
UNIVERSITI PUTRA MALAYSIA

# CARRIAGE OF *Salmonella* spp. and *Escherichia coli* BY HOUSE FLIES AT THREE EATERIES AROUND UNIVERSITI PUTRA MALAYSIA (UPM) AND THREE POULTRY FARMS IN SELANGOR

E ALVWINA JULIAN

# CARRIAGE OF *Salmonella* spp. and *Escherichia coli* BY HOUSE FLIES AT THREE EATERIES AROUND UNIVERSITI PUTRA MALAYSIA (UPM) AND THREE POULTRY FARMS IN SELANGOR

**E ALVWINA JULIAN**

**A project paper submitted to the**

**Faculty of Veterinary Medicine, Universiti Putra Malaysia**

**In partial fulfillment of the requirement for the**

**DEGREE OF DOCTOR OF VETERINARY MEDICINE**

**Universiti Putra Malaysia Serdang, Selangor Darul Ehsan**

**March 2017**

## CERTIFICATION

It is hereby certified that we have read this project paper entitled "Carriage of *Salmonella* Spp. And *Escherichia coli* by House Flies At Three Eateries around University Putra Malaysia (UPM) and Three Poultry Farms in Selangor", by E Alvwina Julian and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4990 – Final Year Project.

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## ACKNOWLEDGEMENTS

I would like to express my utmost gratitude to my family for their love and support throughout my study and for giving me endless supports while conducting this project.

A million thanks to my supervisor, Dr Nur Indah Ahmad and my co-supervisor, Prof. Dr. Saleha Abdul Aziz for their kind guidance throughout the completion of this project.

Sincerest gratitude to Madam Fauziah Nordin and Dr. Saleh Jajere of Public Health Laboratory for their kindest guidance throughout the completion of my project.

Warmest gratitude to Miss Krishnammah Kuppusamy, Miss Rabiatuladawiyah Rosli and Mister Mohd. Azri Roslan of Bacteriology Laboratory for their generous help during my project.

I would also like to extend my gratitude to Mister Arrifin of Large Animal Ward for driving us for sampling at the poultry farms. Also, to all FYP-classmates in Bacteriology Lab for their advices and supports.

To my dearest roommate, Hazlini Shafie, thank you so much for your warm supports. Lastly, to the awesome rotamates of Group 6, DVM 2017 and all lecturers and staffs of Faculty of Veterinary Medicine.

Thank you.

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## ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Final Year Project.

**CARRIAGE OF *Salmonella* spp. AND *Escherichia coli* BY HOUSE FLIES AT THREE EATERIES AROUND UNIVERSITY PUTRA MALAYSIA (UPM) AND THREE POULTRY FARMS IN SELANGOR**

**By**

**E Alvwina Julian**

**2017**

**Supervisor: Dr. Nur Indah Ahmad**

**Co-supervisor: Prof. Dr. Saleha Abdul Aziz**

House flies are of public health concern for their high potential to be carriers of communicable diseases to both human and animals. There is an urgency to explore if the house flies can carry disease–causing bacteria in their gastrointestinal tract that can greatly amplify the risk of human and animal exposure to diseases. The aim of the present study is to study the carriage of *Salmonella* spp. and *Escherichia coli* by house flies collected from three local eateries that are frequented by students and staffs of University Putra Malaysia (UPM) and from another three poultry farms in Selangor. This study also aims to investigate the evidence of antibiotic resistance of the isolated

bacteria. A total of 60 samples of house flies were caught using sticky papers from three local eateries around UPM and three poultry farms in Selangor. The finding reveals only 3.33% of the total 60 house flies samples collected from both eateries and poultry were confirmed positive for *Salmonella* spp. The positive samples were shown to exhibit antibiotic resistance towards Amoxicillin. This study also revealed that 56.67% of the total 60 house flies samples caught from the three local eateries around UPM and three poultry farms in Selangor carry *Escherichia coli*. Most of the *Escherichia coli* isolates were found to be resistant to Amoxicillin, Streptomycin and Ceftriaxone. House flies are evidently proven to be able to carry both *Salmonella* spp. and *Escherichia coli* and these bacteria do potentially carry antibiotic resistance gene based on the resistant patterns exhibited.

*Keywords*: house fly, *Salmonella* spp., *Escherichia coli*, antibiotic resistance

## ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir Pelajar

**PENGANGKUTAN *Salmonella* spp. DAN *Escherichia coli* OLEH LALAT DI TIGA KEDAI MAKAN DI SEKITAR UNIVERSITY PUTRA MALAYSIA (UPM) DAN TIGA LADANG AYAM DI SELANGOR**

**Oleh**

**E Alvwina Julian**

**2017**

**Penyelia: Dr. Nur Indah Ahmad**

**Penyelia bersama: Prof. Dr. Saleha Abdul Aziz**

Lalat rumah menimbulkan kerisauan terhadap kesihatan awan kerana potensinya yang tinggi sebagai pembawa pelbagai penyakit terhadap manusia dan juga haiwan. Oleh sebab itu, terdapat keperluan untuk menyelidiki sama ada lalat rumah mampu mengangkut patogen di dalam salur pencernaannya. Kemampuan seumpama ini mampu meningkatkan risiko penyebaran penyakit-penyakit bawaan lalat rumah kepada manusia dan haiwan yang sering kali terdedah kepada lalat rumah. Tujuan kajian ini adalah untuk menyelidiki sama ada terdapat pembawaan *Salmonella* spp.

dan *Escherichia coli* oleh lalat rumah di kawasan-kawasan kajian iaitu tiga gerai makan di sekitar Universiti Putra Malaysia dan tiga ladang ayam di sekitar Selangor. Kajian ini juga bertujuan untuk menyiasat sama ada bakteria-bakteria tersebut memperlihatkan bukti adanya daya tahan terhadap antibiotik. Sebanyak 60 sampel terkumpul lalat rumah ditangkap menggunakan kertas lekit di kawasan-kawasan kajian. Sebanyak 3.33% daripada 60 sample tersebut membuktikan terdapat pembawaan *Salmonella* spp. oleh lalat rumah. Sampel-sampel yang positif itu turut memperlihatkan bukti terdapatnya tanda-tanda daya tahan terhadap antibiotik Amoxicillin. Sebanyak 56.67% daripada 60 sampel yang sama turut membuktikan bahawa terdapat pembawaan *Escherichia coli* oleh lalat rumah di kawasan-kawasan kajian. Sampel-sampel positif-*Escherichia coli* juga menunjukkan terdapatnya bukti daya tahan terhadap tiga jenis antibiotik yang berbeza iaitu Amoxicillin, Streptomycin dan juga Ceftriaxone. Lalat rumah sememangnya terbukti mampu membawa *Salmonella* spp. dan *Escherichia coli* dan bakteria-bakteria ini menunjukkan bukti-bukti daya tahan terhadap beberapa jenis antibiotik.

*Kata kunci*: lalat rumah, *Salmonella* spp., *Escherichia coli*, daya tahan antibiotic

## 1.0 INTRODUCTION

House fly is a well-known pest capable of transmitting numbers of diseases to both humans and animals (Keiding, 1986). House flies are non-biting arthropods that usually breed in animals manure during warm day. Thus, they can easily be found around the world. Studies conducted by Bouamama in Tangier, Morocco in 2010 revealed successful isolation of various bacteria including *Escherichia coli* from the external body of the house flies. However, among all the houseflies caught, only one sample was confirmed positive for *Salmonella* spp. This indicates low carriage of *Salmonella* spp. by house flies in Tangier, Morocco during the sampling period.

