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Evaluation of different microbial pathogens associated with the external surfaces of houseflies and to determine the antibiotic susceptibility pattern of recovered bacterial pathogens in Owo

Abdulwasiu Oladele Hasssan[1], Emmanuel Ifeanyi Obeagu[2,3] and Blessing Oyeromi Oluwasinmile[1]

[1]Department of Medical Laboratory Science, Achievers University, Owo, Ondo State, Nigeria
[2]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria
[3]Department of Medical Laboratory Science, Madonna University Nigeria

Abstract

The aim of this research is to evaluate the different microbial pathogens associated with the external surfaces of houseflies and to determine the antibiotic susceptibility pattern of recovered bacterial pathogens in Owo, Ondo state, Nigeria. The study was conducted in Owo. A total of 100 houseflies were collected from four different sites with sterile sweep net, namely, refuse dump sites, meat stand, fruits and vegetable stand, palm wine joints all located within Owo. The proportion of houseflies collected from each location and the frequency of the bacteria and parasite isolate from the houseflies were analyzed using statistical package for social sciences (SPSS) 25.0 versions for the analysis of the data appropriately. Oneway analysis of variance (ANOVA) was used for comparison within the groups. Spearman correlation was used to test the association between variables. Data was presented using mean ± standard deviation (mean ± SD) for all quantitative values. The level of significance was taken at 95% confidence interval and $P<0.05$ was considered significant**.** The houseflies collected from refuse dump sites, meat stand, fruits and vegetable stand, palm wine joints all located within Owo town are potential vectors of a wide range of pathogenic organisms like Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa, Staphylococcus aureus and Citrobacter spp.Only two parasites; Ascaris lumbricoides and Entamoeba histolytica were recovered in this study.Antibiotics such as Ciprofloxacin, Perfloxacin, Sparfloxacin, Streptomycin and Cefuroxime are relatively highly sensitive to bacteria isolates in this study but there are still reasons for public health concerns. This study shows that houseflies collected from different environmental locations are all capable of carrying antimicrobial resistant bacteria at a high level. Houseflies may play a significant role in the dissemination of antimicrobial resistance to various environments. This study showed the persistence of resistant bacteria in the Owo environment.

Keywords: pathogens, houseflies, antibiotic susceptibility pattern

Introduction

Houseflies (*Musca domestica L.*) are members of the insect family *Muscidae*, which contains a number of species considered as vectors of disease. They are considered filth flies due to their association with organic substrates such as household waste and manures (Moon *et al.*, 2002). The housefly is known to have originated from Central Asia and spread throughout the whole world, and they are found in both rural and urban areas of tropical and temperate climates (Hussein and John, 2014).Houseflies accounts for about 90% of all the flies in human habitation worldwide (Nmorsi *et al*., 2006). There are about 170 genera and 4200 species in the family Muscidae, some are of medical importance including the housefly, *Musca domestica* (Service, 2012).The house fly belongs to a group often referred to as “filth flies” the other members belong to the families Calliphoridae and Fanniidae (Szalanski *et al.*,2004*)*.

The house fly has been in existence since the origin of human life and is well adapted to life in human habitations/dwelling (WHO, 2008). The house fly is known to carry pathogens that can cause serious diseases in humans and animals. Over 100 pathogens including bacteria, viruses, fungi and parasites (protozoan and metazoans) have been associated with the insect (Tsagaan *et al.*, 2015). Molecular analysis revealed that house flies carry very diverse groups of microorganisms (Bahrndorff *et al.*, 2017). Evidence supporting the role of the house fly in transmission of diseases are inconclusive, with the strongest evidence pointing to the correlation between the rise in incidence of diarrhoea and an increase in the fly population (Farag *et al.*, 2013, AHA *et al.*, 2014). House fly causes mechanical transmission of pathogens, which is the most widely recognized mechanism (Fisher *et al.*, 2017). This occurs when pathogens are transmitted from one vertebrate hosts to another without amplification or development of the organism within the vector (Sarwar, 2015).Houseflies are the most ubiquitous insects and are widely distributed all over the world, but more adapted to tropical areas (Goulson *et al*., 2005). House flies transmit more than hundred human and animal diseases.

