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Research Article

Bacterial species associated with houseflies (*Musca domestica*) and blowflies (*Lucilia cuprina and L. sericata*) at a market dumpsite and possible disease risk in Benin City, Nigeria

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Special Issue

A Themed Issue in Honour of Professor Onukwuli Okechukwu Dominic (FAS).

This special issue is dedicated to Professor Onukwuli Okechukwu Dominic (FAS), marking his retirement and celebrating a remarkable career. His legacy of exemplary scholarship, mentorship, and commitment to advancing knowledge is commemorated in this collection of works.

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Bacterial species associated with houseflies (*Musca domestica*) and blowflies (*Lucilia cuprina* and *L. sericata*) at a market dumpsite and possible disease risk in Benin City, Nigeria

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Abstract
Blowflies and houseflies are common fly species in Africa. Their lifestyle and external features allow them to do more than just be a nuisance in the environment; they also act as vectors for major diseases including bacterial. This study aimed to identify bacterial species associated with common flies—specifically, *Musca domestica*, *Lucilia cuprina*, and *L. sericata*—sampled from the dumpsite at Uselu Market in Benin City, Edo State, Nigeria. Flies were collected from the market dumpsite using sterilized equipment, sorted, and identified. Microbial analysis of the fly samples included bacterial isolation, identification, evaluation of phenotypic virulence properties, antimicrobial susceptibility, and calculation of the multiple antibiotic resistance (MAR) index. Statistical analyses were performed using Microsoft Excel and SPSS. Identified bacterial isolates include *Escherichia coli, Bacillus subtilis, Enterobacter cloacae*, *Pseudomonas aeruginosa*, , *Proteus vulgaris* and *Serratia marcescens*. Heterotrophic Bacterial Count (HBC) varied significantly among fly species ($P < 0.05$). *Lucilia cuprina* had the highest HBC (12,200 ± 1,555.64 cfu/g), while variation in the Coliform Count was significant ($P < 0.05$) only between *L. cuprina* and *L. sericata*. Determinants of phenotypic virulence in bacterial isolates differed across species. *Enterobacter*, *Serratia* and *Proteus* exhibited a multiple antibiotic resistance (MAR) index of 0.44, while other bacteria had MAR index of 0.33. The detection of antibiotic restistant bacteria from the flies is worrisome considering the risk it poses

Keywords: Bacterial species, *Lucilia sericata*, *L. cuprina*, *Musca domestica*, antibiotics resistance

1. Introduction

Blowflies and houseflies are usually the initial arthropods to appear on carcasses, decomposing organic material and feces, where they consume, reproduce, and deposit their eggs (Junqueira *et al*., 2017; Tomberlin *et al*., 2017; Park *et al*., 2019). They thrive in decaying organic matter such as animal manure, human waste, open privies, litter, dumpsites and waste from food and vegetable processing. These areas are rich with microorganisms and can be carried by blowflies when they contaminate their mouthparts, wings, feet and limbs (Singh *et al*., 2015; Tomberlin *et al*., 2017; Junqueira *et al*., 2017; Poudel., 2019). As ubiquitous and synanthropic flies, they play a significant role in disseminating microorganisms across both urban and natural environments (Singh *et al*., 2015; Tomberlin *et al*., 2017; Poudel *et al*., 2019).

Blowflies and houseflies are among the most medically important member species of the Calliphoridae and Muscidae respectively (Service, 2012). Due to their constant interractions with humans, they can act as mechanical or biological vectors, spreading pathogenic and multi-drug-resistant bacteria (Poudel *et al*., 2019). Flies that carry pathogenic and drug-resistant bacteria can come into contact with food, and potentially transfer same to humans (Singh *et al*., 2015; Tomberlin *et al*., 2017). This increases the risk of infections that are difficult to treat with standard antibiotics, leading to more severe illness and higher healthcare costs (Poudel *et al*., 2019). Despite decades of efforts to use antimicrobials judiciously, antimicrobial resistance remains a serious public health threat.

