Pathogenic Microorganisms Associated With Flies Within Uyo Metropolis During The Wet Season

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Abstract: This study reports on pathogenic microorganisms associated with flies within Uyo metropolis during the wet season. Flies were collected from ten (10) different sites within Uyo metropolis in the month of July. The sites were characterized into institutional areas, market areas, congested areas and affluent areas. The pour plate method was employed for the determination of microbial load of samples using standard methods. The microbial counts of the various fly samples analyzed for the months of July and August showed that the total heterotrophic bacterial counts ranged from 4.8 x 10^4 cfu/g to 18.9 x 10^4 cfu/g. Total coliform ranged from 4.9 x 10^4 cfu/g to 13.0 x 10^4 cfu/g, *Salmonella/Shigella* count ranged from 4.1 x 10^4 cfu/g to 2.16 x 10^4 cfu/g. *Vibro* count was 2.6 x 10^4 cfu/g to 18.3 x 10^4 cfu/g, *Staphylococcus* count was 2.5 x 10^4 cfu/g to 3.2 x 10^4 cfu/g and 1.1 x 10^4 cfu/g to 1.9 x 10^4 cfu/g for fungal count. For the month of August, the total heterotrophic bacterial count ranged from 4.6 x 10^4 cfu/g to 17.4 x 10^4cfu/g, 8.2 x 10^4cfu/g to 15.3 x 10^4 cfu/g, for total coliform, 5.4 x 10^4 cfu/g to 15.6 x 10^4cfu/g for *Salmonella/Shigella* count, 2.7 x 10^4cfu/g to 14.1 x 10^4cfu/g for *Vibro* count, 3.1 x 10^4cfu/g to 5.4 x 10^4cfu/g for *Staphylococcus* count and 1.4 x 10^4cfu/g to 3.1 x 10^4cfu/g for fungal count. The frequency of occurrence of the isolates showed that bacteria (57.8%) were more predominant than fungi isolates (42.2%). It showed that *Salmonella* spp., [18 (34.6%)] was the most prevalent bacterial isolates. This was followed by *Shigella spp*. [10 (19.2%)], *Vibrio cholerae* [8 (15.4%)], *Vibrio parahaemolyticus* [6 (11.5%)], *Escherichia coli* [4 (7.7%)], and *Staphylococcus aureus* [3 (5.8%)]. *Pseudomonas aeruginosa* [1(1.9%)], *Bacillus* spp., [1(1.9%)] and *Aeromonas spp*. [1 (1.9%)] were less prevalent. The frequency of occurrence of fungal isolates showed that *Penicillium spp*. [16(42.1)] was most prevalent fungi isolates among the flies studied, followed by *Aspergillus fumigatus* [10(26.3%)], *Aspergillus niger* [7(18.4%)] and *Verticillium* spp., [2 (5.3%)] while *Paecilomyces spp*. [1(2.6%)], *Mucor spp*. [1(2.6%)] and *Aspergillus spp*. [1(2.6%)] were less prevalent. This study has so far established that flies pose a possible health risk to both man and his environment. Thus, the introduction of strict public health measures is however needed in homes, public places and the environment at large.
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1. INTRODUCTION

An Entomologist defines a fly as any species of insect of the order Dipetra, (Service, 1980). Man has through the ages lived in frequent direct and indirect contacts with certain species of flies. The biology of general fly species is collectively and generally known as filth flies (Armed Forces Pest Management Board, 2003). The "filth flies" refer to several species of true flies (Dipetra) belonging primarily to the families Muscidae, Calliphoridae and Sacrophagidae. Filth flies have been implicated as disease causing vectors of plant and human (Cayol et al., 1994).

The housefly, *Musca domestica*, acts as mechanical vectors for various microorganisms in the environment that are associated with animal feces and manure. They have been shown to feed on selections and other human wastes, making them ideal carriers for transmitting several pathogenic microorganisms. When feeding, house flies regurgitate liquid from the stomach which enhances the process of the food to be dissolved and then use their sponging mouth parts to suck it up. They leave fecal spots where they have traded and disseminate pathogens to humans through this agency. The likelihood of human excrement being transmitted flies is great (Gangarosa and Bcisel, 1960). Large populations of *Musca domestica* may reduce yields and contribute to substantial public health problems when they enter a nearby human habitation (Axtell and Arends, 1990; Howard and Wall, 1996).