Musca domestica is synanthropic and endophilic species, i.e. it lives in close association with human being and is able to complete its lifecycle within habitations of domestic animals and humans (Smallegange *et al*, 2007). They are day active and are normally found around human dwellings. House flies can harbor pathogenic microorganism, excreting viable isolates in their vomits and feces (*Joyner et al*., 2013), and can spread them mechanically to various hosts (Wang *et al.*, 2013). House flies are able to transport numerous pathogens from one place to another, therefore posing as risks to humans (Gaugler, 2016). Many Diptera play a remarkable role in the transmission of bacteria and parasites and can harbor different species of pathogenic microorganisms and is known to play a role in the epidemiology of many infectious diseases. Houseflies are one of the highest successful animals because of some major factors. They are found in all kinds of habitat and in all parts of the world. They feed on vast varieties of plant or animal material and have been incriminated as major cause of diseases for centuries. Insects are regarded as vectors when they transmit pathogenic organisms from human to humans or from animals to humans. Without the vector, the life cycle of parasites would not be completed and the pathogen will not live. Vectors can cause injury in a lot of ways. They may cause diseases, and this may happen through the consumption of food that contain human entero-pathogens, mechanically transmitted by houseflies (Gehad and Sherbini, 2010).

Houseflies have always been associated with human and domestic animals due to the plenty of food resources found in human homes and domestic garbage. These houseflies are of major concern due to their ability to act as vectors of several pathogenic organisms such as protozoa cysts, helminthes parasites, pathogenic bacteria, and enterovirus (Graczyk *et al.*, 2001). Flies are found both indoors and outdoors. Houseflies persist on decaying animal bodies, and in areas where feces, and a lot of garbage are left exposed. Flies have always been known to be attracted to dirty and contaminated environments. Evidence abound that the Nigeria environment is characterized by dirt, thus encouraging prolific breeding of the insects like houseflies (Tatfeng *et al*., 2005; Adeleke *et al.*, 2017).

Several studies have shown that eggs of *Ascaris lumbricoides, Trichuris trichiura,* hook worm, *Enterobious vermicularis,Taenis sp., Hymenolepis nana*, *Toxocara canis*, hook worm larvae, and *Strongyloides stercoralis*, protozoan cysts and trophozoites such as Entamoeba histolytica, Giardia species, Trichomonas species, Taenia species, Hymenolepsis species, Dipylidium species, Diphyllobothrium species and bacteria such as Shigella species, *Escherichia coli*are transferred by many species of house flies (Getachew *et al.*, 2007). A report has been described on the significant correlation between the prevalence of gastrointestinal diseases, such as diarrhea, and a seasonal increase in population of houseflies, which can be stopped by controlling the population of such flies using different approaches (Pava-Ripoll *et al.*, 2015). Studies have also revealed house flies as carriers of *Salmonella* species (the cause of typhoid, food poisoning, and diarrhea) from slaughter houses to the fruit and food markets as well as residential areas (Olse and Hammack, *2010*).

Materials and Methods

Study area

The study was conducted in Owo. *Owo* is a city in Ondo state in the south-western part of Nigeria, *Latitude*: 7° 11' 46.32" N and *Longitude*: 5° 35' 12.52" E. It is at the southern edge of the Yoruba hills, and at the intersection of roads from Akure, Kabba, Benin City. Owo is situated halfway between the towns of Ile Ife and Benin City.

Sample collection and study sites

A total of 100 houseflies was collected from four different sites with sterile sweep net, namely, refuse dump sites, meat stand, fruits and vegetable stand, palm wine joints all located within Owo. The collection sites were duplicated, to cover different parts of Owo. An average of 20 houseflies was collected from each site at different intervals but all in the day time between the hours of 10.00 am and 4.00 pm and placed in sterile containers and then transported to the laboratory for further analysis.

Study design

The study adopted a field survey study design to assess the parasites and microbial load of housefly from major market.

Study Duration

The duration of this study was 4 months between June 2021 to August 2021.

Sample and sampling technique

A total of 100 houseflies was collected using sweep net. Collected housefly samples were preserved in disinfected disposable Petri dishes and transported to the Microbiology Laboratory, Department of Medical Laboratory Science, Achievers University, Owo for microbial and parasitological analysis.

Bacteriological analysis of the housefly

The house flies were kept in the universal bottles and 2ml of sterile normal saline was added to the bottles and shaken vigorously for 5 minutes and left for some minutes to dislodge debris associated with the house flies. 0.01ml of the sample was taken from each container with the use of sterile wire loop and cultured on the Blood agar, chocolate agar, MacConkey agar and incubated for 24hours at 37°C. Chocolate agar plate was incubated in CO_2 environment. The bacteria were identified using standard microbial procedures which include macroscopic morphology, gram staining and biochemical tests.