Similar studies aimed to investigate the species of bacteria harbored by house flies internally and/or externally were conducted in different countries; India (Gupta *et al*., 2011), Iran (Kassiri *et al*., 2012) and Iraq (Ahmed *et al*., 2012) revealed that *Escherichia coli* were among the commonly isolated bacteria from external and/or internal of the house flies' body. However, there was no *Salmonella* spp. was isolated from either study. This suggested low occurrence of *Salmonella* spp. at all the sampling sites from the mentioned studies. However, these findings should not be overlooked as house flies can still potentially transmit *Salmonella* spp.

Based on the data acquire from the Ministry of Health Malaysia, from the year 2010 to 2015, among all the listed food borne diseases such as cholera, dysentery, food poisoning, typhoid and Hepatitis A; food poisoning was recorded having the highest incidence rate of more than 44% . This data showed that there were high numbers of food poisoning cases every year due to various causes. Hence, this study was designed to study the carriage of *Salmonella* spp. and *Escherichia coli* by house flies at three

local eateries which were frequented by students and staffs of University Putra Malaysia (UPM) and three poultry farms in Selangor. In addition, this study also aimed to investigate whether the positive isolates of *Salmonella* spp. and *Escherichia coli* exhibit resistance toward several selected antibiotics.

## 2.0 LITERATURE REVIEWS

### 2.1 *Salmonella* spp.

Morphologically, *Salmonella* spp. is a gram negative, rod-shaped bacterium when stained using Gram stain. This bacterium is commonly found in the intestines of healthy birds and mammals (EFSA, 2014). This bacterium can be classified into many serotypes depending on the variations of antigens that were found on the cell membrane surface and the flagella. *Salmonella* spp. is known to be the second most predominant pathogen that causes food-borne gastroenteritis and the first being *Campylobacter* spp. (Mahmoud, 2012) it is hardy and able to resist desiccation in the environment thus enabling it to survive for an extended period in the environment outside a host. Over 90 000 salmonellosis cases were reported every year in European Union (EFSA, 2014).

### 2.2 *Escherichia coli*

Morphologically, *Escherichia coli* are gram negative, rod-shaped bacterium when stained using Gram stain. This bacterium is a normal flora of the gastrointestinal tract of humans and animals (Welch, 2006). Escherichia coli is also preferably used as an indicator of fecal contamination in the assessment of food and water safety. Harmless *Escherichia coli* strains are commensals of the gastrointestinal tract and do not cause diseases. Less pathogenic strains *Escherichia coli* only cause milder form of gastroenteritis which is self-limiting in nature. However, more pathogenic strains of *Escherichia coli* can rapidly bring down the infected victims downhill. Thus, antibiotic therapy is needed to resolve the infection (Macovei *et al*., 2008).

### 2.3 House flies (*Musca domestica*)

Morphologically, adult house fly can be identified as grey-colored, 6-9 mm long with distinguishable four dark stripes running lengthwise on the dorsal part of the body. House fly is commonly found worldwide. Often found at close proximity to places with abundant food sources such as human foods, garbage and excreta including sweat and animal dung (Keiding, 1986). The female deposited eggs in decaying organic materials or feces of human or animals. House fly can fly up to two miles from its original site to find foods thus; it is a highly convenient vector to various pathogens including *Salmonella* spp. and *Escherichia coli* (Macovei *et al*., 2008). A study by Esten & Mason in 1908 stated that a single house fly can carry approximately 6 000 000 bacteria on the exterior surface of its body.

### 2.4 Method of house flies sampling

Similar studies conducted in Morocco (Bouamama *et al.*, 2010), India (Gupta *et al*., 2011), Iran (Kassiri *et al*., 2012) and Iraq (Ahmed *et al*., 2012), in regards of using house flies as sample, primarily utilize sterilized insect nets to catch live house flies for bacterial isolation and identification. These studies were all aimed to map out different species of bacteria that can be isolated from the surface of the flies' body as well as within the alimentary tract of the flies. However, to this date, there has been no published study that utilizes sticky paper as primary method of collecting the house flies for similar purpose. Sticky papers are adhesive, baited papers that are conveniently used by the public to reduce the number of flies. It is cheap and easy to use.

**2.5 Carriage of *Salmonella* spp. and *Escherichia coli* by house flies in Malaysia.**

In Malaysia, a thorough epidemiological study on the prevalence of Salmonella spp. and Escherichia coli has yet to be conducted. In 2000, a study by Sulaiman *et al*. in Chow Kit area, Kuala Lumpur, various types of bacteria were successfully isolated from different types of flies. In 2005, a study by Nazni *et al*. had successfully isolated *Escherichia coli* from the house flies collected at various sampling sites such as food courts, dumping grounds, food processing plants and poultry farms in different states in Malaysia. However, no *Salmonella* spp. was isolated. This indicates low carriage of *Salmonella* spp. by house flies. In 2010, Choo in her study had successfully isolated *Campylobacter* spp. and *Salmonella* spp. from both external and internal part of the flies' body which were sampled from the Large Animal Ward of the Faculty of Veterinary Medicine, a cafeteria and a poultry farm. In all of the studies mentioned, antibiotic susceptibility test was not done.

## 3.0 MATERIAL AND METHODS

### 3.1 Sampling Site

Samplings of house flies were done at six sampling sites. Three eateries which were frequented by staffs and students of University Putra Malaysia (UPM) were chosen. The eateries chosen were Sri Serdang, Padang and Tasik. Another three sampling sites were three poultry farms in Selangor. One of the chosen poultry farms was located in Banting and another two poultry farms were both located in Jenderam. All three poultry farm rear 'ayam kampon' or village chicken (*Gallus gallus*) in free range production system.

### 3.2 Sample

A total of sixty (60) samples of house flies were caught from all sampling sites by using sticky paper. Ten (10) house flies were caught from each sampling site. In this study, a pooled sample was used. One sample was equivalent to five (5) house flies pooled together. The purpose of using pooled sample of house flies was to increase the chance of isolating the targeted bacteria; *Salmonella* spp. and *Escherichia coli*. Each sampling site was sampled only once and the time of sampling of all sampling site ranged from 9.30am to 12.30pm.

### Isolation and Identification

### 3.3 Isolation of *Salmonella* spp.

Sticky papers from the sampling site were brought to Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, UPM for sample processing. For each pooled sample, five (5) house flies were collected from the sticky paper using sterilized

forceps and transferred to Bijou bottle containing 10ml of Buffered Peptone Water (BPW) and incubated at 37°C for 24 hours. The house flies were then crushed using sterilized forceps against the wall of Bijou bottle.

Refer to Plate 1.

1ml of the aliquot was transferred into 10ml of Rappaport Vasiliadis (RV) broth and incubated at 42°C for 24 hours. The next day, 1 loopful of the aliquot from RV broth was culture onto selective media used to culture *Salmonella* spp., Xlyose Lysine Deoxycholate XLD agar. The agar was incubated at 37°C for 24 hours. After 24 hours, suspected white colonies with black center was identified and subculture onto nutrient agar at 37°C for 24 hours to obtain pure culture of *Salmonella* spp.

Refer to Plate 2 to 4.

Gram staining of the pure culture of suspected *Salmonella* spp. was done to confirm the cell morphology as gram negative, rod in singles. Biochemical tests comprising of Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) and Serum Agglutination Test (SAT) using Polyvalent 'O' antisera were carried out to confirm whether the isolates is *Salmonella* spp. All *Salmonella* spp. isolates were subjected to antibiotic susceptibility test.

