BACTERIA AND FUNGI ASSOCIATED WITH HOUSEFLIES COLLECTED FROM CAFETERIA AND FOOD CENTRES IN SOKOTO

Supported by tetfund TERTIARY EDUCATION TRUST FUND

I. Awache[1*] and A.A. Farouk[2]

[1]Department of Microbiology, Federal University Wukari, Taraba State, Nigeria

[2]Department of Microbiology, Usmanu Danfodiyo University Sokoto, Nigeria

*Corresponding author: awachedason2@yahoo.com;

Abstract: Bacterial and fungal species associated with common filth houseflies (*Musca domestica*) in cafeteria and food centers in Sokoto state were investigated. A total of one thousand and fifty nine (1,059) adult houseflies were collected with the aid of clean sweep net (1 m×25 cm×45cm). The samples were mixed with distilled water in a conical flask. Samples from the conical flask were serially diluted. Isolation and identification of bacteria and fungi were done using standard techniques. The bacterial count at the various sampling sites ranged from 2.1×10^{7} – 2.67×10^{9} cfu/ml. The predominant bacterial species associated to the flies were *Escherichia coli* (16.67%), *Bacillus* species (50%), *Citrobacter freundii* (16.67%) and *Moganella morgana* (16.67%). The predominant fungal isolates were *Aspergillus* species (85.71%) and *Mucor circinelloides* (14.29%). Most of these isolates are potential pathogens and may pose a public health hazard to the community. There is need for routine sanitation and disinfection of food centres in Sokoto State to prevent transmission of pathogenic organisms via food by flies.

Keywords: Bacteria, Fungi, *Musca domestica* and food centres.

Introduction

Housefly, *Musca domestica*, has long been in existence since the beginning of human life (Waheed *et al*., 2014). It is said to have originated from the savannahs of Central Asia, but now disseminated throughout the all inhabited continents of the world, in both the rural and urban areas of tropical and temperate climates either indoors or outdoors (Hussein and John, 2014; Oyindo *et al.*, 2014).

Musca domestica are readily found to be in close association with humans and have adapted to life in human settlements (WHO, 2005). They are often found in abundance in areas of human activities such as; hospitals, food markets, slaughter houses, food centers or restaurants, poultry and livestock farms where they pose problems such as disease vectors and as nuisance to humans, poultry, livestocks and farm animals. They play key role in the mechanical transmission of disease pathogens ranging from viruses, bacteria, fungi, protozoa, and nematodes amongst animals and humans (Babak *et al.*, 2008; Davari *et al.*, 2012; Szalanski *et al.*, 2004). They are potential vehicle for etiological agents such as *Salmonella typhi* and *paratyphi*, *Shigella dysentriae*, *Vibrio cholerae*, *Campylobacter*, *Esherichia coli*, *Enterococcus*, *Chlamydia* and many other species of public health significance (Hussein and John 2014). Furthermore, they enhance the spread of diseases such as; typhoid and paratyphoid fever (enteric fever), cholera, bacillary dysentery, conjunctivitis, poliomyelitis, hematic carbuncles, and bovine mastitis (garget) and many others amongst humans' population as well as their livestock (Isabel, 2015).

The role of flies in pathogen transmission is mainly related to their biology as decomposers with polyphagus habits, and endophilic behavior. Their morphology and behavioral feeding habits enhance the process of disease transmission (Sukontason *et al.*, 2006). Their pair of wings aid in flight movement across different surfaces while their spongy-like and toothless enables them to suck up liquid foods; which usually is their favorite. Also, flies can make use of solid food by dissolving the substrates through vomiting or spitting on it or readily dissolving it in the salivary gland secretions or in the crop (Waheed *et al.*, 2014). The houseflies are attracted to waste due to their strong odor, and they feed on all type of human food, sweat, excreta, garbages as well as animal dungs. They pick-up pathogenic microorganisms from these sources and then transferred on their mouth parts, through their vomits, faeces and contaminated external body parts to humans' food and animal feed (Babak *et al.*, 2008). This paper reports the bacterial and fungal species associated with houseflies collected in cafeteria and food centres in some parts of Sokoto, Nigeria.

Materials and Methods

Sample collection/study area

A total of 1,059 adult houseflies were caught with the aid of a clean sweep net twice daily, morning (10:00h) and Afternoons (15:00h) from two major food centers (IBB and Students' cafeteria) and male students hostel of the Usmanu Danfodiyo University, Sokoto. The samples were aseptically transferred into a marked specimen bottle. Using sterile disposable hand gloves, various species of the houseflies were sorted out as described by Dipeolu (1977). A total of 443 adult houseflies were caught from the male hostel, 295 from student cafeteria and 331 from the IBB food centers.