Several pathogens are associated with flies causing disease conditions in humans and animals such as bacillary dysentery, cholera, typhoid and paratyphoid, anthrax, shigellosis, bovine mastitis, conjunctivitis, and poliomyelitis, tuberculosis and infantile diarrhea, as well as parasitic worms. Pathogenic microorganisms are picked up by flies from

refuse, sewage and other body parts, through their vomit, faeces and contaminated external body parts to human and animal food (Graczyk et al., 2001). Transmission of human pathogens can be through different types of flies. For instance, the Mediterranean fruit fly (*Ceratitis capitata)* possesses the ability to contaminate commercial and wild fruits with pathogenic bacterial of humans according to researchers in Israel. This fly is considered as a menace and danger to commercial fruit industry world wide. The fruit fly feeds on animal feces for protein in order to produce eggs, they then lay eggs in the fruit by puncturing the skin and injecting them.

Outbreaks of food-borne disease associated with fresh produce consumption are rapidly increasing, reinforcing the need to identify the source of contamination. Reports of concurrent increases in fly populations and the incidence of diarrhea in Northern Africa and Middle Eastern Military campaigns during world wars I and II are numerous (Levine and Levine, 1991). Ledingham (1990) found a strong correlation between fly density and the incidence of dysentery. Vectors like rodents and insect, especially flies have been reported as carriers of yeast and filamentous fungi. The association of insects and fungi has been confirmed by several reports (Steinhaus, 1986). Dirt, soil body discharges and excreta from animals in holding pens are the main of fungal contamination of flies. The objective of this study is to isolate, characterize and identify the pathogenic microorganisms associated with flies from different locations in Uyo metropolis.

2. MATERIAL AND METHODS

2.1. *Study Areas*

Flies were collected from ten (10) different sites within Uyo metropolis in the month of July. The sites were characterized into institutional areas, market areas, congested areas and affluent areas. The sites included; residential apartments on C.C.C. Road, Ikpa Road, Urua Ekpa Road, Oron Road, Aka Road, Okokon Etuk Street, Etuk Street, and Abattoir at Iba Oku, refuse dumping sites at Udoh Street and Ewet housing Estate, University of Uyo hostel Canteen, and Uniuyo Small Market. Ten other different sites were visited in the month of August, 2008. These sites include; Uyo Main Market, residential apartment on Okokon Etuk Street, Oron Road, restaurants along Nwaniba Road, Akpan Andem Market, a refuse dumping site at Udo Uwana Street, Fresh fish Selling point on Orun Road, a residential building on Ekpanya Street, a two storey apartment and University of Uyo Small Market.

2.2. *Collection of Samples (Flies)*

All fly collections were carried out by using a standard sweep net provided with a heavy dusty aerial bag. Aiming at the swarming flies one or two quick sweeps were made to collect a good number of flies. The bag containing flies was closed with rubber bands prior to removing it from the ring assembly. The entire net bag with flies was transferred to the laboratory within 30-45 minutes of their collection. The adult muscoid flies were collected on a warm sunny day at temperatures between 28-30^{o}C allowing for ample fly activity. On reaching the laboratory, the flies were immobilized by suffocating them with Carbon Tetrachloride for about 5 minutes. Identification of flies' species was made by examining the flies under a low power field microscope.

2.3. *Enumeration, Isolation and Identification of Bacteria and Fungi Isolates*

The pour plate method was employed for the determination of microbial load of samples using different solid media. Multiple tube fermentation procedure (also known as the Most probable number procedure); a quantitative analysis of food and water samples was employed to give a statistical estimate of the number of bacteria that would give the observed result. It was used in the enumeration of coliforms especially feacal coliforms. Tenfold serial dilutions of the samples were made and 10^{-5} dilution of the samples from different location were plated out on Nutrient agar, MacConkey, Salmonella-Shigella agar, Sabouraud 4% Dextrose Agar (SDA), Mannitol Salt Agar, Buffered Peptone water, Triple Sugar Iron (TSI) Agar and Kovac's Indole reagent using the spread plate technique. These plates were incubated for 24 hours at 37^{0}c in the incubator. Sabourand dextrose agar and potato dextrose agar (PDA, Difco) were used for the total fungal counts and incubated at 28 ± 1^{O}C for 5 days under 12 h photoperiod. After incubation, observed colonies were counted and then isolated. The bacterial isolates were further examined for their ability to ferment sugar, carbohydrate production of indole from tryptophan, citrate utilization, catalase production and oxidase test. The bacterial isolates were also identified by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994) and Oyeleke and Manga (2008). The pure isolated fungi were identified using cultural and morphological features according to the most documented keys in fungal identification (Samson and Varga, 2007).

