Academia Journal of Microbiology Research 9(2): 033-039, April 2021
DOI: 10.15413/ajmr.2021.0105
ISSN 2315-7771

Academia Publishing

Research Paper

Isolation and characterization of bacteria from blowflies *Chrysomya chloropyga* collected from two different abattoirs in two communities

Olatubi I. V.[1], Ashipa O. S.[2], Aromolaran O.[2], Akinkunle V. A.[3] and Adeleke O. A.[2]

[1]Department of Pure and Applied Biology, Bowen University, Iwo, Nigeria.
[2]Department of Microbiology, Bowen University, Iwo, Nigeria.
[3]Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Nigeria.

*Corresponding author. E-mail: olatubivictoria@gmail.com. Tel: +2347039014087.

Accepted 2nd June, 2021

ABSTRACT

The objective of this study was to isolate and identify bacteria found on the body surface of blowfly *Chrysomya chloropyga* collected from two different abattoirs. In this study, adult blowflies were obtained from two different abattoirs of two towns, the first set were collected from Iwo (Odori) abattoir while the second set were collected from Oluponna (Alaya) abattoir. Serial dilution of the whole-body homogenate was done to obtain the bacteria in them. The bacteria were sub cultured to get pure colony. These colonies were Gram stained and viewed under the microscope for identification. Several biochemical tests such as sugar test, starch hydrolysis, citrate test, MRVP test and, indole test were subsequently carried out. The sub culturing, Gram staining and Biochemical tests were done to determine the possible organisms that could be isolated from blowflies. The organisms that were identified from Iwo abattoir were all Gram positive with *Staphylococcus* sp. (81.80%) being the highest in frequency, while those identified from Oluponna abattoir were a composition of both Gram positive and Gram-negative bacteria with *Bacillus* sp. and *Citrobacter* sp. (32%) being the highest in frequency. This study however concludes that *C. chloropyga* had a wide range of bacteria on its body surface with Gram positive organisms being dominant as compared with gram negative, and Iwo abattoir blowflies had the highest number of pathogenic bacteria while Oluponna abattoir blowflies had the highest diversity of bacteria.

Key words: Abattoir, bacteria, characterization, *Chrysomya chloropyga*, isolation.

INTRODUCTION

There is an age long African adage that says “the noise of flies does not prevent the meat seller from making sales” this explains how closely associated flies are to abattoir and the Blowflies are one of the most abundant and important group of insects present, serving as vectors of some of the diseases affecting humans (Pava-Ripoll et al., 2012). Blowfly belongs to family Calliphoridae and order Diptera with more than 1,100 species included in about 150 genera worldwide (Rognes, 1991). These flies are cosmopolitan and well distributed in all continents (Triplehorn and Johnson, 2005) with about 80% of the species found in the Old World with Africa being especially diverse (Shewell, 1987). *Chrysomya chloropyga* is a medium to large sized blowfly with metallic blue green coloration and having dark L markings on the thorax as a diagnostic feature. This blowfly and relatives have been implicated in the transmission of serious diseases such as anthrax, typhoid fever, cholera, tuberculosis and have been demonstrated to harbor or transmit other pathogenic bacteria including *Salmonella* sp., *Shigella* sp., *Klebsiella* sp., *Chlamydia* sp., *Helicobacter pylori*, and the causative agent of gastric ulcer (Aigbodion et al., 2018; Singh et al., 2015) and for centuries, they have been constant companions of humans with both desirable and undesirable impacts (Harrison, 1978) as they are commonly associated with human surroundings such as food shops, markets, village sundry shops (Chaiwong et al.,

