of food borne illness vary from pathogen to pathogen. Complications due to illness are often found in the elderly, young children and immune suppressed (Kendall et al., 2006; Koehler et al., 2006). The predominant bacterial flora involved in food spoilage or toxin production are as follows: Salmonella spp., Shigella spp., S. aureus, C. jejuni,
Enterococcus spp., E. coli, L. monocytogenes, Y. enterocolytica, Aeromonasaerophila, V. cholerae and V. parahaemolyticus. Salmonellosis is generally a self-limiting gastroenteritis mostly caused by non–typhoidal Salmonella such as Salmonella typhimurium, Salmonella enteritidis, Salmonella cholerasuis, Salmonella hader, Salmonella virchow, Salmonella dublin etc. with a tendency to produce severe illness in immunocompromised individuals (Sakai and Chalermchaikit, 1996; Hohmann, 2001). Despite the strong link between food of animal origin and human salmonellosis, it has been noticed that infection may also be acquired by cross contamination through contact as well as by ingestion of vegetables and fruits (Neto et al., 2010). There have been evidences to suggest that as low as 10-100 cells of Salmonella may initiate an infection under certain conditions and with some foods, Septicemia occurs as a complication of gastroenteritis which can be fatal in immune compromised hosts (D’ Aoust, 1994).

2.1.4 Disease transmission in health care facilities

The acquisition of nosocomial pathogens in health care facilities is a complex interaction between the host, pathogen and environment (Rutala and Weber, 1987). Infections may be caused by a microorganism acquired from another person in the hospital (cross-infection) or may be caused by the patient’s own flora (endogenous infection) especially in severely immune compromised persons. Various related studies have also suggested a possible link between the microorganisms present in the hospital environment and hospital acquired infections (exogenous flora). Environmental sources of exogenously acquired pathogens include inanimate objects in the hospital environment or substances contaminated from another human source. Dissemination of pathogens from an animate or inanimate reservoir to the patient may occur by one or more of several different routes such as airborne, contact, ingestion, infusion or through arthropod vectors, such as (houseflies, mosquitoes, ticks). The acquisition of infection in hospital through contact may be director indirect. Direct contact transmission occurs if there is a close physical Contact between the source and the patient. The longer a nosocomial pathogen persists on a surface, the lengthier act as a source of infection noticed. (Jawad et al., 1998).
Gram negative bacteria have been described to persist longer than grampositive bacteria (Hirai, 1991). Bacteria such as klebsiella sp. aeruginosa, E. coli were found to be surviving long under humid conditions (Wenzel, 1987; Williams et al., 2005; Vogel et al., 2010). Though disinfecting surfaces in the immediate environment of the patient have been described to reduce acquisition of certain nosocomial bacterial pathogens routine treatment of clean floors with various types of surface disinfectants have no influence on the incidence of nosocomial infection (Dharan et al., 1999; Hayden et al., 2006). The bacteria commonly encountered in hospital acquired infections are Staphylococcus spp., Enterococcus spp., P. aeruginosa, E. coli Klebsiella spp., Citrobacter spp., Enterobacterspp., Serratia spp., Proteus spp. and Acinetobacter spp.

2.2. Staphylococcus .

The main species of medical importance is: Staphylococcus aureus, several other species may also cause disease; Staphylococcus epidermidis and Staphylococcus saprophyticus. Staphylococci are widely distributed in the environment. They form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract.(Monica 1984)

2.2.1. Staphylococcus aureus:

Gram-positive bacteria, cluster-forming coccus, non-motile, non-spore forming, facultative anaerobe, Staph aureus ferment glucose and produces lactic acid, in addition to fermentation of mannitol, have catalase, coagulase and golden yellow colony on agar(Kenneth Todar, 2012)