Refer to Plate 5 to 7.

**3.4 Isolation of *Escherichia coli***

Sticky papers from the sampling site were brought to Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, UPM for sample processing. For each pooled sample, five (5) house flies were collected from the sticky paper using sterilized

forceps and transferred to Bijou bottle containing 10ml of Buffered Peptone Water (BPW) and incubated at 37°C for 24 hours. The house flies were then crushed using sterilized forceps against the wall of Bijou bottle.

1 looopful of the aliquot was cultured onto selective culture media, Chromocult agar and incubated at 37°C for 24 hours. *Escherichia coli* colonies grew as a dark blue to violet colony on Chromocult agar. 1 ml of KOVACS *Indole* reagent was added onto the suspected *Escherichia coli* colony and positive result revealed the KOVACS Indole reagent changed color from yellow to cherry red color when in contact with *Escherichia coli* colony. The similar dark blue to violet *Escherichia coli* colonies were sub-cultured onto nutrient agar at 37°C for 24 hours to obtain pure culture of *Escherichia coli* for antibiotic susceptibility test.

Refer to Plate 8 to 11.

**3.5 Antibiotic Susceptibility Test**

All positives isolates of *Salmonella* spp. and *Escherichia coli* were subjected to antibiotic susceptibility test. Kirby Bauer disc diffusion method, 0.5 McFarland turbidity standards and Mueller-Hinton agar were used in this study. Six different types of antibiotics were used in this study; Enrofloxacin, Amoxicillin, Streptomycin, Chloramphenicol, Nalidixic acid and Ceftriaxone. The diameter of the zone of inhibition in Millimeter (mm) was measured and recorded. The inhibition zone diameter interpretative, standards for both *Salmonella* spp. and *Escherichia coli* were used to determine if the recorded zone of inhibition was sensitive, intermediate or resistant towards the antibiotics tested.