3. RESULTS ANALYSIS

The samples of flies were found to carry several species of pathogenic bacteria as well as fungi. The bacteria isolated from the flies were identified *Bacillus* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Vibro cholerae, Vibro parahaemolyticus* and *Aeromonas* spp., The fungi isolated were identified as *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., *Verticillum* spp., *Paecilomyces* spp., *Aspergillus niger and Aspergillus fumigatus*.

Table 1 shows the microbial counts per gram of the various fly samples analyzed for the months of July and August. The media used for each sample is also matched against each count. The total heterotrophic bacterial counts, ranged between 4.8 x 10^4 cfu/g to 18.9 x 10^4 cfu/g, 4.9 x 10^4 cfu/g to 13.0 x 10^4 cfu/g for the Coliform count, 4.1 x 10^4 cfu/g to 2.16 x 10^4 cfu/g for *Salmonella/Shigella* count, 2.6 x 10^4 cfu/g to 18.3 x 10^4 cfu/g for *Vibro* count, 2.5 x 10^4 cfu/g to 3.2 x 10^4 cfu/g for counts on MSA and 1.1 x 10^4 cfu/g to 1.9 x 10^4 cfu/g for fungal count all in the month of July. The lowest count of 4.8 x 10^4 cfu/g was obtained from species A5 on the total heterotrophic count, 4.9 x 10^4 cfu/g from species "A1" on coliform count, 4.1 x 10^4 cfu/g from species "A1" on fungal count. The highest counts of 18.9 x 10^4 cfu/g, 13.0 x 10^4 cfu/g, 21.6 x 10^4 cfu/g and 18.3 x 10^4 cfu/g obtained from species "A4" on the bacterial count coliform count, *Salmonella/Shigella* count and *Vibro* count respectively, while 3.2 x 10^4 cfu/g was obtained from species "A2" On MSA count and 1.9 x 10^4 cfu/g was obtained from species "A3" on fungal count.

For the month of August, the total heterotrophic bacterial count ranged between 4.6 x 10^4 cfu/g to 17.4 x 10^4cfu/g, 8.2 x 10^4cfu/g to 15.3 x 10^4 cfu/g, for Coliform count, 5.4 x 10^4 cfu/g to 15.6 x 10^4cfu/g for *Salmonella/Shigella* count, 2.7 x 10^4cfu/g to 14.1 x 10^4cfu/g for *Vibro* count, 3.1 x 10^4cfu/g to 5.4 x 10^4cfu/g for total count on MSA and 1.4 x 10^4cfu/g to 3.1 x 10^4cfu/g for fungal count. The lowest counts of 4.6 x 10^4 cfu/g was obtained from species B5 on bacteria count 8.2 x 10^4 cfu/g from species B5 on coliform count 5.4 x 10^4 cfu/g from species B2 on *salmonella/Shigella* count 2.7 x 10^4 cfu/g from species B2 on Vibro count, 3.1 x 10^4 cfu/g from species B2 on MSA count and 1.4 x 10^4 cfu/g from species B2 on fungi count. The highest counts of 17.4 x 10^4 cfu/g, 15.3 x 10^4 cfu/g and 1.4 x 10^4 cfu/g were obtained from species B4 on bacteria count, coliform count, S*almonella/Shigella c*ount and Vibro count MSA count while 2.1 x 10^4 cfu/g from species B3 on fungi count.