Gram staining technique

Gram staining procedure was first developed by the Hans Christian Gram in 1844. As a differential staining method, it differentiates gram positive and gram-negative bacteria. Colonies from different pure culture plates were emulsified into a drop of distilled water on a slide and a thin preparation was made. The smear was allowed to air dry, covered with crystal violet stain for 60 sec and was rapidly washed off with clean water. Lugol's iodine was added for 60 sec and was washed off. The smear was decolorized with acetone alcohol and washed off rapidly. The smear was counter stained with safranin for 60 sec and washed off. Finally, the smear was examined under the microscope at ×100 objective lens. The gram -negative bacteria shows pink color and gram -positive bacteria shows purple color. The confirmation of each of the microorganism isolated was carried out using different standard laboratory test procedures.

Biochemical Tests

Different types of biochemical tests was performed for the identification of bacteria. Some but not limited to are:

Catalase test

Catalase is an enzyme that breakdown hydrogen peroxide into water and oxygen. Hydrogen peroxide is a form of byproduct of aerobic carbohydrate metabolism. The reagents used is 3% hydrogen peroxide. A loop full of bacteria from pure culture was taken and placed on the slide. In addition two drops of 3% H_2O_2 was added on the slide to check the production of hydrogen peroxide in the bacteria seen as bubbles.

Citrate Utilization Test

Bacterial strains with citrate utilization are called citrate positive and those without citrate utilization are called citrate negative. For this test, 100ml of Simmons citrate solution was prepared. The isolated bacterial strain was inoculated on the Simmons citrate media plates, by taking a loop full of bacteria from each plate. The plates were then incubated in the incubator at 37^{O}C for 48-72 hours. Green color of media turned blue is called as citrate positive other that don't cause color change are citrate negative.

Coagulase Test

Coagulase is an enzyme-like protein that causes plasma to clot by converting fibrinogen to fibrin. Coagulase will differentiate *Staphylococcus aureus* from non-coagulase producing Staphylococcus

Urease test

This test is used to check for utilization of urea by the bacteria. Urea Agar Base [UAB] was prepared and the isolated bacterial strains was inoculated and incubated. The bacterial strains with pink color are urease positive and other that doesn't turn the color into pink is urease negative.

Antibiotic susceptibility test

The antibiotic susceptibility test was carried out using antibiotic discs containing cefuroxime, ceftriaxone, chloramphenicol, Ciprofloxacin, Augmentin, Gentamycin, Amoxicillin, Sparfloxacin, and Pefloxacin. A colony of the test organism inoculated into peptone water using a sterile wire loop. The turbidity was then compared against a reference 0.5 McFarland standard tube. The suspension was streaked on the surface of nutrient agar plate and antibiotic disc was placed on it using a forceps. The plate was incubated at 37^{o}C for 24hours. Zone of inhibition generated by each antibiotic disc was grouped as susceptible and resistant.

Parasitological analysis of the housefly

The experimental procedures for parasitic analysis followed standard technique in Arora, 2010. The second part of the elution of eggs and cysts of parasites from the housefly was done using a concentration method. Each preparation was dispensed into clean centrifuge tubes and centrifuged at 1500 rpm for 5 min. The supernatant was discarded into disinfectant jar and the sediment was mixed with a few of lugol's iodine. A drop was applied on the center of a clean grease-free slide and covered with slip. The slide was examined under the microscope for parasites using ×10 and ×40 objectives. Identification of parasite followed pictorial key in Arora and Brij13.

Statistical analysis

The proportion of houseflies collected from each location and the frequency of the bacteria and parasite isolate from the houseflies were analyzed using statistical package for social sciences (SPSS) 25.0 versions for the analysis of the data appropriately. Oneway analysis of variance (ANOVA) was used for comparison within the groups. Spearman correlation was used to test the association between variables. Data was presented using mean ± standard deviation (mean ± SD) for all quantitative values. The level of significance was taken at 95% confidence interval and $P<0.05$ was considered significant.

Budget

An estimated amount of #150,000 is budgeted for this project.