2.2.2. Pathogenicity of Staph aureus:

S. aureus has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulites’ (Minnesota Department of Health Fact Sheet Revised February, 2010). Staphylococcus aureus has been implicated as a causative agent
in acute food poisoning episodes, toxic shock syndrome, *impetigo, scalded skin syndrome*; It is also a common cause of systemic infections such as infective *endocarditic, osteomyelitis, epiglottitis*, and sinus infections. S. aureus is also responsible for many infective and systemic infections in the health care setting (nosocomial infections). (EHA consulting group 2016)

### 2.2.3. Transmission of disease:

*Staphylococcus aureus* can cause illness by preformed toxin production as well as by infecting both local tissues and the systemic circulation. Disease transmission can occur in the following settings: *Gastrointestinal*; *Staphylococcus aureus* causes acute episodes of food poisoning via preformed *enter toxins*. Food items likely to be infected by *staphylococcal food poisoning* include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, milk and dairy products. Skin and hair infections; *Staphylococcus aureus* commonly colonizes many skin surfaces on the *nasopharynx, and perineum*; but can cause infection of these surfaces particularly if the coetaneous barrier has been disrupted or damaged. Systemic infections; *Staphylococcus aureus* commonly causes infective *endocarditis* in IV drug abusers; *osteomyelitis, sinus infections* in the general population; and *epiglottitis* in young children. *Nosocomial infections*; *Methicillin resistant Staphylococcal aureus (MRSA)* is a strain of the bacteria that is commonly implicated in nosocomial infections. *Risk factors for MRSA colonization or infection* in the hospital settings include prior antibiotic exposure, admission to an intensive care unit, surgical incisions, and exposure to infected patients. (EHA consulting group 2016)

### 2.2.4. Virulence factors:

*S. aureus* expresses many potential virulence factors: (1) surface proteins that promote colonization of host tissues; (2) invasins that promote bacterial spread in tissues (*leukocidin, kinases, hyaluronidase*); (3) surface factors that inhibit phagocytic engulfment (capsule, Protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production); (5) immunological disguises (*Protein A,*
coagulase); (6) membrane-damaging toxins that lyse eucaryotic cell membranes (hemolysins, leukotoxin, leukocidin; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (SEA-G, TSST, ET); and (8) inherent and acquired resistance to antimicrobial agents.

2.2.5. Identification of Staphylococci:

Staphylococci grow well aerobically and in carbon dioxide enriched atmosphere. Most strains also grow an aerobically, but less well. Temperature range for growth is 10-42 with an optimum of 35-37.

*S. aureus* on blood agar and chocolate (heated blood) agar, *S. aureus* produces yellow to cream or occasionally white 1-2 mm in diameter colonies. Some strains are beta - hemolytic when grown aerobically. Colonies are slightly raised and easily emulsified on a slide.

On MacConkey agar, smaller (0.1-0.5 mm) colonies are produced after overnight incubation at 35-37. Most strains are non-lactose fermenting. *Mannitol salt agar* is a useful differential and selective medium for recovering *S. aureus* from fecal specimens when investigating *staphylococcal food-poisoning.*

Staphylococcus are gram positive which after being stained dark purple with crystal violet are not decolorized by acetone or ethanol. (Monica 1984-)

2.3. Other Study:

Houseflies have long been regarded as potential carriers of microorganisms. Since pathogenic microorganisms are widespread in the hospital environment, there is abundant opportunity for flies to become contaminated and, in turn, to contaminate the patient environment. In the present study, an attempt was made to isolate and identify pathogenic bacteria, fungi and parasites from the housefly Musca domestica collected in the surgical ward of the All India Institute of Medical Sciences Hospital and also in a remote residential area located 5 km from the hospital. A total of 113 flies were collected: 65 from a surgical ward (test) and 48 from a residential area for comparison. Ten genera of bacteria were isolated from the test group of flies compared with nine from the control group. In primary isolations, it was observed that the load of bacteria carried by the test group of flies was significantly more (P less than 0.001) than for the control flies.
Pseudomonas aeruginosa, Enterococcus faecalis and viridans streptococci were isolated only from the test flies. The isolation rate of Staphylococcus aureus was significantly higher (P less than 0.001) in test houseflies than in the control houseflies. There was no significant difference in isolation of parasitic ova and cysts from test and control houseflies. Candida spp. were isolated in almost equal numbers from both group, of houseflies, yet none of these was Candida albicans. Houseflies therefore may act as vectors of potentially pathogenic bacteria in a hospital environment. (Fotedar R1, Banerjee U, Singh S, Shriniwas, Verma AK)

