



UNIVERSITI PUTRA MALAYSIA

**OCCURRENCE OF SALMONELLA SPP. AND E. COLI ISOLATES
FROM PERIDOMESTIC COCKROACHES
(PERIPLANETA AMERICANA) AND THEIR ANTIBIOTIC
SUSCEPTIBILITY PATTERNS**

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(*PERIPLANETA AMERICANA*) AND THEIR ANTIBIOTIC
SUSCEPTIBILITY PATTERNS**

By:

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Occurrence of *Salmonella* spp. And *E. coli* Isolates From Peridomestic Cockroaches (*Periplaneta Americana*) And Their Antibiotic Susceptibility Patterns”, by Nurliyana Meor Abdullah and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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DEDICATIONS

This project paper is dedicated to

My parents,

Meor Abdullah Meor Baldin & Badiyah Baharuddin

Whose affection, love, encouragement, and prays of days and night make me able to
get such success and honor,

Along with all hard working and respected

Teachers.

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LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
BGA	Brilliant green agar
XLD	Xylose lysine desoxycholate
EMB	Eosin-methylene blue
LIA	Lysine iron agar
TSI	Triple sugar iron
SIM	Sulfide indole motility
BPW	Buffer peptone water
RV	Rappaport-vassiliadis
MDR	Multiple drug resistant

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek.

**KEHADIRAN *SALMONELLA* DAN *E. COLI* DARI LIPAS PERIDOMESTIK
(*PERIPLANETA AMERICANA*) DAN POLA-POLA KECENDERUNGAN
RESISTAN TERHADAP ANTIBIOTIK.**

oleh

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(2016)

Penyelia: Dr. Yusof Hamali Ahmad

Terdapat banyak lipas di Malaysia yang di dapati membawa pelbagai mikroorganisma merbahaya. Keadaan persekitaran serta kebersihan yang berkaitan dengan demografi/sosio-ekonomi sesuatu kawasan boleh mempengaruhi kehadiran dan jenis patogen pada lipas. Tujuan kajian ini adalah untuk menentukan kehadiran lipas yang membawa mikroorganisma berkaitan kepentingan kesihatan awam dalam persekitaran yang berbeza. Dari 11 Januari-17 Januari 2016, lipas telah ditangkap daripada 4 kawasan yang berbeza pekelilingnya iaitu Serdang, Dengkil dan Kajang. Sebanyak 40 ekor lipas berjaya ditangkap dari semua kawasan. Spesis lipas yang ditangkap dikenal pasti sebagai lipas peridomestic biasa (*Periplaneta americana*).

Sebanyak 40 sampel dari bahagian luaran badan dan 40 sampel dari “homogenate” usus lipas telah dianalisa bagi menentukan kehadiran *Salmonella* (S) dan *Escherichia coli* (EC), dan ujian kerentanan antibiotic (AST). Sampel permukaan luaran badan dan usus “homogenate” lipas telah dipalit ke agar khas dan dibiarkan di dalam inkubator bagi tujuan pengasingan bakteria tertentu. Bakteria yang telah dikenal pasti kemudian perlu melalui ujian AST dengan menggunakan kaedah “Standard Kirby-Beuer (Disk penyebaran)”. Status resistan diukur menggunakan “Zone of Inhibition”. Hasil daripada kajian ini menunjukkan bahawa 6 daripada 80 sampel dianalisa mengandungi S spp. di mana 5.0% terdiri daripada sampel permukaan luar badan dan 10% terdiri daripada sampel usus. Sebanyak 22 daripada 80 sampel yang telah disahkan mengandungi EC, ia itu 42.5% terdiri daripada permukaan luar badan dan 12.5% daripada kandungan usus. *Salmonella* spp. ditemui pada lipas di semua lokasi kecuali lipas di tangkap di bilik pelajar, Fakulti Perubatan Veterinar dan Kolej 17, UPM. Aras keberlakuan *Salmonella* spp. pada semua lokasi yang dikaji adalah rendah. Spesies EC dikenal pasti banyak terdapat di kawasan setinggan dan pasar mini. Kedua-dua bakteria yang dikenal pasti dalam kajian ini didapati resistan terhadap pelbagai ubat antibiotik (MDR) yang digunakan pada manusia. Daripada 6 spesies *Salmonella* yang diasingkan, satu spp didapati positif MDR manakala bagi EC, 5 daripadanya didapati positif MDR. Hasil kajian ini mengesahkan bahawa lipas adalah pembawa pelbagai spesies kuman terutamanya S dan EC, dan bakteria ini didapati mempunyai kesan resistan terhadap beberapa antibiotik. Keberlakuan dan beban kuman jangkitan yang ada pada lipas mungkin berkait rapat dengan keadaan kebersihan kawasan-kawasan yang diujikaji. Malah, lipas juga berpotensi membawa kuman ke rumah dan berupaya meningkatkan risiko jangkitan pada manusia. Untuk

memastikan penghapusan menyeluruh terhadap mikroorganisma ini dalam persekitaran tertentu, pengakawalan lipas merupakan perkara asas yang perlu diberi keutaman.

Kata kunci: *lipas, Salmonella, E. coli, resistan pelbagai ubat*



ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement of the course VPD 4999-Project.

