

HOUSEFLY (*MUSCA DOMESTICA*) AS A CARRIER OF PATHOGENIC MICROORGANISMS IN HOSPITAL ENVIRONMENTS IN CALABAR, NIGERIA

Houseflies and transmission of nosocomial infections in Hospitals

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Abstract

House flies are carriers of microorganisms, some of which are pathogenic. They are present and can be observed in many human-inhabited environments including hospital environments, where they contribute to the nosocomial infections. This work aimed at isolating and identifying potential pathogenic bacteria from flies in 2 prominent hospitals in Calabar, Nigeria. One hundred and fifty flies from each of the hospitals (University of Calabar Teaching Hospital (UCTH) and General Hospital Calabar (GHC)) were sampled and examined bacteriologically for the presence of potentially pathogenic bacteria. Obtained pathogens were subjected to antibiotic sensitivity testing. A total of 137 and 70 pathogens were isolated from the flies obtained from UCTH and GHC, respectively. At both hospitals, *Escherichia coli* was the most prevalent, 46 (34%) at UCTH and 26 (37%) at the GHC, followed by the enteropathogens, *Salmonella* and *Shigella*. Some of the houseflies that were tested harboured more than 1 potential pathogen. At UCTH, 50 of the 150 flies presented this phenomenon, while 20 flies did the same at the GHC. The commonest pattern of polypathogen harbourage was *Escherichia coli*-*Pseudomonas aeruginosa*: 15 (30%) at UCTH and 7 (35%) at the GHC. *Escherichia coli* and *Pseudomonas* isolates obtained at both hospitals were mainly responsive to pefloxacin, ciprofloxacin, and streptomycin but completely resistant to ampicillin/cloxacillin and Septrin. Houseflies are confirmed to be potential vectors of pathogenic organisms within the hospitals in Calabar; therefore, regular hospital hygiene, and fly reduction and elimination programmes should be regularly practised to reduce the rate of fly-borne nosocomial infections.

Keywords: Vectors, contamination, pathogenic bacteria, nosocomial infections

Introduction

The housefly (*Musca domestica*) is considered as a potential reservoir and mechanical vector of pathogenic organisms such as bacteria, protozoa, viruses, and helminth eggs (Dame and Fasulo, 2003). Since pathogenic microorganisms are widespread in the hospital environment, there is abundant opportunity

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for flies to become contaminated and, in turn, to further contaminate the patient environment (Fotedar *et al.*, 1992). The species of housefly always found in association with humans or activities of humans is *Musca domestica* (Mian *et al.*, 2002). It is the common species found at hog and poultry farms, horse stables, and ranches (Hald *et al.*, 2004).

Every fly is able to carry up to 5,000,000 germs including the pathogens of diseases such as typhoid, cholera, dysentery, polio, pneumonia, and foot and mouth disease (Meinhart, 1980). On a suitable substratum, germs multiply very rapidly. Houseflies frequently come into contact with patients' food and excrement and are involved in the dissemination of numerous diseases.

Structurally the fly is well adapted for picking up pathogens (Braverman *et al.*, 1999). Its proboscis is provided with a profusion of fine hairs, and each of the 6 feet of the fly is fitted with hairy structures and pads that secrete a sticky material, thus adding to its pathogen transmission potential.

Flies can be serious pests in managing patients and even those working in the hospital environment (Campbell *et al.*, 1977; Catangui *et al.*, 1997). They can cause financial loss to the patient, give a bad impression to visitors, and can transmit contagious diseases to patients and workers such as meningitis, respiratory reactions, and diarrhoea (Graczyk *et al.*, 2001).

Musca domestica are known to travel up to 20 miles (Murvosh and Taggard, 1966), although more commonly for no more than 2 miles. Transmission of pathogens by adult flies occurs by:

- (1) Mechanical dislodgement from their exoskeleton (for example by the hair and bristles on their legs or by cushion-like structures that are used for adherence to vertical surfaces),
- (2) Faecal deposition, and
- (3) The regurgitation of incompletely digested food (Amano, 1985).

