Jurnal Teknologi

Full Paper

PATHOGENIC BACTERIA ISOLATED FROM COCKROACHES FOUND IN FOOD PREMISES

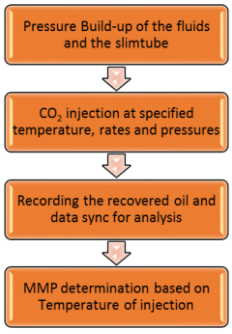
Anis Husna Wahab[a*], Mohd Pozi Mohd Tahir[a], Emida Mohamed[b]

[a]Department of Environmental Health, Faculty of Health Science, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, Selangor, Malaysia
[b]Department of Medical Laboratory Technology, Faculty of Health Science, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, Selangor, Malaysia

Article history
Received
11 June 2015
Received in revised form
18 September 2015
Accepted
17 December 2015

*Corresponding author
2013244818@isiswa.uitm.edu.my

Graphical abstract



Abstract

Cockroaches play an important role in transmission of different diseases either mechanically and biological because they spread and harbor various species of microorganism. This study was carried out to isolate the pathogenic bacteria from external and internal body of cockroaches at food premises. A total of 48 cockroaches, 32 of Periplanetta americana and 16 of Blatta orientalis, were collected at food premises in Shah Alam, Selangor, Malaysia. Medically important bacteria were isolated from external and internal surface by using microbial technique. From the collected Periplanetta americana and Blatta orientalis, E.coli,Salmonella species, Shigella species, and Staphylococcus were isolated and identified in their selective media such as nutrient agar, MacConkey agar, and Xylone Lysine Deoxycholate (XLD) agar using microbial technique. The data obtained from the study emphasized that cockroaches may play an important role in the transmission of pathogenic bacteria to human. The presence of cockroaches at food premises maybe due to unhygienic environment. Thus it may pose as a safety issue as they may carries of pathogenic microorganisms that can threaten the health of individuals.

Keywords: Periplanetta Americana, blatta orientalis, e.coli, salmonella, shigella spp, staphylococcus, pathogenic bacteria

1.0 INTRODUCTION

Cockroaches are among the important pests that are in close contact with human. They can be found in the home and other buildings. They usually search for food at night in places where food is available such as kitchen garbage, sewages, restaurants and grocery stores [1]. These insects are also found in urban and rural areas which can cause serious health problems to the public [2, 3]. Cockroaches can live in different environments such as water pipes, sewage pipes, garbage, wall slits and filthy places. Perhaps they are attracted to food, organic waste and fluids regularly discharged in such sites [4].

The habitats of cockroach are typically the tropical areas as well as in subtropical region and cooler zones. Cockroaches are primitive and only have three stages in their life cycle which are egg, nymph and adult. They can grow by repeatedly shedding their cuticle or skin in stages. After several months to one year, they are fully grown depending on their species.

Cockroaches need food to stay alive but they can survive without food for several days to several weeks. Baumholltz *et al* [5] have documented that an immature cockroach can survive approximately 10 days without food, while adult cockroach can survive until six weeks without food. Humidity is also important in the longevity of cockroach. The finding by

Baumholtz *et al* [5] revealed that adult cockroaches will die in one to four weeks without water but depending on their species. In contrast, they can stay alive at least one year in moist places. In a study by Alzain, B [6] and Jirage R [7], they revealed that cockroaches generally like warm and moist environment with abundant of food.

Cockroaches are arthropods that play an important role as mechanical vector of pathogenic bacteria in health and medical centers [8]. According to Rivault *et al.* [9], cockroaches can transmit bacteria, fungi, protozoa, and helminthes that can cause food poisoning and other infections.

