African Journal of Pure & Applied Sciences

DOI:10.33886/ajpas.v5i1.492

Journal homepage: http://journal.ku.ac.ke/index.php/AJPAS

Research Article Open Access

Bacterial communities of the *Musca domestica* housefly in different urban habitats and their antimicrobial susceptibility profiles

Zainab Adesewa Adebayo[1*], Fatima Ogunyele[1], Oluwadamilare Ganiu Dauda[2], Harun Kolawole Aremu[3], Olawale Olufemi Adelowo[1]

[1]Department of Microbiology, University of Ibadan, Nigeria

[2] Department of Zoology, Osun State University, Osogbo, Nigeria

[3]Department of Biochemistry, Osun State University, Osogbo, Nigeria

*Corresponding author: Zainab Adesewa Adebayo

Email: adebayozainab518@gmail.com

Published online 30th June 2024

Citation: Zainab Adesewa, A., Fatima, O., Ganiu Dauda, O., & Kolawole Aremu, H. Bacterial Communities of the Musca Domestica Housefly in Different Urban Habitats and their Antimicrobial Susceptibility Profiles. African Journal of Pure and Applied Sciences, 5(1). https://doi.org/10.33886/ajpas.v5i1.492

Abstract

Houseflies are a nuisance and can potentially transmit diseases to humans and animals due to their abundance and proximity to human habitats. Hence, this study aims to investigate the bacterial contaminations of house flies in different urban habitats and determine the resistance of these bacteria against various antibiotics. A total of 80 houseflies were collected from four different locations, namely the Bodija abattoir, household, poultry and piggery. The surface microbiota of the houseflies was identified using standard microbiological procedures at the genus level and subjected to antimicrobial susceptibility tests and extended-spectrum beta-lactamases (ESBL) screening. The results showed that there were more bacterial isolates from houseflies in abattoir than those from households, poultry and piggeries. The number of isolates included *Proteus* spp. (n = 30), *Citrobacter* spp. (n = 19), *Enterobacter* spp. (n = 9), *Klebsiella* spp. (n = 3), *Salmonella* spp. (n = 3), *Aeromonas* spp. (n = 3), and *Edwardsiella* spp. (n = 1). Antimicrobial susceptibility assay revealed that *Pseudomonas* spp., *Salmonella* spp., *Edwardsiella* spp., *Aeromonas* spp., *Enterobacter* spp. and *Klebsiella* spp. have relatively high resistance to ceftazidime, ciprofloxacin, trimethoprim, ertapenem, ceftazidime, ceftriaxone, cefpodoxime and cefoxitin as compared to *Citrobacter* spp., and *Proteus* spp. ($p < 0.05$). The results of the ESBL screening showed that all the bacterial isolates were resistant to multiple antibiotics. In conclusion, our study demonstrated that the most harmful bacteria are harboured by houseflies from abattoirs, and these organisms have the ability to spread diseases that are resistant to antibiotics. Specifically, the spread of houseflies poses a risk to public health since they may contribute to the environmental spread of infectious diseases.

Keywords: Antimicrobial resistance, *Musca domestica*, multiple drug resistance, bacterial community

1. Introduction

Houseflies are nuisance and can potentially transmit diseases to humans and animals due to their abundance and proximity to human habitats. Microbes can be transmitted by flies in three ways: by their droppings, through touching infected body parts, and through regurgitation while feeding (Stoffolano, 2019). Alternatively, they can be seen as a measure of environmental quality, as they proliferate in polluted environments and absorb bacteria by ingesting or adsorption to the surface of the exoskeletons (Graczyk *et al.,* 2001). In developing nations, houseflies are key epidemiologic factors that cause trachoma and acute gastroenteritis in infants and young children, as well as nosocomial infections with bacteria that are resistant to several medications in healthcare settings (Onyido *et al.,* 2014).

The species *Musca domestica* L. (Diptera: Muscidae) is a resilient commensal insect that exhibits adaptability and survives in several ecological conditions. This organism inhabits the vicinity of humans and domesticated animals, and is commonly encountered in residences, food markets, agricultural establishments, and livestock farms. Its diet consists of decomposing organic matter, waste, excrement, and human consumption. House flies often defecate while feeding or resting, leaving behind fly specks and other organisms that pass through their digestive tract (Saleha *et al.,* 2011). It is widely distributed around the world and is frequently found in close proximity to dwellings of human beings.

House flies are tightly associated with microorganisms (Sudagidan *et al.,* 2022). Recent findings have revealed the presence of antibiotic-resistant bacteria in flies inhabiting diverse environments, including a multitude of antibiotic-resistant strains of pathogenic bacteria. The natural capacity of flies to absorb bacteria and traverse great distances, may have contributed to the spread of extended-spectrum beta-lactamase (ESBL)-producing bacteria is particularly worrisome (Monyama *et al.,* 2023). Antibiotic resistance is a significant concern in public health due to its detrimental impact on treatment efficacy and heightened risks to public well-being (Musa *et al.,* 2020a). As a result, there is increasing concern regarding the transmission of antibiotic-resistant bacteria via flies.