**Plate 1**: 10ml of Buffered Peptone Water (BPW) in a Bijou bottle

**Plate 2**: 10ml of Rappaport Vasiliadis (RV) broth in Bijou bottle before incubation

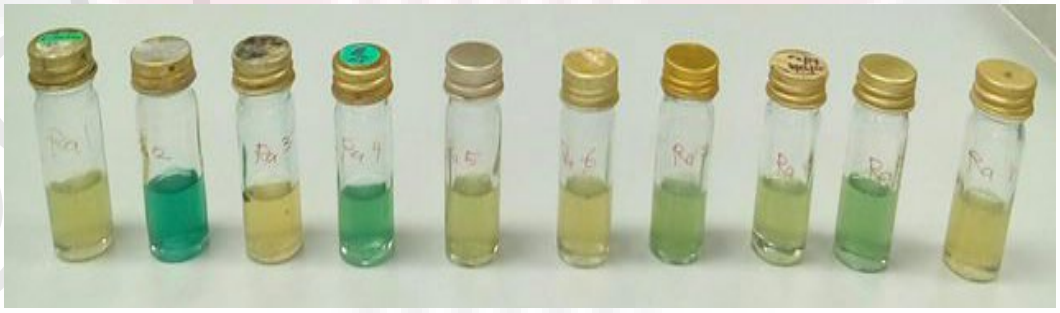

**Plate 3**: 10ml of Rappaport Vasiliadis (RV) broth in Bijou bottle after incubation

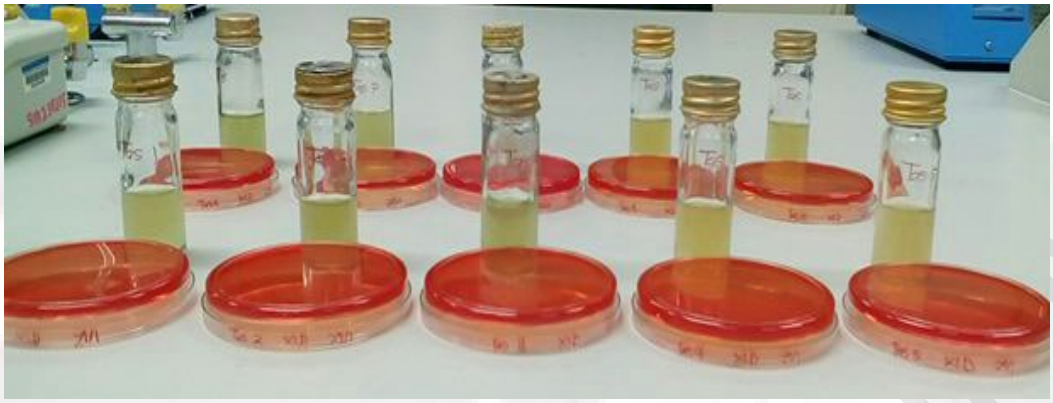

**Plate 4**: Inoculum from Rappaport Vasiliadis (RV) broth was cultured onto Xlyose Lysine Deoxycholate XLD agar.

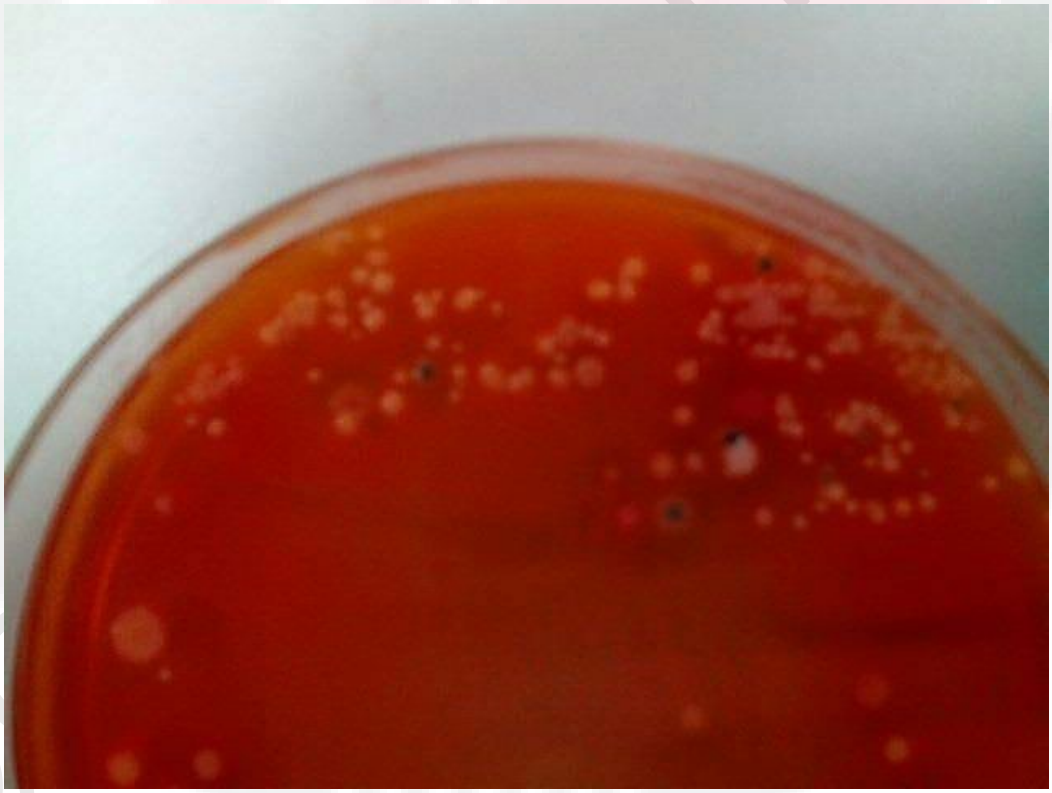

**Plate 5**: Suspected white-colored *Salmonella* spp. colonies with black centers

**Plate 6**: Triple Sugar Iron (TSI)-Left &Lysine Iron Agar (LIA)-Right

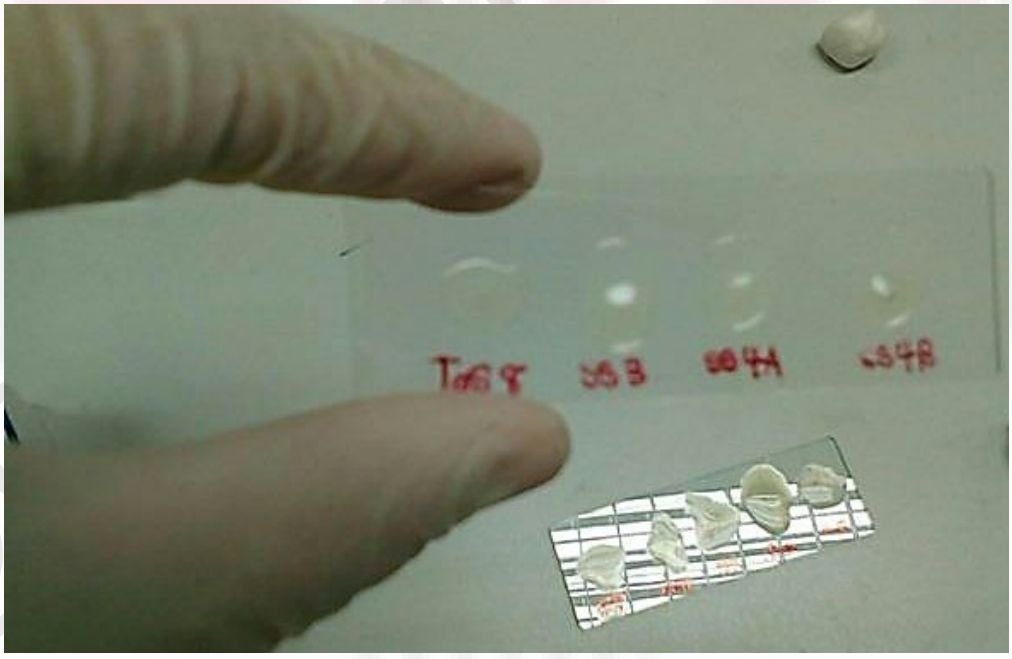

**Plate 7**: Serum Agglutination Test (SAT) using Polyvalent 'O' antisera

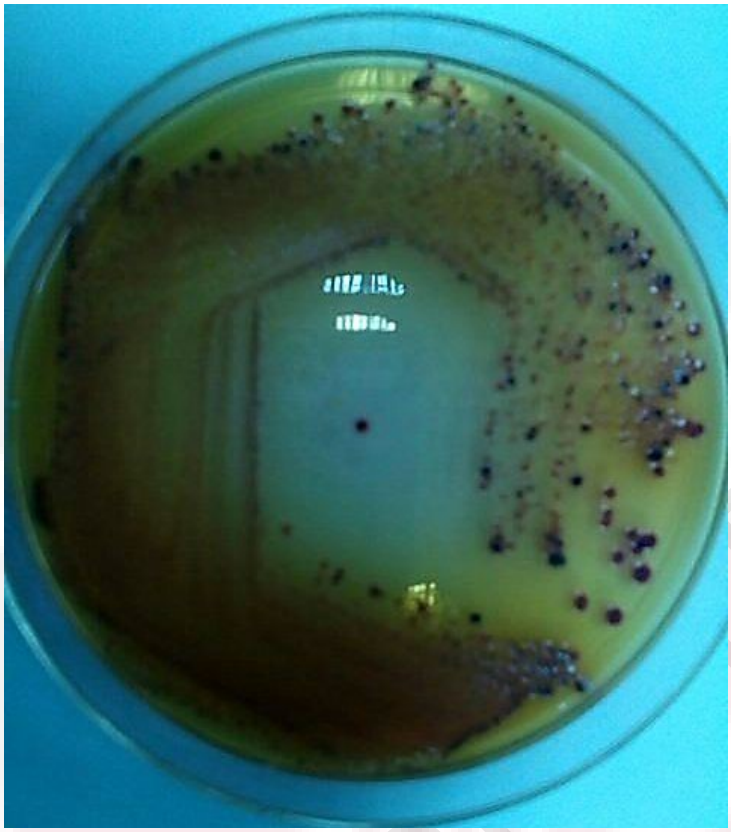

**Plate 8:** Dark blue to violet colonies of *Escherichia coli* on Chromocult agar

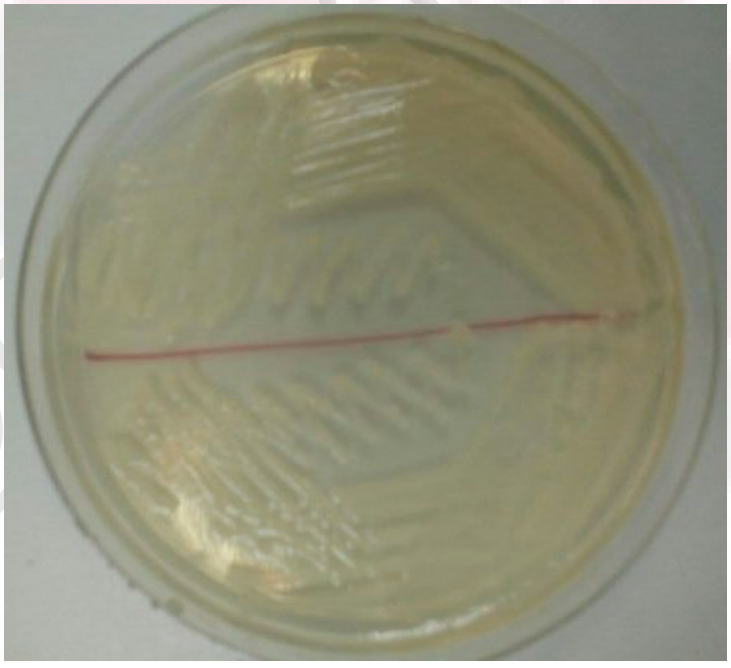

**Plate 9**: Pure Culture of *Escherichia coli* on nutrient agar


**Plate 10:** 0.5 McFarland turbidity standards

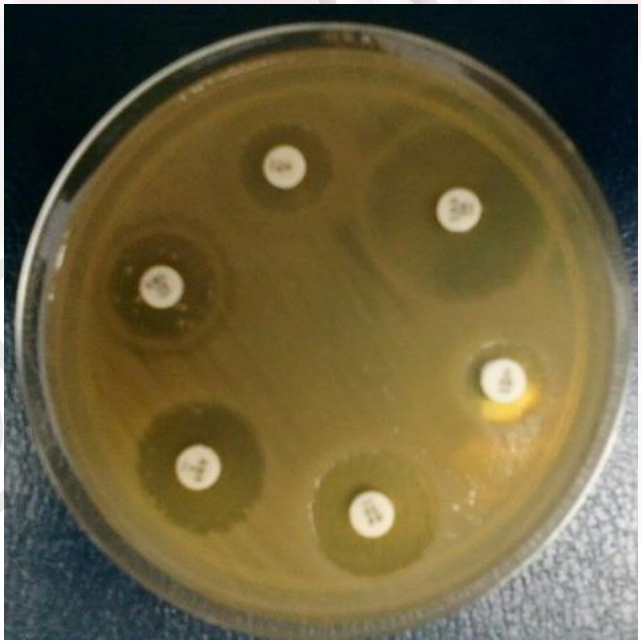

**Plate 11**: Zone of Inhibition (ZOI) of antibiotics on Mueller-Hinton agar

## 4.0 RESULTS

A total of sixty pooled samples each consisted of five house flies were collected from three eateries around Universiti Putra Malaysia (UPM) and three poultry farms in Selangor. *Salmonella* spp. was detected in only two (2) out of sixty (60) samples (3.33%) and *Escherichia coli* were detected in thirty-four (34) out of sixty (60) samples (56.67%).