Houseflies may transmit an ulcer bug, for example *Helicobacter pylori*, a bacterium responsible for some ulcers and which is associated with stomach cancer.

Houseflies are known to carry the protozoa parasite which can cause infection to patients in the hospital environment. These parasites may include *Ascaris lumbricoides*, *Entamoeba histolytica*, *Hookworm/Strongyloides* (Okoronkwo and Onwuli, 1998), *Cryptosporidium parvum* (Graczyk *et al.*, 2001), *Toxoplasma gondii* (Wallace, 1971; Graczyk *et al.*, 2001), and *Sarcocysts* spp. (Markus, 1980). Moreover, these flies have also been reported to transmit several types of viral pathogens to patients including hog

cholera (Dorset *et al.*, 1919). Flies are also involved in the transmission of bacteria pathogen such as *Enterocolytica* (Fukushima *et al.*, 1979), *Salmonella* spp., (Fotedar *et al.*, 1992; Barber *et al.*, 2002; Olsen and Hammack, 2000; Winfield and Groisman, 2003), *Campylobacter* spp. (Ekdahl *et al.*, 2005; Szalanski *et al.*, 2004), and *Shigella* spp.

Houseflies in a hospital environment feed on hospital dumpage and pick up drug-resistant bacteria strains, thus making treatment of infected humans very difficult (Reilly, 2006). Here, flies can be the vectors taking antibiotic resistant bacteria and aiding the transfer of resistant traits to competent bacteria.

Multi-drug resistance is a serious problem plaguing the world today, as the number of antibiotics which are effective at treating human infections continues to decline. Thus, this work aimed at isolating and identifying potential pathogenic and antibiotic resistant bacteria in 2 prominent hospitals in Calabar, Nigeria from houseflies caught in these environments.

Materials and Methods

Description of the Study Area

This research covered 2 major hospitals in the Calabar metropolis (the University of Calabar Teaching Hospital (UCTH) and the General Hospital Calabar (GHC)). Calabar, the capital city of Cross River State of Nigeria, is located at latitude 04° 58'N and longitude 08° 21'E along the coastal plains of Nigeria. It is in the rainforest belt near the Atlantic Ocean. The 2 hospitals are about 1.8 km apart.

Sample Collection

Houseflies were collected from various sites at the hospitals. In UCTH, the samples were collected from a laboratory, the accident/emergency unit, maternity ward, and food selling points. At GHC, flies were collected from the laboratories, operating theatre, and patients' wards (male, female, and paediatric units).

The samples were collected between March and August, 2015 on warm sunny days with an ambient temperature of 28-38°C, to allow for maximum fly activity. The fly collection was done using a standard fly collection bag in combination with a fly attractant. The flies were collected as they swarmed the attractant with 1 or 2 quick sweeps. The entire net bag was then transferred to a well-labeled clean polythene bag. This was transported to the laboratory for immediate analysis.

Sample Preparation, Cultivation, and Identification of Isolates

In the laboratory, the flies were crushed in test tubes containing 1ml of sterile peptone water, sealed with sterile cotton wool, and incubated aerobically for 1 h to revive viable bacteria. They were then cultured onto blood agar, MacConkey agar, and Deoxycholate Citrate agar.

All biochemical analysis was performed using 24-hour-old pure cultures to adequately identify all suspected pathogenic isolates. Some of the biochemical methods were triple sugar iron agar, the oxidase test, motility, indole production, urea, the catalase test, coagulase test, methyl red Voges Proskauer test, and citrate utilization test. The test

results and responses were used to properly identify the isolates by comparing the results to a standard bacterial identification manual, using Bergey's Manual of Systematic Bacteriology.