The microbiota of *M. domestica* has been the subject of recent research, which has specifically examined samples collected from diverse environments and countries (Sudagidan *et al.,* 2022;

Monyama *et al.,* 2023). Proteobacteria, Bacteroidetes, and Firmicutes have been identified as significant phyla within the microbiome of house flies. Some bacteria transmitted by houseflies include *Acinetobacter baumanni*, which is linked with meningitis; *Bacillus pumilus,* a pathogen associated with foodborne illness; and *Enterobacter sakazakii,* a pathogen known to cause infections in the bloodstream, lungs, and urinary tract. (Butler *et al.,* 2010). It might function as a potential conduit for the spread of antimicrobial resistance in human pathogens (Van de Bogaard *et al.,* 2000). Houseflies carrying such bacteria could pose a threat to therapeutic options and increase the spread of resistant bacteria. The burden of antibiotic resistance in the community is significantly increased by the spread of ESBL-producing bacteria (Van den Bunt *et al.,* 2017). Houseflies have been identified as vectors of antibiotic-resistant bacteria in multiple investigations conducted in different communities (Sudagidan *et al.,* 2022; Nayduch *et al.,* 2023; Monyama *et al.,* 2023). Subsequently, this study aimed to investigate the bacterial contaminations of house flies captured from abattoir market in Bodija, as well as from households, poultry and piggery farms in Ibadan, Oyo State, Nigeria and also assess the antimicrobial susceptibility profiles of these bacteria against various antibiotics.

2. Materials and Methods

2.1 Informed consent

The study was approved by Faculty members of the Department of Microbiology, University of Ibadan. No specific authorizations were required to acquire samples. However, consent was obtained from the Market Traders and Farmers Associations prior to collecting the samples from different locations.

2.2 Sample collection

A total of 80 houseflies (20 from each location) were collected separately for 2 months using a sterile sweep net from four different locations (Fig. 1) namely the Bodija abattoir (7°26’06.3”N 3°54’51.6”E); Abdulsalam Abubakr hall, University of Ibadan (7°26’20.6”N 3°53’40.3”E), Poultry, University of Ibadan research farm (7°26’40.6”N 3°54’53.9”E) and Piggery, University of Ibadan research farm (7°24’15.0”N 3°52’54.2”E) between the morning and afternoon each day for 4 weeks when the flies were active. The major activities in the sample sites include piggery and poultry farming, extensive waste dump from household and slaughterhouse. The annual

rainfall ranges from 1 750 to 2 000mm and a mean annual temperature range of 26°C to 28°C. A pool of 20 flies from each location were placed in a labelled universal transparent container and kept in a freezer for 15 minutes for anesthetization. Thereafter, the flies were carefully picked with a sterilized forceps and emulsified in test tubes containing 5 mL of peptone water and incubated at 37°C for 24 hours for enrichment. All analyses were carried out at the Environmental Microbiology and Biotechnology Laboratory Department of Microbiology, University of Ibadan.

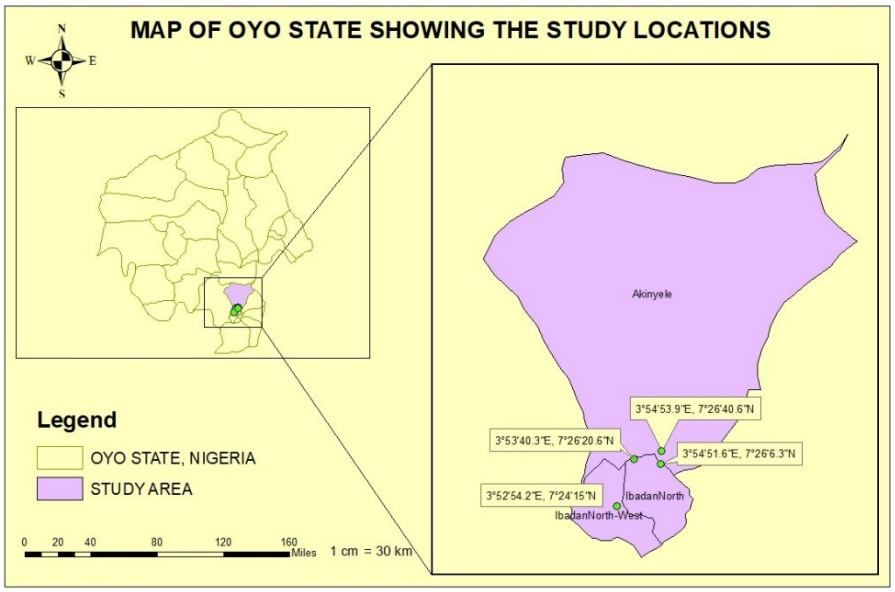

Figure 1: Map of Sample locations

2.3 Bacteria isolation and purification

The total heterotrophic bacteria count from the external surface of the flies was obtained using the pour plate technique according to Khatter (2013). Ten-fold serial dilutions of the prepared samples were carried out and 1 ml of each sample source was picked and plated on Mueller Hinton agar. The plates were incubated at 37°C for 24 hours. Each experiment was carried out in triplicate and the results were expressed as mean colony forming units (CFUs). Total cefotaxime resistant bacteria count was determined using Mueller Hinton agar incorporated with a beta-lactam antibiotic, cefotaxime (8µg/ml) (a 3rd generation cephalosporin). A serially diluted sample (1 ml) was transferred to a freshly prepared plate containing antibiotic in duplicate. The plates

were incubated aerobically at 37°C for 24 hours to determine the number of antibiotic-resistant bacteria (Mir *et al.,* 2016). After 24 hours incubation, the colonies were sub-cultured for another 24 hr at 37°C for pure colonies and stored in cryovials containing nutrient broth with 15 % glycerol awaiting further analysis.