Cockroaches have the ability to carry and transmit bacterial pathogens both on the external and internal of their body surfaces. Alzain B. [6] revealed that cockroach can carry and transmit a lot of infectious microorganisms. Different microorganisms have been isolated and identified from their external and internal body surfaces. The study by Akinjogunla O.J *et al.* [10] found that species of German (*Blattella Germanica)* and American cockroachs (*Planetta Americana)* that were collected in their study, from hospital, human dwellings, and canteens/restaurants carried various types of bacteria on their external body and internal guts. In addition, there were more than 100 species of bacteria that have been isolated from cockroaches [11, 12]. Hence, this study was conducted to determine the presence of bacterial pathogen isolated from external and internal body of cockroaches found in food premises.

2.0 MATERIALS AND METHOD

2.1 Collection and Identification of Cockroach

48 samples of cockroaches were collected from the Food Court of section 2 in Shah Alam, Selangor, Malaysia. The groups of insects were captured (mostly at night or early morning) by using a trap (Ideal Life Insects Specialist). Several traps were left on the floor overnight.

On the next day, each trap containing cockroaches were collected by using sterile zip lock bag and sterile hand gloves. Only cockroaches that are still alive and have their whole body intact were considered in this study. Then the cockroaches in the trap were transported to the laboratory and anaesthetized by putting at 0^0C for about 5 to 10 minutes. Subsequently, the species of cockroaches were identified based on their characteristics such as color, shape, and size.

2.2 Isolation and Identification of Bacteria on the External Body of Cockroach

At the same time, nutrient agar, MacConkey agar and Xylose Lysine Deoxycholate (XLD) agar were prepared to detect the bacteria on the cockroaches. Initially, 0.9% which is 2.0 ml of sterile normal saline was added into a sterile falcon tube containing a cockroach. Then, the falcon tube was thoroughly shaken for 2 minutes. Aliquots (0.1 ml) of the washing (external body homogenate sample) was cultured separately onto the plates of nutrient agar (NA), Xylose Lysine Deoxycholate (XLD), and MacConkey Agar (MCA), was incubated overnight at 37^0C. After overnight incubation, the colonies on positive plates was subcultured on petri dishes containing freshly prepared nutrient media to obtain pure cultures and were incubated aerobically at 37^0C for 18 – 24 hours. Characteristics such as color and colony shape will be noted in the typical appearance of bacteria on solid agar. The results were recordered and the colonies were identified after overnight incubation at 37^0C.

2.3 Isolation and Identification of Bacteria on the Internal Gut of Cockroach

After the external body of cockroach was washed, the decontamination of the external surfaces will be carried out by soaking it in 70% ethanol for 5 minutes. The cockroach was re-washed to remove any traces of ethanol by using sterile normal saline. Then the digestive tract (gut) of each of the cockroaches was removed by using sterilized entomological instruments and the whole gut was homogenized in sterile normal saline and aliquot was obtained. Subsequently, 0.1mL of the aliquot was cultured onto the plates of nutrient agar, MacConkey agar, and Xylone Lysine Deoxycholate Agar (XLD) and was incubated aerobically for 18 – 24 hours at 37^0C. After overnight incubation, the colonies on the positive plates were sub-cultured onto freshly prepared media to obtain pure cultures and incubated aerobically at 37^0C for 24 hours. Typical appearances of the bacteria on solid agar, such as color and colony shape, were noted. The colonies were then identified after overnight incubation at 37^0C.

3.0 RESULTS AND DISCUSSION

A total of 48 cockroaches were collected at food premises in Shah Alam, consisting of 32 (66.66%) *Periplanetta americana* (American cockroach) and 16 (33.33%) *Blatta orientalis* (Oriental cockroach). For the identification of bacterial isolates from the external and internal body of cockroaches, morphology, structural and colony characteristics were taken into consideration [18, 19].