Housefly larvae were cultured on fresh fish and collected for the isolation and identification of microorganisms associated with them. The microbes were cultured from both the gut and body surface of the maggot on nutrient agar (for bacteria) and potato dextrose agar (for fungi) and incubated at about 37°C for 48 h before observations. A variety of microorganisms, which includes the pathogenic Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus tamarii and Bacillus cereus were found. Also, nonpathogenic microbes were recovered including Bacillus subtilis and Streplococcus faecalis (BANJO, A.D., LAWAL, O.A.* and ADEDUJI, O.O. s  2005)

Varies bacterial species were isolated and identified from truefly species collected from market place in Basrah city. Eighteen species of bacteria were isolated from five species of Muscidae, Calliphoridae and Sarcophagidae. The most common bacteria isolated were E. coli, Staphytococus, Streptococcus, Enterobacter, there bacteria were cause disease conditions in human and animals. The mean bacteria number were between 15-2.6 x 10^6 cuf/ml (Mufeed A. Habeeb* and Mohammed A. Mahdi 2012)

There is abundant opportunity for house fly, Musca domestica, to become contaminated and, in turn, to contaminate the patient environment. This study has been conducted with the aim of isolating and identifying the probable pathogenic inoculations of the flies in three hospitals in Ahwaz County, Khuzestan province, south-west of Iran. Methods: Adult flies were collected by means of insect net and then transfers to lab for microbiological process. Results: Totally on all house fly samples the bacteria genus Pseudomonas and Esherichia coli had the significantly highest rate of infection (P<0.05). The genera Klebsiella, Bacillus, and Diphptroid has significant lowest rate of infection. Hot and humid weather of the Khuzestan province which is host of patients coming from
far localities for hospitalization, provide suitable conditions for housefly activities during the year. Conclusions: Thus suitable and applicable control methods such as environmental sanitations should be implemented (Hamid Kassiri 1*, Kamran Akbarzadeh 2, Anvar Ghaderi 2012)

Bacterial pathogens associated with wild-caught houseflies in Awka metropolis, were investigated between April and August, 2012. Locally designed fly traps baited with common materials in the site of fly collection were used to collect flies from the six different ecological locations in the city. A total of 2,157 wild-caught flies were used in the study. They were identified into genera and species using standard taxonomic keys. Eight fly species (Musca domestica, Lucilia cuprina, Chrysomyia bezziana, Fannia canicularis, Wohlfartia species, Stomoxys calcitrans, Musca sorbens and Sarcophaga species) were identified. The internal and external pathogens associated with the flies were isolated and aliquots of the homogenates from both their internal and external contents were inoculated on various agar plates for pathogen isolations and identification. Bacterial pathogens isolated from the flies were Staphylococcus aureus, Pseudomonas species, Proteus mirabilis, Streptococcus species, Salmonella species, Shigella species, Escherichia coli, and Klebsiella species. All the eight genera of bacterial pathogens isolated are known to be pathogenic to man, indicating that wild-caught flies, especially M. domestica, are potential mechanical vectors of bacterial pathogens. This calls for improvement of sanitation/hygiene in both urban and rural areas of the country so as to improve the health of our people (1Onyido, AE, 1Nwangwu, UC, 1Aribodor DN, 1Umeanaeto, PU, 1Ugha, CN and 2Ugwu FM, 3Onwude CO 2014)