Sample preparation and analysis for bacteria

The sample from each study site was mixed with distilled water in a conical flask. Serial dilution of the fresh sample and the digested slurry sample were carried out up to 10^{-6} tubes. Exactly 0.5ml was obtained using sterile syringe from the 10^{-5} test tube and inoculated onto already prepared nutrient agar plates by spread plate and pour plate methods of inoculation. The inoculated plates were incubated at 37^{0}C for 24h. Bacterial colonies that emerge on the plates were counted and recorded as colony forming units per milliliter (cfu/ml) of the sample. The colonies were also sub-cultured repeatedly on fresh plates to obtain pure isolates. The pure bacterial isolates were Gram-stained and subjected to different biochemical tests as described by Cheesebrough (2006). The bacterial isolates were identified by comparing their characteristics with those of known taxa using the schemes of Cowan &

Steel (1993). An aliquot (0.1ml) of the prepared sterile distilled water used for the sample analysis from each of the conical flask was inoculated into a prepared media and incubated accordingly for 24 h at 37^{o}C as control.

Sample preparation and analysis for fungi

The sample from each study site was mixed with distilled water in a conical flask. Serial dilution of the fresh sample and the digested slurry sample were carried out up to 10^6 tubes. Exactly 0.5ml was obtained using sterile syringe from the 10^5 tube and inoculated onto already prepared Saboraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) and was kept at a room temperature in the dark for about 4 to 10 days. After which distinct growth colonies were sub-cultured in the middle of fresh plates of SDA and PDA, so the growth spread-out. Incubations were also done for another 4 to 10 days. Identification was done using the lactophenol cotton blue and slide cultures techniques (Leck, 1999; Anthony and Walkes 1962).

Results and Discussion

The bacterial count ranged from 2.1×10^7 to 2.67×10^9 cfu/ml (Table 1). The bacteria isolated were *Escherichia coli*, *Citrobacter freundii*, *Bacillus megatarium*, *B. sphaericus*, *B. alvei* and *Moganella morgana* (Table 2). The frequency of isolation of bacterial species is shown in Table 3. The fungal species isolated includes *Aspergillus niger*, *A. fumigatus and Mucor circinelloides* (Table 4). And the frequency of isolation of fungal species is shown in Table 5.

Table 1: Mean bacterial count from each sampling site

Sampling site	Mean bacterial load (CFU/ml)
Houseflies from male hostels	2.1×10^7
Houseflies from students cafeteria	2.68×10^9
Houseflies from IBB food centre	1.64×10^8

The bacteria isolated from houseflies in this study include *Escherichia coli,Bacillus megatarium, B. sphaericus, B. alvei,, Moganella morgana and Citrobacter freundii*. Earlier researchers, Banjo *et al.* (2005), isolated *Bacillus cereus and B. subtilis* among other organisms isolated from flies. The isolation of *Bacillus* species as observed in this study can be related to the close association of the flies with the soil. Other researchers have isolated different locations and under varied climatic conditions.

Ugbogu *et al.* (2006) reported the isolation of *Salmonella* and *Shigella* species from houseflies in Uturu. Their study targeted only *Salmonella* and *Shigella* species and there is the likelihood that the flies carried other organisms including those isolated. The studies by Oyindo *et al.* (2014) reported the isolation of *Staphylococcus aureus*, *Proteus* species, *Streptococcus* species, *Klebsiella* species and *Pseudomonas* species which were not isolated in this study. This observation shows that flies carry different organisms at different times, locations and seasons. However, the isolation of *E. coli* in both studies suggests that flies likely carry *E. coli* despite the location and time of study.