Item	Amount
Nutrient agar	20,000
MacConkey Agar	20,000
Mueller Hinton Agar	20,000
Antibiotic Sensitivity Disc	15,000
Petri Dish	5,000
Universal Bottle	1,000
Glass Slide	3,000
Biochemicals	20,000
Miscellanous	30,000

Results

Table 4.1: Frequency of bacteria isolates from the housefly

Bacterial isolates	Number (%) of isolates N = 100
Citrobacter spp.	13 (13%)
Escherichia coli	26 (26%)
Enterobacter spp.	3 (3%)
Klebsiella pneumonia	21 (21%)
Klebsiella oxytoca	4 (4%)
Pseudomonas aeruginosa	15 (15%)
Salmonella spp	3 (3%)
Shigella spp	1 (1%)
Staphylococcus aureus	13 (13%)
Streptococcus spp.	1 (1%)

Data are presented as absolute values with corresponding percentages in parentheses. Where: N = total number of houseflies collected

Figure 4.1: Bacterial isolated from housefly in refuse dumps

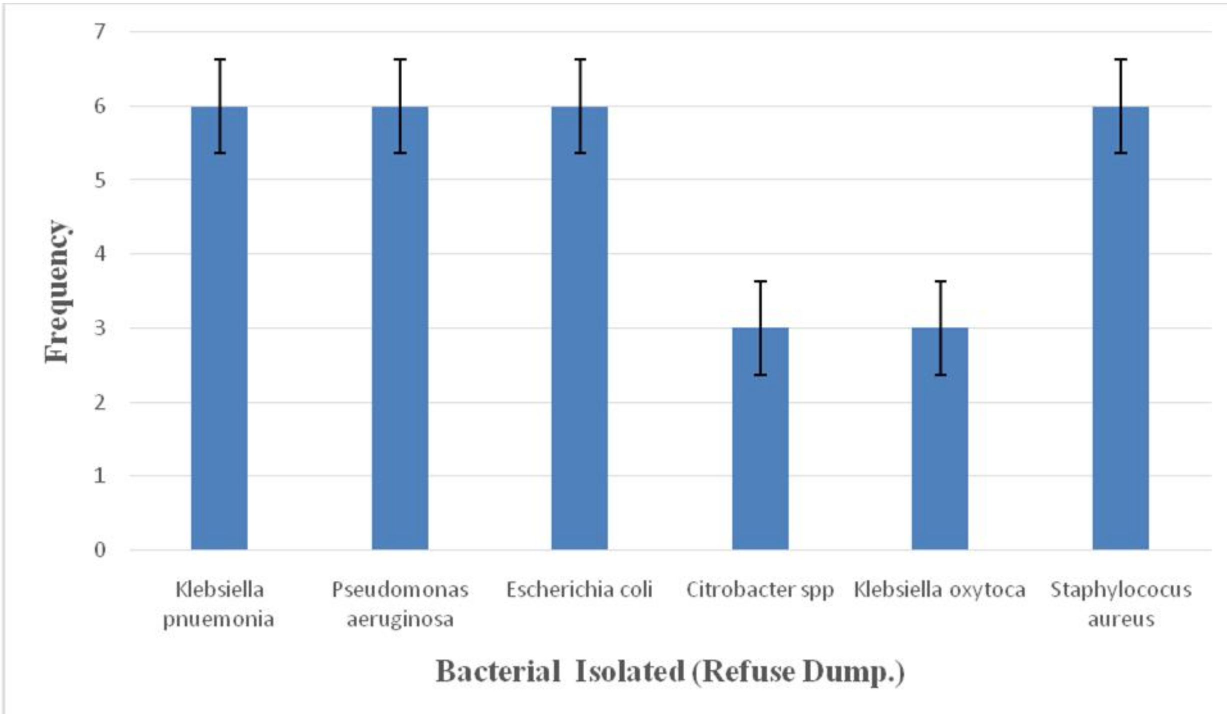

Figure 4.2: Bacterial isolated from housefly in fruit and vegetables

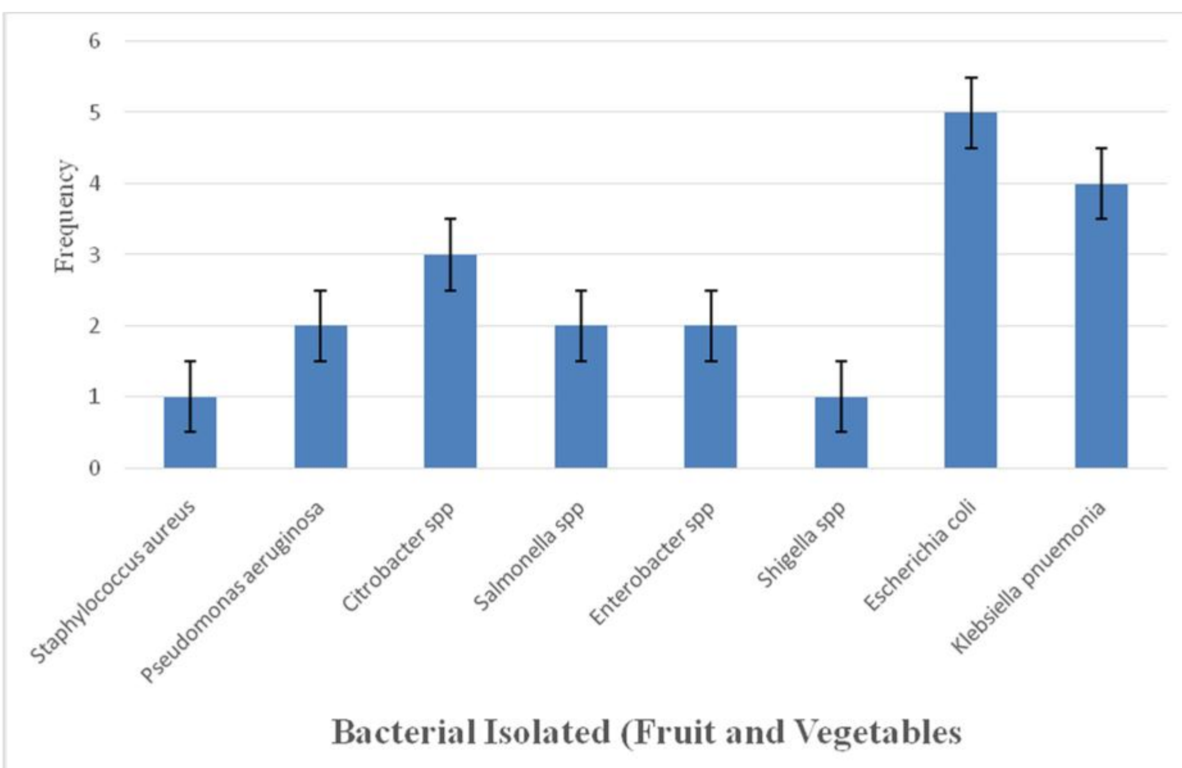

Figure 4.3: Bacterial isolated from housefly in meat stands

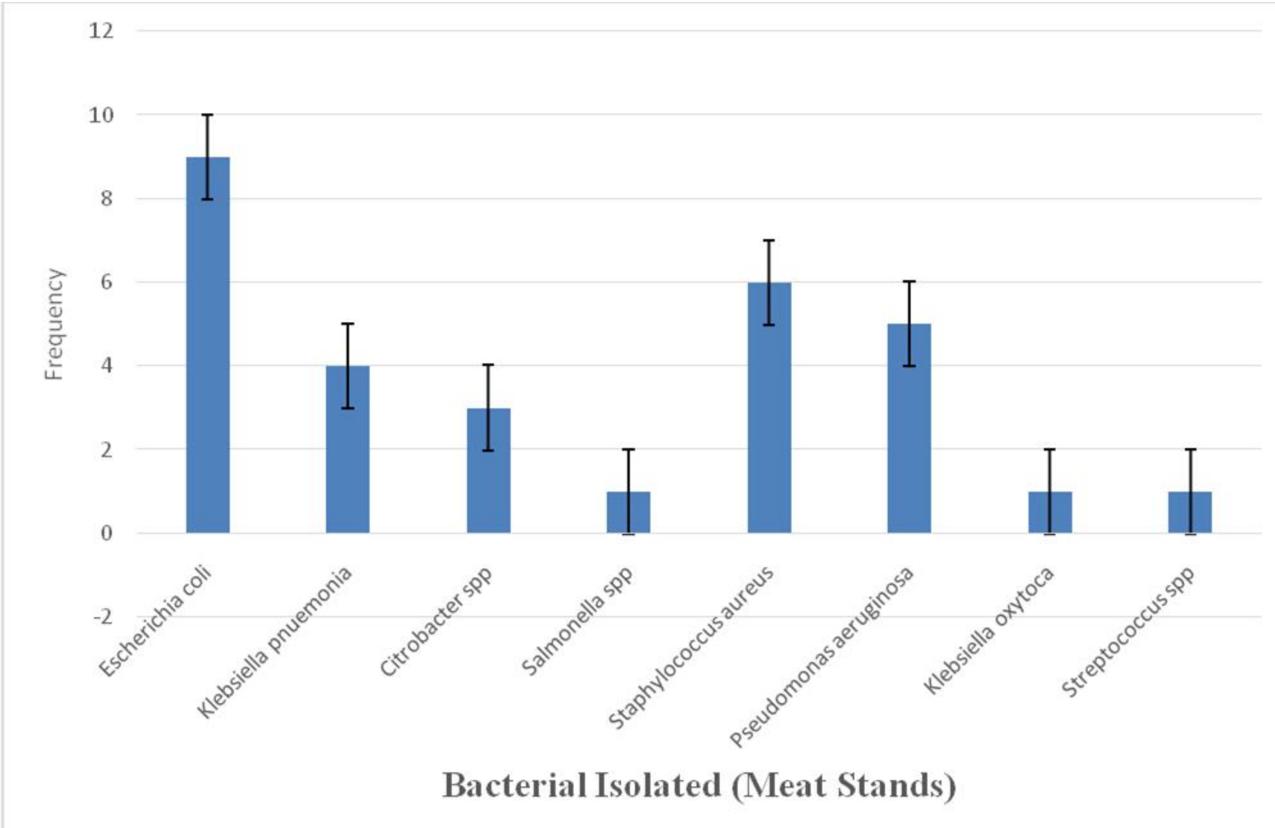