Table 1: Microbial counts per gram of the various fly samples analyzed for the months of July and August

Samples	Total heterotrophic bacterial count (CFU/g)	Total coliform count on MacConkey agar (CFU/g)	Salmonella-Shigella count (CFU/g)	Vibrio count on Thio Citrate Bile Salt agar (CFU/g)	Staphylococcus count on Mannitol Salt Agar (CFU/g)	Total fungal count on Sabouraud Dextrose agar (CFU/g)
Month of July						
A1	6.5 x 10^4	4.9 x 10^4	4.1 x 10^4	2.7 x 10^4	2.6 x 10^4	1.1 x 10^4
A2	14.5 x 10^4	11.1 x 10^4	5.5 x 10^4	2.6 x 10^4	3.2 x 10^4	1.3 x 10^4
A3	10.0 x 10^4	11.8 x 10^4	18.1 x 10^4	4.8 x 10^4	2.7 x 10^4	1.9 x 10^4
A4	18.9 x 10^4	13.0 x 10^4	26.1 x 10^4	18.3 x 10^4	2.5 x 10^4	1.5 x 10^4
A5	4.8 x 10^4	9.6 x 10^4	6.2 x 10^4	11.8 x 10^4	2.9 x 10^4	1.6 x 10^4
Month of August						
B1	7.9 x 10^4	12.5 x 10^4	5.5 x 10^4	5.0 x 10^4	3.5 x 10^4	1.6 x 10^4
B2	12.7 x 10^4	9.6 x 10^4	5.4 x 10^4	2.7 x 10^4	3.1 x 10^4	1.4 x 10^4
B3	9.0 x 10^4	11.4 x 10^4	15.2 x 10^4	12.9 x 10^4	3.2 x 10^4	2.1 x 10^4
B4	17.4 x 10^4	15.3 x 10^4	15.6 x 10^4	14.1 x 10^4	3.6 x 10^4	1.9 x 10^4
B5	4.6 x 10^4	8.2 x 10^4	11.8 x 10^4	4.8 x 10^4	5.4 x 10^4	1.8 x 10^4

Table 2 shows the frequency of occurrence of microbial isolates. It showed that bacteria (57.8%) were more predominant than fungi isolates (42.2%).

Table 2: Frequency of occurrence of microbial isolates

Isolates	No. (%)
Bacteria	52(57.8)
Fungi	38(42.2)
Total	**90(100.0)**

Table 3 shows the frequency of occurrence of bacterial isolates. It showed that *Salmonella* spp., [18 (34.6%)] was the most prevalent bacterial isolates. This was followed by *Shigella spp*. [10(19.2%)], *Vibrio cholerae* [8(15.4%)], *Vibrio parahaemolyticus* [6 (11.5%)], *Escherichia coli* [4 (7.7%)], and *Staphylococcus aureus* [3(5.8%)]. *Pseudomonas aeruginosa* [1(1.9%)], *Bacillus* spp., [1(1.9%)] and *Aeromonas* spp. [1 (1.9%)] were less prevalent (Table 3).

Table 4 shows the frequency of occurrence of fungal isolates. It showed that *Penicillium spp*. [16(42.1)] was most prevalent fungi isolates among the flies studied. This was followed by *Aspergillus fumigatus* [10(26.3%)],

Aspergillus niger [7(18.4%)] and *Verticillium* spp., [2(5.3%)] while *Paecilomyces spp*. [1(2.6%)], *Mucor spp*. [1(2.6%)] and *Aspergillus spp*. [1(2.6%)] were less prevalent (Table 4).

Table 3: Frequency of occurrence of bacterial isolates

Isolates	No. (%)
Pseudomonas aeruginosa	1(1.9)
Bacillus spp.	1(1.9)
Aeromonas spp.	1(1.9)
Shigella spp.	10(19.2)
Salmonella spp.	18(34.6)
Vibrio cholerae	8(15.4)
Vibrio parahaemolyticus	6(11.5)
Staphylococcus aureus	3(5.8)
Escherichia coli	4(7.7)
Total	**52(100.0)**

Table 4: Frequency of occurrence of fungal isolates

Isolates	No. (%)
Paecilomyces spp.	1(2.6)
Mucor spp.	1(2.6)
Aspergillus spp.	1(2.6)
Penicillium spp.	16(42.1)
Aspergillus niger	7(18.4)
Aspergillus fumigatus	10(26.3)
Verticillium spp.,	2(5.3)
Total	**38(100.0)**

