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Isolation and Identification of Pathogenic Microorganisms from Houseflies

By Ibrahim, A. W., Ajiboye. T. O., Akande T. A. & Anibaba, O. O.
Kwara State Polytechnic

Abstract- The housefly, *Musca domestica,* is a fly of the suborder *Cyclorrhapha*. It is an important vector for transmission of pathogenic microorganisms including bacteria, fungi and parasites. A total of 27 houseflies were collected, 9 each from dumpsite, canteen and indoor premise with the use of insect traps. The bacteria isolates obtained are: *Escherichia coli*, *Salmonella* species, *Pseudomonas* species, *Shigella* species, *Klebsiella* species, *Staphylococcus* species, *Streptococcus* species, *Bacillus* species and *Proteus* species. The fungi isolated are *Aspergillus* species, *Penicillium* species, *Alternaria* species and *Fusarium* species. The parasites obtained include *Gairdia lamblia*, *Entamoeba histolytica*, *Enterobius vermicularis* and *Strongyloides* species. Dumpsite had the highest percentage occurrence of microorganisms, followed by indoor and food canteen which had the lowest percentage occurrence. The presence of these pathogenic microorganisms in food canteens, indoor toilets and dumpsite implied a possible risk of transmission of the pathogens from the houseflies to humans thereby causing diseases; hence, the need to step up control measures against the insects.

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Isolation and Identification of Pathogenic Microorganisms from Houseflies

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Abstract- The housefly, *Musca domestica,* is a fly of the suborder *Cyclorrhapha*. It is an important vector for transmission of pathogenic microorganisms including bacteria, fungi and parasites. A total of 27 houseflies were collected, 9 each from dumpsite, canteen and indoor premise with the use of insect traps. The bacteria isolates obtained are: *Escherichia coli*, *Salmonella* species, *Pseudomonas* species, *Shigella* species, *Klebsiella* species, *Staphylococcus* species, *Streptococcus* species, *Bacillus* species and *Proteus* species. The fungi isolated are *Aspergillus* species, *Penicillium* species, *Alternaria* species and *Fusarium* species. The parasites obtained include *Gairdia lamblia*, *Entamoeba histolytica*, *Enterobius vermicularis* and *Strongyloides* species. Dumpsite had the highest percentage occurrence of microorganisms, followed by indoor and food canteen which had the lowest percentage occurrence. The presence of these pathogenic microorganisms in food canteens, indoor toilets and dumpsite implied a possible risk of transmission of the pathogens from the houseflies to humans thereby causing diseases; hence, the need to step up control measures against the insects.

Keywords: housefly; musca domestica, bacteria, fungi, parasites, food canteens, toilets and dumpsites.

I. Introduction

The housefly, *Musca domestica* is the most common fly species found in habitations such as refuse dumps, toilets, domestic waste bins and other areas of poor sanitary conditions. Houseflies enter several places, including contaminated premises because of their own biologic habits for feeding (Service, 2000). It is not only a nuisance pest but also acts as an important vector for lots of pathogenic microorganism including bacteria, protozoa, fungi and viruses among humans and animals (Hussein and John, 2017). Houseflies transmit these disease agents by means of different parts of their bodies (hairs body, appendages and mouth parts) and secretions (regurgitates and faeces) (Babak *et al*., 2008).

Furthermore, they enhance the spread of diseases such as cholera caused by *Vibrio cholerae*, typhoid and paratyphoid fever by *Salmonella typhi* and *Salmonella paratyphi*, bacillary dysentary caused by *Shigella* species, tuberculosis caused by *Mycobacterium tuberculosis*, anthrax caused by *Bacillus anthracis* and many others amongst human's population as well as their livestock (Isabel, 2015). Some of the fungal diseases transmitted by houseflies include: Aspergillosis caused by *Aspergillus* species, Penicilliosis caused by *Penicillium* species, Onychomycosis caused by *Fusarium* species, Alternariosis caused by *Alternaria* species, etc (Davari *et al*., 2012). Houseflies have been identified as vectors of protozoan parasites that causes diseases such as Sarcocystis caused by *Sarcocystis* species, Toxoplasmosis caused by *Toxoplasma gondii*, Isosporiasis caused by *Isospora* species, Giardiasis caused by *Giardia* species, Amoebiasis caused by *Entamoeba histolytica*, etc (Thaddeus *et al*., 2005). Pathogenic organisms are picked up by flies from garbage, sewage and other sources of filth, and then transferred on their mouth parts and other body parts through their vomits, faeces and contaminated external body parts to human and animal food (Babak *et al*., 2008). Macovei and Zurek (2006) have reported which houseflies in food-handling and serving facilities habour and may have the capacity to transfer antibiotic-resistant and potentially virulent strains of pathogenic microorganisms.