Among the sixty samples collected in this study, only two samples were found to be *Salmonella*-positive. Sri Serdang and Tasik each had one *Salmonella*-positive sample. Another eatery, Padang as well as the three poultry farms was revealed negative for house flies carrying *Salmonella* spp. at the time of sampling. The results were as shown in Table 1.

**Table 1**

Carriage of *Salmonella* spp. by House Flies at Three Eateries in Sri Serdang and Three Poultry Farm in Selangor

| Location | Number of Samples | Number of *Salmonella sp.* Positive Plates (XLD agar) | Biochemical Tests (TSI & LIA) | Serum Agglutination Test (SAT) – Polyvalent O | Number of *Salmonella* spp. isolated |
|---|---|---|---|---|---|
| Sri Serdang (SS) | 10 | 3/10 (30%) | 3/3 (100%) | 1/3 (33.33%) | 10% |
| Padang (PD) | 10 | 2/10 (20%) | 1/2 (50%) | 0/1 (0%) | 0% |
| Tasik (TS) | 10 | 6/10 (60%) | 1/6 (16.67%) | 1/1 (100%) | 10% |
| Banting | 10 | 10/10 (100%) | 0/10 (0%) | 0% | 0% |
| Jenderam 1 | 10 | 10/10 (100%) | 0/10 (0%) | 0% | 0% |
| Jenderam 2 | 10 | 10/10 (100%) | 0/10 (0%) | 0% | 0% |
| Total: | 60 | | | | 2/60 (3.33%) |

The six out of ten samples (60%) from Sri Serdang, seven out of ten samples (70%) from Padang, five out of ten samples (50%) from Tasik were positive for *Escherichia coli*. Among the three selected poultry farms in Selangor; Banting had 40% *Escherichia coli*-positive sample, Jenderam 1 and Jenderam 2 both had 60% *Escherichia coli*-positive samples respectively. The results were as shown in Table 2 and Table 3.

**TABLE 2**

Carriage of *Escherichia coli* by House Flies at Three Eateries in Sri Serdang, Selangor

| Location | Number of Samples | Number of *E.coli* Positive Plates (Chromocult agar) |
|---|---|---|
| Sri Serdang (SS) | 10 | 6 (60%) |
| Padang (PD) | 10 | 7 (70%) |
| Tasik (TS) | 10 | 5 (50%) |

**TABLE 3**

Carriage of *Escherichia coli* by House Flies at Three Poultry Farms in Selangor

| Location | Number of Samples | Number of *E.coli* Positive Plates |
|---|---|---|
| Banting (KM) | 10 | 4 (40%) |
| Jenderam 1 (RA) | 10 | 6 (60%) |
| Jenderam 2 (CR) | 10 | 6 (60%) |

Antibiotic susceptibility test of *Salmonella* spp. isolates from eatery in Sri Serdang was found to be resistant to two types of antibiotics; Amoxicillin and Nalidixic acid but susceptible to all the other four types of antibiotics; Enrofloxacin, Streptomycin, Chloramphenicol and Ceftriaxone. Another *Salmonella* spp. isolates was found to be

resistant to Amoxicillin and Streptomycin but susceptible to Enrofloxacin, Chloramphenicol, Nalidixic acid and Ceftriaxone. Antibiotics susceptibility test of *Escherichia coli* isolates from all the sampling sites were found to exhibit resistance towards few types of antibiotics. Most of the isolated *Escherichia coli* isolates exhibited significant resistant towards Amoxicillin, Streptomycin and Ceftriaxone.

**Refer to Table 5 to 9.**

## 5.0 DISCUSSION

In this study, 3.33% of *Salmonella* spp. was isolated from a total of sixty pooled samples of house flies caught from three eateries around Universiti Putra Malaysia (UPM) and three poultry farms in Selangor. The low carriage of *Salmonella* spp. by house flies is a good finding. As such, there is lesser risk of the publics to contract diseases due to *Salmonella* spp. However, the successful isolation of *Salmonella* spp. from two local eateries is alarming. Although these isolates were not confirmed as of pathogenic serotypes, however, this opened the door for possible occurrence of food poisoning due to *Salmonella* spp.

*Escherichia coli* were successfully isolated at 56.67% of the total sixty pooled samples of house flies caught from the sample sampling sites. The fact that *Escherichia coli* are normal flora of mammalian guts could have influenced this finding. All eateries were within close proximity to the housing area in Sri Serdang. Although the sewage system at this housing area is of closed type which prevents human fecal contamination to the environment, however, there were also many roaming pets and strays around the housing areas. The house flies could have accessed to the animals' manures and thus carried the bacterium on or in their body.

Within the period of one week after the samples collection there was no major reported case of food poisoning from around the sampling sites. Clinical signs following ingestion of the bacteria usually appeared within 72 hours (Macovei *et al*., 2008). Clinical signs such as gastroenteritis and diarrhea following ingestion of contaminated foods are usually self-limiting in immunocompetent individuals (Keiding, 1986). Recovery often without antibiotic therapy is common. However,

immunocompromised individuals such as, children less than 5 years old, elderly more than 65 years old, diabetic, cancer and HIV patients are highly at risk developing more severe form of clinical signs (CDC, 2016). Hence, affected individuals in the mentioned categories often need antibiotic therapy to kill off the bacteria that cause gastroenteritis and diarrhea.

Although not all types of existing serotypes or strains of *Salmonella* spp. and *Escherichia coli* can cause food poisoning; prevention and control measures must be practiced to prevent such incident from ever occurring. As such, prevention is always better than cure. It is of utmost importance that personnel who are handling foods that are meant for consumption to practice good hygiene at all times. Responsible eateries workers must wear suitable protective attires and disinfect properly before handling, preparing and serving foods. Poultry farms workers should disinfect properly before and after handling the birds or the fecal materials. Both *Salmonella* spp. and *Escherichia coli* are known to be quite hardy and able survive outside a host for a considerable amount of time.

In this study, it was observed that often eateries workers did not clean the food scrapes and tables right away due to over-flowing customers especially during peak hours and leftovers were often left in exposed nearby trash bags. Responsible wastes management must be practiced to reduce the optimum breeding environments for house flies. Eateries workers are recommended to immediately dispose all wastes after food preparation and leftovers after all customers left. Prolonged exposure to the environment will attract house flies that potentially contaminate the exposed foods

with the harmful bacteria that they carry on their body surfaces or within their alimentary tract.