OCCURRENCE OF *SALMONELLA* SPP. AND *E. COLI* ISOLATES FROM PERIDOMESTIC COCKROACHES (*PERIPLANETA AMERICANA*) AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS

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Cockroaches are abundant in Malaysia and are seen to harbour an array of pathogens. Environmental and sanitary conditions associated with demographic/socio-economic settings of an area could contribute to the prevalence of disease pathogens in cockroaches. The aim of this study was to determine the incidence of bacteria of public health interest transmitted by cockroaches in different environments. From 11th January to 17th January 2016, cockroaches were trapped in 4 different areas i.e. Serdang, Dengkel and Kajang. A total of 40 cockroaches were collected in all areas. The cockroach species of interest were identified to be the common peridomestic cockroaches (*Periplaneta americana*). A total 40 external body surface and 40 gut homogenates of the cockroaches were analysed for *Salmonella* (S) and *Escherichia coli* (EC), and subjected to antibiotic susceptibility test (AST). The

external body surface and gut homogenates samples were streaked onto special agars and incubated for isolation of the specific bacteria. After the bacteria were identified, they were then subjected to AST using standard Kirby-Bauer Method (Disk diffusion). The status of resistance were measured using Zone of Inhibition. Results from this study showed that 6 out of 80 specimens of cockroaches analysed harboured *S* spp. which comprised of 5% recovered from external body surfaces and 10% from gut contents. For EC, a total of 22 out of 80 specimens were confirmed as EC isolates which comprised of 42.5% recovered on external body surfaces and 12.5% from gut contents. *Salmonella* spp. were found in all cockroaches caught from all areas except those trapped in a student room, Faculty of Veterinary Medicine and college 17, UPM. The occurrence of *S* spp. from all samples studied was low. Samples with EC were mostly analysed from cockroaches collected from squatters and mini market. Both *S* and EC isolates in this study were found to be multi-drug-resistant (MDR) to antibiotics used in human. Among the 6 *S* spp. isolates, one of them was found to be MDR positive whereas for EC, 5 out of 22 isolates were found to be MDR positive. Results from this study confirmed that cockroaches were carrier for a number of bacterial species especially *S* and EC, and these bacteria were found to be resistant to some antibiotics. The prevalence and burden of infection in cockroaches is likely to be a reflection of the sanitary conditions of these areas. Also, cockroaches in these areas making incursions in homes may increase the risk of human infections with these disease agents. To ensure adequate elimination of these microorganisms in specific environments, the control of cockroaches appears to be fundamental.

Keywords: *cockroach*, *salmonella*, *E. coli*, *antibiotic*, *multi-drug-resistance*

1.0 INTRODUCTION

Cockroaches have been on earth for more than 270 million years, and are the world's most usual insects. Approximately thirty of over 4,500 identified species of this insect in the world live close to humans (Chamavit *et al.*, 2011). As a potential mechanical vector of human diseases, many pathogenic organisms have been associated with cockroaches. These include poliomyelitis viruses, bacteria, fungi, protozoa, and helminthes (Baumholtz *et al.*, 1997; Fathpour *et al.*, 2003; Saichua *et al.*, 2008; Tاتفeng *et al.*, 2005).

Cockroaches not only spoil food but also transfer pathogens and cause allergic reactions and psychological distress (Brenner, 1995) They have been found to harbour a number of pathogenic and potentially pathogenic bacteria which were carried either on the cuticle or in the gut (Cloarec *et al.*, 1992). The bacterial loads were up to 14 million on the bodies, and 7 million in each of their faecal droppings (Bennett, 1993). Reports have proved that cockroaches are the carrier of 40 different species of bacteria which are pathogenic to vertebrates (Roth and Willis 1957, 1960; Burgess *et al.*, 1973a, 1973b, 1974; Artyukhina & Evokimov 1973; Ulewicz and Zawistowski 1973; Klowden & Greenberg 1976; Ash & Greenberg, 1980; Cornwell and Mendes, 1981).

American cockroaches are known to transport pathogenic bacterial agents in their intestine and their body surface (Kassiri *et al.*, 2014). This germ may be transferred to food and other items that come in contact with the cockroaches. Various bacteria universally associated with these insects are recognised to inure diarrhoea, dysentery, and food intoxication (Cornwell, 1968; Cornwell and Mendes, 1981). *Shigella dysenteriae*, *Salmonella typhi*, and toxigenic strains of *Escherichia coli* can

be retained in the gut of cockroaches for up to several days. Thus, these insects also play a role in transmitting food-borne disease (Lamiaa *et al.*, 2007; Chaichanawongsaroj *et al.*, 2004)

Earlier study by Paul *et al.*, (1992) demonstrated that cockroaches also been proposed as likely transmitters of drug-resistant pathogens. In a study conducted by Mpuchane *et al.*, (2006), more than 33.3% of cockroach isolates from the experiment have shown resistance to antimicrobials.

Thus, this investigation was conducted to evaluate the potential risk related to presence of cockroaches and their capacity for disseminating bacteria (*Salmonella* spp. and *E. coli* spp.) and their antibiotic susceptibility patterns in four different sampling areas: Student Room at the Faculty, Squatters in Dengkil, Selangor, College 17 UPM, and mini market at Kajang, Selangor.

2.0 LITERATURE REVIEW

2.1 The Role of cockroaches in Disease Transmission

Cockroaches have become most vital pest, are commonly found in association with man in areas where food is prepared and stored, such as restaurants, kitchens, bakeries, and grocery stores (Rivault *et al.*, 1993a, Vythilingam *et al.*, 1997). Approximately 99% of the 4000 species of cockroaches described are wild and do not represent a risk to human health while the remaining 1% which are domestic have become a considerable nuisance and pose public health threat (Ramirez, 1989). The remainder are free-living insects that are found mainly in the topical regions of the world (Roth & Willis, 1960).