Musca domestica is capable of carrying a variety of bacteria, viruses, fungi and parasitic diseases over its body appendages and can therefore pose a threat to the societal health, but despite the awareness of the dangers posed by houseflies, the inability to maintain a good sanitation leads, improper handling of food, indiscriminate refuse dumping and little or no care of toilet facilities have led to an increase in the population of houseflies. Therefore, the aim of this study was to isolate and identify bacteria, fungi and parasites picked up by houseflies in the food handling setting, dumpsite and indoor premises.

II. Materials and Methods

a) Sample collection

A total of 27 houseflies were collected, 9 samples each, from dumpsite, canteens and indoor premises in Ilorin, Kwara state. The method of collection was by the use of an insect trap to capture the houseflies, and then the houseflies were killed by exposure to chloroform for few minutes in the traps and then placed in sterile universal bottles individually, properly labeled and transported immediately to the laboratory.

Author α σ ρ ω: Department of Science Laboratory Technology, Microbiology unit, Kwara State Polytechnic, Kwara State, Nigeria.
e-mail: tezyakande@yahoo.com

b) Preparation of media

All media were prepared in the Erlenmeyer flask according to the manufacturer's instructions. The media used were MacConkey agar, Nutrient agar, Potato dextrose agar, Citrate agar, Sulphide Indole Motility (SIM) agar and Triple Sugar Iron (TSI) agar. The media used were sterilized in the autoclave at 121°C for 15 minutes.

c) Isolation and maintenance of the isolates

The houseflies were each placed in a test tube containing 1.0ml sterile normal saline by using a pair of forceps, the flies were gently rinsed by stirring with a glass rod in order to wash the microbial flora on the external parts of the houseflies into the normal saline then a drop of the normal saline from each tube was inoculated on Nutrient, Potato dextrose and MacConkey agar plates by streaking with the use of a flame sterilized inoculating loop, this was done in duplicates and around the flame to maintain aseptic condition.

The remnant was centrifuged and decanted to obtain the concentrate which was later used to make a wet mount and was examined using the 10X and 40X objective of the microscope for the presence of parasites.

The houseflies were then collected from the test tubes and washed in ethyl alcohol to decontaminate their surfaces. They were then washed in normal saline to wash off excess alcohol that may affect the internal microbial flora during dissection. The flies were then each placed on a sterile slide where they were dissected under a dissecting microscope. The guts were obtained and placed in test tubes containing 1.0ml of normal saline and homogenized. The resulting mixture was cultured and incubated in the same way as the external body surface.

Inoculated plates were then incubated in an inverted position aerobically at 37°C for 24 hours. After 24 hours of incubation, distinct colonies were selected randomly and subcultured on nutrient agar and potato dextrose agar plates to obtain pure cultures. This is then incubated aerobically in an inverted position at 37°C for 24 hours. For maintenance of the isolates, 24 hours pure culture of the isolates were transferred to nutrient agar and potato dextrose agar slant and stored at 0-4°C.

d) Identification of the bacterial isolates

The cultured bacterial isolates were identified using morphological characteristics namely: Colonial morphology, Gram staining and Endospore staining and biochemical characteristics namely: Catalase test, Oxidase test, Coagulase test, Citrate utilization test, sugar fermentation test (lactose, glucose and sucrose), hydrogen sulphide production, indole production and motility test as described by Cheesebrough (2006) and Fawole and Osho (2007).. Triple Sugar Iron (TSI) agar and Hydrogen Sulphide, Indole and Motility (SIM) agar were used for sugar fermentation, H_2S production, Indole production and Motility tests respectively. The bacterial isolates were identified based on their biochemical characteristics using Bergey's Manual of Determinative Bacteriology (Bergey and John, 1994).

e) Identification of the fungal isolates

The cultured fungal isolates were identified using colonial morphology and microscopically using Lactophenol blue staining.

f) Identification of the parasites

The parasites were identified microscopically by making wet mount of the parasites and examinining them using the 10X and 40X objective of the microscope for the presence of parasites.