All of the three selected poultry farms practiced free range production system. This type of production system allowed the birds to roam freely within a perimeter of fenced area. The open space allowed plenty of direct interactions between the house flies and the birds. House flies hovering on the birds' droppings as well as sick and injured birds were not a rare sighting. The manures were found to be rarely on regular basis and could serve as a possible harbor for house flies' larvae to grow. Farmers are encouraged to clean the farm regularly to reduce chances of contracting various diseases including salmonellosis and colibacillosis. Surprising, none of the farms had sustained severe and devastating *Salmonella* spp. or *Escherichia coli* infection outbreak since the beginning of establishment.

The important clue of warding off such nasty infection from crippling the entire production system lies on the breed superiority. All of the poultry farms rear 'ayam kampong' or village chicken (*Gallus gallus*) which is known to be a breed native to certain parts of Asia including Malaysia and Indonesia. This native chicken breed possessed superior immunity as compared to those fast growing commercialized breeds commonly used in intensive production system. Studies have shown that 'ayam kampong' is more resistant against *Salmonella* spp. infection. A study in 2013 by Ulupi *et al*. revealed that 'ayam kampong' had natural antibodies against *Salmonella entertidis* which was induced by natural exposure to *Salmonella enteritidis* in the free range rearing system.

Extensive poultry production did not practice regular cleaning of the fecal materials as practiced in intensive poultry farming. Hence, farms practicing extensive production system are recommended to be cleaned regularly to reduce incidence of house flies as well as to reduce chances of contracting various diseases borne by the free moving arthropods. Excessive layers of fecal materials on the ground can create optimum, warm environment for house flies to lay eggs and complete their life cycles. Consequently, this can lead to propagation of house flies within the farm vicinity. In order to prevent this, collected fecal materials must be covered and the surface must be kept dry (Keiding, 1986).

*Salmonella* spp. and *Escherichia coli* isolates in this study were both shown to exhibit resistant pattern toward Amoxicillin. A study by Belmar-Liberato *et al*. in 2011 addressed the same problem. The emergence of Amoxicillin-resistant bacteria is a concerning matter. Amoxicillin is one of the most common antibiotics prescribed to both human and animals as broad-spectrum antibacterial against infections by many Gram-positive aerobic and anaerobic bacteria and Gram-negative aerobic bacteria. Bacteria such as *Salmonella* spp. and *Escherichia coli* are capable of acquiring antibiotic resistant genes via conjugation with other bacterium possessing resistant genes. Irresponsible usage of Amoxicillin might have contributed to the development of resistance against this antibiotic.

Human and animals that came down with bacterial infections and were treated with Amoxicillin can be the potential source that contributes to this finding. Dispensed antibiotics must be taken as per indicated by the medical officers or veterinarians. Under dosed antibiotic will not achieve the desired therapeutic level and primed the

target bacteria to the antibiotic thus leading to development of antibiotic resistance. It should be brought to attention that University Putra Malaysia (UPM) is within close proximity to few clinics and hospitals, in-campus farms as well as an established veterinary school whereby the prescription and usage of Amoxicillin is a common practiced. Though this is not proven, this can be speculated to be one of the contributing factors of resistance against Amoxicillin. Most *Escherichia coli* isolates from all sampling sites were also demonstrated to exhibit prominent resistant patterns toward other commonly used antibiotics; Streptomycin and Ceftriaxone.

## 6.0 CONCLUSION AND RECOMMENDATIONS

*Salmonella* spp. and *Escherichia coli* were successfully isolated from three eateries around Universiti Putra Malaysia and three poultry farms in Selangor. The house flies at the sampling sites carry low number of *Salmonella* spp. (3.33%) but high number of *Escherichia coli* (56.67%). Both eateries and poultry farms workers need to practice good hygiene before, during and after handling the foods and birds respectively. Good wastes management can significantly reduce the number of flies within the vicinity; hence, this reduces the chance of vector-borne diseases transmission. Responsible antibiotic usage must be practiced as an effort against development of antibiotic resistance.

It is recommended for future studies to conduct sampling during sunny day as to increase the chance of catching more house flies. House flies are most active at temperature of 20-25°C, low humidity and calm air. If the researcher intends to study the carriage of bacteria from both external and internal body of the house flies, it is recommended to use sterilized insect net. In this study, collection method using sticky paper had made it unreliable to study the carriage of *Salmonella* spp. and *Escherichia coli* by house flies from the external parts of the flies' bodies.

Based on the findings from this study, similar study can be conducted to investigate the types of bacteria carried by house flies at different animal units and cafeterias within Universiti Putra Malaysia (UPM) campus and to investigate the antibiotic susceptibility profiles of the isolates.

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**TABLE 4:**

Reference Range for Determining Antibiotic Susceptibility

| Antibiotic | Resistant (mm) | Intermediate (mm) | Susceptible (mm) |
|---|---|---|---|
| Enrofloxacin | ≤15 | 16-20 | ≥21 |
| Amoxicillin | ≤13 | 14-17 | ≥18 |
| Streptomycin | ≤11 | 12-14 | ≥15 |
| Chloramphenicol | ≤12 | 13-17 | ≥18 |
| Nalidixic acid | ≤13 | 14-18 | ≥19 |
| Ceftriaxone | ≤13 | 14-20 | ≥21 |

**Table 5:**

Antibiotic Susceptibility Test (AST) of samples which are tested positive for *Salmonella* spp.

| Antibiotics | Diameter of Zone of Inhibition (mm) | | | Interpretation |
|---|---|---|---|---|
| | SS4 | TS8 | Average | |
| Enrofloxacin | 22 | 24 | 23.000 | **Gen: Susceptible**<br>R: -<br>Ir: -<br>S: 100% |
| Amoxicillin | 0 | 0 | 0 | **Gen: Resistant**<br>R: 100%<br>Ir:-<br>S:- |
| Streptomycin | 17 | 0 | 17.000 | **Gen: Susceptible**<br>R: 50%<br>Ir:-<br>S:50% |
| Chloramphenicol | 28 | 25 | 26.500 | **Gen: Susceptible**<br>R:-<br>Ir:-<br>S:100% |
| Nalidixic acid | 0 | 21 | 21.000 | **Gen: Susceptible**<br>R: 50%<br>Ir:-<br>S:50% |
| Ceftriaxone | 21 | 0 | 21.000 | **Gen: Susceptible**<br>R: 50%<br>Ir:-<br>S:50% |

**Table 6:**

Antibiotic Susceptibility Test (AST) for *Escherichia coli* isolates from Sri Serdang

| Antibiotics | Diameter of Zone of Inhibition (mm) | | | | | | | Interpretation |
|---|---|---|---|---|---|---|---|---|
| | SS1 | SS3 | SS4 | SS6 | SS9 | SS10 | Average | |
| Enrofloxacin | 0 | 14 | 20 | 24 | 10 | 18 | 14.333 | **Gen: Resistant**<br>R: 50%<br>Ir: 33.33%<br>S: 16.67% |
| Amoxicillin | 0 | 0 | 0 | 0 | 8 | 12 | 3.333 | **Gen: Resistant**<br>R: 100%<br>Ir: -<br>S: - |
| Streptomycin | 13 | 13 | 14 | 15 | 16 | 12 | 13.833 | **Gen: Intermediate**<br>R: -<br>Ir: 66.67%<br>S: 33.33% |
| Chloramphenicol | 24 | 22 | 21 | 27 | 24 | 29 | 24.500 | **Gen: Susceptible**<br>R: -<br>Ir: -<br>S: 100% |
| Nalidixic acid | 0 | 0 | 0 | 20 | 0 | 16 | 6.000 | **Gen: Resistant**<br>R: 66.67%<br>Ir: 16.67%<br>S: 16.67% |
| Ceftriaxone | 0 | 0 | 17 | 0 | 0 | 0 | 2.833 | **Gen: Resistant**<br>R: 83.33%<br>Ir: 16.67%<br>S: - |

