Bacterial Pathogens Associated with wild-caught houseflies in Awka metropololis of Anambra State, Southeastern Nigeria

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Abstract: Bacterial pathogens associated with wild-caught houseflies in Awka metropolis, were investigated between April and August, 2012. Locally designed fly traps baited with common materials in the site of fly collection were used to collect flies from the six different ecological locations in the city. A total of 2,157 wild-caught flies were used in the study. They were identified into genera and species using standard taxonomic keys. Eight fly species (*Musca domestica, Lucilia cuprina, Chrysomyia bezziana, Fannia canicularis, Wohlfartia species, Stomoxys calcitrans,Musca sorbens* and *Sarcophaga species*) were identified. The internal and external pathogens associated with the flies were isolated and aliquots of the homogenates from both their internal and external contents were inoculated on various agar plates for pathogen isolations and identification. Bacterial pathogens isolated from the flies were *Staphylococcus aureus, Pseudomonas species, Proteus mirabilis, Streptococcus species, Salmonella species, Shigella species, Escherichia coli,* and *Klebsiella species.* All the eight genera of bacterial pathogens isolated are known to be pathogenic to man, indicating that wild-caught flies, especially *M. domestica*, are potential mechanical vectors of bacterial pathogens. This calls for improvement of sanitation/hygiene in both urban and rural areas of the country so as to improve the health of our people.
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1. Introduction

Houseflies are among the most common and persistent insects found within human homes. They have a strong interdependent relationship with man and will travel with human populations to even the coldest regions of the world (Service, 1980). They are commonly found both indoors and outdoors. They thrive on excrement, dead animal bodies, and contaminated areas where faecal matter, large amounts of organic waste and piles of garbage are left exposed and unattended (Sulaiman *et al*, 1989). Over 50 species of synanthropic flies have been reported to be associated with unsanitary conditions and are involved in dissemination of human pathogens in the environment (Olsen, 1998). These synanthropic flies are major epidemiologic factors responsible for the spread of acute gastroenteritis, trachoma among infants and young children in developing countries and transmission of nosocomial infections with multiple antibiotic-resistant bacteria in hospital environment (Graczyk *et al.,* 2001). Greenberg (1971) demonstrated the role of housefly in the transmission of pathogens and gastrointestinal diseases such as shigellosis, salmonellosis, cholera, and yaws. WHO (1969) showed a correlation between houseflies and enteric fever and flies that were carriers of *Salmonella typhosa* and *S. paratyphi A* and stated that these micro-organisms remain alive for many days in the flies. Richards (1961), cited by WHO (1969), observed that pathogenic bacteria remain alive in houseflies for an appreciable length of time. Flugge (1893) and Buchanan (1897), cited by WHO (1969), had earlier indicated that flies were transmitters of cholera but Flu (1915) was the first to isolate *Vibrio cholera* from flies. Grubel *et al.* (1997) stated that houseflies probably act as vectors in the transmission of *Helicobacter pylori,* through contamination of human food. Esrey (1991) and Cohen *et al*., (1991) observed that there was a correlation between fly population and diarrhea, and diarrhoea and shigellosis incidence, respectively. Emerson *et al.,* (1999) showed that fly control could reduce trachoma and diarrhoea among children in Gambia. Pruss and Mariotti (2000) observed that the spread of trachoma was through person-to-person contact and flies appear to constitute the major transmission pathways. Experiments in which flies were allowed to walk over culture media in sterile dishes have resulted in the growth of over 100 bacterial and fungal colonies from bacterial and fungal spores which the fly deposited (Kobayashi, 1999). Flies are responsible for the spread of such diseases as diarrhoea, anthrax, eye infections and

possibly tuberculosis, typhoid fever, cholera, staphylococcal food poisoning and shigellosis (Connor, 1966; Sack *et al*, 1971).

In spite of increasing urbanization and globalization, bush defaecation and many unsanitary practices are still going on in some areas of Awka metropolis and elsewhere in developing countries (Onyido *et al*, 2009; Onyido *et al*, 2011), and at the same time so many food items are displayed uncovered in the open markets, by unhygienic food vendors thereby creating easy means of disease transmission by synanthropic flies. More so, there is dearth of information on fly pathogens and parasites in Awka and Nigeria as a whole. This study was aimed at determining the bacterial pathogens associated with wild-caught houseflies (*Musca domestica* Linnaeus), in Awka metropolis, Anambra State, South-east Nigeria. The specific objectives included

(1) collection and identification of fly species from different ecological locations.