III. Results

a) Bacteria identification

Out of the 30 bacteria isolates obtained in this study, twenty three (23) of the bacteria were Gram negative (nineteen (19) from the external surface of the houseflies and four (4) from the intestinal guts of the houseflies) while seven (7) of the bacteria isolates were Gram positive (external surface of the houseflies). The bacterial isolates were further characterized based on the morphological and biochemical identifications as shown in table 1, 2 and 3. In all, nine (9) bacterial genera were identified.

The Gram negative bacteria isolates from the external surface of the houseflies were identified as *Escherichia coli* (36.8%), *Salmonella* species (26.3%), *Pseudomonas* species (5.3%), *Shigella* species (26.3%) and *Klebsiella* species (5.3%) as shown in figure 1.

The Gram positive bacteria isolates from the external surface of the houseflies were identified as *Staphylococcus* species (42.9%), *Streptococcus* species (28.6%) and *Bacillus* species (28.6%) as shown in figure 2.

The Gram negative bacteria isolates from the internal surface of the houseflies were identified as *Escherichia coli* (50%), *Klebsiella* species (25%) and *Proteus* species (25%) as shown in figure 3.

The microorganisms isolated from the different study sites were compared to each other, Canteen (26.7%), dumpsite (43.3%) and indoor (30%) as shown in figure 4.

Table 1: Morphological and Biochemical Identification for the Gram Negative Bacteria Isolated from the External Surface of the Houseflies

Isolates	Gram reaction	Morphology	Catalase	Oxidase	H_2S	Indole	Motility	Citrate	Glucose	Sucrose	Lactose	Gas	Probable organisms
CA1	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*
CA2	-	Rods	+	-	+	-	+	-	A	-	-	-	*Salmonella* sp.
CA4	-	Rods	+	-	+	-	+	-	A	-	-	-	*Salmonella* sp.
CA5	-	Rods	+	+	-	-	+	+	-	-	-	-	*Pseudomonas* sp.
CB1	-	Rods	+	-	-	+	-	-	-	-	-	-	*Shigella* sp.
CB2	-	Rods	+	-	+	-	+	-	A	-	-	-	*Salmonella* sp.
DA2	-	Rods	+	-	-	+	-	-	-	-	-	-	*Shigella* sp.
DB1	-	Rods	+	-	-	-	-	+	A	A	A	+	*Klebsiella* sp.
DB2	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*
DB3	-	Rods	+	-	-	+	-	-	-	-	-	-	*Shigella* sp.
DB5	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*
DC1	-	Rods	+	-	+	-	+	-	A	-	-	-	*Salmonella* sp.
DC4	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*
DC5	-	Rods	+	-	-	+	-	-	-	-	-	-	*Shigella* sp.
IA1	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*
IA2	-	Rods	+	-	+	-	+	-	A	-	-	-	*Salmonella* sp.
IB1	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*
IC2	-	Rods	+	-	-	+	-	-	-	-	-	-	*Shigella* sp.
IC3	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*

Table 2: Morphological and Biochemical Identification for the Gram Positive Bacterial Isolated from the External Surface of the Houseflies

Isolates	Gram reaction	Morphology	Spore	Catalase	Coagulase	H_2S	Indole	Motility	Citrate	Glucose	Lactose	Sucrose	Gas	Probable organisms
CA3	+	Cocci	-	+	+	-	-	-	+	A	A	A	-	*Staphylococcus* sp.
DA1	+	Cocci	-	-				-	-	A	A	A	-	*Streptococcus* sp.
DB4	+	Rods	+	+			-	+	+	A	-	A	-	*Bacillus* sp.
DC2	+	Cocci	-	+	+	-	-	-	+	A	A	A	-	*Staphylococcus* sp.
DC3	+	Cocci	-	+	+	-	-	-	+	A	A	A	-	*Staphylococcus* sp.
IB2	+	Rods	+	+			-	-	+	A	-	A	-	*Bacillus* sp.
IC1	+	Cocci	-	-		-	-	-	-	A	A	A		*Streptococcus sp.*

Table 3: Morphological and Biochemical Identification for the Gram Negative Bacteria Isolated from the Intestinal guts of the Houseflies