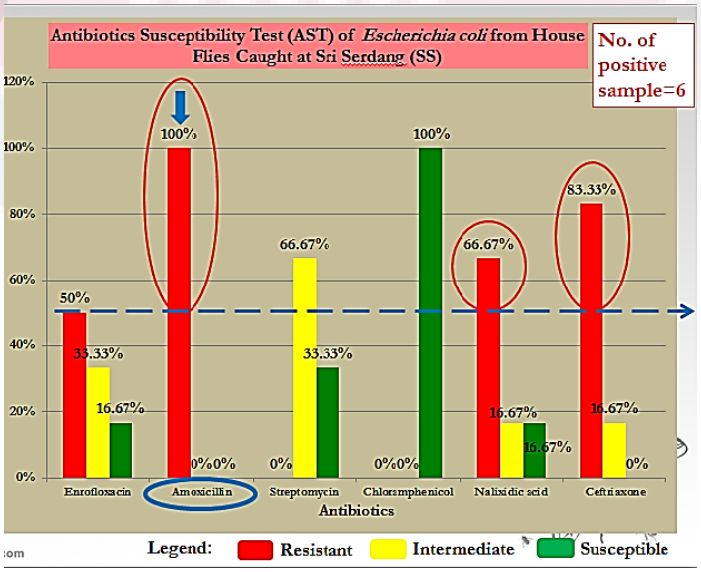

**Table 7:**

Antibiotic Susceptibility Test (AST) for *Escherichia coli* isolates from Padang

| Antibiotics | Diameter of Zone of Inhibition (mm) | | | | | | | | Interpretation |
|---|---|---|---|---|---|---|---|---|---|
| | PD1 | PD2 | PD3 | PD4 | PD6 | PD7 | PD8 | Average | |
| Enrofloxacin | 14 | 13 | 14 | 21 | 18 | 15 | 17 | 16.000 | **Gen:Intermediate**<br>R: 57.14%<br>Ir: 28.57%<br>S: 14.28% |
| Amoxicillin | 0 | 14 | 0 | 21 | 15 | 0 | 0 | 7.143 | **Gen: Resistant**<br>R: 57.14%<br>Ir: 28.57%<br>S: 14.28% |
| Streptomycin | 10 | 13 | 11 | 11 | 13 | 10 | 10 | 11.143 | **Gen: Resistant**<br>R: 71.43%<br>Ir: 28.57%<br>S: - |
| Chloramphenicol | 20 | 23 | 10 | 22 | 22 | 22 | 25 | 20.571 | **Gen: Susceptible**<br>R: 14.28%<br>Ir: -<br>S: 85.71% |
| Nalidixic acid | 13 | 15 | 18 | 19 | 17 | 15 | 15 | 16.000 | **Gen:Intermediate**<br>R: 14.28%<br>Ir: 71.43%<br>S: 14.28% |
| Ceftriaxone | 10 | 20 | 16 | 18 | 11 | 0 | 0 | 10.714 | **Gen: Resistant**<br>R: 57.14%<br>Ir: 42.86%<br>S: - |

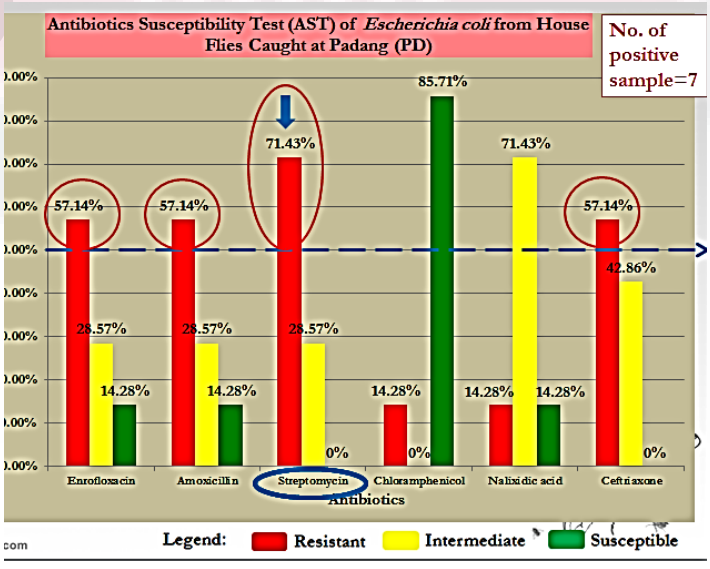

**Table 8:**

Antibiotic Susceptibility Test (AST) for *Escherichia coli* isolates from Tasik

| Antibiotics | Diameter of Zone of Inhibition (mm) | | | | | | Interpretation |
|---|---|---|---|---|---|---|---|
| | TS3 | TS4 | TS5 | TS6 | TS7 | Average | |
| Enrofloxacin | 27 | 28 | 20 | 16 | 21 | 22.400 | **Gen:Susceptible**<br>R: -<br>Ir: 40.00%<br>S: 60.00% |
| Amoxicillin | 15 | 21 | 0 | 0 | 0 | 7.200 | **Gen: Resistant**<br>R: 60.00%<br>Ir: 20.00%<br>S: 20.00% |
| Streptomycin | 14 | 14 | 8 | 11 | 11 | 11.600 | **Gen: Resistant**<br>R: 60.00%<br>Ir: 40.00%<br>S: - |
| Chloramphenicol | 19 | 20 | 22 | 29 | 31 | 24.200 | **Gen:Susceptible**<br>R: -<br>Ir: -<br>S: 100% |
| Nalidixic acid | 29 | 17 | 15 | 0 | 21 | 16.400 | **Gen:Intermediate**<br>R: 20.00%<br>Ir: 40.00%<br>S: 40.00% |
| Ceftriaxone | 19 | 19 | 19 | 0 | 20 | 15.400 | **Gen:Intermediate**<br>R: 20.00%<br>Ir: 80.00%<br>S: - |

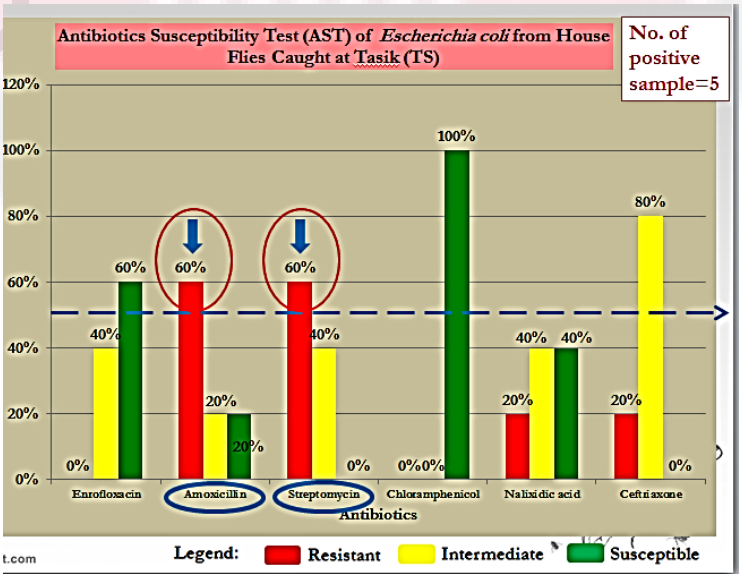

**Table 9:**

Antibiotic Susceptibility Test (AST) for *Escherichia coli* isolates from Banting