During the last decade, the prevalence of insect borne diseases due to contaminated food as well as the outbreaks of diseases due to enterotoxin-producing Staphylococcus aureus (ETSA) strains has increased. Objectives: This study was conducted to determine the prevalence rate, enterotoxigenecity, and antimicrobial resistance of S. aureus isolated from M. domestica collected from the residential areas of nine districts broiler farms in Zabol, Iran. Materials and Methods: The flies were captured with a sterile nylon net and washed twice with distilled water. The contents were streaked onto selective media and S. aureus was identified using the conventional biochemical tests. Antimicrobial susceptibility testing was performed using CLSI guideline.
Staphylococcal enterotoxins (SEs), SEA, SEB, SEC and SED, was detected by the reverse passive latex agglutination (PRLA) method. Coagulase typing was performed with coagulase typing kit. Results: A total of 87 (17%) S. aureus was isolated from 450 samples. The ability to synthesize staphylococcal enterotoxins (SEs) was determined in 62 of 87 (71%) isolates. SE type B was the most common enterotoxin found in the isolated S. aureus (45%), followed by SE type A (26%), SE type C (5%), SE type D (8%), SEA + SEC (7%) and SEA + SEB (7%). Among the antibiotic tested, Penicillin was the most resistant antibiotic tested. Sixty seven percent of isolates belonged to coagulase type III, VI, VII, VIII, IV, and V. Conclusions: Our results suggest that S. aureus, including ETSA, is being carried by house flies such as M. domestica and may contribute to the spread of pathogenic isolates, with an impact on public health (Mehdi Rashki Ghalehnoo 2015)

The Oriental latrine fly, Chrysomya megacephala (Fabricius) (Diptera: Calliphoridae) and the house fly, Musca domestica L., (Diptera: Muscidae) are synanthropic flies which are adapted to live in close association with human habitations, thereby making them likely mechanical vectors of several pathogens to humans. There were two main aims of this study. The first aim was to determine the prevalence of these two fly species from five types of human habitations including: fresh-food markets, garbage piles, restaurants, school cafeterias and paddy fields, in the Muang Ubon Ratchathani and Warin Chamrap districts of Ubon Ratchathani province of Northeast Thailand. Flies collection were conducted monthly from September 2010-October 2011 using a reconstructable funnel trap, containing 1 day-tainted beef offal as bait. A total of 7 750 flies (6 401 C. megacephala and 1 349 M. domestica) were collected. The second aim was to examine the potential of these flies to carry pathogenic bacteria. Bacteria were isolated from 994 individual flies collected using a sweep net (555 C. megacephala and 439 M. domestica). A total of 15 bacterial genera were isolated from the external surfaces, comprising ten genera of gram-negative bacteria and five gram-positive bacteria. The most common bacteria isolated from both species were coagulase-negative staphylococci, followed by Streptococcus group D non-enterococci. Human pathogenic enteric bacteria isolated were Salmonella sp., Shigella sp., Escherichia coli O157:H7, Salmonella typhi, Bacillus sp., and Enterococcus sp., of which S. typhi is the first report
of isolation from these fly species. Other human pathogens included Staphylococcus aureus and Pseudomonas aeruginosa. Not only were the number of C. megacephala positive for bacteria significantly higher than for M. domestica, but they were also carrying ~11-12 times greater bacterial load than M. domestica. These data suggest that both fly species should be considered potential mechanical vectors of bacterial pathogens associated with human habitations year-round in this region of Northeast Thailand (Chaiwong, T.1*, Srivoramas, T.1, Sueabsamran, P.1, Sukontason, K.2, Sanford, M.R.3 and Sukontason, K.L.2 2014)
Chapter Three
3. Methodology

3.1 Study area:
This study was carried out at Medani flies breeding sites and microbiology lab, Faculty of Medical Laboratory Sciences, University of Gezira

3.2. Study design:
This study includes cross-sectional study, observation, and data analysis.

3.3. Study population
This study was carried in *Musca domestica* a population.

3.4. Sample size:
A total of 25 House–flies were collected in batches of flies each from decaying organic material from markets in wad madni city.