Figure 4.4: Bacterial isolated from housefly in palm wine joint

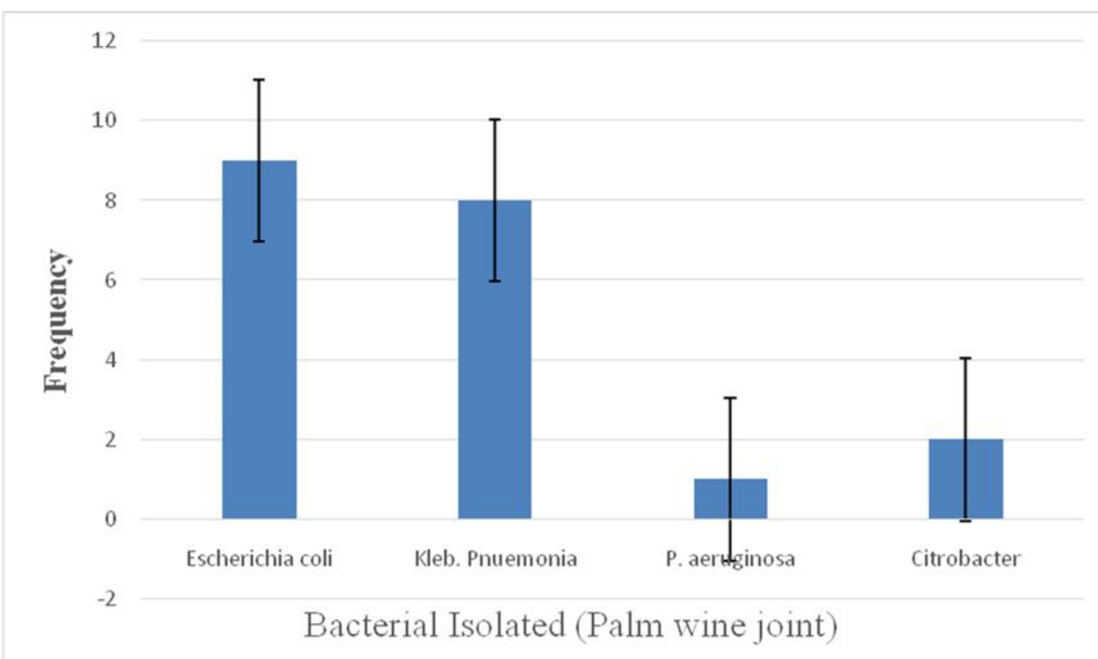

Table 4.2: Frequency of parasites from housefly collected (N = 100)

Parasites	Number (%) of isolates
Ascaris lumbricoides	3 (3%)
Entamoeba histolytica	3 (3%)

Table 4.3: Antibiotics susceptibility pattern of isolates from the houseflies collected

GNB = Gram negative bacteria, AM = Amoxicillin, AU = Augmentin, CH = Chloramphenicol, CN = Gentamicin, CPX = Ciprofloxacin, OFX =Ofloxacin, PRF = Pefloxacin, S=Streptomycin, SP =Sparfloxacin, SXT =Sulfamethoxazole