2.4 Extended-spectrum beta-lactamases (ESBL) screening and bacterial identification

The double-disc synergy method, as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2021), was employed to perform phenotypic testing for the synthesis of ESBL. Every individual sample was placed in sterile saline water, and the clarity was modified to meet the 0.5 McFarland reference standard. A swab stick was used to inoculate the whole surface of a Mueller Hinton agar plate by swabbing in three directions (Ogefere *et al.,* 2015). In the middle, a disk containing 30 µg of amoxicillin-clavulanic acid (Oxoid, England) was positioned. Subsequently, disks containing ceftazidime, cefotaxime, and cefpodoxime were placed 15-20 mm from each other. Thereafter, the plates were incubated at 37°C for 24 hours, and antibiotic effects were assessed. The presence of clavulanate was found to enhance the inhibitory zones of extended-spectrum beta-lactamases, indicating the formation of ESBL- (Ogefere *et al.,* 2015). The organisms present were identified by characterizing the isolates to the genus level using various macroscopic, cultural, morphological, and biochemical characteristics. These characteristics included Gram staining reaction, oxidase, catalase, methyl red, Voges Proskauer, indole production, motility, urease, triple sugar iron, citrate utilization, and sugar fermentation.

2.5 Antimicrobial susceptibility testing

All the isolates under investigation were tested for antimicrobial susceptibility by the disk diffusion method, using the following antibiotics from Oxoid, England; ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), trimethoprim (5 µg), ertapenem (10 µg), cefpodoxime (10 µg), and cefoxitin (30 µg). The 0.5 McFarland standard was used to attune the turbidity of bacterial inoculums. The size of the zone of inhibition (mm) was observed and recorded as resistant, intermediate, or susceptible to different antibiotics based on recommendation by clinical laboratory standards institute (CLSI), (CLSI, 2016).

2.6 Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA). The study employed Duncan post-hoc tests ($p < 0.05$) to compare multiple means and detect disparities among groups with the IBM SPSS 25.0 software package.

3. Results

3.1 Total heterotrophic bacteria and total cefotaxime (8µg/ml) bacterial counts of the houseflies collected from different locations

The mean heterotrophic bacteria count and mean cefotaxime bacteria count for all the houseflies collected from different locations in colony forming units (CFU/ml) are shown in Table 1. The abattoir and poultry have the highest value of THBC with 26.4×10^8 cfu/ml followed by piggery farm with 25.2×10^8 cfu/ml while the lowest value of THBC was obtained from households with 4.9×10^8cfu/ml. Similarly, the highest TCBC value were obtained from the abattoir and poultry farms (4.24×10^8 cfu/ml and 3.86×10^8 cfu/ml respectively), followed by households (0.80×10^8 cfu/ml) while the lowest TCBC value was obtained from the piggery farm (0.41×10^8 cfu/ml).

Table 1: Total heterotrophic bacteria count (THBC) and total cefotaxime bacteria count (TCBC) (×108 cfu/ml) from houseflies collected from different sample sites ($\times 10^8$ cfu/ml)

Sampling point	Range of THBC	Range of TCBC	Mean THBC (±S.E)	Mean TCBC (±S.E)
Piggery	9.02 - 46.02	0.03 - 0.60	25.20 ± 8.50	0.41±0.14
Poultry	21.06 - 36.0	1.05 - 7.09	26.4 ± 3.30	3.86±1.26
Household	0.07 - 18.09	0.01 - 1.67	4.9 ± 4.50	0.80±0.36
Abattoir	10.10 - 50.8	0.20 - 9.08	26.4 ± 9.30	4.24±2.09

S.E- Standard Error

3.2 Detection of ESBL-producing bacteria using the double disk synergy test

Approximately 125 bacterial isolates were obtained from the four locations, 69 (55.2%) of which were positive for ESBL- production with piggery farm 6 (8.7%), poultry farm 16 (23.2%) while

household and abattoir had 18 (26.1%) and 29 (42%) respectively. The frequency of occurrence is shown in Table 2.

Table 2: Frequency of extended-spectrum beta-lactamases production from houseflies collected

Sample Source	**No of orgs obtained from each source**	**No of ESBLs positive from each source**
Piggery	29	6 (8.70%)
Poultry	**35**	16 (23.20%)
Household	**19**	18 (26.10%)
Abattoir	**42**	29 (42.00%)
Total		69 (55.20%)

Key: orgs – organisms; No.- Number

3.3 Frequency of ESBL-producing bacterial isolates

A total of sixty-nine ESBL- producing bacteria were obtained from four locations, piggery farm (6), poultry farm (16), household (18) and abattoir (29). The bacterial isolates belonged to eight genera: *Enterobacter* spp. (13%, 9/69), *Klebsiella* spp. (4.3%, 3/69), *Proteus* spp. (43.5%, 30/69), *Citrobacter* spp. (27.5%, 19/69), *Salmonella* spp. (4.3%, 3/69), *Aeromonas* spp. (4.3%, 3/69), *Edwardsiella* spp. (1.4%, 1/69), and *Pseudomonas* spp. (1.4%, 1/69). The *Proteus* spp. had the highest percentage (43.5%) of occurrence in abattoir, poultry, and piggery, while the *Citrobacter* spp. had a high occurrence in abattoir and households. *Edwardsiella* spp. and *Pseudomonas* spp. had the lowest occurrence among all the locations (Table 3).