3.5 Ethical considerations:
Permission from Ministry of Health, Gezira State.
Ethical Clearance was obtained from the Faculty of Medical Laboratory Science Ethical committee Gezira University.

3.6 Inclusion criteria:
Live house fly

3.7 Exclusion criteria:
Dead house fly
house fly that Collected from out study area.

3.8. Material:
Bifbaf insectied for capturing the house flies.

3.8.1 Culture media:
The Following media was used in culturing the bacteria, blood agar, MacConky agar, nutrient agar, sets of biochemical test.

3.8.2 Nutrient agar
3.8.3 Blood agar
3.8.4 MacConkey agar
3.8.5 Hydrogen peroxide for catalase test
3.8.6 plasma for coagulase test
3.8.7 Disc for antimicrobial sensitivity test
3.8.8 Urease medium
3.8.9 citrate medium
3.8.10 Peptone water
3.8.11 Oxidase

3.9 Method:

3.9.1. Sample collection (houseflies):

The study was confined to the city Wad Medani. House flies were collected in batches of 25 flies each from decaying organic material places. Flies were caught from the selected sites during the period of study with BifBaf insectecid and from 9:00 a.m. to 1:0 p.m., when the flies are active. The collected flies were placed in to sterile vials, and flies were transferred immediately to peptone water as transport media then to bacteriological laboratory.

3.9.2. Bacteria Isolation:

Bacteria from fly samples collection were isolated by using the conventural Isolation technique. Each flies transferred individually with sterile forceps to peptone water and after one hour each sample was cultured on agar plates MacKanky, and Blood Agar Media. For culturing Microbes of the External Body Parts of the musca domestica, a wire loop was flamed red hot, allowed to cool, before using it, then was collected a loopful of the homogenised suspension from the external surfaces of the flies, and streaked on the surfaces of the prepared MacConkey, Blood agar, The media were then incubated at 37oC for 24 hours. all bacteria colonies subculture on to media and further incubated until pure colonies were obtained (Cheesbrough, 2000). And mad smears for
preparing direct gram smear as presumptive detection of bacteria in collected samples. The bacteria were identified to the genus level by colony morphology, texture and Gram staining and species level for some of them by using biochemical test.

3.9.3 Gram staining techniques’:

Fixed the dried smear, the fixed smear was covered with crystal violet stain for 30-60 seconds. and Rapidly washed off the stain with clean water .Then all the water tipped off ,and the smear was covered with lugol iodine for 30-60 seconds, and the iodine washed off with clean water then the iodine Decolorized rapidly with acetone –alcohol .washed immediately with clean water., finally the smear was covered with neutral red stain for 2 minutes, then the stain was washed off with clean water. After that the smear examined microscopically.

3.9.4 Biochemical reactions:

Catalase test:

2-3 ml of hydrogen peroxide solution was pour into a test tube and by Using a sterile wooden stick a good growth of the test organism was removed and immersed it in the hydrogen peroxide solution. If bubbling was seen, the test is positive.

Coagulase test:

This test is used to differentiate staphylococcus aureus which produces the enzyme coagulase, from other species.
A drop of physiologically saline was placed on slide, a colony from culture was emulsified and made thick suspension. Then a drop of plasma was added to suspension ,within 10 seconds clumping was appeared, positive test.

3.10. Antimicrobial Sensitivity test:

Sensitivity testing was performed by using disc diffusion techniques; A disc of blotting paper was impregnated with a known volume and appropriate concentration of 8 antibiotic, and this is placed on a plate of sensitivity testing agar (Mueller Hinton) inoculated with test organism. And after overnight incubation the zone was read.
Antibiotic susceptibility test was performed and the following antibiotics were used in this study tetracycline, Erythromycin, Ciprofloxacin, Gentmycin, Ampicillin Amoxicillin, penicillin, Nalidixic acid, Ferodantin, Amikacin, Rifadin, Chloramphenicol, Augmentin, Fusidic acid.