After 18 – 24 hours of incubation, the nutrient agar became turbid reflecting presence of bacteria. Nutrient agar showed growth as demonstrated in Table 1 (Nutrient Agar). MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the Enterobacteriaceae family. The morphology and colony characteristics showed on MacConkey agar plates are shown in Table 1 (MacConkey). Xylone Lysine Deoxycholate

(XLD) agar is also a selective growth medium used to detect the presence of *Salmonella* and *Shigella spp*. The XLD agar plates showed growth as in Table 1 (Xylone Lysine Deoxycholate (XLD) agar).

Table 1 species of bacteria determined from features of their colonies

Types of Agar	Features of colony	Species of bacteria
Nutrient agar	i) Small, circular, entire, flat, semi opaque or translucent, colorless and moist colonies.	i) *Escherichia coli (E.coli)*
	ii) Opaque, 1 to 2 mm round, smooth, golden yellow and shining colonies with grape like cluster.	ii) *Staphylococcus*
	iii) Small, semi opaque or translucent, smooth, circular, colorless and moist colonies.	iii) *Salmonella sp.*
MacConkey agar	i) Circular, pink color, entire, elevated, opaque and moist colonies.	i) *Escherichia coli (E.coli)*
	ii) Round, flat, translucent, cream white color, and moist colonies.	ii) *Salmonella sp.*
Xylone Lysine Deoxycholate (XLD) agar	i) round, opaque, red-yellow colonies with black centers, the agar itself turn to red color	i) *Salmonella sp.*
	ii) Round, red color	ii) *Shigella sp.*

The presence of cockroaches especially in human dwellings, hospitals and food premises may pose an impact to human health such as nosocomial infection as these cockroaches, that can move freely, may harbor pathogenic bacteria [13]. Cockroaches live in different environments such as water pipes, sewage pipes, garbage, wall slits and filthy places as they are attracted to food, organic waste and fluids regularly discharged in such sites [2, 4]. In this study, it was shown that *Periplanetta americana* and *Blatta Orientalis* collected from food premises were found to carry an array of bacteria on their external and internal guts. Similar finding was shown in a study by Fotedar *et al*. [14], which reported presence of various types of pathogenic bacteria carried by cockroaches collected from domestic, hospitals and restaurants. The nocturnal and filthy habits of these insects make them ideal carries for many pathogenic microorganisms [3]. Similar results were reported by Chaichanawongsoraj *et al.* in 2004 [17]. In another study conducted in the urban area of France, 56 species of bacteria were isolated from cockroaches and 14 species were found to be pathogenic or potentially pathogenic to human and animals [9, 11].

In this study, different species of potentially pathogenic bacteria were isolated from the external and internal surfaces of *Periplanetta americana* and *Blatta orientalis*. Most of the bacteria isolated were medically important, including *E.coli, Salmonella spp, Shigella spp,* and *Staphylococcus spp*. Presence of *E.coli* was supported by a previous study performed in the urban area of Malaysia. A total of 17 species of bacteria were isolated from six species of cockroaches and *E.coli* and *K.pneumonia* were the most important bacteria isolated [1, 11].

Findings of the present study are also in accordance with the result of Vazirianzadeh *et al*. [15] from Khuzestan, which showed presence of *Klebsiella spp., E.coli, Stapylococcus spp., Streptococcus spp., Proteus spp., Pseudomonas spp., Neiserria spp., Salmonella spp., Shigella spp., and Bacillus spp.*[8, 15]. Isolation of pathogenic and potential pathogenic bacteria such as *E.coli, Staphylococcus spp, Salmonella spp* and *Shigella spp* from the external body and internal guts of *P.americana* and *Blatta orientalis* in this study also agrees with Tachbele *et al*. [16]. The presence of *Salmonella spp* in cockroaches collected in this study also confirms the report of Akinjogula *et al* [10]. In their experiment which was performed in 10 districts of Tangier, Morocco, 11 species of bacteria were isolated from 50 cockroaches captured in houses including *Escherichia coli, Salmonella spp., Shigella spp., Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus species, Enterobacter spp., Klebsiella spp., Serratia spp., Proteus spp.,* and *Proteus vulgari* [13, 20].