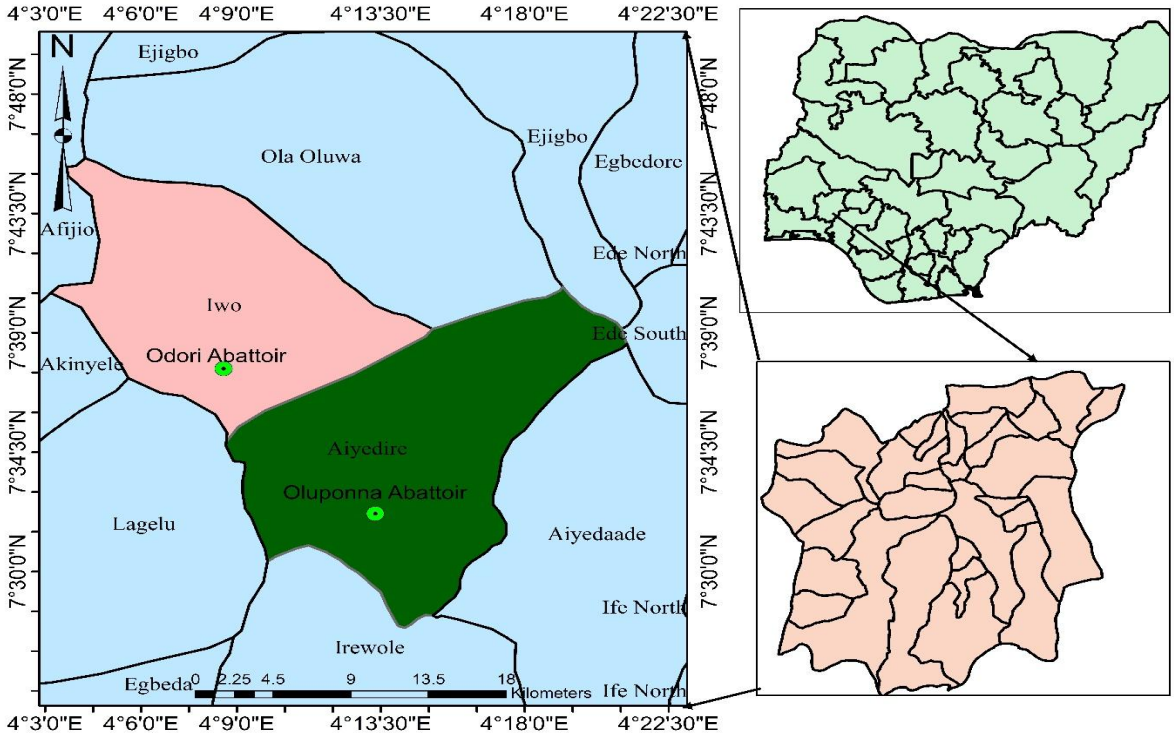

Figure 1: Map showing the sampling sites.

2012, Nurita and Abu Hassan, 2013, Khoso et al., 2015) and even in Abattoir causing problem to the health of the public (Harwood and James, 1989; Gabre and Abouzied, 2003; Forster et al., 2009). Consequently, as mechanical vectors, they bridge the bio-network in the spread of human intestinal parasite and pathogenic microorganisms in un-sanitized developing countries (Oguniyi et al., 2015). This is because they frequently traverse faecal contaminated and filthy sites (Parrish and Ryan, 2014; Mahfouz et al., 1997).

The microorganisms carried by blowfly may vary depending on its exposure, geographical location, weather, climate and soon. Hence the need for this study which seeks to compare the bacteria associated with blowflies collected from Iwo and Oluponna abattoir, respectively.

MATERIALS AND METHODS

Sampling Site

Iwo and Oluponna are adjacent communities both located in Osun State; Southwest Nigeria (Figure 1) sharing the same latitude and longitude of 7° 38'N and 4° 11'E respectively with an altitude of 322 m. These communities are located in the tropics and have a yearly temperature that typically varies from 18.9 to 35°C and is rarely below 15.6°C or above 37.8°C and an average relative humidity of 60 .8%. With a wet season that is harsh and cloudy and a dry season that is muggy and partly.