Table 2: Identification of bacteria isolated from houseflies

S/S	G/R	Colonial Characteristics	CT	UR	CI	IN	MT	H_2S	GP	LT	SC	GL	MR	VP	SS	Organism
A	+Rods	Small raised colonies with rough edges, opaque and creamy in colours.	+	_	+	_	+	+	+	_	+	+	+	_	+	*Bacillus megatarium*
	-Rods	Large circular and transparent colonies that are milky in colours	NA	+	+	_	_	+	+	+	+	+	+	_	_	*Citrobacter freundii*
B	+Rods	Large circular rough edged	+	+	+	_	+	_	_	_	_	_	+	_	+	*Bacillus sphaericus*
	-Rods	Small circular raised and transparent colonies with creamy colours	NA	-	+	+	+	-	-	+	+	+	+	-	-	*Escherichia coli*
C	+Rods	Large raised colonies, opaque and creamy in nature	+	+	_	+	+	+	_	_	_	+	_	+	+	*Bacillus alvei*
	-Rods	Small circular colonies, transparent and creamy in nature swarmed all over the plate	NA	+	+	+	_	_	+	_	+	+	+	_	_	*Morganella morganni*

A= Houseflies from male hostels; MR= Methyl Red; LT= Lactose; CT= Catalase;IN= Indole; SS= Spore staining; B= Houseflies from students cafeteria; VP = Voges Proskauer; G/R= Gram reaction; UR= Urease; MT= Motility; S/S= Sampling site; C= Houseflies from IBB food centers; GL= Glucose; GP= Gas Production; CI= Citrate; SC= Sucrose;NA= Not Application; += Positive;- = Negative

Table 3: Percentage frequency of occurrence of bacteria isolated from houseflies

Organism Isolated	Frequency	(%)
Escherichia coli	16.67	
Citrobacter freundii	16.67	
Bacillus species	50	
Morganella morgana	16.67	

Table 4: Showing morphological characteristics and identification of fungi isolated from houseflies

S/S	Media used	Colour of a special hyphae	Colour of substrate hyphae	Nature of hyphae	Shape and type of asexual structure	Presence of special structure	Apperance of sporangiophore/ conidiophore	Characteristics of spore head	Maximum days	Organism Isolated
A	PDA, SDA	Black	Brown	Non septate	Globose black conidiophore	Round column-like present	Non septate	Multinucleate	4	*Aspergillus niger*
	PDA, SDA	Clayish green (tight green)	Grayish blue	Non Septate	Globose grayish conidiophore	Foot cell present	Long erect and non-septate	Radiating	4	*Aspergillus niger*
	PDA, SDA	Cotton grey	White or bloused	Non septate	Globose black conidiophore	Branched sporangiosphore	Ellipsoidal sporangiosphore	Long spine	8	*Mucor circinelloides*
B	PDA, SDA	Black	Brown	Non septate	Globose black conidiophore	Round column-like present	Non septate	Multinucleate vesicle	4	*Aspergillus niger*
	PDA, SDA	Grayish green	Grayish blue	Septate	Globose black conidiophore	Foot cell present	Long erect and non-septate	Radiating sterigma	4	*Aspergillus fumigatus*
C	PDA SDA	Black	Brown	Non septate	Globose black conidiosphore	Round colum-like present	Long erect –non septae	Multinucleate vesicle	4	*Aspergillus niger*
	PDA SDA	Grayish green	Grayish blue	Septate	Globose gray conidiophore	Foot cell present	Long erect and non-septate	Radiating sterigma	4	*Aspergillus fumigatus*

A= Houseflies from students cafeteria B= Houseflies from Male hostels C= Houseflies from IBB food centres SS= Sampling sites

Table 5: Showing percentage frequency of occurrence of fungi isolated from houseflies

Organism Isolated	Frequency (%)
Aspergillus species	85.71
Mucor circinelloides	14.29

The fungal species isolated were *Aspergillus niger, A. fumigatus and Mucor circinelloides.* In a similar research, Davari *et al.* (2012) in Iran isolated *Aspergillus* species (66%), *Penicillium* species. (14%), *Fusarium* species (11.3%), *Alternaria* species (6%) and *Microsporum gypseum* (8.6%) were identified. Again, Banjo *et al.* (2005), isolated the following fungi from houseflies: *Alternaria* species, *Cladosporium* species, *Fusarium oxysporum, Aspergillus tamari* and *Penicillum axalicum.* In addition, Majid *et al.* (2007) isolated *Aspergillus, Penicillium, Yeasts, Cladosporium* and *Fusarium* and *Microsporum gypseum* and *Trichophyton mentagrophytes* (dermatophytes). The isolation of *Aspergillus* speciesfrom all locations may be as a result of the close association of the flies with the soil. The difference in species of fungi isolated in this study may be the result of differences in time, location and season as earlier stated.

Conclusion

The isolation of potential pathogens from flies in these locations investigated has validated the fact that flies are harbingers and serve as vectors for dissemination of pathogens especially via food. This calls for improved sanitation and maintenance of good hygiene in food centres in the tropical region where flies are abundant in the environment.