(2) Isolation and identification of bacterial pathogens harboured by the flies using various microbial techniques for culturing and identifying them.

2. Materials and Methods

2.1 Study Area

Awka is the political capital of Anambra State, South-east of Nigeria. Historically, Awka was the seat of the ancient Nri civilization governed by titled men known as Ozo and Ndichie who were accomplished individuals in the community. They held general meetings or "Izu Awka" either at the residence of the oldest man (Otochalu Awka) or at a place designated by him. He was the "Nne Uzu" or the Master Blacksmith. The city still preserves its traditional systems of governance with Ozo titled men often consulted for village and community issues and a paramount traditional ruler, the "Eze Uzu", who is elected by all "Ozo" titled men by rotation amongst different villages to represent the city even at state functions.

Awka is geographically situated between latitude 6.22^{o}N and longitude 7.07^{o}E (Iloeje, 2001). It lies within the flood plain of Udi escarpment and is drained into the River Niger by the Omambara River and its tributaries (Iloeje, 2001). It is within the rainforest zone of Nigeria and has marked wet and dry seasons. The area has about eight months of rainfall (April - October), with an annual rainfall range of 2000 – 3000mm. The dry season lasts from November to March, with dry harmattan winds blowing for four to six weeks, between December and January or even February. It also has a daily temperature range of about 22.3 – 32.1 ^{0}C and a relative humidity range of about 68 – 79%.

Awka has a population of 1,130,020 inhabitants (NPC, 2006).The inhabitants of Awka are mainly Igbos, although there are few other groups, like the Hausas and Yorubas, who constitute a small percentage of the populace. It has three institutions of higher learning, including Nnamdi Azikiwe University, Paul's University, and Anambra State University Teaching Hospital. Also, it has 34 secondary schools and 76 primary schools. Other institutions in the city include the Anambra Broadcasting Service (ABS), a Television and Radio Station located in the city centre, while the privately owned Silver Bird Television and Rhythm FM stations are located in the outskirts of the city. A number of Federal Institutions including the Central Bank of Nigeria, the NTA Awka Media Station, and branch offices of the Federal Inland Revenue Service, Federal Road Safety Commission, Nigerian Immigration Service, and Corporate Affairs Commission are also present in the city. Major Nigerian Banks such as Access Bank, Fidelity Bank, Mainstreet Bank, Keystone Bank, Ecobank, First Bank, UBA, Union Bank, Zenith Bank, and Guaranty Trust Bank, have branches in the city.

Occupationally, Awka was famous for blacksmith and woodwork – carving of objects like masks, stools and statues before the 20th century and their products were highly prized throughout the region (Iloeje, 2001). They also produced farming implements like matchets, hoes, cutlasses, guns and tools. Awka was the seat of Nri civilization that produced the earliest documented bronze works in Sub-Saharan Africa around 800 A.D. (Iloeje, 2001). In addition to craftsmanship, Awka is an agrarian community, practicing crop farming with keeping of animals, mostly at subsistence level. In recent years, several new businesses have been established in the city. Various organizations have erected fascinating new buildings that have largely changed the face of the city. The partly state-owned Orient Petroleum Resources Limited has the headquarters in Awka. The company has a refinery at Igbariam for refining the huge crude oil deposits in the Anambra River Basin. Also Juhel Nigeria Limited has a huge manufacturing plant for the production of Parenteral drugs in the city.

2.2 Collection of Houseflies

Six ecological locations were selected for collection of flies based on human activities in the area especially trading on food items. The locations include: Bishop Crowther Seminary Area, Regina-Caeli Junction Area, Aroma Junction Area, Unizik Temporary Site Area, Abattoir (Kwatta) Area and Eke Awka Market. The flies were collected with a specially designed trap made up of a transparent plastic bucket, about 10 cubic litres, manually perforated with tiny holes to provide adequate

ventilation for the flies and a fly bait. The fly baits were varied according to site. Dry and fresh fishes were used as baits in fish stalls, tomatoes were used as bait in tomato stalls, and pieces of meat were used as bait in the abattoir and meat stalls. Each trap (bucket with fly bait) was placed at a strategic place in the selected sites. As the flies were attracted to the bait inside the bucket, it was swiftly covered with its lid. The trapped flies were taken to the Parasitology and Entomology Laboratory of Nnamdi Azikiwe University, for identification and processing.