Table 3: Frequence of ESBL- producing bacterial isolates at different sample sites

Organism	Sample Site				
	Piggery	Poultry	Household	Abattoir	Total
***Enterobacter* spp.**	0	1	6	2	9 (13%)
***Klebsiella* spp.**	1	0	1	1	3 (4.3%)
***Proteus* spp.**	4	8	3	15	30 (43.5%)
***Citrobacter* spp.**	1	4	6	8	19 (27.5%)

***Salmonella* spp.**	0	3	0	0	3 (4.3%)
***Pseudomonas* spp.**	0	0	1	0	1 (1.4%)
***Aeromonas* spp.**	0	0	1	2	3 (4.3%)
***Edwardsiella* spp.**	0	0	0	1	1 (1.4%)

3.4 Antibiotic susceptibility pattern of bacteria isolated from all four locations to selected antibiotics

Antibiotic susceptibility test revealed that the highest percentage of isolates were resistant to ceftazidime (94.2%, 65/69) followed by cefpodoxime (91.3%, 63/69), ceftriaxone (84.1%, 58/69), trimethoprim (79.7%, 55/69) while the lowest percentage was observed for ciprofloxacin (44.9%, 31/69) followed by cefoxitin (42%, 29/69), and ertapenem (30.5%, 28/69). Ertapenem (33.3%, 23/69) was the most sensitive against test bacteria followed by cefoxitin (26.1%, 18/69) as shown in Fig.. 2.

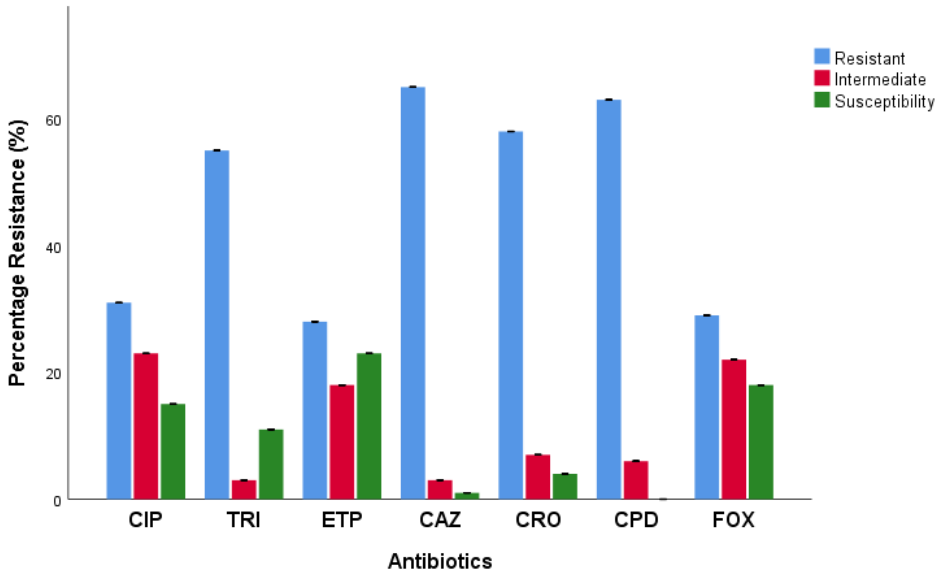

Figure 2: Antibiotic susceptibility profile of ESBL-producing bacteria. CIP: ciprofloxacin, TRI: trimethoprim, ETP: ertapenem, CAZ: ceftazidime, CRO: ceftriaxone, CPD: cefpodoxime, FOX: cefoxitin

3.5 Phenotypic antibiotic resistance pattern of ESBL-producing bacterial isolates (n=69)

Table 4 shows the resistance profiles of the isolated ESBL-producing bacteria to different selected antibiotics where the isolates showed resistance to two or more types of antibiotics. Approximately 97% (29/30) *Proteus* spp. identified were resistant to both ceftazidime and cefpodoxime, while 87% (26/30) were also resistant to both trimethoprim and ceftriaxone. All the *Enterobacter* strains 100% (9/9) were resistant to the three third-generation cephalosporins tested i.e. ceftazidime, ceftriaxone and cefpodoxime. However, *Klebsiella, Citrobacter, Edwardsiella, Aeromonas,* and *Salmonella* species also showed resistance to ceftazidime, ceftriaxone, cefpodoxime and trimethoprim at different proportions.

Table 4: Phenotypic antibiotic resistance pattern of ESBL-producing bacterial isolates

		Number of Resistant isolates (Percentage; %)						
Isolate	Total	CIP	TRI	ETP	CAZ	CRO	CPD	FOX
Klebsiella spp.	3	0 (0)	2 (67)	1 (33)	3 (100)	2 (67)	2 (67)	1 (33)
Proteus spp.	30	15 (50)	26 (87)	10 (33)	29 (97)	26 (87)	29 (97)	11 (37)
Enterobacter spp.	9	5 (56)	7 (78)	3 (33)	9 (100)	9 (100)	9 (100)	4 (44)
Citrobacter spp.	19	4 (21)	14 (74)	9 (47)	18 (95)	14 (74)	15 (79)	7 (37)
Salmonella spp.	3	3 (100)	1 (33)	2 (67)	2 (67)	3 (100)	3 (100)	1 (33)
Edwardsiella spp.	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Aeromonas spp.	3	2 (67)	2 (67)	2 (67)	2 (67)	2 (67)	3 (100)	2 (67)
Pseudomonas spp.	1	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)

CIP: ciprofloxacin, TRI: trimethoprim, ETP: ertapenem, CAZ: ceftazidime, CRO: ceftriaxone, CPD: cefpodoxime, FOX: cefoxitin

3.6 Prevalence of resistant bacteria against selected antibiotics

The resistance profiles of the bacterial isolates against selected antibiotics are shown in Table 5. The resistance patterns of the isolated bacteria varied significantly ($P < 0.05$). For instance, *Salmonella* spp., *Edwardsiella* spp., *Aeromonas* spp., *Pseudomonas* spp., *Enterobacter* spp. and *Klebsiella* spp. have relatively high resistance to ceftazidime, ciprofloxacin, trimethoprim,

ertapenem, ceftazidime, ceftriaxone, cefpodoxime and cefoxitin as compared to *Citrobacter* spp., and *Proteus* spp. ($P < 0.05$).