Their presence in these habitats is of epidemiological significance. At least 22 species of pathogenic human bacteria, viruses, fungi, and protozoans, as well as five species of helminthic worms, have been isolated from field collected American cockroaches (Rust *et al.*, 1991). Transmission could occur by cockroach regurgitation or fecal pellet deposition into human foodstuffs (Bell *et al.*, 2007).

Cockroaches are indiscriminate feeders and often times subsist on human faeces thus can serve as potential carrier of parasites, fungi and bacteria (Fotedar and Banerjee, 1992; Rivault *et al.*, 1993a; Doiz *et al.*, 2000; Pai *et al.*, 2003).

The role of cockroaches in the transmission of parasites such as *Entamoeba histolytica*, *Toxoplasma gondii*, *Sarcocystis*, *Giardia lamblia* and other protozoan parasites had been emphasised (Graczyk *et al.*, 2005).

2.1.1 Location of infestation

Cockroaches are tropical in origin so they live in parts of houses and other buildings i.e. Bathrooms, kitchens, sewerage systems etc., where warmth, moisture, and food are adequate (Cloarec *et al.*, 1992; Rivault, 1993b; WHO, 1997).

In the daytime they hide in cracks and crevices in walls, door frames, and furniture, and in secure places in bathrooms, cupboards, steam tunnels, animal houses, basement, television, radio and other electronic devices, drains and sewer systems. If the light is turned on in an infested kitchen at night the cockroaches will run from dishes, utensil, working surface and the floor toward shelters (Rivault *et al.*, 1993a).

When the cockroaches in houses find suitable conditions for growth and development, they create severe problem in houses (Cochran, 1999). Having poor sewerage was the biggest reason for the severe problem created by cockroaches in houses (Oyedele, 2009).

In homes and in places with poor standards of hygiene, serious infestations by cockroaches like peridomestic American Cockroach (*Periplaneta americana*) and also the domestic German cockroach (*Blattella germanica*) (Feizhaddad *et al.*, 2012) can occur.

Area size and age of the house describes why lesser number of houses have good sanitary conditions. Houses with larger build up area will result in more difficulty in maintaining hygienic conditions and also older houses have more cracks and crevices for cockroaches multiplication (English, 1987; Olanrewaju & Akinbamijo, 2002).

In different types of environment, they can act as mechanical vectors, carrying pathogenic agents, such as fungi and bacteria. (Lemos *et al.*, 2006; Pai *et al.*, 2005).

2.1.2 Public health significance of cockroaches

Cockroaches are described as unpleasant insects of undoubted public health importance (Roth & Willis, 1957). According to Fathpour *et al.*, (2003), cockroaches are known to carry a diverse pathogenic bacteria flora, different protozoan, pathogenic worms, fungi, and viruses.

Cockroaches have been found positive for diarrhoea-causing pathogens (including cholera and typhoid fever). Cockroaches collected from a paediatric hospital ward in Malaysia were examined for the presence of different bacterial pathogens, and 101 out of 104 cockroaches analysed were found to be positive for at least one bacterial strain in their gut (Oothuman *et al.*, 1989)

Some common examples of human diseases known to be transmitted by cockroaches are bubonic plague, dysentery, boils and abscesses, diarrhoea in children; enteric fevers and gastroenteritis, infections of the urinary tract, infections of the urogenital tract and intestine; leprosy; puss formation, food poisoning and typhoid fever (Cornwell, 1968; Cornwell and Mendes, 1981).

In addition, a study done by Chaichanawongsaroj *et al.*, (2004) found that cockroaches have been related with an outbreak of dysentery. Cockroaches have long been known as vectors of food poisoning and infectious organisms (Rueger and Olson 1969). A wide spectrum of bacteria including common food spoilage and pathogenic organisms such as opportunistic pathogens like non-pathogenous

Staphylococcus, *E.coli*, *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp. and *Serratia* spp. were isolated from cockroaches (Vahabi, 2011).

In an investigation in Nigeria, the bacteria isolated from American and German cockroaches captured in hospitals, human dwellings and restaurants were *Salmonella* spp, *Shigella* spp, *Staphylococcus aureus*, *coagulase-negative Staphylococcus* spp, *Bacillus cereus*, *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Morganella morganii*, *P. vulgaris*, *Proteus mirabilis*, *Enterobacter cloacae* and *Providencia* spp.

2.2 Common Cockroaches Species in Malaysia

Cockroaches can be split into two groups: Domestic and peridomestic. Domestic cockroaches live their entire lives inside homes or buildings. An example is the German cockroach. Roaches that live indoors and out (or may be indoors occasionally) are peridomestic.

In peninsular Malaysia, *P. americana* is the largest domiciliary species. It is widely distributed but is rarely found or absent from Orang Asli (local aborigines) villages (Oothuman *et al.*, 1984; Sulaiman *et al.*, 1995; Jeffery *et al.*, 1999). *Periplaneta americana* was the dominant species in several surveys (Oothuman *et al.*, 1984; Yap *et al.*, 1991; Lee *et al.*, 1993; Lee & Lee, 2000). Studies by Jeffery *et al.*, (2003) reported that *Periplaneta brunnea* in the past had been confused with *P. americana* in Peninsular Malaysia. Both these large *Periplaneta* spp. share superficial morphological similarities in the adult stages; however, are very distinct in the oothecal stage (Jeffery *et al.*, 1982).