Sample collection

Blowflies were collected from Iwo (Odori) abattoir and Oluponna (Alaya) abattoir, respectively using a sterile sweep net at the early hours of the day when killing of cattle is done, these flies were transferred into sterile universal bottles and taken to the laboratory, freeze killed, sorted and then identified using standard morphological techniques (Hoell et al.,1998; Resh and Carde, 2009). Upon identification, the flies *Chrysomya chloropyga* from the two communities were collected and then used.

Isolation and identification of bacteria from blowflies collected from Iwo and Oluponna abattoir

Bacteria were isolated from whole body homogenate of the blowflies collected from Iwo and Oluponna abattoir, respectively by standard pour plate techniques. Tenfold serial dilution was prepared; using 9 ml sterile distilled water in test-tubes and aseptically plated on Nutrient agar (NA). The plates were incubated overnight at 37°C for 24 h. discrete colonies were picked from the plates and repeated streaking was done to obtain pure cultures. Various biochemical tests were carried out on the bacterial isolates, such as Gram staining, catalase test, methyl red, Voges-Proskauer, indole, citrate utilization and sugar fermentation and identified as described by Bergey's Manual of Systematic Bacteriology (Garrity et al., 2004).

Table 1: Morphological characteristics of bacteria isolated from the body of blowflies collected from Iwo Town Abattoir.

Isolation Code	Appearance	Size	Colony	Optical characteristics
A1	Cocci	Small	Clusters	Opaque
A2	Cocci	Small	Clusters	Opaque
A3	Cocci	Small	Clusters	Opaque
A4	Cocci	Tiny	Clusters	Translucent
A5	Rod	Small	Distinct	Opaque
A6	Cocci	Small	Clusters	Opaque
A7	Cocci	Small	Clusters	Opaque
A8	Cocci	Small	Clusters	Opaque
A9	Cocci	Tiny	Clusters	Opaque
A10	Cocci	Large	Distinct	Opaque
A11	Cocci	Small	Clusters	Opaque

Table 2: Morphological characteristics of bacteria isolated from the body of blowflies collected from Oluponna Abattoir.

Isolate code	Appearance	Size	Colony	Surface colony	Optical characteristics
B1	Rod	Small	Distinct	Smooth	Opaque
B2	Rod	Small	Distinct	Rough	Opaque
B3	Rod	Large	Distinct	Rough	Opaque
B4	Rod	Small	Clusters	Rough	Opaque
B5	Rod	Small	Distinct	Smooth	Translucent
B6	Rod	Small	Clusters	Rough	Opaque
B7	Rod	Small	Distinct	Rough	Opaque
B8	Rod	Small	Clusters	Rough	Opaque
B9	Rod	Large	Distinct	Smooth	Opaque
B10	Rod	Small	Distinct	Rough	Opaque
B11	Rod	Large	Distinct	Smooth	Opaque
B12	Rod	Small	Clusters	Rough	Opaque
B13	Rod	Small	Distinct	Smooth	Translucent
B14	Rod	Small	Distinct	Rough	Opaque
B15	Rod	Small	Clusters	Rough	Opaque
B16	Rod	Small	Distinct	Rough	Opaque
B17	Rod	Large	Distinct	Rough	Opaque
B18	Cocci	Small	Distinct	Rough	Opaque
B19	Rod	Small	Distinct	Smooth	Opaque