Cockroaches dwelling in food establishments or food premises could be dangerous if they carry

bacteria that are pathogenic [17]. Cockroaches are a health hazard not only because they are a nuisance to the environment [18] but also due to the risks posed by cockroach antigens to asthma sufferers. Most importantly, they can carry disease-causing bacteria. Among potentially pathogenic bacteria that can be carried on their body are *E.coli, Stapylococcus aureus*, *Salmonella sp., Shigella sp.* and other organisms that are associated with dangerous illness such as food poisoning, dysentery, typhoid disease, hepatitis, tuberculosis and others. In addition, the behavior of cockroaches that feed on feces and filth can disseminate infections by the fecal – oral route [13].

Based on the present study, the cockroaches were detected as carriers for *E.coli, Salmonella sp, Shigella sp,* and *Staphylococcus spp*. Presence of these potentially pathogenic bacteria can be associated with variety of health problems. As early as 1949, Mackerras and Mackerras have revealed that *Salmonella species* carried by cockroaches can cause gastro-enteritis outbreak [17, 21]. As for *Staphylococcus aureus* carried by cockroaches, they have been reported to be the most common cause of nosocomial infections [16]. Study by Tachbele *et al.* 2006 indicated that *Shigella species* obtained from restaurant cockroaches were resistant to seven antimicrobials [16]. According to Bauamama, L., et al., *Staphylococcus aureus*, and *E.coli* carried by cockroaches can cause diarrhea [13, 20].

4.0 CONCLUSION

The presence of cockroaches in domestic areas especially food establishments poses a risk to health as they play a role for the transmission of a wide range of pathogenic bacteria. The persistence presence of a range of microorganisms, pathogenic or potentially pathogenic, on cockroaches from various food-related environments is undeniable. Hence, this underlines the need to study the hygiene plans implemented in food-related environments to curb this problem.

Acknowledgement

Deepest gratitude to all who facilitated our field work; special appreciation is given to Tn.Hj Mohd Pozi Mohd Tahir, for his supervision and support in various issues. And a special thank is given to Dr. Emida Mohamed, Department of Medical Laboratory Technology and laboratory staff from Department of Environmental Health , for their guidance in the bacterial identification part.

References

[1] Vythillingam, I., Jeffery, J., Oothuman, P., Razak, A. R. A., and Sulaiman, A. 1997. Cockroaches from Human Dwellings: Isolation of Bacterial Pathogens and Control. *Southeseast Asian Journal of Tropical Medicine and Public Health.* 28: 218-222.

[2] Graczyk, T. K., Knight, R., Gilman, R., and Cranfield, M. 2001. The Role of Non-Biting Flies in the Epidemiology of Human Infectious Diseases. *Microbes and Infection.* 3(3): 231-235.

[3] Graczyk, T. K., Knight, R., and Tamang, L. 2005. Mechanical Transmission of Human Protozoan Parasites by Insects. *Clinical Microbiological Reviews.* 18: 128-132.

[4] Alloytey, J., Mpuchane, S., Gashe, B. A., Simpanya, M., and Matsheka, I. 2009. Trapping Of *Blatella Germanica* (L) Populations in Human Dwellings in Gaborone, Botswana. *Journal of Applied Zoological9 Researches*. 20(2): 175-188.

[5] Baumholltz, M. A., Parish, L. C., Witkowski, J. A., and Nutting, W. B. 1997. The Medical Importance of Cockroaches. *Internet Journal of Dermatology*. 36: 90-96.

[6] Alzain, B. 2013. Cockroaches: Transmission of Medically Important Parasites. *ARPN Journal of Science and Technology*. 3(5): 533-537.

[7] Jirage, R. 2011. What-do-cockroaches-eat. Retrieved October, 19, 2013 from http://www.buzzle.com/articles/what-do-cockroaches-eat.html.

