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Microbiological Investigations of Selected Flies of Public Health Importance from a Waste Dump Site in Port Harcourt, Nigeria

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Abstract:*Bacteria associated with flies of public health importance in Nigeria are not well known and their ecology is also not well understood. We aim to determine the bacteria associated with flies of waste dump site. Three flies of public health significance were collected from a waste dump site of the Rivers State University of Science and Technology, Port Harcourt. The three dipterous flies were Luciliasericata, Chrysomyasp and Musca domestica..The three flies were all of medical importance.The microbial load on three species of flies was investigated using standard plate count methods. The fly samples were collected from the Post Graduate Entomology Laboratory was cultured to isolate and identify the microbes associated with them. The samples were analyzed for total heterotrophic bacteria and fungi counts. The study revealed high heterotrophic bacteria and fungi counts in all three species of the flies used, with Musca domestica having the highest count of 2.9 X* 10^9*Cfu/gram and Chrysomyasp with the least count of 3.4 x 10* ^{5}*Cfu/g and fungi counts ranged from 3.1 X* 10^3*Cfu/g to 2.9 X* 10^5*Cfu/g. The bacteria isolated from these samples includes: Escherichia coli, Pseudomonas*sp,, *Bacillus*sp, *Enterobacter*sp, *Staphylococcus*sp,*Salmonella*sp, *Proteus*sp, *and Klebsiella*sp, *while the fungi species isolated includes: Penicilliumsp, Aspergillus*sp,*Rhizopus*sp,*Cladosporium*sp,*Aspergillusflavus, Aspergillusniger, Fusarium*sp *and Trichoderma sp.*

Keywords:*Flies, bacteria, public health, waste dump.*

I. Introduction

All true flies are of the Order Diptera possessing just a pair of wings, most of them are involved in the spread of one disease or the other apart from those found infesting crops, some are also important in medico-legal investigations serving as useful tools in forensics. Some flies are attracted to garbage, rotting and decomposing materials even to animal carcasses and wounds. Some are facultative being found on more than one or two sources or diets. In the infection of vertebrate tissues, the myiasis-causing flies are also associated with foul odour, and waste sites [1] and they include *Cordylobiaanthropophaga*, ,*Luciliasericata*, *Sarcophagavilosa*, *Musca domestica* and *Chrysomya* species [1, 2]. Some flies may carry antibiotic-resistant bacteria found in wastes [3]. Most flies are available where animal manure is produced whether in poultry, goat sheds, swineor cattle operations. According to a 2013 report by the Centres for Disease Control and Prevention [4], at least 2 million people are infected with antibiotic-resistant bacteria and at least, 23,000 people die yearly as a direct result of the infections in the U.S.[5].

Flies are notorious agents of so many diseases; many bacteria are carried by flies and the bacteria cause illnesses that ranged from food poisoning to respiratory infections in both temperate and tropical environments. At the same time, some flies are good pre-eminent recyclers of wastes generated through anthropogenic activities. Prominent among them are *Hermetiaillucens* larvae which are good bioconversion agents that thrive in Oxygen-depleted environments, salts, ammonia, alcohols and in a variety of food toxins. In the Niger Delta environments they inhabit the depths of oil palm wastes including their shafts [6].Flies are carriers of pathogens which transmit diseases that could result in deathof man and other farm animals. Some fungi and bacteria were isolated from larvae of *Musca domestica*[7]. Diseases such as typhoid fever(caused by *Salmonella typhi*), Cholera(caused by *Vibrio cholera*), Staphylococcus food poisoning (caused by *Staphylococcus aureus*), and Shigellosis (caused by *Shigella*sp) [8, 9] can be deadly affecting man and his animals.This study is an investigation aimed at identifying and characterizing bacteria from external surfacesof flies of a waste dump site in Port Harcourt.

II. Materials And Methods

Entomological Surveys

Sampling surveys were carried out twice weekly[9am-12pm] using sweep nets to collect adult flies at the waste dump site of the female hostel at the University of Science and Technology, Port Harcourt. The wastes were evacuated every 48h and samplings were done prior to evacuation time at1pm.Samplings were maintained two times weekly for four months [June to September]. Flies collected were immediately transferred to a 5L capacity polyethylene transparent bag and were taken to the Post Graduate Entomology Laboratory for identification and recordings. The flies were transferred into sterile Petri dishes and immediately taken to the Microbiology laboratory. The Petri dishes were refrigerated overnight to immobilize the flies. The rationale for the selection of only three flies for microbiological analysis was their availability at homes and at waste dump sites; most of them were facultative feeders.

Statistical Analysis

We adopted purposive sampling and we used analysis of variance along with Duncan Multiple Range Test [DMRT] for means separation and SNK, which is a furtherance of Duncan to establish significant differences among the means.Results obtained from bacterial counts were subjected to statistical analysis employing the student t-test at 95% and 99% probability levels.