The test reactions of the organism were reported to each antibiotic as sensitive, intermediate and resistant.
Chapter Four

Results

From the 25 house flies collected from decaying organic material, house flies were found to carry several species of bacteria on their external surfaces;

4.1. Colonial morphology and biochemical:

The bacteria were identified to the genus level by colony morphology, texture and Gram staining and species level for some of them by using biochemical test as shown in

Table 4. 1. colony morphology and biochemical reactions of various bacterial species isolated from decaying material by the house fly:

<table>
<thead>
<tr>
<th>s/n</th>
<th>Organism</th>
<th>shape</th>
<th>gramstain</th>
<th>coagulase</th>
<th>catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staph aureus</td>
<td>Cocci</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Strepto,species</td>
<td>Cocci</td>
<td>+ve</td>
<td>+ve</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>s/n</th>
<th>Organism</th>
<th>shape</th>
<th>gramstain</th>
<th>Indole</th>
<th>citrate</th>
<th>urease</th>
<th>Oxidase</th>
<th>Motility</th>
<th>coagulase</th>
<th>catalase</th>
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<tbody>
<tr>
<td>1</td>
<td>Proteus</td>
<td>Rod</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella</td>
<td>Rod</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>3</td>
<td>E.coli</td>
<td>Rod</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>
Aerobic culture of samples yielded different species of bacteria, the total number of bacteria isolated was 25 (100%). From all isolates, (56%) were Gram positive bacteria and isolates (24%) were Gram negative bacteria, see (table below).

4.2. Gram-positive bacteria:
The total numbers of G+ve bacteria isolated was 14 (56%). The most common G+ve organisms isolated in this study were *staphylococcus aureus*, see fig 1. Gram-positive bacteria isolated were *staphylococcus species* (64%) and *streptococcus* was 5 (36%), see table 2 below.

![Bar chart](chart.png)

**Figure (4-1) Gram-positive bacteria isolated**

4.3. Gram-Negative Bacteria
The total numbers of G-ve bacteria isolated in this study was 6(24%) isolates. The most common G-ve organisms isolated in this study was *Proteus* 3 (50%). The Gram-negative bacteria isolated in this investigation were 3 *proteus* species (50%), 2*Kelebsela*(33 %), and 1 *E.coli* species (17 %), see table3
The percentage of all bacteria isolated from these sites were *Staph aureus* (45%), Then Strept species (25%), proteus species (15%) Klebiela species (10%), E.coli (5%). The most frequent bacteria isolated from decaying organic material are Staphylococcus aureus (Table 4-3).

**Figure (4-3) Frequency of all bacteria isolated**

### 4.4. Antibiotic Susceptibility Test:

Antibiotic susceptibility test was performed and the following antibiotics were used in this study gentamycin, chloramphenicol, Fusidic acid, Penicillin, Vancomycin, Augmentin, Erythromycin, Ciprofloxacin.
Staph. aureus was found Sensitive 100% to gentamycin. Resistant 33.3% to chloramphenicol, Fusidic acid and penicillin. Resistant 22.2% to vancomycin and Augmentin.

Streptococcus species was found
Sensitive 100% to gentamycin
Resistant 60% to Erythromycin, penicillin, and Augmentin.
Resistant by 40% to Fusidic acid and vancomycin.

Three gram negative bacteria cultures obtained from house flies; their disc sensitivity or resistance to 8 commonly used antibiotics;
Proteus resistant 100% to chloramphenical and 50% to piperacillin.
Klebsiella resistant 100% to chlornphenicol, tetracycline, piperacillin, and Aztreonam.
E.coli resistant 100% to ciprofloxacin, cefazidime, tetracycline, piperacillin, and Aztreonam.