Table 5: Resistance profile of bacteria isolated from *M. domestica*

Isolate	CIP	TRI	ETP	CAZ	CRO	CPD	FOX
Citrobacter spp.	3.67 ± 0.33^{a}	14.67 ± 0.67^{c}	8.33 ± 0.67^{b}	18.33 ± 0.33^{d}	13.33 ± 0.67^{c}	14.67 ± 0.33^{c}	8.00 ± 0.57^{b}
Enterobacter spp.	5.00 ± 0.00^{ab}	6.00 ± 0.58^{c}	3.00 ± 0.00^{a}	9.67 ± 0.33^{d}	8.67 ± 0.33^{d}	9.00 ± 0.00^{d}	4.33 ± 0.33^{ab}
Proteus spp.	15.00 ± 0.58^{b}	26.33 ± 0.33^{cd}	10.67 ± 0.67^{a}	28.67 ± 0.33^{d}	25.00 ± 0.57^{c}	28.67 ± 0.33^{d}	11.00 ± 0.58^{a}
Klebsiella spp.	0.00 ± 0.00^{a}	2.67 ± 0.67^{cd}	1.00 ± 0.00^{ab}	3.33 ± 0.33^{d}	2.33 ± 0.33^{cd}	2.67 ± 0.67^{cd}	1.67 ± 0.67^{abc}
Pseudomonas spp.	1.33 ± 0.33^{ab}	1.00 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.33 ± 0.33^{d}	1.67 ± 0.67^{d}	1.33 ± 0.33^{ab}
Aeromonas spp.	2.33 ± 0.33^{a}	2.67 ± 0.33^{a}	2.67 ± 0.67^{a}	2.33 ± 0.33^{a}	1.67 ± 0.67^{a}	3.00 ± 0.57^{a}	2.33 ± 0.33^{a}
Edwardsiella spp.	1.00 ± 0.00^{a}	1.33 ± 0.33^{a}	1.33 ± 0.33^{a}	1.33 ± 0.33^{a}	1.67 ± 0.33^{a}	1.67 ± 0.67^{a}	1.67 ± 0.67^{a}
Salmonella spp.	3.33 ± 0.33^{b}	1.33 ± 0.33^{a}	2.00 ± 0.00^{ab}	2.33 ± 0.33^{ab}	2.00 ± 0.57^{ab}	3.00 ± 0.00^{b}	1.00 ± 0.00^{a}

CIP: ciprofloxacin, TRI: trimethoprim, ETP: ertapenem, CAZ: ceftazidime, CRO: ceftriaxone, CPD: cefpodoxime, FOX: cefoxitin

4. Discussion

Houseflies are not just nuisance but also pose significant health risks as mechanical vectors. Due to their widespread presence and ubiquitousness in human environments, various locations have been scrutinized for potential contamination by fly-borne pathogens. Sites such as garbage dumps, cattle barns, poultry farms, slaughterhouses, and hospitals are prime breeding grounds for houseflies, fostering close interactions between flies and humans. Given their interactions with

humans, food, manure, and animal waste, flies are suspected reservoirs and carriers of infections for both humans and animals. Additionally, houseflies can easily acquire and spread antibiotic-resistant bacteria, facilitating transmission between humans and animals. Research by Zurek and Gorham (2008) suggested that houseflies may act as a crucial link between human populations and other animal species. Furthermore, Butler et al. (2010) reported that houseflies are sources of various bacterial diseases in different regions worldwide.

The findings presented a culture-dependent characterization of the microbiota found in a common *M. domestica*, a vector of significant public health concern. The highest total heterotrophic count (THBC) a value of 26.4×10^8 CFU/ml was recorded from abattoir and poultry houseflies. Additionally, 25.2×10^8 CFU/ml was observed from houseflies collected from piggery farm while the lowest value 4.9×10^8 CFU/ml was observed from household houseflies. Earlier study by Ogbalu and Douglas (2015) reported a lower heterotrophic bacteria count range of 3.4×10^5 to 2.6×10^6 from houseflies collected from dumpsites which could be a a result of differences in sample sites. The increased heterotrophic count observed in abattoir and poultry houseflies may be due to high amounts of animal waste such as dung and faecal waste material. The primary aim of this investigation was to identify bacteria at the genus level. *Klebsiella*, *Proteus*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Edwardsiella*, *Aeromonas*, and *Pseudomonas* species were among the genera that were shown to be prevalent. It is well-established that these genera serve as hosts for potentially harmful species that hold clinical and veterinary importance. The findings shown here are consistent with prior studies conducted by Khamesipour *et al.* (2018) and Monyama *et al.* (2023), who similarly documented the presence of predominant pathogenic bacteria in houseflies obtained from diverse habitats. Vazirianzadeh *et al*. (2008) reported the isolation of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* spp., and *Proteus* spp., which aligns with the findings of this study, wherein *Proteus* spp. and *Pseudomonas* spp. were also isolated. In addition, Ugbogu *et al.* (2006) successfully isolated *Salmonella* (61.7%) and *Shigella* (100%) species from *M. domestica* samples collected at various sampling sites. In contrast, this study observed a lower occurrence of *Salmonella* spp. among the isolated samples. Additionally, a study conducted by Park *et al*. (2019) revealed a greater prevalence of the genera *Streptococcus* and *Escherichia-Shigella* in housefly specimens collected from hospitals and farms, respectively.