2.2.1 Cockroaches of Interest: The American cockroaches (*Periplaneta americana*)

There are two cockroach species commonly found infesting in domestic area, these are the German cockroach (*Blattella germanica*), and the American cockroach (*Periplaneta americana*) (Rivault *et al.*, 1993a, Gratz 1999, Graczyk *et al.*, 2005, Mpuchane *et al.*, 2005, Bell *et al.*, 2007). American cockroaches can become a public health problem due to their association with human waste and disease and their ability to move from sewers into homes and commercial establishments (Barbara, 2000).

The American cockroach is a vector of bacteria. Over 100 species of bacteria have been isolated domestic cockroaches (Cruden & Markovetz 1987). German cockroach is more prevalent inside the home while the American cockroaches are common around the home and associated with water drainage systems and water pipes (Gratz 1999, Graczyk *et al.*, 2005, Mpuchane *et al.*, 2005, Bell *et al.*, 2007, Allotey *et al.*, 2009)

2.3 The Bacteria of Interest

2.3.1 Prevalence and Persistence of *Salmonella* in Cockroaches

Organisms that are thought to be likely carried by cockroaches and responsible for human infections are those belonging to the *Salmonella* group (Cornwell, 1968). Oothuman *et al.*, (1989) and Fathpour *et al.*, (2003) had successfully isolated *Salmonella* spp. From their samples. Olson and Rueger (1950) were able to show that *Salmonella enteriditis* fed to *B. germanica* could be recovered from the digestive tract

up to nine days after feeding infected feed. In addition, their study also concluded that *salmonella* species can persist at least 4 years in the feces of cockroaches

According to Mackerras & Mackerras, (1949), *salmonella* species also can be carried by cockroaches and have been found to cause a gastro-enteritis outbreak. This study supported by Popoff *et al.*, (2004) that members of the genus *Salmonella* can be found in the intestine of cockroaches. In a study about the incidence of bacteria of public health interest transmitted by cockroaches in various food-related places in Spain, the presence of a number of bacteria, including *Salmonella* were confirmed (Garcia *et al.*, 2012).

2.3.2 Prevalence and Persistence of *E. coli* in Cockroaches

E. coli had the most common frequency bacteria isolated from cockroaches (Vahabi 2011). *Escherichia coli* can be found on both internal and external surfaces of cockroaches (Rivault *et al.*, 1993a).

Chaichanawongsaroj *et al.*, (2004) indicated *E. coli* levels on cockroaches coincided to *E. coli* levels in the environment. Devi & Murray, (1991) studied more than 200 cockroaches and they isolated *Salmonella* from 4.1% of those cockroaches. In the late 1940s, enteropathogenic *E. coli* strains were identified as a cause of gastroenteritis (Fratamico *et al.* 2006). At present there are six different categories of pathogenic *E. coli* that produce diarrhea. The mechanisms by which diarrhoea is produced is vary for each type of pathogenic *E. coli* and include bacterial attachment to the host cells, invasion of intestinal cells, and production of toxins. Oothuman *et al.*, (1989), Jeffery *et al.*, (1997), and Sulaiman *et al.*, (2003) were able to isolate Coliform; *Enterobacter* spp., and *E. coli* from cockroaches in their study.

2.4 Antibiotic Resistant

Cockroaches are mechanical carriers or perfect vector for harbouring and transmitting virulent bacteria associated with food born infections. Some of them demonstrate resistance to antibiotics (Paul *et al.*, 1992, Rivault *et al.*, 1993a, Mpuchane *et al.*, 2005).

Cockroaches may act as reservoirs for antibiotic resistant virulent bacteria found on domestic environment, which may cause serious health problems for Human beings (Pai *et al.*, 2005, Ahmad 2011). Over 4% of cockroaches collected from hospitals, houses, animal sheds, grocery stores, and restaurants in India harboured multiple drug resistant *Salmonella* (Devi & Murray, 1991). More than 33.3% of cockroach isolates have shown resistance to antimicrobials (Mpuchane *et al.*, 2006). There are also many different virulence factors, which are usually encoded by plasmids, chromosomes, or bacteriophages (Tham, 2012).

Based on a study conducted by Elgderi *et al.*, (2006) 403 cockroaches are reported to have multiple resistance, to at least six different antibiotics, was more commonly observed among the enteric bacteria isolated from the hospital cockroaches than among those recovered from the household cockroaches. Overall, more than 30% of the isolates of Enterobacteria recovered were each resistant to at least four antimicrobial agents, and 95% of the *Pseudomonas* isolates were each resistant to at least eight such agents.

3.0 MATERIALS AND METHOD

3.1 Sample Collection

Cockroaches in this study were captured manually using net, cage trap with food bait (Plate 1) and by using food bait attached to adhesive sticky glue trap (Plate 2). The cage trap was made of a plastic container with trapped door. Baits which consist of biscuits, minced chicken, cheese slice, dried prawns were placed on the cage sticky glue trap. Cockroaches were trap in four different sites i.e., Student Room at Faculty of Veterinary Medicine (FPV, UPM) (Plate 3), squatters in Dengkil, Selangor (Plate 4), Mini market at Kajang (Plate 5) and College 17, UPM (Plate 6). The traps were left for a week and the traps were check out for cockroaches every morning. Live cockroaches caught were taken to the Veterinary Public Health Lab for further processing.