| Antibiotics | Diameter of Zone of Inhibition (mm) | | | | | Interpretation |
|---|---|---|---|---|---|---|
| | KM3 | KM6 | KM7 | KM9 | Average | |
| Enrofloxacin | 23 | 23 | 16 | 26 | 22.000 | **Gen: Susceptible**<br>R: -<br>Ir: 25.00%<br>S: 75.00% |
| Amoxicillin | 18 | 17 | 19 | 19 | 18.250 | **Gen: Susceptible**<br>R: -<br>Ir: 25.00%<br>S: 75.00% |
| Streptomycin | 13 | 11 | 11 | 11 | 11.500 | **Gen: Resistant**<br>R: 75.00%<br>Ir: 25.00%<br>S: - |
| Chloramphenicol | 21 | 26 | 24 | 25 | 24.000 | **Gen: Susceptible**<br>R: -<br>Ir: -<br>S: 100% |
| Nalidixic acid | 21 | 13 | 14 | 19 | 16.750 | **Gen:Intermediate**<br>R: 25.00%<br>Ir: 25.00%<br>S: 50.00% |
| Ceftriaxone | 24 | 24 | 10 | 22 | 20.000 | **Gen:Intermediate**<br>R: 25.00%<br>Ir: -<br>S: 75.00% |

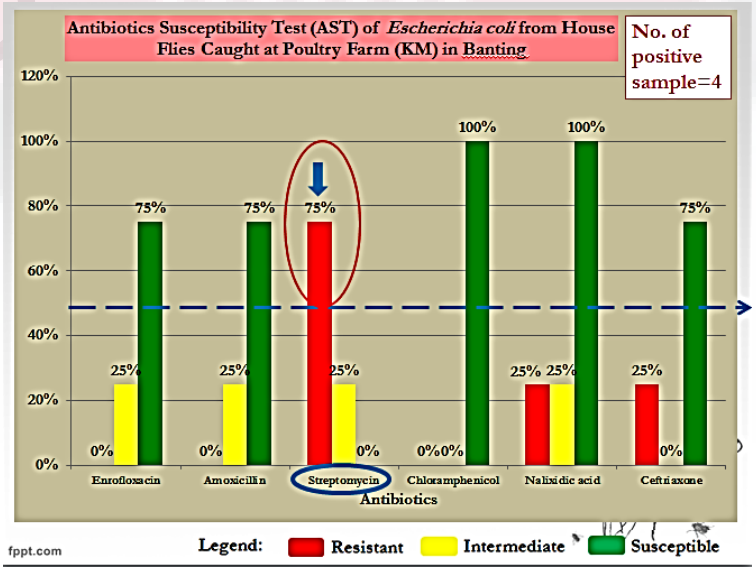

**Table 10:**

Antibiotic Susceptibility Test (AST) for *Escherichia coli* isolates from Jenderam 1

| Antibiotics | Diameter of Zone of Inhibition (mm) | | | | | | | Interpretation |
|---|---|---|---|---|---|---|---|---|
| | RA1 | RA2 | RA3 | RA4 | RA6 | RA9 | Average | |
| Enrofloxacin | 25 | 21 | 22 | 19 | 21 | 27 | 22.500 | **Gen: Susceptible**<br>R: -<br>Ir: 16.67%<br>S: 83.33% |
| Amoxicillin | 19 | 10 | 0 | 0 | 15 | 0 | 7.333 | **Gen: Resistant**<br>R: 66.67%<br>Ir: 16.67%<br>S: 16.67% |
| Streptomycin | 13 | 17 | 12 | 12 | 10 | 18 | 13.667 | **Gen:Intermediate**<br>R: 16.67%<br>Ir: 50.00%<br>S: 33.33% |
| Chloramphenicol | 26 | 24 | 22 | 30 | 26 | 29 | 26.167 | **Gen: Susceptible**<br>R: -<br>Ir: -<br>S: 100% |
| Nalidixic acid | 21 | 8 | 0 | 20 | 18 | 19 | 14.333 | **Gen:Intermediate**<br>R: 33.33%<br>Ir: 16.67%<br>S: 66.67% |
| Ceftriaxone | 19 | 8 | 8 | 8 | 22 | 14 | 13.167 | **Gen: Resistant**<br>R: 50.00%<br>Ir: 33.33%<br>S: 16.67% |

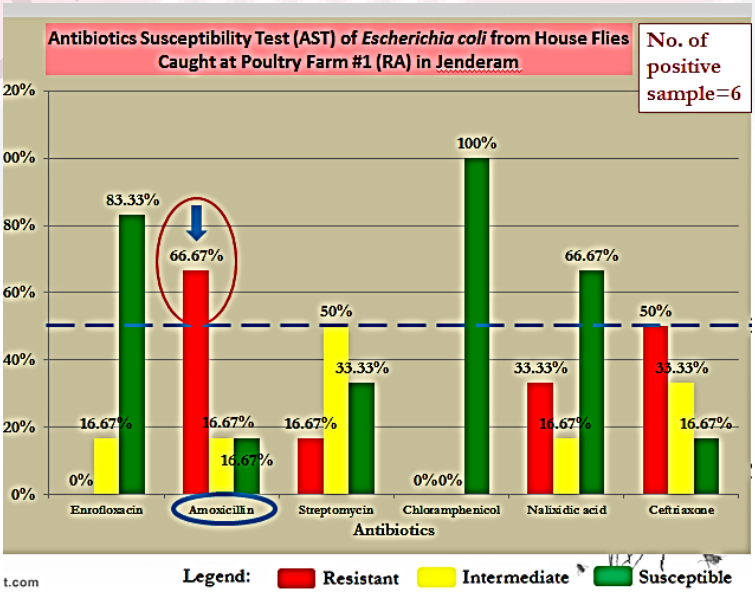

**Table 11:**

Antibiotic Susceptibility Test (AST) for *Escherichia coli* isolates from Jenderam 2

| Antibiotics | Diameter of Zone of Inhibition (mm) | | | | | | | Interpretation |
|---|---|---|---|---|---|---|---|---|
| | CR3 | CR4 | CR7 | CR8 | CR9 | CR10 | Average | |
| Enrofloxacin | 16 | 18 | 18 | 14 | 19 | 16 | 16.833 | **Gen:Intermediate**<br>R: 16.67%<br>Ir: 83.33%<br>S: - |
| Amoxicillin | 16 | 0 | 17 | 18 | 21 | 18 | 15.000 | **Gen:Intermediate**<br>R: 16.67%<br>Ir: 33.33%<br>S: 50.00% |
| Streptomycin | 12 | 12 | 12 | 10 | 10 | 13 | 11.500 | **Gen: Resistant**<br>R: 33.33%<br>Ir: 66.67%<br>S: - |
| Chloramphenicol | 23 | 22 | 24 | 22 | 20 | 26 | 22.833 | **Gen: Susceptible**<br>R: -<br>Ir:-<br>S: 100% |
| Nalidixic acid | 16 | 15 | 15 | 12 | 28 | 0 | 14..333 | **Gen:Intermediate**<br>R: 33.33%<br>Ir: 50.00%<br>S: 16.67% |
| Ceftriaxone | 0 | 8 | 12 | 0 | 0 | 0 | 3.333 | **Gen: Resistant**<br>R: 100%<br>Ir: -<br>S: - |