The genus *Proteus* spp., identified as the most prevalent among detected genera, consists of Gram-negative, facultative anaerobic, heterotrophic rods that are ubiquitous in nature and serve as opportunistic pathogens in humans (Drzewiecka, 2016). These bacteria play significant roles in human pathogenesis, acting as virulence factors that enable them to colonize various niches within the host organism and persist. Infections caused by *Proteus* spp. primarily affect individuals with compromised immune systems, often leading to complex urinary tract and wound infections, as well as nosocomial infections (Bittar *et al.,* 2014). The high prevalence of *Proteus* spp. observed in abattoir settings in this study aligns with existing literature. Notably, *Proteus* spp. are considered undesirable members of the intestinal microflora because they can also contribute to diarrhoea and are used as indicators of faecal pollution (Kefalas *et al.,* 2013).

Furthermore, this study investigated the presence of antibiotic resistance genes within the bacterial communities of houseflies. The highest value of total cefotaxime resistant bacteria count (TCBC) value of 4.2×10^8 CFU/ml and 3.86×10^8 CFU/ml was observed from abattoir and poultry houseflies respectively while the value 0.8×10^8 CFU/ml was observed from houseflies collected from the household. The lowest value 0.4×10^8 CFU/ml was observed from piggery houseflies. The present study has an ESBL-producing organism prevalence of 55.2% in houseflies collected from different sites. Iroha *et al.* (2009) reported ESBL-producing organism at a prevalence of 44.6% in Enugu and 6.7% in Ebonyi from clinical samples which implies a high prevalence from environmental samples.

Among the 125 gram-negative bacteria isolated from the four locations, approximately 69 (55.2%) were ESBL- producers. ESBL-production was found to be high in the genera *Proteus* (30), *Citrobacter* (19), and *Enterobacter* (9). Contrary results have been observed in a Clinical setting where *Klebsiella* species and *E. coli* were found to be the dominant ESBL-producing species present. Fatemeh *et al.* (2012) reported that 26.5% of *E. coli* and 43% of *K. pneumoniae* strains tested positive for ESBL. Their findings underscored a notable prevalence of ESBL-producing Enterobacteriaceae, particularly among inpatients.

The occurrence of ESBL in *Proteus* species (43.5%) are implicated to cause severe human infection and therefore important to public health. *Citrobacter* species (27.5%) are of great concern because infections mostly caused by these bacteria include urinary tract infection, sepsis, and infant meningitis. *Klebsiella*, *Salmonella, Aeromonas* produced ESBL at 4.3% each, while *Edwardsiella tarda* had 1.4% observed to be the lowest. These findings demonstrated a high

incidence of diseases in flies caught near animal habitats. In their study, García-Sánchez *et al.* (2018) reported that houseflies possess the capacity to transmit *Escherichia coli* O157:H7 to the digestive tract of cattle. This finding suggests that houseflies play a role in the ecological dynamics of this foodborne disease, which is prevalent among humans, within the context of cow production. Furthermore, these findings are consistent with those of Graham et al. (2009), who hypothesized that flies collected near broiler poultry operations could help spread drug-resistant bacteria from such facilities, increasing the risk of human exposure to drug-resistant strains.

The increase in acquired antibiotic resistance in bacteria has raised considerable concerns. This type of resistance is currently documented not only in pathogenic bacteria, but also in commensal bacteria, although antimicrobial medicines do not specifically target the latter category. Although it is relatively simple to detect multidrug-resistant bacteria in farm animal faeces, this method does not provide quantitative data or allow comparisons between animal populations (Faldynova *et al.,* 2013). Animal production, which is frequently characterized by massive antibiotic usage, is acknowledged to significantly affect the evolution of new antibiotic resistance combinations. The concerns include the increasing incidence of ESBL-producing bacteria and the advent of strains that are extensively resistant to third-generation cephalosporins. The lack of effective antibiotics substantially limits the treatment choices available for these bacterial species.

Among the studied antimicrobial agents, ertapenem (33.3%.) revealed as the only effective antibiotics against the entire ESBL-producing organism in this study, even though approximately 40% were resistant to this antibiotic. Carbapenems such as ertapenem have been found to be effective in the treatment of ESBL Gram-negative bacteria and are regarded as last resort antibiotic. Among the beta-lactam antibiotics used in this study, the ESBL-producing Enterobacteriaceae isolates showed resistance to ceftazidime (94.2%), cefpodoxime (91.3%), ceftriaxone (84.1%) possibly because the isolates acquired or possessed resistance genes against the effects of the given antibiotics. This finding aligns with a study conducted by Nasreen *et al.* (2015), which revealed that all the isolates exhibited complete resistance to third-generation cephalosporins. Additionally, Knudsen *et al.* (2014) highlighted that the prior use of antimicrobial agents, particularly cephalosporins and fluoroquinolones, has been identified as a risk factor linked with the emergence of ESBL. The isolates also showed resistance to other antibiotics such as trimethoprim (79.7%) and ciprofloxacin (44.9%) but low resistance was

observed for ertapenem (40.5%) and cefoxitin (42%). The resistance observed may be due to the excessive use of antibiotics in farmhouses, slaughterhouses and among humans in the treatment of bacterial infection. Antibiotics in the environment can exert prolonged selective pressure, contributing to the spread of these resistant bacteria (He *et al.,* 2020; Musa *et al.,* 2020b).