Antibiotic Susceptibility Testing

After 18-24 h overnight incubation at 35-37°C, well-isolated colonies of distinctive morphology were harvested with a wire loop and transferred to a tube of sterile peptone water broth. A sufficient quantity of bacterial growth was emulsified in the broth to match the 0.5 McFarland standards in turbidity.

The antibiotic discs, usually kept at 4°C in the refrigerator, were allowed on the bench to reach room temperature. Then, using sterile forceps, the

Table 1. Commercially prepared antibiotic discs used in this study

S/N	Antibiotics	Code	Disc Potency (µg/ml)
1	Pefloxacin	PEF	10
2	Gentamycin	CN	10
3	Ampiclox	APX	30
4	Zinnat	Z	20
5	Amoxicillin	AM	30
6	Rocephin	R	25
7	Ciprofloxacin	CPX	10
8	Streptomycin	S	30
9	Septin	SXT	30
10	Erythromycin	E	10

Standard strain of *Escherichia coli* (ATCC 25922) was used as control

Table 2. Number of flies and their density per square metre obtained from the departments/units of both hospitals

UCTH			GHC		
Department/ Unit	No of flies collected	Density of flies/m ²	Department/ Unit	No of flies collected	Density of flies/m ²
Accident/Emergency	25	2-4	Out Patient Unit	30	1-3
Laboratory section	35	3-5	Female ward	20	5-7
Maternity	30	7-9	Paediatric ward	25	10-12
Food selling points	60	10-12	Male ward	25	5-7
			Laboratory section	25	3-5
			Operating Theatre	25	8-10
Total	150		Total	150	

antibiotics discs were applied to the plates as soon as possible after inoculation, and within 10 min. Then, all antibiotic sensitivity plates were incubated at 37°C for 18-24 h. The diameters of the zones of complete inhibition were measured including the diameter of the discs, and results were recorded in millimetres. The control organisms were included in each batch. Table 1 shows the antibiotics and their dosages used in this study.

Results

A total number of 300 flies were studied from UCTH and GCH (150 flies for each location). At UCTH, the food selling points, laboratory, maternity ward which also encloses the postnatal ward and baby care units, and the accident and emergency unit were studied. The highest load of flies was seen at the food selling points with up to 10-12 flies/m² at the time of the study. Table 2 shows the fly density in all sections studied at UCTH.

At the GHC, the out-patient department, clinical wards, laboratory, and theatre areas were studied; the paediatric unit showed the highest fly burden of 10-12/m² and the data are presented in Table 2.

At UCTH, a total of 137 pathogenic bacteria were isolated from the 150 flies analyzed. Sixty of these pathogens were isolated and identified from flies obtained from the food selling points, while the flies from the accident and emergency unit had the lowest number of pathogens (19) from 25 flies studied. Table 3 presents the data from other units of the hospital. At the GHC, 70 pathogens were isolated from the 150 flies. The flies obtained from the outpatient department and the male ward showed the highest incidence of 14 pathogens from the 30 and 25 flies analyzed, respectively. The flies from the female ward gave the least number of pathogens, 8 from the 20 flies, as shown in Table 3. At both hospitals, *Escherichia coli* was the most prevalent potential pathogen, 46 (34%) at UCTH and 26 (37%) at the GHC. The next most prevalent organisms were the enteropathogens, *Salmonella* and *Shigella*, (18% and 19%) at UCTH and (31% and 9%) at GHC, respectively.