RESULTS

Isolation and characterization

Bacteria were isolated from the body of blowflies. Tables 1 and 2 show the morphological features of the bacteria isolated which include the appearance, size, colony, surface colony and optical characteristics. Tables 3 and 4 show the biochemical characteristics of bacteria isolated from blowflies. All isolates were gotten from the body of *C. chloropyga* blowflies. From the first set of blowflies, isolates A1, A2, A3, A4, A6, A7, A8, A9 and A10 were identified as *Staphylococcus* sp. and were cocci in shape. Isolate A5 was identified as *Bacillus* sp. and was rod in shape while Isolate 11 was identified as *Micrococcus* sp. and was cocci in shape, respectively. Isolates B1, B9, B10, B12, B13 and B14 were identified as *Bacillus* sp., they were rod in shape. Isolates B2, B4, B7, B11, B16 and B19 were identified as *Citrobacter* sp., these isolates were also rod in shape. Isolates B3 and B5 were identified as *Klebsiella* sp. and also rod in shape. Isolates B8 and B15 were rod in shape and identified as *Providencia* sp. Lastly, B17 was identified as *Proteus* sp. and was rod shaped. Based on the biochemical test, the isolates were identified as *Staphylococcus* sp., *Bacillus* sp., *Micrococcus* sp., *Citrobacter* sp., *Klebsiella* sp., *Providencia* sp. and *Proteus* sp. Summarily, the most occurring organism

Table 3: Biochemical characteristics for bacteria for the blowflies collected from Iwo Town Abattoir.

Isolates code	Gram's reaction	Morphology	GLU	SUC	LAC	MAN	IND	CIT	MR	VP	SH	Probable Organism	CIT	CIT	Methyl red	Voges Proskauer	Starch hydrolysis
A1	+	Cocci	AG	AG	AG	AG	+	+	+	-	+	*Staphylococcus* sp	+	+	Positive	Negative	Positive
A2	+	Cocci	AG	AG	AG	AG	+	+	+	-	+	*Staphylococcus* sp	+	+	Positive	Negative	Positive
A3	+	Cocci	AG	AG	AG	AG	+	+	+	-	+	*Staphylococcus* sp	+	+	Positive	Negative	Positive
A4	+	Cocci	AG	AG	AG	AG	+	+	+	-	+	*Staphylococcus* sp	+	+	Positive	Negative	Positive
A5	+	Rod	AG	AG	AG	AG	+	+	+	-	+	*Bacillus* sp	+	+	Positive	Negative	Positive
A6	+	Cocci	AG	AG	AG	AG	+	+	+	-	-	*Staphylococcus* sp	+	+	Positive	Negative	Negative
A7	+	Cocci	AG	AG	AG	AG	+	+	+	-	-	*Staphylococcus* sp	+	+	Positive	Negative	Negative
A8	+	Cocci	AG	AG	AG	AG	+	+	+	-	-	*Staphylococcus* sp	+	+	Positive	Negative	Negative
A9	+	Cocci	AG	AG	AG	AG	+	+	+	-	-	*Staphylococcus* sp	+	+	Positive	Negative	Negative
A10	+	Cocci	AG	AG	AG	AG	+	+	+	-	-	*Staphylococcus* sp	+	+	Positive	Negative	Negative
A11	+	Cocci	AG	AG	AG	AG	-	+	+	-	-	*Micrococcus* sp	+	+	Positive	Negative	Positive

KEY: += Positive, -= Negative, AG= Acid and Gas production, GLU= Glucose, SUC= Sucrose, LAC= Lactose, MAN= Mannitol, CIT= Citrate utilization, MR= Methyl Red, VP=Voges Proskauer, SH= Starch hydrolysis, IND= Indole

Table 4: Biochemical test results for isolates (B Samples) collected from Oluponna Abattoir