Isolates	Gram reaction	Morphology	Catalase	Oxidase	H_2S	Indole	Motility	Citrate	Glucose	Sucrose	Lactose	Gas	Probable organisms
C1	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*
D1	-	Rods	+	-	-	-	+	+	A	A	A	+	*Klebsiella* sp.
I1	-	Rods	+	-	+	-	+	+	A	-	-	+	*Proteus* sp.
I2	-	Rods	+	-	-	+	+	-	A	A	A	+	*Escherichia coli*

KEY
C= Canteen
D= Dumpsite
I= Indoor
+ = Positive
- = Negative
A = Acid production

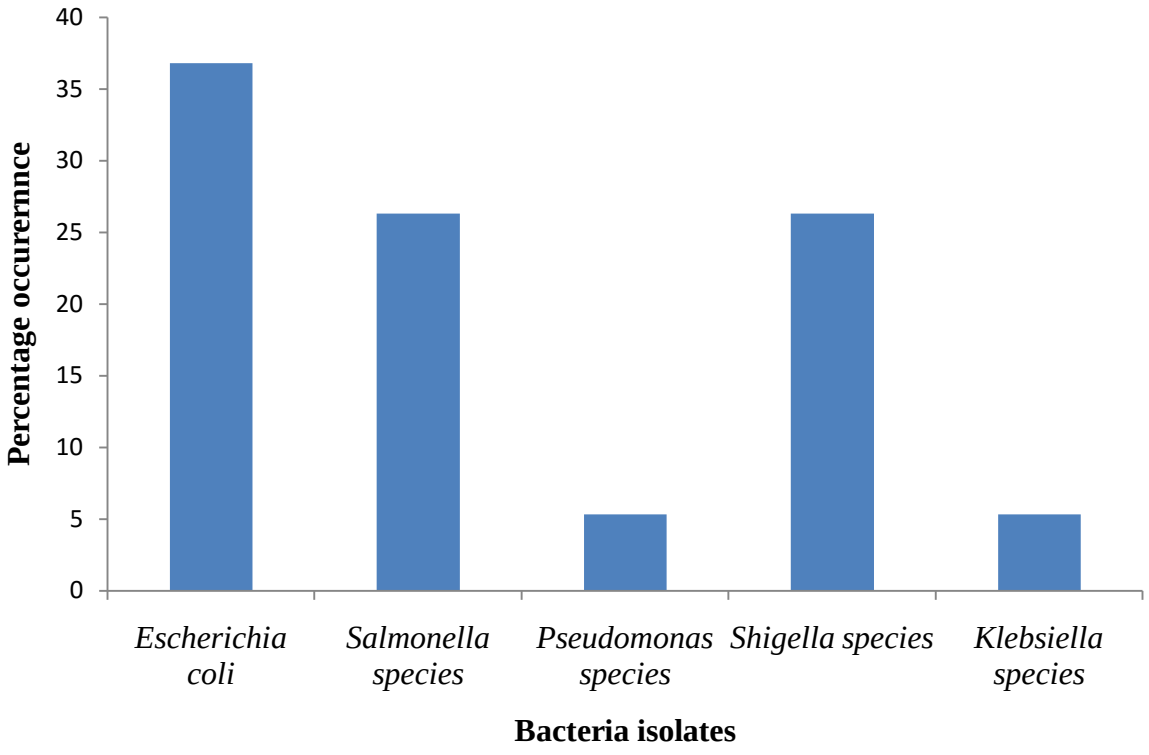

Figure 1: Percentage occurrence of Gram negative organisms from the external surface of the houseflies