Some of the houseflies that were tested harboured more than 1 potential pathogen. At UCTH, 50 of the 150 flies presented this polypathogen phenomenon, while 20 flies did the same at the GHC. The commonest pattern of polypathogen harbourage was *Escherichia coli*-*Pseudomonas aeruginosa*: 15 (30%) at UCTH and 7 (35%) at the GHC. Also, *Salmonella* and *Shigella* co-existed in 12 flies studied

Table 3. The Distribution of Pathogens isolated from the different sections of UCTH and GHC

Pathogen Isolated	UCTH				Total number of flies (n=150)	GHC						
	Laborat-ory (n=35)	Accident/ Emergency (n=25)	Maternity (n=30)	Food selling points (n=60)		Out patient (n=30)	Male ward (n=20)	Female ward (n=25)	Paediatric ward (n=25)	Laborat-ory section (n=25)	Operating theatre (n=25)	Total number of flies (n=150)
<i>Shigella</i> spp.	4(11)	2(8)	7(23)	13(22)	26(18)	2(7)	1(5)	1(4)	-	1(4)	1(4)	6(4)
<i>Salmonella</i> spp.	-	3(12)	9(30)	13(22)	25(17)	5(17)	4(20)	2(8)	3(12)	4(16)	4(16)	22(15)
<i>Pseudomonas</i> spp.	1(3)	6(24)	7(23)	11(18)	25(17)	1(3)	1(4)	1(4)	1(4)	1(4)	1(4)	6(4)
<i>Escherichia coli</i>	12(34)	7(28)	9(30)	18(30)	46(31)	3(10)	7(35)	3(12)	4(16)	4(16)	5(20)	26(17)
<i>Klebsiella</i> spp.	2(6)	1(4)	1(3)	2(34)	6(4)	3(10)	1(4)	1(4)	2(8)	1(4)	2(8)	10(7)
<i>Aeromonas</i> spp.	6(17)	-	-	3(3)	9(6)	-	-	-	-	-	-	-
Total number of pathogen per hospital unit	25	19	33	60	137	14	14	8	10	11	13	70

at UCTH but in only 3 flies at the GHC (Table 4).

The antibiotic sensitivity profile of the isolates obtained from flies at UCTH showed good responses to ciprofloxacin, pefloxacin, and streptomycin (85-100%), followed by cefuroxime, gentamycin, and ceftriazone (50-100%). Other antibiotics like cotrimoxazole, erythromycin, and ampicillin were resisted by the isolates obtained in that environment. At the GHC, all the isolates were sensitive to streptomycin (100%), followed by ciprofloxacin, perfloxacin, gentamycin, and ceftriazone (Table 5).

Discussion

Houseflies (*Musca domestica*) are widely recognized as potential reservoirs and vectors of a wide range of pathogenic organisms. Within the hospital environment, houseflies present a special hazard in harbouring and transmitting agents of nosocomial infections (Campbell *et al.*, 1977). In this study, high fly densities were generally observed around/in the hospital wards with the highest density at food selling points. The 137 and 70 pathogens obtained from UCTH and the GHC, respectively, indicated the poor state of the hospitals' hygiene. This status reveals the potential source of some of the hospital-acquired infections. Similar findings have been made by others (Fotadar *et al.*, 1992; Rady *et al.*, 1992; Rahuma *et al.*, 2005). *Escherichia coli* was the most prevalent isolate in both hospitals. *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* (Kaper *et al.*, 2004). This explains why flies

that harbour these pathogens remain a serious threat in the hospitals. The presence of enteropathogens such as *Salmonella*, *Shigella*, and *Aeromonas* 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 (Nayduch *et al.*, 2002). Additionally, the finding of *Pseudomonas*, a chronic hospital infectious agent, in the flies shows why this organism continues to spread in the hospitals (Fotadar *et al.*, 1992).

A unique feature of this study was the observation of multiple pathogens within the same flies at both hospitals. These polypathogen harbouring flies are therefore double health hazards to the patients in the hospitals.

The antibiotic sensitivity pattern of the isolates showed good response to the aminoquinolones like ciprofloxacin, perfloxacin, cefuroxime, and ceftriaxone. Pathogens from both hospital environments were resistant to older antibiotics such as ampicillin and Septrin.

Conclusions

The houseflies collected from the hospital environments in Calabar are potential vectors of a wide range of pathogenic organisms like *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Pseudomonas aeruginosa*, and *Aeromona*. Since the flies are present in significant counts per square metre of the hospital environments, they present a serious health hazard of nosocomial infections to the patients and hospital workers.