Microbiological analysis of sample

For the determination of Heterotrophic bacteria counts and isolation from the external surfaces of flies, tenfold serial dilution of the sample was done. 1g of each fly sample was weighed out and added to 9ml of sterile physiological saline (prepared by dissolving 8.5g of NaCl in 1 litre of distilled water). This was allowed to stand for 15 minutes and vigorously agitated at intervals to dislodge all the organisms on the flies. 1ml was pipetted into another test tube containing 9ml physiological saline, this was done up to 10^{-6}. 0.1ml aliquots of the dilutions were inoculated on the surface of the Nutrient agar and Potato Dextrose agar respectively using the spread plate, for the enumeration of bacteria and fungi respectively. chloramphenicol was added to the PDA to inhibit bacterial growth.The plates were incubated at 37^0C for 24 hours for bacteria and 4-5days for fungi at also 37^0C[7, 8]. Discrete colonies that developed were counted and recorded as total heterotrophic counts of bacteria and fungi.

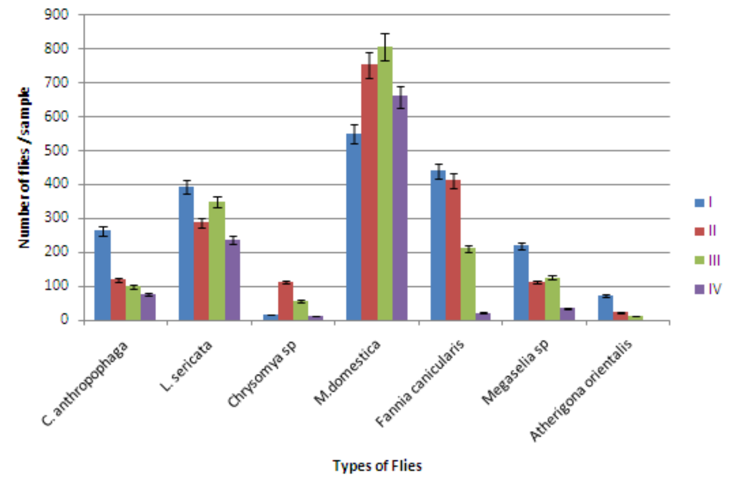
MacConkey Agar (MAC) which is a differential plating medium, selective for Gram negative organisms, was used primarily for detection and isolation of enteric bacteria. Eosin-methylene blue [EMB] agar was also used.The colonies that emerge from the Nutrient agar plates were purified by sub-culturing and pure colonies stored on nutrient agar slants. Pure bacterial isolates were identified based on their cultural, morphological and biochemical characteristics(5, 6,7). The fungi isolates were also purified by successive sub-culturing of the growing fungal colony on PDA and further identified by removing small portion of the growth and placed on a grease free slide and a drop of lactophenol– cotton blue was added and viewed under x10 and 40 objective lenses(8).

III. Results

Entomological surveys.

Different flies were collected from the waste dump site [Fig. 1] and our survey showed *L. sericata*, *C. anthropophaga*,*Chrysomya*sp and *Musca domestica*to be more prominent. Some microbes had been associated with *C. anthropophaga*[9].Results from all the samples showed the houseflies [*Musca domestica*] as a prominent waste visitor. In all the samples *M.domestica* population was significantly higher [548.5-805.6 per week for four months] than all other fly populations [Fig.1;DMRT, P=0.05]. Population of *L. sericata* [237.4-393.4] was also high *C. anthropophaga*.*Atherigonaorientalis* had the lowest population at the dump site.

Fig. 1. Distribution of Flies at a Port Harcourt Dump Site, 2014.



Microbiological analysis

The results of the Microbiological analysis showed that the total heterotrophic bacterial counts for*Musca domestica* ranged from 3.4×10^{5} to 2.6×10^{6}, that of *Chrysomya*sp flies ranged from 4.2×10^{8} to 2.9×10^{9}Cfu/gram while the *Luciliasericata* ranged from 5.4×10^{7}Cfu/gram to 3.0×10^{8}cfu/g. in addition, the total heterotrophic fungi counts ranged 3.1×10^{3}Cfu/g to 2.4×10^{4}Cfu/g for the *Musca domestica*, 3.0×10^{4} to 2.9×10^{5}Cfu/g for green flies and 1.7×10^{3} to 4.2×10^{4}Cfu/g for theLuciliasericata. The bacteria isolated includes: *Escherichia coli*,*Pseudomonas*sp, *Bacillus*sp, *Enterobacter*sp, *Staphylococcus*sp, *Salmonella*sp, Proteussp, and Klebsiellasp, while the fungi species isolated includes:*Penicillium*sp, *Aspergillus*sp, *Rhizopus*sp, *Cladosporium*sp,*Aspergillusflavus*, *Aspergillusniger*, *Fusarium*sp and *Trichoderma* sp. Table 1 shows the occurrence of the isolates in the three fly species. *Bacillus*sp, *Enterobacter*sp, *Escherichia coli*,*Pseudomonas*sp and Staphylococcussp were found in all three species, *Salmonella*sp and *Klebsiella*sp were isolated from the *Chrysomya*sp while the *Proteus*sp was isolated from*Luciliasericata* only. Table 2 also shows the distribution of the isolates from flies.It was observed that *Penicillium*sp, *Aspergillusniger* and *Rhizopus*sp were isolated from all the samples. *Aspergillusflavus* and *Trichoderma*sp occurred in*Chrysomya*spand *Luciliasericata* flies. *Cladosporium*sp was isolated from *M. domestica*only, while*Fusarium*sp occurred in *Chrysomya*sp and *M. domestica*only.