3.2 Identification of cockroaches

Cockroaches of interest were identified based on anatomical and physical characteristics that categorized them into certain species. Identification was done by referring to entomology book and online notes on the internet.

3.3 Sample preparation

In the laboratory, alive cockroaches are sacrificed in a sterile jar using diethyl ether soaked cotton (Plate 7). Cotton soaked with diethyl ether was put inside the sterile jar to sacrifice the cockroaches. For isolation of bacteria from the external body surface of cockroaches, 5 ml of sterile peptone water is added to a stomacher bag. Each stomacher bag contain one cockroach (Plate 8). The sample was vortex for 2

minutes to wash off any bacteria from the insect's external body. Vortexing was performed at the lowest possible speed to prevent insects from vomiting and contaminating the content. The washing solution will be collected as external body homogenate sample. After external body washing, the cockroaches are disinfect with 90% ethanol to decontaminate their external surfaces. After sample is dry off, they are re-washed with peptone water to remove traces of ethanol, and the alimentary tract is aseptically dissected.

Lateral dissection of the cockroach abdomen was conducted to get accessed into the gastrointestinal tract (GIT). The GIT was removed at the point of crop until the end of hindgut (Plate 9) using a sterilized scalpel and a pair of scissors that were sterilized with 70% alcohol and flame between dissections. The excised gut was removed and placed in a stomacher bag containing 5ml of sterile peptone water. The gut content was then squashed using fingers on the outer part of the bag.

A total of 80 samples were prepared, 40 of the samples were taken as external body homogenate and another 40 samples were taken as excised gut homogenates. All 80 samples were subjected to isolation and identification of bacteria of interest.

3.4 Isolation and Identification of Bacteria of Interest

3.4.1 Isolation and subculture of *Salmonella* spp.

For primary enrichment, 1 ml of each homogenate (external body homogenate and excised gut homogenate) is inoculated separately into 9 ml of buffered peptone water (BPW) (Plate 10) and incubated at 37°C for 24 hours. Then, 1ml of sample which had been previously incubated in BPW was transferred into Rappaport-

Vassiliadis (RV) Enrichment Broth (Oxoid) and was incubated at 42°C for 24 hours for secondary enrichment. A loopful of samples in the RV enrichment broth was streak onto Xylose lysine desoxycholate (XLD) agar and Brilliant Green Agar (BGA) and incubated at 37°C for 24 hours. Typical *salmonella* colonies on XLD will appear as pink colonies with or without black centres (Plate 11). However, many cultures of *Salmonella* may produce colonies with large, glossy black centres or may appear as almost completely black colonies on XLD. For BGA, typical *salmonella* colonies (except *Salmonella typhi*) will appear red pink to white opaque coloured colonies surrounded by brilliant red zones in the agar (Plate 12) Isolates were subculture again onto XLD and BGA, and incubated at 37°C for 24hours to purify the culture. The pure colonies were then sub cultured by streaking onto Nutrient Agar and incubated at 37°C for 24 hours before biochemical test (Plate 13) were conducted.

3.4.1.1 Lysine Iron Agar (LIA, Merck)

A single suspected *Salmonella* spp. Colony on BGA and XLD agar were inoculated into LIA in test tube where the butt was centrally stabbed and the slant surface was streaked. Tubes were incubated at 37°C for 18 hours. Typical reaction of *Salmonella* spp. will cause the slant and the butt to turn violet with hydrogen sulphate (H₂S) production which was exhibited by the black discolouration.

3.4.1.2 Triple Sugar Iron (TSI, Merck)

A suspected colony was inoculated into TSI in the test tube. The butt was centrally stabbed while the slant surface was streaked. It was incubated at 37°C up to 48 hours. Typical reaction to *Salmonella* showed acid butt (yellow), alkaline slant (red) with or without hydrogen sulphate (black) and gas production.

3.4.1.3 Urease (Merck)

This medium was recommended to differentiate between *Proteus* and *Salmonella*. A suspected colony was inoculated into Urease broth by spreading it on the surface of the agar. It was incubated at 37°C up to 48 hours. Typical *Salmonella* spp. will cause urea negative and therefore the broth will remain yellow in colour.

3.3.1.4 Sulfide Indole Motility (SIM, Merck)

Sulfide indole and motility medium is recommended for use in qualitative procedures for differentiation of enteric gram-negative bacilli. Suspected colonies were inoculated into this medium by stabbing down the centre of the medium to within the bottom 1/3 of the tube. *Salmonella* spp. caused sulphide production positive by blackening the medium, motility positive by diffuse growth outwards from the stab line and indole reaction negative which indicate by no red or pink colour development.

3.3.1.5 Citrate (Merck)

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the colour change of a pH indicator. Suspected colonies were streaked into the culture medium and incubated for 24-48 hours at 37°C. Colour changes from green to blue indicates positive for *Salmonella*.

3.4.2 Isolation of *E. coli*

A loopful of sample was obtained from the stomacher bag containing each homogenate (external body homogenate and excised gut homogenate). It was directly streaked onto eosin-methylene blue (EMB) agar (Plate 14) and incubated at 37°C for 24 hours. *E. coli* colonies will give a distinctive metallic green sheen colonies on the plate. Isolates were sub cultured again onto EMBA agar to purify the cultures.

3.4.2.1 Identification of *E. coli*

The pure colonies from EMBA plate were then subjected to biochemical test (SIM) to allow detection of sulfide production, motility and indole characteristic (Plate 15). For *E. coli*, the motility and indole will be positive except for sulfide production.