Isolate code	Gram's reaction	Morphology	GLU	SUC	LAC	MAN	IND	CIT	MR	VP	SH	Probable organisms
B1	+	Rod	AG	AG	AG	AG	-	+	+	-	-	*Bacillus* sp
B2	-	Rod	AG	AG	-	AG	-	+	+	-	-	*Citrobacter* sp
B3	-	Rod	AG	AG	AG	AG	+	+	+	+	-	*Klebsiella* sp
B4	-	Rod	AG	AG	AG	AG	+	+	+	-	-	*Citrobacter* sp
B5	-	Rod	AG	AG	AG	AG	+	+	-	-	+	*Klebsiella* sp
B6	-	Rod	AG	AG	AG	AG	-	+	+	+	+	*Enterobacter* sp
B7	-	Rod	AG	AG	-	-	+	+	+	-	+	*Citrobacter* sp
B8	-	Rod	AG	AG	-	AG	+	+	-	-	+	*Providencia* sp
B9	+	Rod	AG	AG	AG	-	+	+	+	-	+	*Bacillus* sp
B10	+	Rod	AG	AG	-	-	+	+	+	+	+	*Bacillus*sp
B11	-	Rod	AG	AG	AG	AG	+	+	+	-	+	*Citrobacter* sp
B12	+	Rod	AG	AG	-	AG	+	+	+	-	-	*Bacillus* sp
B13	+	Rod	AG	AG	-	AG	+	+	+	-	-	*Bacillus* sp
B14	+	Rod	AG	-	-	AG	+	+	+	-	-	*Bacillus* sp
B15	-	Rod	-	-	-	AG	-	+	-	-	-	*Providencia* sp
B16	-	Rod	AG	AG	AG	AG	+	+	+	-	-	*Citrobacter* sp
B17	-	Rod	AG	-	-	-	+	+	+	-	-	*Proteus* sp
B18	+	Cocci	AG	AG	AG	-	-	-	-	+	-	*Staphylococcus* sp
B19	-	Rod	-	AG	AG	AG	-	+	+	-	-	*Citrobacter* sp

KEY: += Positive, -= Negative, AG= Acid and Gas production, GLU= Glucose, SUC= Sucrose, LAC= Lactose, MAN= Mannitol, CIT= Citrate utilization, MR= Methyl Red, VP= Voges Proskauer, SH= Starch hydrolysis, IND= Indole.

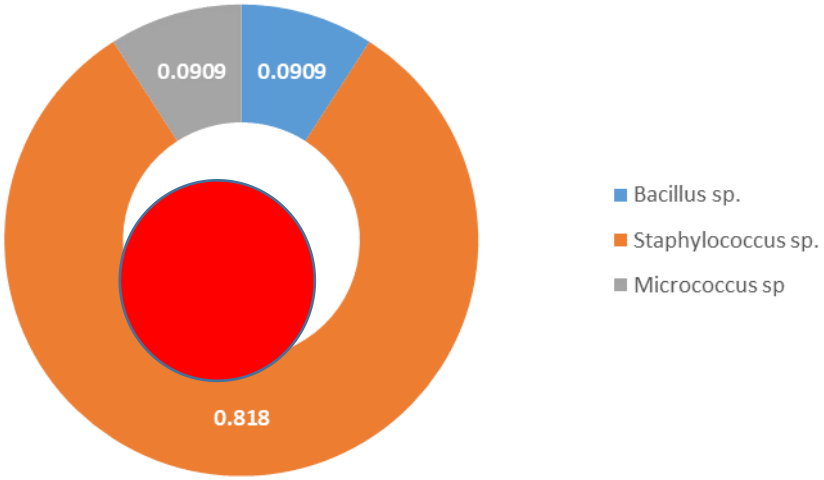

Figure 2: Percentage occurrence of bacteria Isolated from Iwo Abattoir.