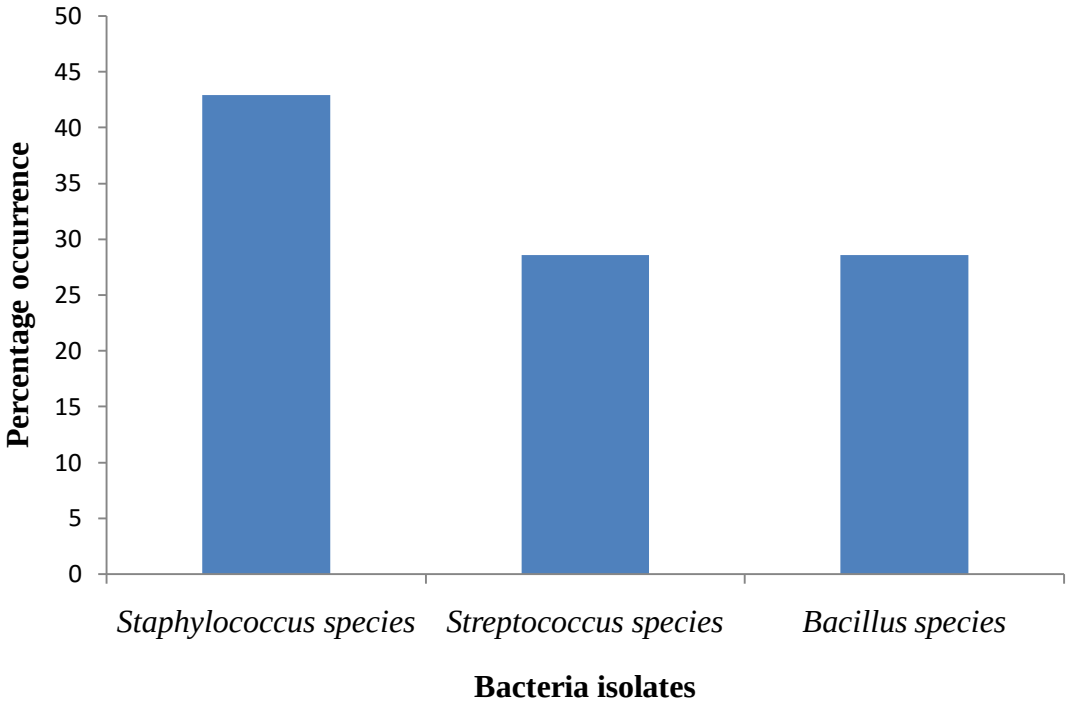

Figure 2: Percentage occurrence of Gram positive bacteria from the external surface of the houseflies

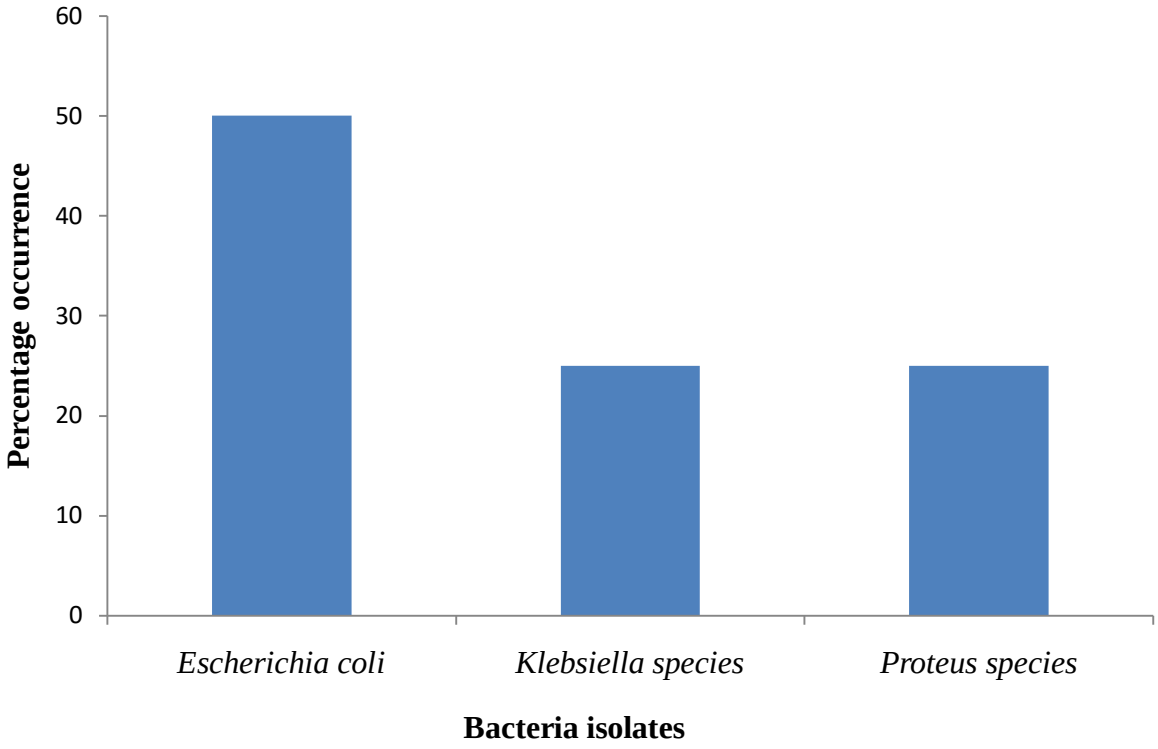

Figure 3: Percentage occurrence of bacteria isolates from the intestinal guts of the houseflies