3.5 Antibiotic susceptibility test

The disk diffusion method was used to determine the bacterial susceptibility to antimicrobials (Plate 16). In this methods, a pure colony obtained from nutrient agar was mixed in 4ml tryptic soy broth until its turbidity was similar to turbidity of 0.5 McFarland. The dilution was then streaked evenly onto Mueller Hinton agar using a sterile swab and left to semidry. The antibiotic disk used were Ampicillin, Amoxicillin/clavulanic acid, Ceftriaxone, Ceftaxidime, Ciprofloxacin and Trimethoprim/ sulphamethoxazole. After incubation of 24 hours at 37 C, antibiotic activity was determined by the width of zone of no growth around the antibiotic disc (Plate 17).

4.0 RESULTS

4.1 Frequency Distribution of Cockroaches in Various Studied Locations

A total of 40 cockroaches were captured in this study. All cockroaches sampled in this study were identified to be *Periplaneta americana*. Number of cockroaches captured from different areas in this study were shown in table 1.

Table 1. Number of cockroaches trapped at various locations.

Location	No. of cockroaches collected
Student Room, FVM UPM	5 (12.5%)
Squatters, Dengkil, Selangor	15 (37.5%)
College 17, UPM	12 (30%)
Mini Market, Kajang	8 (20%)

The number of cockroaches trapped at student room at FPV UPM, squatters at Dengkil, college 17 UPM and mini market in Kajang were 5, 15, 12, and 8 respectively. The highest number of cockroaches were trapped in the squatter's area (37.5%) and the lowest number were recorded in Student Room at the FPV UPM. Overall, the number of cockroaches trapped were low.

4.2 Isolation of *Salmonella* spp.

Table 2. The number of occurrence of *Salmonella* spp. from collected cockroaches in various sampled locations.

Locations	No. of samples	Presumptive <i>salmonella</i> colonies (Morphological)		Confirmed <i>Salmonella</i> spp. by biochemical test		Confirmed <i>Salmonella</i> spp. By Slide Agglutination test	
		*ExtP	Gut	*ExtP	Gut	*ExtP	Gut
Student room	5	1 (20%)	0	0	0	0	0
Squatters	15	7 (46.6%)	9 (60%)	1 (6.6%)	2 (13.3%)	1 (6.6%)	2 (13.3%)
College !7	12	4 (33.3%)	2 (16.2%)	0	0	0	0
Mini market	8	6 (75%)	5 (62.5%)	2 (25%)	2 (25%)	1 (12.5%)	2 (25%)
Total	40	18 (48.6%)	16 (43.2%)	3 (8.1%)	4 (10.8%)	2 (5.0%)	4 (10%)

*ExtP = External parts of cockroach

Table 2 show the external and gut sample isolates of cockroaches with positive *salmonella* colonies. Eighteen (48.6%) of external body sample and 16 (43.2%) of gut isolates samples were found to have typical *Salmonella* colonies morphology. These isolates were identified as presumptive positive for *Salmonella* and were subjected to biochemical test. Thus, a total of 34 (45.9%) presumptive *Salmonella* were identified based on colony morphology on BGA and XLD plate.

All of the 34 presumptive *Salmonella* were subjected to biochemical test (TSI, LIA, Urease, Citrate, and SIM) (Plate 13). Out of 34 presumptive *Salmonella*, 3 (8.1%) samples isolated from external body parts and 4 samples isolated from gut were confirmed positive through biochemical test. Out of 3 (8.1%) samples isolated

from external body, 1 (16.6%) cockroaches was caught from squatters' area and the remaining 2 (25%) cockroaches were caught from mini market site. However, none of the presumptive *Salmonella* caught from student room at the FVM and College 17 UPM were confirmed positive after completed biochemical test. Thus, out of 74 samples analysed from both external body and gut samples of the cockroaches, a total of 7 (9.5%) samples were confirmed positive for *salmonella* contamination after biochemical test.

Finally, all positive presumptive *salmonella* isolates by biochemical test were tested for *Salmonella* somatic (O) antigen using slide agglutination method (Plate 18). Out of 7 isolates, 6 isolates were confirmed as *Salmonella* spp. by slide agglutination test, comprising 2 isolates from external body of the cockroach and 4 isolates from the gut of the cockroaches.

4.3 Isolation of *E. coli* spp.

Table 3. The number of occurrence of *E. coli* from collected cockroaches in various sampled locations.

Locations	No. of samples	Positive <i>E. coli</i> (Morphologically)		Confirmed <i>E. coli</i> through selective media (Chromacult Agar)	
		*ExtP	Gut	*ExtP	Gut
Student room	5	1 (20%)	1 (20%)	1 (20%)	0
Squatters	15	13 (86.6%)	5 (33.3%)	9 (60%)	2 (13.3%)
College 17	12	6 (50%)	3 (25%)	4 (33.3%)	0
Mini market	8	4 (50%)	3 (37.5%)	3 (37.5%)	3 (37.5%)
Total	37	24 (64.9%)	12 (32.4%)	17 (45.9%)	5 (13.5%)

*ExtP = External parts of cockroach

Table 3 shows external body parts and gut samples of cockroaches positive for *E. coli*. From 40 sample of cockroaches analysed for *E. coli*, 24 (64.9%) samples isolated from external body of the cockroaches and 12 (32.4%) samples isolated from gut of the cockroaches were found to have typical *E. coli* colonies morphology on EMBA plate. The highest number were 13 (86.6%) which were isolated from the external body parts of cockroaches caught in squatters area, followed by 6 (50%) from the external body parts of the cockroaches caught from the college 17. Thus, a total of 36 (45%) positive presumptive *E. coli* were identified based on colony morphology EMBA plate. Out of 80 samples analysed, 22 (27.5%) isolates were confirmed positive for *E. coli* after the biochemical test.