[8] Kassiri, H., and Kazemi, S. 2012. Cockroaches [*Periplaneta americana* (L.), Dictyoptera; Blattidae] as Carriers of Bacterial Pathogens, Khorramshahr Country, Iran. *Jundishapur Journal of Microbiology*. 5(1): 320-322.

[9] Rivault, C., Cloarec, A., and Leguyader, A. 1993. Bacterial Load of Cockroaches In Relation To Urban Environment. *Epidemiology and Infection.* 110: 317-325.

[10] Akinjogunla, O. J., Odeyemi, A. T., and Udoinyang, E. P. 2012. Cockroaches (*Periplaneta Americana* and *Blatella germanica*): Reservoirs Of Multi Drug Resistant (MDR) Bacteria In Uyo, Akwa Ibom State. *Science Journal of Biological Science*. 1(20): 19-30.

[11] Pai, H. H., Chen, W. C., Peng, C. F., 2005. Isolation of Bacteria Wit Antibiotic Resistance from Household Cockroaches (*Periplanetta Americana* and *Blattella Germanica*). *Acta Tropica*. 93: 259-265.

[12] Cruden, D. L. and Markoverts, A. J. 1987. Microbiol Ecology Of The Cockroach Gut. *Ann Rev. Microbial*. 41: 617-643.

[13] Feizhaddad, M. H., Kassiri, H., Sepand, M. R., and Ghasemi, F. 2012. Bacteriology Survey of American Cockroaches in Hospital. *Middle-East Journal of Scientific Research.* 12(7): 985-989.

[14] Fotedar, R., Shriniwas, U. B., and Verma, A. 1991. Cockroaches (*Blatella Germanica*) As Carriers of Microorganisms of Medical Importance in Hospitals. *Epidemiology and Infection.*107: 181-187.

[15] Vazirianzadeh, B., Mehdinejad, M., and Dehghani, R. 2011. Identification of Bacteria Which Possible Transmitted By Polyphaga Aegyptica (Blattodea: Blattidae) In the Region of Ahvaz, SW Iran. *Jundishapur Journal of Microbiology.* 2(1): 36-40.

[16] Tachbele, E., Erku, W., Gebre-Michael, T., and Ashenafi, M. 2006. Cockroach-Associated Food-Borne Bacterial Pathogens from Some Hospitals and Restaurants in Addis Ababa, Ethiopia: Distribution and Antibiograms. *Journal of Rural and Tropical Public Health*. 5: 34-41.

[17] Chaichanawongsaroj, N., Vanichayatanarak, K., Pipatkullachat, T., Polrojpanya, M., and Somkiatcharoen, S. 2004. Isolation of Gram-Negative Bacteria from Cockroaches Trapped from Urban Environment. *The Southeast Asian Journal of Tropical Medicine and Public Health.* 35(3): 681-684.

[18] Malik, K., Jamil, A., Arshad, A. 2013. Study of Pathogenic Microorganisms in the External Body Parts of American

Cockroach (*Periplanatta Americana*) Collected from Different Kitchens. *Journal of Pharmacy and Biological Sciences*. 7(6): 45-48.

[19] Bagde, U. S., Gopi, U., Prasad, R. 2013. Isolation and Characterization of Gut – Associated Microbes In Cockroach. *African Journal of Microbiology Research*. 7(9): 2034-2039.

[20] Bouamama, L., Sorlozano, A., Laglaoui, A., Lebbadi, M., Aarab, A., Gutierrez, J. 2010. Antibiotic of resistance patterns of bacterial strains isolated from *Periplanetta americana and Musca Domestica* in Tangier. Morocco. *J Infect Dev Ctries*. 4(4): 194-201.

[21] Mackerras, I. M., Mackerras, M. J. 1949. An Epidemic of Infactile Gastroenteritis Cause by Salmonella Bovis Morbificans. *J Hyg.* 47: 166-81.