Bacterial isolates (GPC)	**Number of tested**	**NUMBER** Data are presented as absolute values with corresponding percentages in parentheses Where: **(%) OF ISOLATES SENSITIVE TO**									
		AM	CN	CPX	PEF	S	COT	CXM	E	APX	CRO
Staphylococcus aureus	13	6 (46.2%)	6 (46.2%)	12 (92.3%)	9 (69.2%)	11 (84.6%)	8(61.5%)	12 (92.3%)	6 (46.2%)	1 (7.7%)	1 (7.7%)
Streptococcus spp.	1	0 (0.0%)	1(100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	0 (0%)	1 (100.0%)	0 (0%)	0 (0%)	0 (0%)

Data are presented as absolute values with corresponding percentages in parentheses
Where
GPC = Gram positive cocci
AM = Amoxicillin
CN = Gentamicin
CPX = Ciprofloxacin
PEF =Pefloxacin
S = Streptomycin
COT =Cotrimoxazole
CXM = Cefuroxime
E = Erythromycin
APX =Ampiclox
CRO =Cefriaxone

Discussion

As already stated, *Musca domestica* is a worldwide-distributed pest and the dominant synanthropic fly species in animal production, homes, and restaurants. *Musca domestica*, is one of the most common fly species found worldwide. The constant movement of the housefly back and forth from faeces (or other animal waste) to food and drinking water therefore places humans and animals at risk of infection. The frequent isolation of pathogens from the body surfaces of the flies makes it plausible that when house flies transmit pathogens, they only act as mechanical vectors (Sarwar, 2015; Khamesipour *et al.,* 2018). Unlike in biological transmission, there is no multiplication (amplification) of the pathogen in the host in mechanical transmission. House flies habitually feed on faeces, animal manure, overripe fruits, vegetables, carrion and other decaying organic matter. In the process of feeding, pathogens stick on their mouth parts, wings, legs and other body surfaces, which they carry back to human habitations and animal farms, where they live and complete their lifecycle. However, the fly has been demonstrated to carry sufficient quantity of pathogens on its body surface, enough to cause an infection, faeces and vomitus may also serve as a major route of transmission of pathogens (Pava-Ripoll *et al.,* 2015).

A systematic review revealed a total of at least 130 pathogens that have been isolated from the house fly. Bacterial pathogens were by far the most frequently reported, suggesting the house fly may play an important role as vector of bacterial diseases. Fungi were the second most frequently isolated pathogens followed by parasites, and viruses were the least frequent (Khamesipour *et al.,* 2018). The results of this study demonstrated that all house flies were capable to carry at least one type of bacteria which corroborate findings from a study in Iran.

In this study, 10 bacterial pathogens and 2 parasites of medical importance were isolated from the house flies. Houseflies were collected from four different sites with sterile sweep net, namely, refuse dump sites, meat stand, fruits and vegetable stand, palm wine joints all located within Owo town, Ondo state. The isolates which are bacteria genera of medical importance include *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas spp*., *Klebsiella spp*., *Citrobacter spp, Salmonella spp*., and *Shigella spp*. (Table 1). This observation is in accordance with the findings of other researchers (Kassiri *et al.,* 2012, Ahmed *et al.,* 2013; Nwankwo *et al.,* 2019). In this present study, enteric bacteria were the most frequently isolated bacteria. This is corroborated by the result of (Bahrndorff*et al.,* 2017; Songe *et al.,* 2107) also supported by (Nwankwo *et al.,* 2019). This could be due to the fact that house flies feed mainly on faeces and other animal waste, which is a rich source of enteric bacteria.

Escherichia coli was the most prevalent (26%) bacteria isolate in this study (Figure 1), similar pattern was observed in a study done in two hospitals in Calabar, Nigeria (Akpan *et al.,* 2017). *Escherichia coli* has also evolved to be a multisystem infectious pathogen exhibiting various forms like enteropathogenic, enterotoxigenic, enterohaemorrhagic, enteroaggregative, enteroinvasive, meningitis-associated, and uropathogenic *E. coli.* This explains why flies that harbour these pathogens remain a serious threat to the community.

Of the studied antimicrobial agents, Amoxicillin was the one with the lowest efficacy (42% resistance) for the total of the pathogens isolated from the flies. Ciprofloxacin revealed itself as the most effective antimicrobial agent (93% susceptibility) against the studied pathogens (Table 3). Augmentin is the least effective antimicrobial agent (63% resistance) for Gram negative organism isolated in this study. The increase in the number and emergence of new bacterial strains resistant to antimicrobial agents is the result of the frequent and uncontrolled use of these agents in medicine and food animal production. When animals are treated in any way with antimicrobial agents, the pathogens that become resident in the animal and, therefore, are present in their faeces are likely to be resistant to those agents. Flies live and develop in close proximity with these animals, slaughterhouses, meat stand and are often present in the animal manure and have unrestricted movement.