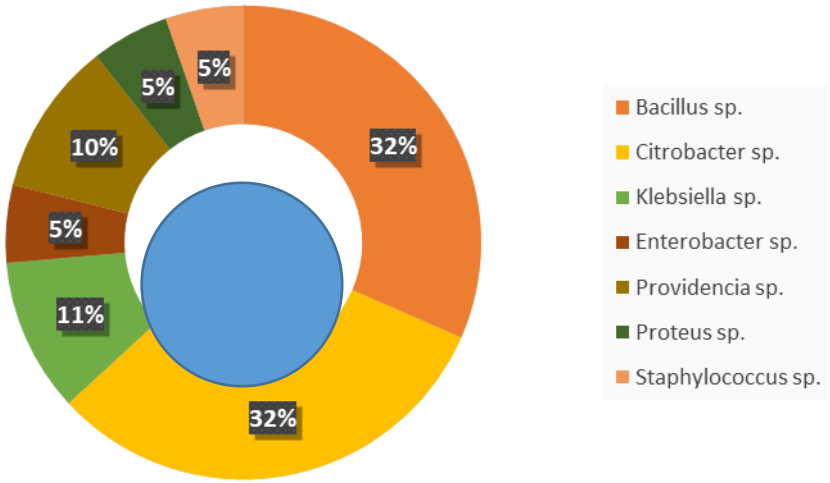

Figure 3: Percentage occurrence of bacteria isolated from Oluponna Abattoir.

was *Staphylococcus* sp. from Iwo abattoir (Figure 2) while *Bacillus* sp. and *Citrobacter* sp. (Figure 3) were the most occurring organisms from Oluponna.

DISCUSSION

From this study, the blowflies (*Chrysomya* sp.) collected were seen to have numerous microorganisms, as a wide range of bacteria were isolated from these blowflies collected from the Iwo and Oluponna abattoirs, respectively. Three bacteria were isolated from Iwo abattoir and they were all Gram positive with *Staphylococcus* sp. being the highest with 81.8% frequency while seven different species were isolated from Oluponna abattoir with a composition of both Gram positive and Gram-negative bacteria. *Bacillus* sp. and *Citrobacter* sp. were found to be the highest in frequency (31.6%). The finding is similar to the report of Caballero et al. (1996) who isolated *Escherichia sp., Proteus sp., Providencia* sp., *Staphylococcus* sp. *and Streptococcus* sp. from *Chrysomya hominivorax* during sheep myiasis. Epling et al. (1993) on the other hand isolated *Salmonella* sp. (12% - 20%) from swabbed ham surfaces. In a previous study, the surface bacteria inoculation on these flies in human dwelling showed *Escherichia coli* an organism which has been implicated to have the highest rate of infection in humans and animals (Vazirianzadeh et al., 2008) while in the hospital environment it was found that *Pseudomonas* sp. had the greatest rate of bacterial surface infection on these flies in addition to *Escherichia coli*. In another study, some of the bacterial genera such as *Bacillus* sp., *Escherichia* sp.

and *Klebsiella* sp. were isolated from the flies of poultry farms in Malaysia (Nazni et al., 2005). Similarly, *Staphylococcus* sp., *Bacillus* sp. and *Escherichia coli* known to cause diarrhea were obtained from external and internal parts of house fly body (Bouamama et al., 2010; Nazni et al., 2005). Sulaiman et al. (2000) in their research, isolated *Aeromonas hydrophilia*, *Klebsiella oxytoca, Burkholderia pseudomallei* in Malaysia but Sukontason et al. (2007) isolated *Escherichia coli*, *Klebsiella pneumonia*, *Morganella morganii, Enterobacter cloacae*, *Proteus mirabilis* from *Chrysomya megacephala* and *Musca domestica* in Thailand. Previous studies have also established that these flies are involved in transmission of pathogens such as helminthes and protozoan parasites (Getachew et al., 2007) in addition to microorganisms. *Staphylococcus* sp. which are known to be pathogenic were the abundant bacteria isolated (81%) from the Iwo abattoir, this could be because of the poor hygienic practices at this abattoir (personal observation). The Oluponna abattoir on the other hand had 5% occurrence of *staphylococcus* and the reason is not far-fetched as the abattoir was observed to be very neat and well organized with very good waste disposal practices as only a few of these flies were seen around. Despite the good hygiene practices at the Oluponna abattoir, a wide range of bacteria were still isolated from the blowflies collected which are of public health importance.