Table (4.2): Percentage of Antibiotic susceptibility tests of Staph aureus
identified from 25 samples of house fly picked up from decaying organic material in supermarkets of wadmadeni:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Staph aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Gen</td>
<td>%100</td>
</tr>
<tr>
<td>Cip</td>
<td>%88.8</td>
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<tr>
<td>E</td>
<td>%77.1</td>
</tr>
<tr>
<td>C</td>
<td>%66.6</td>
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<tr>
<td>Fc</td>
<td>%33.3</td>
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<tr>
<td>VA</td>
<td>%77.7</td>
</tr>
<tr>
<td>P</td>
<td>%66.6</td>
</tr>
<tr>
<td>ANC</td>
<td>%33.3</td>
</tr>
</tbody>
</table>
Table (4.3): Percentage of Antibiotic susceptibility tests of Strepto species identified from 25 samples of house fly picked up from decaying organic material in supermarkets of wadmadeni:

<table>
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<th>Antibiotic</th>
<th>Strepto species</th>
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<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen</td>
<td></td>
<td>%100</td>
<td>%0</td>
<td>%0</td>
</tr>
<tr>
<td>Cip</td>
<td></td>
<td>%80</td>
<td>%20</td>
<td>%0</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>%40</td>
<td>%0</td>
<td>%60</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>%80</td>
<td>%20</td>
<td>%0</td>
</tr>
<tr>
<td>Fc</td>
<td></td>
<td>%60</td>
<td>%0</td>
<td>%40</td>
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<tr>
<td>VA</td>
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<td>%0</td>
<td>%40</td>
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<td>%40</td>
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<td>ANC</td>
<td></td>
<td>%40</td>
<td>%0</td>
<td>%60</td>
</tr>
</tbody>
</table>

Table (4.4): Antibiotic susceptibility tests of Proteus identified bacteria from house fly of decaying organic material in supermarkets of wadmadeni:

<table>
<thead>
<tr>
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<th>I</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Cip</td>
<td></td>
<td>%100</td>
<td>%0</td>
<td>%0</td>
</tr>
<tr>
<td>CAZ</td>
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<td>%100</td>
<td>%0</td>
<td>%0</td>
</tr>
<tr>
<td>Ak</td>
<td></td>
<td>%33.3</td>
<td>%66.6</td>
<td>%0</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>%100</td>
<td>%0</td>
<td>%0</td>
</tr>
<tr>
<td>IPM</td>
<td></td>
<td>%100</td>
<td>%0</td>
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<td>TE</td>
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<td>%0</td>
<td>%100</td>
</tr>
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Table (4.5): Antibiotic susceptibility tests of E.coli identified bacteria from house fly of decaying organic material in supermarkets of wadmadeni:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>E.coli</th>
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<tbody>
<tr>
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</table>

Table (4.6): Antibiotic susceptibility tests of Klebsiela identified bacteria from house fly of decaying organic material in supermarkets of wadmadeni:

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<tr>
<th>Antibiotic</th>
<th>Klebsiela</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
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<td>%0</td>
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</tr>
<tr>
<td>CAZ</td>
<td>%100</td>
<td>%0</td>
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<tr>
<td>Ak</td>
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<tr>
<td>C</td>
<td>%0</td>
<td>%0</td>
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</tr>
<tr>
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<tr>
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Chapter Five