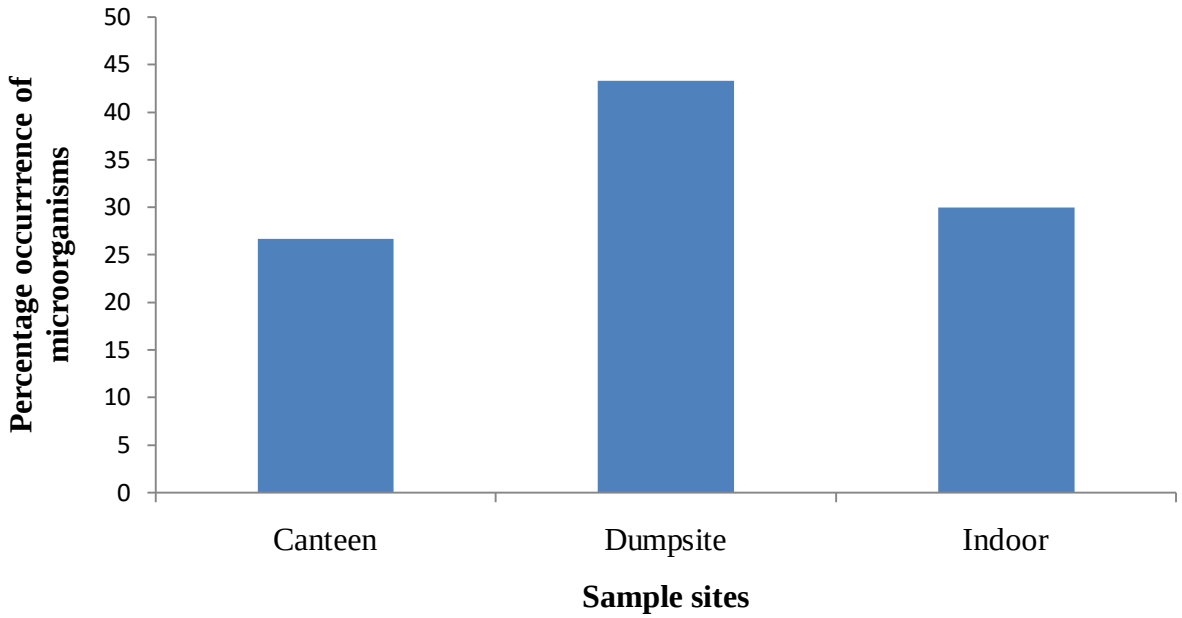

Figure 4: Percentage occurrence of microorganisms in the different sample sites

IV. Fungi Identification

Out of 10 fungi isolates, three (3) were *Aspergillus* species, four (4) were *Penicillium* species, one (1) was *Alternaria* species and two (2) were *Fusarium* species as shown in figure 5. In all, four (4) fungal genera were isolated. They were identified based on microscopic examination.