Multiple drug resistance patterns were observed in which the isolates were resistant to combinations of two or three more antibiotics such as trimethoprim, ceftazidime, ceftriaxone, and cefpodoxime. The work of Rahuma *et al*. (2005) coincides with the present study in which bacterial strains from households showed multidrug resistance to antibiotics. The findings of this study provide confirmation that houseflies poses significance beyond being mere nuisances, as they have the potential to pose substantial health hazard as mechanical vectors. A notable proportion of houseflies collected from both human and animal habitats exhibited the presence of ESBL-producing bacteria. These findings indicated a risk of human exposure and the occurrence of multidrug resistance which could limit therapeutic options for the treatment of infections caused by these ESBL-producing bacteria**.**

Conclusion

Houseflies possess diverse and extensive bacterial microbiotas on their external surfaces, with specific microorganisms varying depending on their habitat. Culture-based analysis has shown that houseflies carry bacteria resistant to antibiotics, posing a substantial threat to antibiotic efficacy and contributing to the spread of resistance determinants. Consequently, it is imperative to enforce appropriate safety measures to prevent food contamination and infections transmitted by houseflies.

Acknowledgments

The Laboratory unit at the Department of Microbiology, University of Ibadan, Nigeria is graciously appreciated for their support.

References

Bittar, F., Keita, M. B., Lagier, J.-C., Peeters, M., Delaporte, E., & Raoult, D. (2014). Gorilla gorilla gorilla gut: A potential reservoir of pathogenic bacteria as revealed using culturomics and molecular tools. Scientific Reports. https://doi.org/10.1038/srep07174

Butler, J., Garcia-Maruniak, F. A., Meek, F., & Maruniak, J. E. (2010). Wild Florida house flies (Musca domestica) as carriers of pathogenic bacteria. Florida Entomological Society, 93(2), 218–223.

Clinical and Laboratory Standards Institute (CLSI). (2016). Performance standards for antimicrobial susceptibility testing: Eighteenth informational supplement (CLSI Document M100-S27). Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute (CLSI). (2021). Performance standards for antimicrobial susceptibility testing (31st ed.). CLSI supplement M100. Wayne, PA: CLSI.

Drzewiecka, D. (2016). Significance and roles of Proteus spp. bacteria in natural environments. Microbial Ecology, 72, 741–758. https://doi.org/10.1007/s00248-015-0720-6

Faldynova, M., Videnska, H., Havlickova, F., Sisak, P., Juricova, H., Babak, V., … Rychlik, I. (2013). Veterinarni Medicina, 58(6), 298–304.

Fatemeh, A., Emran, A., Elnaz, K., Mohammad, J. G. S., & Mahboubeh, N. (2012). The frequency of extended spectrum beta-lactamase (ESBL) in Escherichia coli and Klebsiella pneumonia: A report from Mashhad, Iran. Journal Medical Bacteriology, 1(3), 12–19.

García-Sánchez, L., Melero, B., & Rovira, J. (2018). Campylobacter in the food chain. Advances in Food and Nutrition Research, 86, 215–252. https://doi.org/10.1016/bs.afnr.2018.04.005

Graczyk, T. K., Knight, R., Gilman, R. H., & Cranfield, M. R. (2001). The role of non-biting flies in the epidemiology of human infectious diseases. Microbes and Infection, 3, 231–235.

Graham, J. P., Price, L. B., Evans, S. I., Graczyk, T. K., & Silbergeld, E. K. (2009). Antibiotic-resistant Enterococci and Staphylococci isolated from houseflies collected near confined poultry feeding operations. Science of the Total Environment, 407(8), 2701–2710.

He, Y., Mathieu, J., Stadler, L., Senehi, N., Sun, R., & Alvarez, P. J. J. (2020). Antibiotic resistance genes from livestock waste: Occurrence, dissemination, and treatment. Clean Water, 3, 4.

Iroha, I. R., Adikwu, M. U., Esimone, C. O., Aibinu, I., & Amadi, E. S. (2009). Extended spectrum beta-lactamase (EBSL) in E.coli isolated from a tertiary hospital in Enugu state, Nigeria. Pakistan Journal of Medical Science, 25(2), 279–282.

Kefalas, E., Castritsi-Catharios, J., & Miliou, H. (2003). Bacteria associated with the sponge Spongia officinalis as indicators of contamination. Ecological Indicators, 2, 339–343.

Khamesipour, F., Lankarani, K. B., Honarvar, B., & Kwenti, T. E. (2018). A systematic review of human pathogens carried by the housefly (Musca domestica L.). BMC Public Health, 18, 1049.

Khatter, N. A. (2013). Transmission of bacteria pathogens by the housefly Musca domestica vicina. American Journal of Research Communication, 1(7), 1–12.