4. DISCUSSION

It was observed that the bacterial isolates were mostly Gram negative bacterial while a few others were Gram positive. The bacterial isolates were found to be mainly rod-shaped bacterial (bacilli) and sphere-shaped bacterial (cocci) and they were aerobic and anaerobic. The fungi isolated were *Aspergillus niger, Aspergillus fumigatus*, *Mucor* spp. *Paecilomyces* spp. *Aspergillus spp*., and *Penicillium* spp., Flies are known to be mechanical vectors of pathogens that cause disease (Nichols, 2005). The results obtained from this study were in accordance with other reports which highlight the importance of flies in caring various pathogens (Koura and Kamel, 1990; Fotedar et al., 1992; Rivault et al., 1993; Echeverria et al., 1997; Kobayashi et al., 1999; Pai et al., 2003).

The role of flies in the transmission of pathogens and gastrointestinal diseases had already been established (Greenberg, 1973). Although whether these organisms were carried externally or internally was not investigated in this study. Other studies have shown that infection of flies could be externally or internally (Suleiman et al., 2000). Isolation of a variety of pathogenic microorganisms from flies including *Musca domestica* by Ugbogu et al. (2006) also showed that flies pooled from refuse dump sites propagate disease causing agents.

Microbes such as *Staphylococcus aureus*, *P. aeruginosa*, *Aspergillus* spp. and *Bacillus* spp. are liable and can cause various infectious diseases in human. S. *aureus* is capable of causing toxigenic food poisoning and some other infectious disease which would result in diarrhoea (Nawigen and Koenig, 1981; Akonai et al., 1991). From the study, the bacterial isolates identified included: *Pseudomonas aeruginosa, Bacillus* spp. *Staphylococcus aureus, Aeromonas* spp. *Salmonella* spp. *Vibrio cholerae, Vibrio parahaemolyticus and Escherichia coli*. The fungi isolated were: *Aspergillus niger, Mucor* Spp. *A.fumigatus, Penicillum* spp. *Paecilomyces* spp., *and Aspergillus* spp., The finding of this study indicated that flies can transmit pathogenic microorganisms.

The fact that *Shigella species* were isolated from the fly samples is enough cause for worry since its mode of transmission is mostly food and water borne and species like Housefly, Latrine fly and Lesser fly are easily found in infections and are major causes of illness and death worldwide (WHO, 2002; Rosek et al., 2003).

The presence of *Bacillus spp*. can be said to be as a result of the prevalence of spores in the environment. The presence of *Escherichia coli* indicates fresh faecal contamination which could easily be carried by most of the fly species in the environment. *Pseudomonas aeruginosa* enters the body through ingestion of contaminated substance; the presence of *Staphylococcus aureus* indicates food and water since they are found in food and water.

Salmonella is often pathogenic for humans or animals when acquired by the oval route. Its presence indicates enteritis, systemic infection and enteric fever. They are typically transmitted by faecally contaminated food and water and the species could cause salmonellosis*:* enteric fever (typhoid), resulting from bacterial invasion of the blood stream and acute gastroenteritis, resulting from food borne infection/intoxication and their principal habitat is the intestinal tract of the human because they can only multiply mostly in the digestive tracts. *Salmonella* are species which are responsible for enteric fevers and enterocolitis. Also, the fact that *Vibrio* species like *V. cholerae* and *V. parahaemolyticus* was isolated from the fly species calls for serious concern since Vibrio implies gastrointestinal infection caused by *V. cholerae* and cholera is one of the most rapidly fatal illness known which can cause death within 18 hours of onset of signs

and symptoms. The Vibrios are found in marine and surface waters. They are however transmitted to humans by water and food (that is inadequately cooked or raw seafood).

The fungal isolates identified are known to produce toxins which are collectively termed as mycotoxin and they are implicated in causing serious illness. These findings highlight the potential of flies in carrying human pathogens and also to serve as mechanical vectors for the transmission of food borne diseases and other infectious diseases. These findings further strengthen the need to carry out further investigations in other to evaluate the actual epidemiological potential of flies to transmit human pathogens.

5. Conclusion

This study has so far established that flies pose a possible health risk to both man and his environment. Thus, the introduction of strict public health measures is however needed in homes, public places and the environment at large.

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