2.3 Bacteriological Techniques

(a) Preparation of culture media

Owing to the variety of bacterial pathogens reportedly transmitted by housefly (Thirumalai *et al*, 2008), the following media were used in culturing the microorganisms; MacConkey agar, Nutrient agar, and Blood agar. This was to ensure that both fastidious and non-fastidious microorganisms were taken care of. The various media were prepared according to manufacturer's instructions.

(b) Culturing Of the Microbes in Various Growth Media

Microbes on the External Body Parts

For the external microbes, a wire loop was flamed red hot, allowed to cool, before using it to collect a loopful of the homogenised suspension from the external surfaces of the flies, and streaked on the surfaces of the prepared MacConkey, Blood and Nutrient agars respectively. The media were then incubated at 37^{o}C for 24 hours. The emergent colonies were sub-cultured for another 24 hours at 37^{o}C to produce the pure cultures of the pathogens and examined for the presence of distinct colonies (Cheesbrough, 2000).

Microbes in The Internal Body Parts

For the internal microbes, the wire loop was flamed red hot, cooled, and used to collect an aliquot of the homogenised suspension of the internal contents of the crushed flies that were streaked on the surfaces of the prepared MacConkey, Blood

and Nutrient agars respectively. The media were then incubated at 37^{o}C for 24 hours. The emergent colonies were sub-cultured for another 24 hours at 37^{o}C and examined for the presence of distinct colonies (Cheesbrough, 2000).

2.4 Identification of Bacterial Pathogens

The cultured organisms were identified to various genera and species where possible, using Colony morphology, gram staining, motility, as well as catalase, coagulase, indole and oxidase tests.

3. Results

The bacterial pathogens isolated from the flies are shown in Table 1. Medically important bacteria were isolated from flies collected in all the six different ecological sites. *Staphylococcus aureus* was isolated from flies caught at Bishop Crowther area, Regina Caeli area, Unizik Temporary Site, and Abattoir; *Salmonella species* from flies caught at Regina Caeli junction area, Aroma junction area, Unizik Temporary Site, and Eke Awka Market; *Proteus mirabilis* isolated from flies caught at Bishop Crowther, Regina Caeli, Aroma junction, and Eke Awka market areas; *Pseudomonas species* was isolated from flies caught at Bishop Crowther, Regina Caeli, Abattoir, and Eke Awka market areas.

Escherichia coli and *Klebsiella species* were isolated from flies caught from two different locations. *E. coli* was isolated from flies caught from Unizik Temporary Site junction and Eke Awka, while *Klebsiella species* was isolated from flies caught from the Abattoir and Eke Awka markets. *Streptococcus* was isolated only from flies caught from Bishop Crowther Area. In summary, Eke Awka market accounted for the highest number of diverse bacteria species, 6 (75%) of the 8 species, isolated from the flies in the study; Aroma junction recorded the least, 3 (37.5%) of the 8 species.

Colony morphology and biochemical reactions of the various bacterial pathogens isolated from the flies are shown in Table 2. *Salmonella species, Shigella species,* and *Proteus mirabilis,* had colourless pale colony masses, *Staphylococcus aureus* had pale ash coloured colony mass, *E. coli* had a smooth pinkish-red colony mass; *Pseudomonas species* had a greenish colony; *Klebsiella* had a pinkish-red colony mass, while *Streptococcus* had a pale-whitish (cloudy) mass colony.

All the bacterial pathogens were rod-shaped except *Staphylococcus aureus,* and *Streptococcus species* that were cocci. Also, all the bacteria pathogens isolated were gram negative bacteria except *Staphylococcus aureus,* and *Streptococcus species* that were gram positive.

All the isolated bacteria were indole and oxidase negative. *Proteus mirabilis* and *Klebsiella species* were urease positive, while others were negative. Except for the *Staphylococcus aureus* that were both coagulase and catalase positive, others were negative. *Streptococcus* was coagulase positive but catalase negative

The bacteria isolated from the external and internal organs of the flies are shown in Table 3. All the bacteria isolates were obtained from the suspensions made from both the external and internal organs of the flies. Of particular interest is *M. domestica* which haboured all the bacteria both in its internal and external body parts.