Awareness of the dangers posed by flies and their link to poor sanitation exists, yet practices like indiscriminate refuse dumping, open waste disposal, improper food handling and poorly located food vendors still persist in Nigeria (Ifeoluwa, 2019; Odipe *et al*., 2019). The wide spread of pathogenic microbes in our environment makes way for abundant opportunities for these flies to become contaminated and, in turn, contaminate the environment (Junqueira *et al*., 2017; Tomberlin *et al*., 2017; Poudel *et al*., 2019). Thus, the spread of drug-resistant bacteria by flies from domestic and market settings is a significant public health concern (Nazari, 2017; Tomberlin *et al*., 2017; Ogofure *et al*., 2018; Ogofure *et al*., 2019; Poudel *et al*., 2019). Houseflies have the capacity to mechanically transport antibiotic-resistant bacteria from dumpsites to human dwelling areas, posing significant health risks to residents. While studies highlight the role of blowflies and houseflies in spreading bacteria (Junqueira *et al*., 2017), there is limited information on their antimicrobial resistance status in the environment (Poudel *et al*., 2019).

Antibiotic resistant strains of *Pseudomonas*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus*, *Citrobacter* and *Enterococci* have been documented to be transported by flies (Akter *et al*., 2020; Odetoyin, 2020; Onwugamba, 2020). While separate investigations worldwide have underscored housefly's contribution to the spread of bacteria, information is scarce regarding their roles, density/population of bacteria transmitted to food or other materials and their pathogenic pedigree in Nigeria (Nazari, 2017). With the numerous reports of blowflies widespread and close association with humans (Poudel *et al*., 2019; Tomberlin *et al*., 2017; Williams *et al*., 2014), additional information is still needed regarding their microbial transmission potential (Singh *et al*., 2015). In this study, we enumerated the population of bacteria from *M. domestica*, *Lucilia cuprina* and *L. sericata* obtained from dumpsites of a major trade market in Benin City and evaluated their pathogenic and potential public health implications in their multiple antibiotic resistance index.

2.0 Material and methods

2.1 Study Area

The study was carried out in Uselu market, a major trade market in Egor which is one of the metropolitan Local Government Areas (LGAs), in Edo State, Nigeria. The market lies between latitude 6°22'25" N and longitude 5°36'50" E. The LGA is situated in the south-southern region of the state, covering a total area of 93 square kilometres. It has its headquarters in the Uselu town. The climate is tropical, with two major seasons: rainy and dry seasons, respectively.

The Uselu market hosts traders who deal in a wide variety of items, including food (both raw and cooked), beverages, clothing, fashion wares, fabrics, vegetables, fish, meat, fruits, cooking utensils, cosmetics, and more. The market features a primary dumpsite located at an open space at the rear. It lies between latitude 6°22'25.93" N and longitude 5°36'45.51" E. This area serves as a dumping ground for the traders in the market. Information from traders indicates that the piled dumps are evacuated by local authorities weekly. Upon surface evaluation, the dumpsite mainly consists of fresh and decaying food waste, including meat, fish, fruits, vegetables, and other types of waste (see Plate 1). The nearest trader to it is a food vendor located approximately 20 feet away.

2.2 Collection, sorting and Identification of Blowflies

Proir to sampling, sweep nets, personal protective equipment (PPEs) and laboratory materials such as petri dishes, glass wares and bottles were sterilized to avoid contamination from external source. Species of flies were obtained from two randomly selected stations (sections) of the refuse pile, which were approximately 12 feet apart. Adult flies were collected using entomological sweep net and transferred to an ethyl acetate killing jar. Samples of dead flies were subsequently transferred to the Entomology Unit of Animal and Environmental Biology Department, University of Benin for sorting and identification. Flies were identified morphologically according to taxonomic keys as described by CDC (1966) and Service (2012). Only species of interest such as *Musca domestica*, *Lucilia sericata*, *L. cuprina* were retained for microbial analysis.

Plate 1: Dumpsite at Uselu market, Edo state.

2.3 Microbial Analysis

2.3.1 Sample Collection and Preparation

The species of flies were separated. A 0.5 g sample of the flies was placed into 4.5 ml of sterile saline diluent. Samples were serially diluted and 0.1 ml of each dilution was spread onto nutrient agar for heterotrophic bacteria counts and MacConkey agar for coliform counts (Ogofure and Ologbosere, 2023). The plates were kept at room temperature (28°C) for 24 hours to allow incubation.

2.3.2 Bacterial Isolation and Identification

Following the initial incubation, subculturing was carried out to isolate pure bacterial colonies. The identification of bacterial isolates was performed using conventional biochemical methods. The isolates were then purified for further analysis (Ogofure *et al*., 2022).