Table 1.Bacterial Isolates Found on three Fly SpeciesFrom a Waste Dump site in Port-Harcourt, Nigeria.

Isolates	*Chrysomya*sp	*Musca domestica*	*Luciliaseriicata*
Escherichia coli	+	+	+
*Bacillus*sp	+	+	+
*Enterobacter*sp	+	+	+
*Pseudomonas*sp	+	+	+
Staphylococcus aureus	+	+	+
Salmonella sp	-	+	+
*Klebsiella*sp	-	+	-
Proteus sp	-	-	+

Key= + Positive, - negative

Table 2; Fungal Isolates Found on three Fly Species from a Waste Dump site in Port Harcourt, Nigeria.

Isolates	*Chrysomya*sp	*Musca domestica*	*Luciliasericata*
*Penicillum*sp	+	+	+
Aspergillusniger	+	+	+
Aspergillusflavus	-	+	+
*Rhizopus*sp	+	+	+
*Cladosporium*sp	-	+	-
*Fusarium*sp	+	+	-
*Trichoderma*sp	-	+	+

IV. Discussion

The isolation and identification of bacteria and fungi from a waste dump site in a female hostel showed high microbial load with the *Musca domestica* having the highest microbial counts, followed by *L. sericata*and the *Chrysomya*sp having the least counts. The high counts observed in this study for *M. domestica* and *L. sericata*flies may be due to their feeding habits; they are facultative feeders having other sources of contaminating pathogens. Both flies have been found on cadavers, in pit latrines. Some facultative flies including the ones found in this study are myiasis-producing flies. *M. domestica*,*L. sericata* and *Chrysomya* species including *Cordylobiaanthropophaga* and *Sarcophaga*species adults are synathropic flies that associate with human dwellings and can easily come in contact with human food after perching on feacal matters and decomposing animals at waste dump sites.

Eight genera of bacteria which includes:*Escherichia coli*, *Pseudomonas*sp, *Bacillus*sp, *Enterobacter*sp, *Staphylococcusaureus*, *Salmonella*sp, *Proteus*sp , and*Klebsiella*sp and six genera of fungi species including: *Penicillium*sp, *Aspergillus*sp, Rhizopussp, *Cladosporium*sp, *Aspergillusflavus*, *Aspergillusniger*, *Fusarium*sp and *Trichoderma*sp were isolated, which implies that house flies are also carriers of fungi spores.*Aspergillus* species.,*Penicillium*sp., Yeasts,*Cladosporium*sp. and *Fusarium* sp. were also the most commonly isolated (4). Previous workers(9) also reported the presence of *Bacillus*sp, and *Staphylococcus aureus* on the external of thehousefly.From their work, they also isolated *Micrococcus*sp, *Streptococcus*sp and *Acinetobacter*spwhich were not found in this study. Other workers (10)also isolated ten bacteria genera from their work. The fungi isolated like *Aspergillusniger*,*Cladosporium*sp, *Fusarium*spand *Aspergillusflavus*, are all potential pathogenic fungi since they are capable of producing mycotoxins and allergens (1). The presence of *Escherichiacoli* confirms fecal contamination and the presence of organisms of public health importance. A variety of bacterial diseases are transmitted by housefly which includes; typhoid fever, cholera, staphylococcal food poisoning (caused by *Staphylococcus aureus*) and Shigellosis caused by *Shigella*. Their feeding and filthy habits make them efficient vectors and transmitters of human enteric pathogens. These implicate the flies as reservoirs of various human pathogens which confirms the incrimination of these flies in human diseases such as diarrhea, typhoid and gastroenteritis [11].

It has also been reported that some flies may carry antibiotic resistant bacteria found in wastes[2, 19,20]**.** We therefore conclude that flies are mechanical vectors in the spreading of diseases; the presence of the flies would indicate sanitary deficiency and unhygienic conditions in an environment. In order to prevent possible outbreak of infection, all possible breeding sites of flies should be eliminated and flies prevented from gaining access to contaminate human food[12, 21]. Fly control is still an important public health measure, which helps in disease transmission, especially in the developing countries. Proper environmental sanitation should be implemented around any waste dumpsites to reduce breeding ground for flies. .Recently, the flies sampled in this work were also associated with septic omphalitis in neonates in the rural villages of the Niger Delta and some eastern states of Nigeria [22].

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