Table 4. Polypathogen harbouring status of the houseflies obtained from the 2 hospitals

Polypathogens	UCTH No (%)	GHC No (%)
<i>Shigella-Salmonella</i>	12 (24)	3 (15)
<i>E. coli-Shigella</i>	2 (4)	2 (10)
<i>E. coli-Salmonella</i>	4(8)	2 (10)
<i>E. coli-Aeromonas</i>	6 (12)	-
<i>E. coli-Pseudomonas</i>	15 (30)	7 (35)
<i>E. coli-Klebsiella</i>	5 (10)	3 (15)
<i>Klebsiella-Pseudomonas</i>	1 (2)	2 (10)
Greater than two pathogens	5 (10)	1 (5)
TOTAL	50	20

Recommendation

1. The hospital administrators should regularly ensure the maintenance of good sanitary care within and around their healthcare facility.

2. The hospital waste management system should not provide any conducive breeding environment for flies. Hospital waste involves foul smelling products of health care such as blood, tissues, used laboratory culture media, fomites, plasters, soiled cotton wools, etc.; hence, adequate methods of collecting, treating, storing, transporting, and disposing of garbage should be functional at all times.

3. The hospital environments (gutters, drainages, and refuse dumps) should be regularly fumigated to destroy the larval stages of houseflies and mosquitoes with long-lasting residual insecticides on surfaces and their breeding places.

4. The food selling points should be adequately built and protected with insect nets to prevent flies coming into contact with the patients' food and eating utensils. Also, commercial food vendors permitted to sell food in the hospitals should be adequately trained to maintain high hygienic practices.

5. The hospital wards and operating theatre must be regularly treated with insecticides and fitted with protective insect barriers. Also, electric ultraviolet light fly traps should be installed in these units to eliminate flies that have entered the wards.

6. The Federal and State Ministries of Health should regularly inspect, repair, maintain, and adequately provide hospitals with sanitary equipment and provide personnel to keep the hospitals clean and tidy, as this will discourage the presence and breeding of flies.

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Table 5. Antibiotic sensitivity testing of the pathogens isolated from the flies at the University of Calabar Teaching Hospital

Pathogens Isolated	PEF (%)		CN (%)		APX (%)		Z (%)		AM (%)		R (%)		CPX (%)		S (%)		SXT (%)		E (%)	
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
<i>Shigella</i> (n=26)	22	(85)	26	(100)	13	(50)	22	(85)	9	(33)	17	(67)	26	(100)	26	(100)	4	(17)	0	(0)
<i>Salmonella</i> (n=25)	16	(67)	21	(83)	13	(48)	21	(83)	4	(17)	21	(83)	23	(92)	25	(100)	4	(17)	0	(0)
<i>Pseudomonas</i> (n=25)	25	(100)	25	(100)	0	(0)	25	(100)	0	(0)	25	(100)	25	(100)	0	(0)	0	(0)	0	(0)
<i>Klebsiella</i> (n=6)	6	(100)	3	(50)	0	(0)	6	(100)	3	(50)	3	(50)	6	(100)	6	(100)	0	(0)	0	(0)
<i>Escherichia coli</i> (n=46)	23	(50)	46	(100)	23	(50)	35	(75)	0	(0)	35	(75)	46	(100)	46	(100)	0	(0)	0	(0)
<i>Aeromonas</i> (n=9)	9	(100)	9	(100)	9	(100)	9	(100)	9	(100)	9	(100)	9	(100)	9	(100)	9	(100)	0	(0)

KEY: PEF—Pefloxacin, CN—Gentamycin, APX—Ampiclox, Z—Zinnat, AM—Amoxicillin, R—Rocephin, CPX—Ciprofloxacin, S—Streptomycin, SXT—Septin, E—Erythromycin n- number of each pathogen tested

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