2.3.3 Evaluation of Phenotypic Virulence Properties

The purified bacterial isolates were evaluated for phenotypic virulence properties, including the production of hemolysin, deoxyribonuclease (DNAse), gelatinase, and lipase, using standard protocols. Standardized bacterial inocula, prepared according to the McFarland turbidity standard (1.5 x 10^8 CFU/ml), were used for these evaluations (Pascoal *et al*., 2018; Sharma *et al*., 2019).

2.3.4 Antimicrobial Susceptibility Testing

The antimicrobial activity of the bacterial isolates was evaluated against antibiotics from eight distinct classes using the Kirby-Bauer disc diffusion technique as described by Ogofure *et al*. (2022). The antibiotics discs, obtained from Oxoid (UK), included: Gentamicin (GEN; 10 µg), Colistin (CS; 10 µg), Imipenem (CB; 30 µg), Metronidazole (M; 5 µg), Augmentin (AG; 30 µg), Clindamycin (CD; 2 µg), Ciprofloxacin (CIP; 30 µg), Tetracycline (TE; 30 µg) and Erythromycin (E; 15 µg). Prior to the susceptibility testing, pure cultures of the isolates were grown overnight in nutrient broth and standardized to 0.5 McFarland's scale corresponding to 1.5 x 10^8 CFU/mL. The level of susceptibility, intermediate resistance, or outright resistance was evaluated using the Clinical Laboratory Standard Institute CLSI (2020) guidelines. Sensitivity results were recorded after 24 hours of incubation by measuring the inhibition zones with a meter rule.

2.3.5 Multiple Antibiotic Resistance (MAR) Index

The MAR index was calculated following the method described by Ogofure *et al*. (2022). The MAR index was calculated using the formula:

$$MAR\ index = \frac{y}{nx}$$

$where\ y = number\ of\ resistance\ scored,$
$n = number\ of\ isolates\ and$
$x = total\ number\ of\ antibioitics$

Davis and Brown (2016) state that an index of 0.2 or higher signifies a high-risk contamination source or indicates that the bacteria pose a significant public health concern.

2.5 Data analysis

All descriptive statistics, Jaccard similarity index, charts and tables were computed in Microsoft-Excel. Analysis of Variance (ANOVA) ($\alpha = 0.05$) computed in SPSS version 23, was used to compare the heterotrophic bacterial count and coliform count across the fly species

3.0 Results and Discussions

Six (6) species of bacteria were isolated from the flies (including *L. sericata*, *L. cuprina* and *M. domestica*) in this study. The isolates include *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Serratia marcescens* and *Proteus vulgaris* and in different concentrations (Table 1). Among the isolated bacteria, *Pseudomonas* and *Escherichia* are recognized for their potential to harbour pathogenic strains (Patel *et al*., 2014; Tuipulotu *et al.*, 2021), while *Enterobacter* and *Bacillus* are typically considered less pathogenic to humans (Davin-Regli *et al*., 2019). *P. aeruginosa* is associated with healthcare-associated pneumonia, urinary tract infections, and ear infections (Bassetti *et al*., 2018). *E. coli* is known to induce symptoms such as diarrhea, abdominal pain, and vomiting (Gomes *et al*., 2016). Prior studies by Olatubi *et al*. (2021) identified elevated levels of *Bacillus* species in *Chrysomya chloropyga* at the Oluponna abattoir in Osun state, Nigeria. Of particular concern was the isolation of *Bacillus cereus*, a known causative agent of foodborne diseases. *B. cereus*, which is often associated with food-borne diseases, was also found in blowflies by Akter *et al*. (2021) in Dhaka, Bangladesh. These findings have significant public health implications.