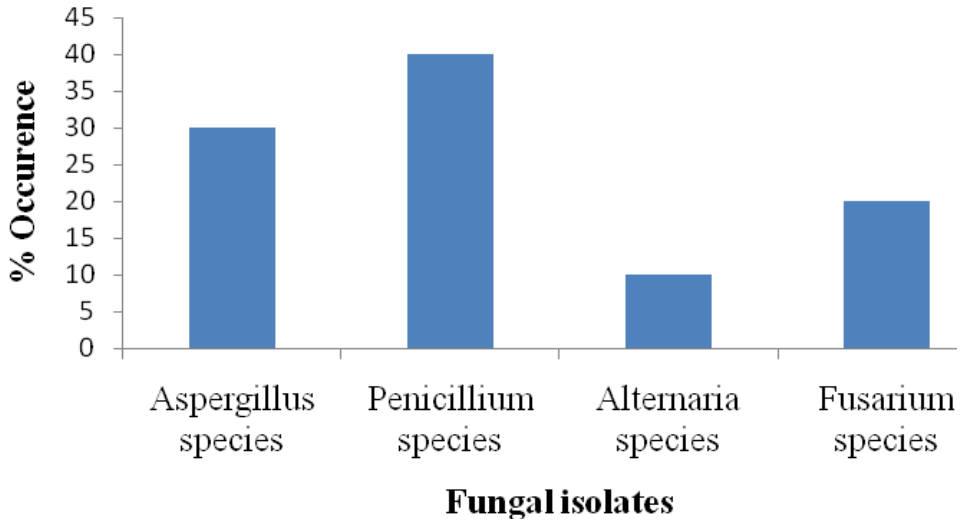

Figure 5: Percentage occurrence of fungi isolates from the external surface of the houseflies

V. Parasites Identification

A total of twenty two (22) parasites were identified based on microscopic examination, eight (8) were *Entamoeba histolytica* (36.36%), eight (8) were *Giardia lamblia* (36.36%), two (2) were *Enterobius vermicularis* (9.09%) and four (4) were *Srongyloides* spp (18.18%) as shown in table 4 and figure 6. In all, four (4) parasites were identified.

Table 4: Parasites identified and their percentage occurrence

Species of parasites	Class	No. Isolated	Percentage
Entamoeba histolytica	Protozoa	8	36.36
Girdia lamblia cyst	Protozoa	8	36.36
Enterobius vermicularis egg	Nematode	2	9.09
Strongyloides spp. egg	Nematode	4	18.18

VI. Discussion

The result of this study confirmed the mechanical transmission of pathogenic microorganisms by housefly, *Musca domestica*. Some of the bacteria genera isolated in this study such as *Klebsiella* species, *Bacillus* species, *Staphylococcus* species and *Pseudomonas* species correlates with the findings of Hamid *et al*., (2012). The bacteria species isolated from the outer parts of the houseflies include *Escherichia coli* (which was the most frequently occuring), *Staphylococcus* species, *Shigella* species, *Streptococcus* species, *Salmonella* species, *Klebsiella* species and *Pseudomonas* species is similar to the findings of Mawak and Olukose (2006) and Babak *et al*., (2008). The isolation of Salmonella is quite notable because salmonellosis is currently regarded as one of the most common food – borne zoonotic infections in the world causing diarrhea (Songe *et al*., 2017). From the intestinal parts of the houseflies, *Escherichia coli*, was the most frequently occurring and *Klebsiella* species and *Proteus* species being the least frequently occuring is in agreement with the findings of Mawak and Olukose (2006). The isolation of some bacteria from houseflies in this study not only corroborate the findings of some earlier studies, but also raises the possibilities of spread of antibiotic resistant pathogens as some of the similar pathogens isolated in other studies have been shown to have antibiotic resistance. Hemmatinezhad *et al*., (2015) reported the isolation of antimicrobial resistant strain of Pseudomonas aeruginosa in Iran. β -lactamase-producing *Escherichia coli* was isolated from houseflies in Spanish broiler farms (Solar-Gines *et al*., 2015) highlighting the potential contribution of houseflies to the rise and spread of virulence and resistance genes into different ecological niches. *Aspergillus* species, *Penicillium* species, *Fusarium* species and *Alternaria* species were isolated in this study and this is similar with the findings of Davari *et al*., (2012). The detection of *Giardia lamblia*, *Entamoeba histolytica*, *Enterobius vermicularis* and *Strongyloides stercoralis* correlates with Mawak and Olukose (2006).

VII. Conclusion

The presence of these pathogens which include *Escherichia coli*, *Staphylococcus* species, *Klebsiella* species, *Entamoeba histolytica*, *Girdia lamblia*, *Aspergillus* species, etc. in houseflies found in food canteens, dumpsite and indoor coupled with their intimacy with man and their highly motile nature implies a possible risk of transmission of the pathogens from the houseflies to humans thereby causing diseases. To prevent this, control measures against the houseflies must be employed such as enforcing strict legislatory standards to ensure hygienic condition of places like food canteens, public toilets and dumpsite, proper hygiene and environment sanitation should be practised and the public should be enlightened on the dangers of poor sanitation. Insecticides, fly traps, etc. should be used as control measures against these houseflies.

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