Discussion and Conclusion & Recommendations

5.1 Discussion:

The biology and ecology of Musca domestica make it an ideal mechanical vector of human 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 five species of bacteria were isolated from Musca domestica (Table - 1). Our results are in accordance with other reports which highlight the importance of house flies in caring various pathogens [Gurbel et al, 1997, Kobayashi et al 1999, Koura et al 1990, Pai et al 2003]. Many scientists indicated that the external organs of Musca domestica (legs, wings, and mouth parts) constituted a large source of bacteria they isolated [Kobayashi et al 1999, Graczyk et al (1999)]. The results of this study indicated that Musca domestica could play a great role as a mechanical carrier of bacteria in this study, most of the bacteria isolated were medically important, including, Staph. aureus, E.coli, Klebsiella, proteus, and Streptococci. These findings agree with the results of [Vazirianzadeh et al 2008, Moosa et al 2010] which presence E.coli, P. aeruginosa, K. pneumonia, and Proteus mirabilis on the external surface of house fly collected from slaughterhouse, zoo, and hospitals. There are more studies which confirm the role of house flies on transmission of different bacteria as a world wide agent transmission of bacteria is using different insect species and different methods of transmission. [Oisen et al 2000] isolated Salmonella enteritidis, S. infantis and S. heidelberg from house flies over poultry houses. 18 species of entero-pathogenic bacteria from different cyclorrhaphan flies were isolated in Malaysia [Sulaiman et al 2000]. The isolates of E.coli, Staphylococcus aureus, Pseudomonas spp., Proteus spp., in Ahvazc city [Moosa et al 2010]. This confirm that the housefly's body can act as a mechanical vector either bacteria or many microorganisms. We examined the antibiotic susceptibility and resistance patterns of five bacteria obtained from the houseflies by using 15 commonly used antibiotics (Table 2). The types of bacteria in general were resistance to all antibiotics. These findings are in agreement with the finding of other studies which show
that the enter bacteria, E. coli, Klebsiella, isolated from house flies collected in hospital were found to be resistant to significantly more of the commonly used antibiotics that were tested than the Enterobacteria isolated from the flies caught in the streets. Insects such as house flies Musca domestica L. that develop in decaying organic material may transmit antibiotic-resistant bacteria from manure of animals and other decaying organic substrates to residential setting [Graczyk et al 2001, Alam et al 2004, Zurek et al 2000]. Multidrug resistance in clinical isolates has become a serious problem due to a progressive decline in the number of antibiotics that are effective for treatment of human infections [Witte, W, 1999]. It has been suggest that there is a connection between the antibiotic resistance of food animal origin, the antibiotic resistance of clinical isolates, and community health [Eaton et al 2000, Salyers, A, A 2002, Smith et al 2002]. However, this remains a controversial issue [Philips 2004, Turnidge, J 2004] because the ecology of antibiotic resistance and virulence genes in the environment is not well understood. The present study indicates that the house fly Musca domestica poses a possible health risk to communities proved that the isolated strains of bacteria were resistance to various antibiotics.

5.2 Conclusion:

Isolation of Staphylococcus aureus can transmitted by housefly musca domestica collected from flies breeding sites showed that, The highest isolation rate was Staphylococcus 9 spp (45%) from all the collected samples. And many species of bacterial were isolated. Important pathogens such as Proteus, E.coli, Klebsiella, Pseudomonas, Strepococcus were isolated from all type of samples examined. When antimicrobial sensitivity testing was carried some strains of gram positive and negative showed resistant to certain antibiotics.

5.3 Recommendations:

1. To control house flies in Wad Medani city: the following should be considered: using traps, and insecticides, but in some instances integrated fly control should be implement
2. Reduction or elimination of fly breeding sites.
3. Reduction of sources that attract flies from other areas.
4. Prevention of contact between flies and disease-causing germs.
5. Protection of food, eating utensils and people from contact with flies.
6. Killing adult flies may reduce the infestation, but elimination of breeding areas is necessary for good management.
7. Ultraviolet light traps can be used to assess population levels, but also serve as a non-chemical control technique that can be used indoors in both agricultural and non-agricultural areas.
8. The use of biological control in fly management.
9. Chemical control, when the house fly is a may or pest in commercial egg production facilities, the control of this insect is by the application of adulticides, or larvicides to directly or indirectly suppress adult densities.
10. Garbage cans and dumpsters should have tight-fitting lids and be cleaned regularly.
11. Dry garbage and trash should be placed in plastic garbage bags and sealed up.
12. All garbage receptacles should be located as far from building entrances as possible.
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