In general, the Heterotrophic Bacterial Count (HBC) exhibited significant variation based on the fly species (Figure 2), as evidenced by the markedly higher HBC in *L. cruprina* (12200 ± 1555.64 cfu/g) compared to both *L. stericata* ($P < 0.001$) and *M. domestica* ($P < 0.001$). Furthermore, there was no significant difference ($P > 0.05$) in HBC between *M. domestica* (6800 ± 496.66 cfu/g) and *L. stericata* (5775 ± 247.49 cfu/g). Additionally, the Coliform Count differed significantly ($P < 0.05$) exclusively between *L. cuprina* (1200 ± 424.26 cfu/g) and *L. sericata* (400 ± 70.71 cfu/g) (Figure 3). The elevated counts of heterotrophic bacteria and coliforms observed in *Lucilia cuprina* specimens indicate a significant interaction with organic materials present in the dumpsite environment. This finding strongly suggests that *L. cuprina* may have a higher propensity for encountering and carrying pathogenic microorganisms. The data align with the hypothesis that *L. cuprina* has an increased potential for transmitting coliform bacteria, particularly when coming into contact with food or food preparation surfaces. This behaviour elevates the risk of foodborne illnesses, emphasizing the need for stringent hygiene practices to mitigate potential health hazards. (Tomberlin *et al*., 2017; Ogofure *et al*., 2018). However, all bacterial species found in *L. cuprina* were also present in other flies. This was expected, since as all species were picked from the same area. Junqueira *et al*. (2017)'s study also supports the evidence that both houseflies and blowflies share overlapping bacterial community.

Table 1: Cultural, morphological and biochemical characteristics of bacterial isolates from flies

MORPHOLOGICAL						
Elevation	Flat	Raised	Flat	Flat	Flat	Raised
Margin	Undulate	Entire	Undulate	Undulate	Entire	Entire
Colour	Cream	Lemon	Cream	Cream	Cream	Cream
Shape	Irregular	Circular	Irregular	Irregular	Circular	Circular
Size	Large	Medium	Large	Large	Medium	Medium
Gr. diff. agar	EMB	PCA	EMB	BCA	SSA	EMB
Colour	Purple	Green	green	Straw	black	opaque
STAINING						
Gram stain	-	-	-	+	-	-
Cell type	Rod	Rod	Rod	Rod	rod	rod
Arrangement	Disperse	Disperse	disperse	disperse	disperse	disperse
Colour	Pink	Pink	Pink	purple	pink	red
Spore staining	-	-	-	+	-	-
BIOCHEMICAL						
KOH String Test	+	+	+	-	+	+
Catalase	+	+	+	+	+	+
Indole	-	-	+	-	+	-
Citrate	+	+	-	+	+	+
Oxidase	-	+	-	-	-	-
Motility	+	+	+	+	+	+
Urease	-	+	-	-	+	-
Glucose	+	-	+	+	+	+
Sucrose	+	-	-	+	-	+
Lactose	+	-	+	+	-	+
Mannitol	-	-	-	+	-	+
Gas formation	+	-	+	-	-	-
H_2S formation	-	-	-	-	+	-
TSI (Slant/Butt) reaction	A/AG	K/K	A/AG	A/A	K/A H_2S	K/A (*A/A)
Esculin Hydrolysis	(+/-)	-	-	-	+	-
Identity	*Enterobacter cloacae*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Bacillus subtilis*	*Proteus vulgaris*	*Serratia marcescens*

Key: + = Positive, - = Negative, TSI = triple sugar iron agar test, Gr. Diff. Agar = Growth on differential agar, KOH = Potassium hydroxide, Gr. Diff. Agar = Growth on differential agar, PCA = Pseudomonas cetrimide agar, EMB = Eosin methylene blue agar, SSA = Salmonella shigella agar, BCA = Bacillus cereus agar, TSI = Triple sugar iron agar,

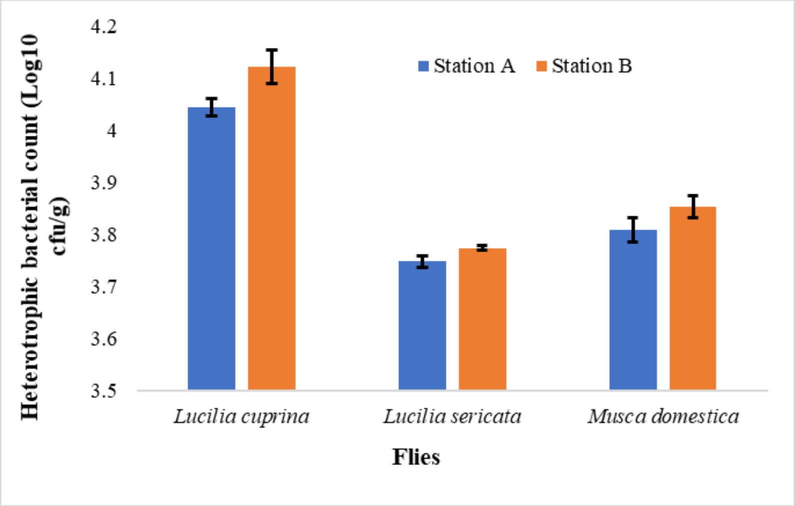

Figure 2: Heterotrophic bacterial count in CFU/g of flies (Log$_{10}$ Transformed)