4.4 Antibiotic susceptibility test

Table 4. Number of *Salmonella* isolates sensitive, intermediates and resistance towards selected antibiotics.

Antibiotic	No of sensitive	No. of intermediate	No. of resistance
Ampicillin 10 µg	1	3	2
Amoxicillin/clavulanic acid 30 µg	3	2	1
Ceftriaxone 30 µg	2	4	0
Ceftazidime 30 µg	6	0	0
Ciprofloxacin 5 µg	4	2	0
Trimethoprim/sulphamethoxazole 25 µg	3	0	3

Table 4 shows that *Salmonella* isolates in this study were resistant to Trimethoprim/Sulphamethoxazole, followed by Ampicillin and Amoxicillin/clavulanic acid. However, the isolates show no resistance at all against Ceftriaxone, Ceftazidime, and Ciprofloxacin indicates wider activity against these antibiotics. This result showed that *Salmonella* isolates in this study are multiple drug resistant which is resistant to at least one agent in three or more antimicrobials categories.

Table 5. Number of *E. coli* isolates sensitive, intermediates and resistance towards selected antibiotics.

Antibiotic	No of sensitive	No. of intermediate	No. of resistance
Ampicillin 10 µg	2	4	16
Amoxicillin/clavulanic acid 30 µg	12	10	0
Ceftriaxone 30 µg	8	8	6
Ceftazidime 30 µg	13	5	4
Ciprofloxacin 5 µg	19	3	0
Trimethoprim/sulphamethoxazole 25 µg	12	2	8

Table 5 shows that most of the *E. coli* isolates in this study were resistant the most to Ampicillin, followed by Trimethoprim/Sulphamethoxazole, Ceftriaxone and Ceftazidime. However, the isolates have a wider activity against Amoxicillin/Clavulanic acid and Ciprofloxacin which shows no resistance at all. However, result from the study showed that *E. coli* isolates are multiple drug resistant which is resistant to at least one agent in three or more antimicrobials categories.

5.0 DISCUSSION

Cockroaches pose a significant public health threat owing to their ability to harbour diverse pathogenic bacteria and other disease-causing microorganisms. As indiscriminate feeders, cockroaches consume both the food and the faeces of man and domestic animals. In this way, pathogen of the alimentary canal of a cockroach may be readily transferred from animal to animal and from animal to man; cockroaches make them ideal carriers of various pathogenic microorganisms (Allen, 1987; Graczyk *et al.*, 2005, Pai *et al.*, 2005)

The presence of *Salmonella* spp. in the range of 4.1% to 47.5% have been reported in study conducted by Devi & Murray (1991) and Fathpour *et al.*, (2003). However in this study 7.5% out of 80 cockroach samples harboured *Salmonella* spp. which indicated that the occurrence of *Salmonella* from cockroaches trapped in this study sites is low. The low occurrence could be due to the environmental factors that may inhibit *Salmonella* multiplication. Besides that, although the selective media such as XLD agar is used to detect the presence of *Salmonella*, other bacteria such as *Shigella* may growth on the same agar and shows similar appearance like *Salmonella* spp.

For *E. coli*, the presence has been reported in the study of microbial carriage of cockroaches by Tetteh-Quarcoo *et al.*, (2013), Vahabi *et al.*, (2011), and Jeffery *et al.*, (1997) and it range between 11.8% to 76.9%. Vahabi *et al.*, (2011) in his study to evaluate bacterial contaminations in domestic cockroaches able to isolated 76.9% *E. coli* from external surface of cockroaches however there are no internal (gut) contamination reported. In this study, both external and internal contamination found

on the cockroaches examined which comprised of 42.5% from external body surface and 12.5% from gut content.

It is found that the cockroaches infected with *Salmonella* spp. and *E. coli* in this study belongs to the cockroaches samples collected from squatters and mini market areas. The abundance of cockroaches has been linked to various environmental factors such as cleanliness (Shah *et al.*, 1996), human density (Rivault & Cloarec 1996), and humid environment (Rozendaal, 1997). Heavier levels of infestations were found in dwellings with poor standards of hygiene (Shah *et al.*, 1996). Heavy cockroach infestation is also associated with adequate food resources within a dwelling (Shah *et al.*, 1996). This is supported by study done by Chaichanawongsaroj *et al.*, (2004) that cockroaches are likely to be encountered in favourable environmental conditions with a ready source of food.

From the analysis of sample in this study, squatters were found to have a very low level of hygiene which make it favourable environment for cockroach infestation with disease-causing bacteria. Whereas, mini market was the second site with most location with high occurrence of *Salmonella* and *E. coli* and this was probably due to the favourable environment (available food sources, warm, and humid condition) which is highly preferred by cockroaches. The student room at the Faculty of Veterinary Medicine recorded least number of cockroaches infestation and none of the sample collected were positive either for *Salmonella* or *E. coli*. This could be due to good hygiene level and presence of activities which might controlled the pest multiplication (Rivault, 1993b).