The presence of enteropathogens such as *Salmonella* and *Shigella* species in the flies also lends credence to the fact that flies may participate as vectors in the transmission of nosocomial gastroenteritis, food poisoning, and diarrhoea (Khamesipour *et al.,* 2018).The antibiotic sensitivity pattern of the isolates showed good response to the some antibiotics like ciprofloxacin (93%), perfloxacin (84%), sparfloxacin (75%), streptomycin (77%), and cefuroxime (93%) sensitivity was observed in Gram positive isolates (Table 6).

The importance of limiting fly breeding by employing proper sanitation is of crucial importance. It is clear that without the use of proper sanitation methods, flies will continue to replicate and disperse from adjacent areas and will undermine any control measures. Good sanitation will result in a reduction in fly population. *Salmonella* was isolated from commercial dairies and poultry ranches, demonstrating that houseflies are potential carriers of *Salmonella* organisms and pose a possible health risk to communities living in close proximity to animal operations that harbour heavy fly population (Barreiro *et al.,* 2013). Graham *et al.,* 2009 reported that flies collected near broiler poultry operations may be involved in the spread of drug resistant bacteria from these operations and may increase the potential for human exposure to drug-resistant bacteria. The same conclusion was reported by Davari *et al.,* 2010 in houseflies collected in hospitals and slaughterhouses indirectly implicating house flies as a potential source of the contamination.

The bacterial isolates tested showed a large population with antimicrobial resistance that can be carried by the flies into the human habitat. Reports of the isolation of viruses from wild-caught flies are very rare. However, house flies were reported to be capable of carrying a number of viruses in laboratory experiments. The majority of these viruses were of veterinary importance. One study demonstrates the ability of the house fly to carry the Ebola virus in laboratory experiments (Haddow *et al.,* 2017).

Ascaris lumbricoides and *Entamoeba histolytica* were recovered in this study similar to (Balla *et al.,* 2014; Ibrahim *et al.,* 2018) although more parasites were recovered from houseflies in both studies. Another report also in Umuahia, Nigeria (Okore *et al.,* 2013) observed that cysts of *Entamoeba histolytica* and *Giardia. Lamblia* and ova of A*scaris lumbricoides* have high frequency of occurrence on houseflies that are found around broken sewage. This compares favourably with the reports from this study.Our results contributes to showing the persistence of resistant bacteria in the environment and highlight the reservoir of resistance associated with the use of antibiotics as a feed additive animal industry. Further, the carriage of antibiotic resistant bacteria by flies in environment increases the potential for human exposure to drug-resistant bacteria.

There is enough evidence to show that house flies can carry pathogens capable of causing serious diseases in humans and domestic animals, and should therefore be controlled. The control of the house fly can be achieved by physical (such as composting manure), chemical and biological methods. The use of chemical pesticides, which is the most common method today, is fast losing grounds due to the growing resistance by the house fly and other pests, couple to the effects they may have on non-target organisms, have led to the consideration of other methods, including biological control. Biological control agents including fungi of the genera Metarhizium and Beauveria, and bacteria including Bacillus thuringiensis can be used to control the housefly (Kwenti, 2017; Khamesipour *et al.,* 2018)

The combination of different methods for control and prevention or eradication of houseflies should be implemented to stop human or animal diseases. In high-risk areas, health education, proper environmental sanitation, and personal hygiene are strongly advocated.

Conclusion

The houseflies collected from refuse dump sites, meat stand, fruits and vegetable stand, palm wine joints all located within Owo town are potential vectors of a wide range of pathogenic organisms like *Escherichia coli*, Klebsiella spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Citrobacter spp. Only two parasites; *Ascaris lumbricoides* and *Entamoeba histolytica* were recovered in this study. Antibiotics such as Ciprofloxacin, Perfloxacin, Sparfloxacin, Streptomycin and Cefuroxime are relatively highly sensitive to bacteria isolates in this study but there are still reasons for public health concerns. The bacteria isolates have significant level of resistance to antibiotics such as Amoxillin, Augmentin, Chloramphenicol, Gentamicin, Cefriaxone, Erythromycin and Ampiclox.

This study shows that houseflies collected from different environmental locations are all capable of carrying antimicrobial resistant bacteria at a high level. Houseflies may play a significant role in the dissemination of antimicrobial resistance to various environments.This study showed the persistence of resistant bacteria in the Owo environment.

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