Knudsen, J. D., & Andersen, S. E., for the Bispebjerg Intervention Group. (2014). A multidisciplinary intervention to reduce infections of ESBL- and AmpC-producing, Gram-negative bacteria at a university hospital. PLoS ONE, 9(1), e86457. https://doi.org/10.1371/journal.pone.0086457

Mir, R. A., Weppelmann, T. A., Johnson, J. A., Archer, D., Morris, J. G. Jr, & Jeong, K. C. (2016). Identification and characterization of cefotaxime resistant bacteria in beef cattle. PLoS ONE, 11(9), e0163279. https://doi.org/10.1371/journal.pone.0163279

Monyama, M. C., Taioe, O. M., Nkhebenyane, J. S., van Wyk, D., Ramatla, T., & Thekisoe, O. M. M. (2023). Bacterial communities associated with houseflies (Musca domestica L.) inhabiting hospices in South Africa. Microorganisms, 11(6), 1440. https://doi.org/10.3390/microorganisms11061440

Musa, D. A., Aremu, K. H., Adebayo, Z. A., & Pellicano, R. (2020a). Molecular detection of main resistance genes by nested PCR in Salmonella spp. isolated from raw meat and stool samples in Niger State, Nigeria. Minerva Biotecnol, 32, 58–63.

Musa, D., Aremu, H., Ajayi, A., & Smith, S. (2020b). Simplex PCR assay for detection of blaTEM and gyrA genes, antimicrobial susceptibility pattern and plasmid profile of Salmonella spp. isolated from stool and raw meat samples in Niger State, Nigeria. Microbiology and Biotechnology Letters. Korean Society for Microbiology and Biotechnology. https://doi.org/10.4014/mbl.1911.11008

Nasreen, M., Sarker, A., Malek, M. A., Ansaruzzaman, M. D., & Rahman, M. (2015). Prevalence and resistance pattern of Pseudomonas aeruginosa isolated from surface water. Advances in Microbiology, 5, 74–81.

Nayduch, D., Neupane, S., Pickens, V., Purvis, T., & Olds, C. (2023). House flies are underappreciated yet important reservoirs and vectors of microbial threats to animal and human health. Microorganisms, 11, 583. https://doi.org/10.3390/microorganisms11030583

Ogbalu, O. K., & Douglas, S. (2015). Microbiological investigations of selected flies of public health importance from a waste dump site in Port Harcourt, Nigeria. IOSR Journal of Pharmacy and Biological Sciences, 10(1), 75–78.

Ogefere, H. O., Aigbiremwen, P. A., & Omoregie, R. (2015). Extended-spectrum beta-lactamase (ESBL)–producing Gram-negative isolates from urine and wound specimens in a tertiary health facility in Southern Nigeria. Tropical Journal of Pharmaceutical Research, 14(6).

Onyido, A. E., Nwangwu, U. C., Aribodor, D. N., Umeanaeto, P. U., Ugha, C. N., Ugwu, F. M., & Onwude, C. O. (2014). Bacterial pathogens associated with wild-caught houseflies in Awka metropolis of Anambra State, Southeastern Nigeria. New York Science Journal, 7(12).

Park, R., Dzialo, M. C., Spaepen, S., Nsabimana, D., Gielens, K., Devriese, H., … Lievens, B. (2019). Microbial communities of the house fly Musca domestica vary with geographical location and habitat. Microbiome, 7, 147.

Rahuma, N., Ghenghesh, K. S., Ben Aissa, R., & Elamaari, A. (2005). Carriage by the housefly (Musca domestica) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospital and other urban environments in Misurata, Libya. Annals of Tropical Medicine & Parasitology, 99, 795–802.

Saleha, A. A., Wai, S. S., Choo, L. C., & Fauziah, N. (2011). Isolation of Campylobacter and Salmonella from houseflies (Musca domestica) in a university campus and a poultry farm in Selangor, Malaysia. Tropical Biomedicine, 28(1), 16–20.

Stoffolano, J. G. (2019). Fly foregut and transmission of microbes. Advances in Insect Physiology, 57, 27–95. https://doi.org/10.1016/bs.aiip.2019.10.001

Sudagidan, M., Ozalp, V. C., Can, Ö., Eligül, H., Yurt, M. N. Z., Tasbasi, B. B., … Koçak, O. (2022). Surface microbiota and associated staphylococci of houseflies (Musca domestica) collected from different environmental sources. Microbial Pathogenesis, 164, 105439. https://doi.org/10.1016/j.micpath.2022.105439

Ugbogu, O. C., Nwachukwu, N. C., & Ogbuagu, U. C. (2006). Isolation of Salmonella and Shigella species from house flies (Musca domestica I.) in Uturu, Nigeria. African Journal of Biotechnology, 5(11), 1090–1091.

Van Den Bogaard, A. E., & Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: Links between animals and humans. International Journal of Antimicrobial Agents, 14, 327–335. https://doi:10.1016/S0924-8579(00)00145-X

Van den Bunt, G., Liakopoulos, A., Mevius, D. J., Geurts, Y., Fluit, A. C., Bonten, M. J., … Willems, R. J. L. (2017). ESBL/AmpC-producing Enterobacteriaceae in households with children of preschool age: Prevalence, risk factors and co-carriage. Journal of Antimicrobial Chemotherapy, 72(2), 589–595. https://doi.org/10.1093/jac/dkw443

Vazirienzadeh, B., Shams, S., Rahdar, M., & Mehdinejad, M. (2008). Identification of bacteria which are possibly transmitted by Musca domestica in the region of Ahvaz, SW Iran. Jundishapur Journal of Microbiology, 1(1), 28–31.

Zurek, L., & Gorham, J. R. (2008). Insects as vectors of foodborne pathogens. In J. G. Voeller (Ed.), Wiley handbook of science and technology for homeland security (pp. 1–16). Wiley.