The high incidence of *Staphylococcus* in the blowflies collected from Iwo abattoir is suggestive of unaccepted level of contamination and can give rise to enterotoxins as these flies' perch on the meats to be sold contaminating them while these same flies could also perch on our food contaminating them likewise and may not necessarily alter the appearance of the food. It is important to note that such contaminated meat or food may constitute serious public health hazard as reported by Itah and Opara (1997). The incidence of *Staphylococcus* (5%) and some other bacteria in Oluponna abattoir despite their hygiene practices is indicative that these blowflies harbor a wide range of microorganism and measure should be put in place to reduce them so as to keep our consumables safe. With the very low occurrence of *Staphylococcus,* it therefore means that with improved hygienic practices, these abattoirs can be free of the microorganisms.

CONCLUSION

The present study identified a wide range of bacteria isolated from blowflies collected from Iwo abattoir to be gram positive while those isolated from blowflies collected from Oluponna abattoir were a combination of both gram positive and gram negative bacteria with *Staphylococcus* sp. being the highest in frequency from Iwo abattoir and *Bacillus* sp. and *Micrococcus* sp. being the highest in frequency from Oluponna abattoir. The predominance of food poisoning organisms such as *Staphylococcus* is indicative of gross contamination and constitutes potential hazard to consumers and sellers. Therefore, a clean surrounding, improved hygienic practices and proper waste disposal is non-negotiable to reduce the incidence of pathogenic bacteria isolated from blowflies collected from abattoirs thereby reducing the rate of infections following the degree at which these microorganisms are becoming resistant to antibiotics.

REFERENCES

Aigbodion FI, Egbon IN, Obuseli AJ (2018). Pathogens of Medical Importance Isolated from *Phaenicia Lucilia sericata* (Diptera: Calliphoridae) in Benin City Nigeria Pak. J. Biol. Sci. 16(23):1791-1795.

Bouamama L, Sorlozano A, Laglaoui A, Lebbadi M, Arab A, Gutierrez J (2010). Antibiotic resistance patterns of bacterial strains isolated from Periplaneta Americana and Musca domestica in Tangier, Morocco. J. Infect. Dev. Ctries 4(4):194-201.

Caballero M, Hernandez G, Poudevigne F, Ruiz-Martinez I (1996). Isolation and identification of bacteria associated with the screwworm fly Cochliomyiahominivorax, Coquerel and its myiasis Ann, NY. Acad. Sci. 791:248-254.

Chaiwong T, Srivoramas T, Sukontason K, Sanford MR, Moophayak K, Sukontason KI (2012). Survey of the Synanthropic flies associated with human habitations in Uboh Ratchathani province of Northern Thailand J Parasitol 2012:132-163

Epling LK, Carpenter JA, Blankership LC (1993). Prevalence of Campylobacter and Salmonella spp. On pork carcasses and the reduction effected by spraying with lactic acid. J. Food Prot. 53:536-537.

Forster M, Sievert K, Messler S, Klimpel S, Pfeffer K (2009). Comprehensive Study on the Occurrence and Distribution of Pathogenic Microorganisms Carried by Synanthropic Flies Caught at Different Rural Locations in Germany. J. Med. Entomol. 49:1164-1166.

Gabre RM, Abouzied EM (2003). Sarcosaprophagus flies in Suez province Egypt II Synanthropic and abundance degrees. Bull. Entomol. Soc. Egypt 80:125-132.

Garrity GM, Bell JA, Lilburn TG (2004). Taxonomic outline of the prokaryotesbergey's manual of systematic bacteriology, 2nd edition Release 50 Springer-Verlag, New York, May 2004:1-399.

Getachew S, Gebre-Michael I, Erko B, Balkew M, Medlin G (2007). Non-Biting Cyclorrhaphan Flies (Diptera) as Carriers of Intestinal Human Parasites in Slum Area of Addis Ababa Ethiopia. Acta Trop. 103:186-194.