Result of antibiotic sensitivity test showed that both *Salmonella* and *E. coli* identified in this study were found to be multi-drug-resistant (MDR) to antibiotics

used in human. Among the 6 *Salmonella* spp. isolates, one of them was found to be MDR. According to study conducted by González-Sanz *et al.*, (2009), the drugs of choice for the treatment of complicated salmonellosis are usually ampicillin, amoxicillin/ clavulanate, third-generation cephalosporins, or fluoroquinolones, but the increasing emergence of resistance to these antimicrobials limits the therapeutic choices. However in this study, the resistance towards antibiotics were observed only for ampicillin, amoxicillin/ clavulanate, as well as Trimethoprim / sulphamethoxazole. Whereas, for *E. coli* isolates, 5 out of 22 isolates were found to be MDR. All *E. coli* isolates in this study have a wider activity against Amoxicillin/Clavulanic acid and Ciprofloxacin and this is in accordance with study conducted by Wannigama *et al.*, (2014).

This findings have importance implications for clinicians to choose antibiotic for treatment. According to Beceiro *et al.*, (2013), the resistance to various antimicrobials may be due to presence of some virulence gene, involvement of secretion machinery of multi drug efflux proteins, through mutations in bacterial genome or by gaining additional genes through horizontal gene transfer or by physiology dependent resistance.

The present study indicates that American cockroach possess a possible health risk to communities due to their ability to harbour *Salmonella* and *E. coli* on external surface and gut content. This study also proved that the isolated strains of bacteria were resistant to some of the antibiotics.

The presence of cockroaches in human dwelling areas is never desirable, and must be taken into consideration. Control efforts need to be undertaken looking at the potential hazard the cockroaches pose. The trapping methods that were used in this

study can be used to control the cockroach numbers. Further studies are recommended by increasing the sample size, area of sample collection and usage of individual traps to collect cockroaches to avoid possible cross contamination between cockroaches.

6.0 CONCLUSION

In conclusion, this study documented the occurrence of *Salmonella* spp. and *E. coli* in cockroaches under different sampling areas, namely, faculty student room, squatters, mini market, and college. It is suggested that cockroaches act as carrier for *Salmonella* & *E. coli* because of their ability to harbour these organisms on external surface and gut. Both *Salmonella* and *E. coli* isolates in this study also were found to be multi-drug-resistant (MDR) to some of the antibiotics used in human.

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APPENDICES



Plate 1: Cage trap with food bait



Plate 2: adhesive sticky glue trap with food bait



Plate 3: Student Room in Faculty of Veterinary Medicine, UPM Serdang



Plate 4: Squatters in Dengkil, Selangor



Plate 5: Mini Market at Kajang Selangor



Plate 6: Living room at college 17, UPM Serdang

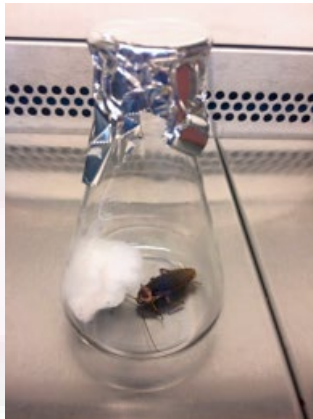


Plate 7: Alive cockroaches killed in sterile jar using diethyl ether soaked cotton

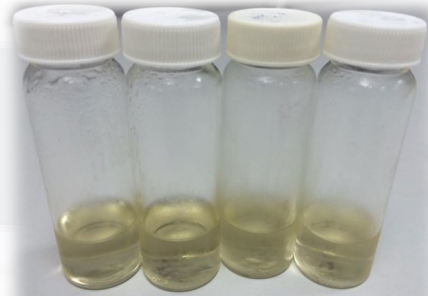


Plate 10: Buffered Peptone Water



Plate 8: A stomacher bag containing one cockroaches



Plate 11: Typical *Salmonella* reaction on XLD plate



Plate 9: GIT tract of cockroach



Plate 12: Typical *Salmonella* reaction on BGA plate



Plate 13: A set of biochemical test for
Salmonella spp.



Plate 16: Disk Diffusion Method for
antibiotic susceptibility test

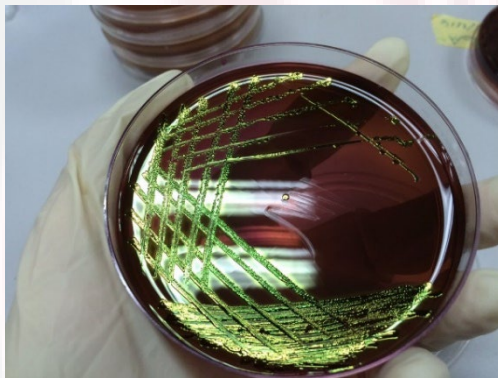


Plate 14: *E. coli* on EMB agar



Plate 17: Antibiotic activity on
Mueller Hinton Agar

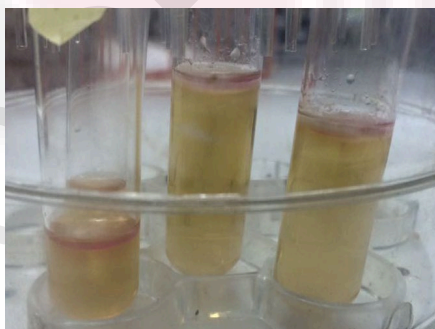


Plate 15: Sulfide indole motility
(SIM) test for *E. coli*



Plate 18: Slide Agglutination test for
Salmonella spp,