Acknowledgements

The authors wish to thank Dr. O. C. Ugbogu and Mr. A. C. Ogodo who out of their tight schedules availed themselves each time we consulted them. God bless you.

References

Anthony EH & Walkes AC 1962. An improvement in slide culture technique. *Cana. J. Microbio*., 8(6): 926-930.

Banjo AD, Lawal OA & Adeduji OO 2005. Banteria and fungi isolated from housefly (*Musca domestica*) larvae. *African J Biotech*., 4(8): 780-784.

Babak V, Setareh SS, Mahmoud R, Reza H & Manijeh M 2008. Identification of bacteria which possible transmitted by *Musca domestica* (Diptera: Muscidae) in the region of Hvaz, SW Iran. *Jundisshapur J. Microbi*., 1(1): 28-31.

Cheesbrough M 2000. District Laboratory Practices in Tropical Countries. Cambridge University Press, pp. 182-184.

Davari B, Khodavaisy S & Ala F 2012. Isolation of fungi from housefly (*Musca domestica*) at slaughter house and hospital in Sanandaj, Iran. *J. Medical Hygiene,* 53: 172-74.

Hamid K, Kamran A, Ansar G 2012. Isolation of pathogenic bacteria on the housefly, *Musca domestica L*. (Diptera: Muscidae), Body surface in Ahwaz hospitals, Southwestern Iran. *Asian Pacific Journal of Tropical Biomedicine*, (12)S1116-S1119.

Hussein SA & John LC 2014. Housefly, Musca domestica Linnaeus (Insecta: Diptera: Muscidae). *The Institute of Food & Agric Scie*., 47: 1-7.

Isabel CC 2005. Enterobacteria isolated from Synanthropic flies (Diptera, Calyptratae) in *Medellin, Colombia Caldasia*, 37(2): 319-332.

John BG 1952. The housefly (*Musca domestica* Linn) as a vector of *Salmonella pollorum* (Retteger) Bergy, the agent of white diarrhea of chickens. *The Ohio J. Sci*., 52(5): 287-290.

Leck A 1999. Preparation of Lactophenol Cotton Blue Slide Mounts. *Community Eye Health J*., 12(30): 24.

Majid Z, Babak V, Setareh SS, Ali ZM & Mahmoud R 2007. Isolation of fungi from Housefly (*Musca domestica*) in Ahwaz Iran. *Pak. J. Med Sci., (Part-II),* 23(6): 917-919.

Najat AK 2013. Transmission of bacterial pathogens by the house fly *Musca domestica vicina*. *Amer. J. Res. Comm*., 1(7): 1-12.

Nazni WA, Seleena B, Lee HL, Jeffery JIT, Rogayah TAR & Sofian MA 2005. Bacteria fauna from the house fly *Musca domestica* (L). *J. Trop. Biomedicine.* 22(2): 25-231.

Onyido AE, Nwangwu UC,Aribodor DN, Umeabaeto PU, Ugwu FM & Onwude CO 2012. Bacterial pathogens associated with wild-caught houseflies in Awka metropolis of Anambra State, South Eastern Nigeria. *New York Sci. J*., 7(12).

Szalanski AL, Owens CB, Mckay C & Steelman CD 2004. Detection of *Camphylobacter* and *Escherichia coli* O157:H7 from filth flies by Polymerase Chain Reaction. *Med. Vet. Entomol*., 101: 243-246.

Ugbogu OC, Nwachukwu NC & Ogbuagu UN 2006. Isoaltion of *Salmonella* and *Shigella* species from house flies (*Musca domestica* L.) in Uturu, Nigeria. *African J. Biotech*., 5(11): 1090-1091.

Waheeda I, Muhammad FM, Muhammad KS, Iqra A, Nadia Iram, Aqsad R. 2014. Role of housefly (*Musca domestica*, Diptera; Muscidae) as a disease vector. *J. Entomol. & Zool*., 2(2): 159-163.

Wasala I, Talley JL, Desilva U, Fletcher J & Wayadande A 2013. Transfer of Escherichia coli O157:H7 to spinach by houseflies, *Musca domestica* (Diptera: Muscidae). *Physiopathology,* 103: 373-380.

WHO. 2005. Guidelines for the Control of Shigellosis, Including Epidemics of Child and Adolescent Health and Development, Geneva.