Harrison G (1978). Mosquitoes, Malaria and Man: A History of the hostilities 1st edition John Murray: London

Harwood RF, James MT (1989). Entomology in human and animal health 3rd edition Macmillan publishing co-operation, New York

Hoell HV, Doyen JT, Purcell AH (1998). Insect Biology And Diversity, 2nd Ed Oxford University Press, Osford, UK.

Itah AY, Opara AA (1997). Enterotoxin production by Staphylococcus aureus strains contaminating canned foods in Calabar, Nigeria J Sci Eng Technol 4(2):740-750.

Khoso FN, Wong SK, Chia SL, Lau WH (2015). Assessment of non-biting Synanthropic flies associated with fresh markets J. Entomol. Zool. Stud. 3:13-20

Mahfouz AAR, El-Morshedy H, Farghaly A, Khalil A (1997). Ecological Determinants of Intestinal Parasitic Infections among School Children in an urban Squatter Settlement of Egypt. J. Trop. Pediatr. 43:341-344.

Nazni WA, Seleena B, Lee HL, Jeffery J, Rogayah TAR, Sofian MA (2005). Bacteria fauna from the house fly, *Musca domestica* (L). Trop. Biomed. 2005(2):225-231

Nurita AT, Abu Hassan A (2013). Filth flies associated with municipal solid waste and impact of delay in over soil application on adult filth fly emergence in a sanitary landfill in Pulau Pinang Malaysia. Bull. Entomol. Res. 103:296-302.

Oguniyi TAB, Joshua SO, Oyelade OJ (2015). Parasites Associated with Non-Biting Flies in Ile-Ife, Nigeria. J. Med. Biol. Sci. Res. 9:124-129.

Pava-Ripoll M, Goertz Pearson ER Miller KA, Zlobro CG (2012). Prevalence and Relative Risk of *Cronobacter* spp, Salmonella spp, and Listeriamonocytogenes Associated with the Body Surfaces and Guts of Individual Filth Flies. Appl. Environ. Microbiol. 78(22):7891/7902

Pava-Ripoll M, Goertz Pearson ER Miller KA, Zlobro CG (2015). Detection of Foodborne Bacterial Pathogens from Individual Filth Flies. J. Visual. Exp. 2015(96):52372-52379.

Resh VH, Carde RT (2009). Encyclopedia of Insects, 2nd Ed Academic Press, Cambridge, MA.

Rognes K (1991). Blowflies (Diptera: Calliphoridae) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica pp. 24-272.

Shewell, JE (1987). Calliphoridae Pp 1133-1145 In Manual of Nearctic diptera Vol2 Research Branch, Agriculture Canada Monograph 291332 pp.

Singh B, Crippen TL, Zheng L, Fields AT, Yu Z, Ma Q, et al (2015). A Metagenomic Assessment of the Bacteria Associated with Lucilia sericata and Lucilia cuprina (Diptera: Calliphoridae). Appl. Microbiol. Biotechnol. 99(2):869-883.

Sukontson KL, Bunchoo M, Khandawa B, Plangjai R, Somsak Y, Sukontson K (2007). Comparison between Musca domestica and Chrysomyamegacephala as Carriers of Bacteria in Northern Thailand. South East Asia J. Tropical Med. Public Health 38(1): 8-44.

Sulaiman S, Othman MZ, Aziz AH (2000). Isolations of enteric pathogens from Synanthropic flies trapped in downtown Kuala Lumpur. J. Vector Ecol. 25:90-93.

Triplehorn CA, Johnson NF (2005). Borror and Delong's Introduction to the study of Insects 7th ed Thomson Brooks/ Cole Belmont, California 864 pp.

Vazirianzadeh B, Shams Solary S, Rahdar M, Hajhossien R, Mehdinejah M (2008). Identification of bacteria which possible transmitted by Musca domestica (Diptera: Muscidae) in the region of Ahvaz, SW Iran Jundishapur. J. Microbiol. 2008(1):28-31.