Table 1: Bacterial pathogens isolated from various locations

S/n	Locations in Awka metropolis	Nature of Site	Bacterial Pathogens isolated	Number of Times Sampled	Number of species	Frequency (%)
1.	Aroma junction	Open-air defecation sites, fruit stalls, restaurants and a motor park	*Salmonella species, Shigella species,* and *Proteus mirabilis.*	5	3	37.5
2.	UNIZIK Temporary Site	Concentration of Eateries, restaurants and motor parks, drainages littered with faeces	*Staphylococcus aureus, Salmonella species, Shigella species,* and *Escherichia coli.*	5	4	50
3.	Bishop Crowther	Open-air defecation sites, human homes, fruit stalls, Paediatric hospital with huge undisposed refuse dump	*Staphylococcus aureus, Pseudomonas species, Proteus mirabilis,* and *Streptococcus species*	5	4	50
4.	Abattoir (Kwatta)	State capital's main meat stall, surrounded by bushes utilized for indiscriminate defecation	*Pseudomonas species, Shigella species, Proteus mirabilis, Klebsiella species.*	5	4	50
5.	Regina Caeli Junction	Concentration of popular restaurants	*Staphylococcus aureus, Pseudomonas species, Salmonella species, Proteus mirabilis,* and *Shigella species.*	5	5	62.5
6.	Eke Awka Market	Fruit and vegetable stalls, restaurants, food vendors and assorted food stuffs	*Staphylococcus aureus, Pseudomonas species, Salmonella species, Shigella species, Escherichia coli,* and *Klebsiella species.*	5	6	75

Table 2: Colony morphology and biochemical reactions of various bacterial species isolated from the flies

S/n	Organism	Shape	Gram Stain	Indole	Urease	Motility	Oxidase	Coagulase	Catalase
1.	*Salmonella species*	Rod	-ve	-ve	-ve	+ve	-ve	-ve	-ve
2.	*Shigella species*	Rod	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	*Proteus mirabilis*	Rod	-ve	-ve	+ve	+ve	-ve	-ve	-ve
4.	*Pseudomonas species*	Rod	-ve	-ve	-ve	+ve	-ve	-ve	-ve
5.	*Staphylococcus aureus*	Cocci	+ve	-ve	-ve	-ve	-ve	+ve	+ve
6.	*Escherichia coli*	Rod	-ve	+ve	-ve	+ve	-ve	-ve	-ve
7.	*Klebsiella species*	Rod	-ve	-ve	+ve slow	-ve	-ve	-ve	-ve
8.	*Streptococcus species*	Cocci	+ve	-ve	-ve	-ve	-ve	+ve	-ve

Table 3: Bacterial pathogens isolated from the external and internal body parts of flies

S/n	Bacterial or parasite pathogens isolated	External body surface of *M. domestica*	Internal body parts of *M. domestica*	External body surfaces of other flies	Internal body parts of other flies
1.	*Staphylococcus aureus*	+	+	+	-
2.	*Salmonella species*	+	+	+	+
3.	*Shigella species*	+	+	+	+
4.	*Proteus mirabilis*	+	+	+	-
5.	*Pseudomonas species*	+	+	-	+
6.	*Escherichia coli*	+	+	+	+
7.	*Klebsiella species*	+	+	+	-
8.	*Streptococcus species*	+	+	-	+
	Presence of Bacterial species	External Body Surface of *M. domestica* (n=8) Frequency (%)	External Body Surface of other flies (n=8) Frequency (%)	*t*-value (independent)	P-value
	+	100	75	-1.528	0.170
	-	0	25		
	Presence of Bacterial species	Internal Body Surface of *M. domestica* (n=8) Frequency (%)	Internal Body Surface of other flies (n=8) Frequency (%)	*t*-value (independent)	P-value
	+	100	62.5	-2.049	0.080
	-	0	37.5		

Statistically significant at p-value <0.05

Key

\+ Indicates presence of a bacterial pathogen

\- Indicates absence of a bacterial pathogen

4. Discussion

All the bacteria isolated from the flies in this study are of great medical and public health importance. *Staphylococcus* species is a known causative agent for diarrhoea and urinary tract infections in various parts of the world (Curran and Al-Salihi, 1980). *Klebsiella* species, if present in the respiratory tract in small proportions, causes bacterial pneumonias. It occasionally produces urinary tract infection and bacteremia with focal lesions in debilitated patients (Carbapenem, 2007). *Klebsiella* species also causes hospital acquired infections and is also associated with inflammatory conditions of the upper respiratory tract (Carbapenem, 2007). *Proteus mirabilis* is also known to cause urinary tract infection (Brooks *et al.,* 1995). *Shigella* and *Salmonella* species are well known causes of diarrhoea throughout the world (WHO, 2005). The genus *Streptococcus* causes rheumatic fever, pneumonia, impetigo, and sore throat and can also lead to kidney infections. *Pseudomonas species* cause bacterial keratitis, endocarditis, urinary tract infections, and respiratory infections mostly in immunocompromised individuals (Rossolini and Mantengoli, 2005). On its part, *E. coli* is known for its association with infantile diarrhoea, gastroenteritis and several other diseases particularly in immunocompromised humans (Kobayashi *et al*, 1999). Infection with *E. coli* O157:H7 is potentially lethal, especially in young, elderly, and immunocompromised patients who may develop haemolytic uremic syndrome culminating in acute kidney failure (Szalanski *et al*, 2004).

Some of the bacteria isolates in this study belong to the family Enterobacteriaceae. Different members of this family cause sepsis in 30–35% of all cases, 70% of all urinary tract infections, and in most of gastrointestinal diseases (Förster *et al*, 2007). Among the Enterobacteriaceae, numerous species of the genera *Escherichia*, *Salmonella*, and *Shigella* are considered as dangerous pathogens (Förster *et al*, 2007).

Flies were collected from 6 locations in Awka where food items, fruits and vegetables were sold in both raw and cooked states. This shows that the flies

are abundant in the area, probably due to the existence of favourable breeding sites and tropical climate which increases their rate of reproduction and development. Sualiman *et al* (1989) observed that houseflies thrive on excrement, dead animal bodies, and contaminated areas where faecal matter, large amounts of organic waste and piles of garbage are left exposed and unattended.

Eight fly species were collected in the study with *M. domestica* being the most prevalent (84.60%), while *S. calcitrans* was the least (0.20%). This shows that the environment can support different species of flies leading to high species diversity in the area. Olsen (1998) showed that over 50 species of synanthropic flies have been reported to be associated with unsanitary conditions and are involved in dissemination of human pathogens in the environment.

Graczyk *et al.,* (2001) observed that houseflies among other synanthropic flies are major epidemiologic factors responsible for the spread of many diseases among infants and young children in developing countries and transmission of nosocomial infections in hospital environments. In another study, Banjo *et al.,* (2005) also showed that flies are natural carriers of pathogens and play a considerable role in transmission of bacterial pathogens in different regions of the world. The results of this study revealed that *M. domestica* harboured the 8 bacteria species isolated in this study both in their external and internal organs. These included *Staphylococcus aureus, Pseudomonas species, Salmonella species, Proteus mirabilis, Shigella species, Streptococcus species, Escherichia coli* and *Klebsiella species*. The housefly, *Musca domestica,* has long been considered as a potential agent for disease transmission, and bacteria have been isolated from faeces, vomitus, external surfaces, and internal organs of this species (De-Vos and Bryden, 1998). This result is in agreement with the works of Watt and Lindsay, (1948), Graczyk *et al*., (2001), Zarin *et al*, (2007), Thirumalai *et al*, (2008), and Barin *et al*, (2010), who isolated different bacteria from the organs of *M. domestica.*

All other flies used in this study were also observed to harbour several medically important bacteria. This agrees with the work of Dragon, (1999), and Greenberg, (1973) which showed that filth flies in general are major transmitting agents of human and animal diseases. In another study in Chow Kit area, Kuala Lumpur, Sulaiman *et al* (2000), isolated eighteen species of bacteria from *M. domestica*, 12 species of bacteria from *M. sorbens*, 12 species from *Chrysomya megacephala* and 5 species from *Chrysomya rufifacies*. The collection of bacteria from flies other than *M. domestica* shows that fly species other than the housefly pose potential threat to humans and animals. This is in agreement with the work of De-Jesus *et al*, (2004), which indicated that synanthropic flies are carriers of bacteria and can be important vectors for food-borne pathogens. Also, Iwasa *et al.,* (1999), Kobayashi *et al.*, (1999), and Szalanski *et al.*, (2004) have all demonstrated that the housefly as well as other flies may transmit *E. coli,* among other bacteria.

Awka is a rapidly growing urban city with large population of youths. This is evident in the number of secondary schools, higher institutions, and parks within it. It is also a sprawling city with inadequate planning and infrastructure. So, with increasing urbanisation, overcrowding and congestion of squalor areas, there are apparent poor sanitary conditions and solid waste disposal arrangements. Onyido *et al.,* (2009) observed that this condition may predispose the area for breeding of the flies and exposure of the inhabitants to fly-borne diseases. Improved sanitation is advocated to protect this urban population from epidemics related to poor sanitary conditions of the environment.

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