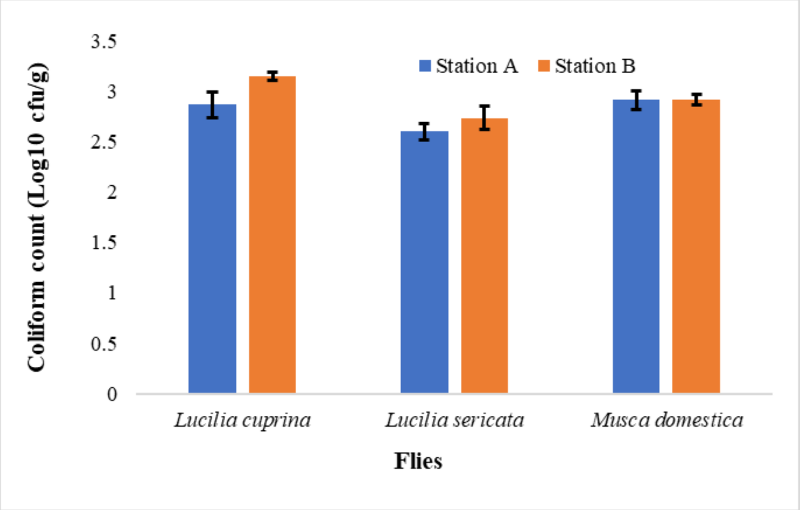

Figure 3: Coliform counts in CFU/g of flies from dumpsites (Log$_{10}$ Transformed)

The determinants of phenotypic virulence in the bacterial isolates varied by species (Table 2). *Bacillus* sp. was phenotypically DNAse, gelatinase and lipase positive. *Enterobacter* and *Escherichia* were negative for DNAse and gelatinase phenotype. On the contrary, *Serratia* sp., *Pseudomonas* sp. and *Proteus* sp. were only positive for DNAse and gelatinase. The results of the antibacterial sensitivity testing for bacterial isolates are presented in Table 3. All bacterial isolates, except *Serratia* were resistant to erythromycin. Whereas no isolate had resistance to gentamicin and ciprofloxacin, all the isolates were susceptible to clindamycin except for *Ecscherichia coli*. *Enterobacter*, *Serratia* and *Proteus* had multiple antibiotic resistance (MAR) index of 0.44, while other bacteria had MAR index of 0.33 (Table 3). Our findings regarding *E. coli* susceptibility to ciprofloxacin, gentamycin, and tetracycline, were consistent in part with the results presented by Haghi *et al*. (2018). Haghi *et al*. (2018) isolated six species of Gram-negative Enterobacteriaceae from *L. sericata* flies, with *Proteus mirabilis* (7.8%) and *Enterobacter aerogenes* (11.1%) being notable bacteria. However, our study additionally found that *E. coli* was resistant to metronidazole, clindamycin and erythromycin. This further highlights the need for good antimicrobial stewardship and constant environmental monitoring so as to ensure that multi-resistant bacteria are kept at bay by every means possible (good sanitation and hygiene and other monitoring measures).

Table 2: Phenotypic virulence determinants of bacterial isolates from flies in dumpsites

Isolates	Hemolysin	DNAse	Gelatinase	Lipase
Bacillus	β	+	+	+
Enterobacter	α	-	-	+
Escherichia	Γ	-	-	+
Serratia	β	+	+	-
Pseudomonas	Γ	+	+	-
Proteus	α	+	+	-

Table 3: Antibacterial sensitivity of bacterial isolates

Isolates	**GEN**	**CS**	**CB**	**M**	**AG**	**E**	**CIP**	**TE**	**CD**	**MAR index**
Bacillus sp.	S	S	R	S	S	R	S	R	S	0.33
Enterobacter sp.	S	R	R	S	S	R	S	R	S	0.44
Escherichia sp.	S	S	S	R	S	R	S	S	R	0.33
Serratia sp.	S	R	S	R	R	S	S	R	S	0.44
Pseudomonas sp.	S	S	S	R	R	R	S	S	S	0.33
Proteus sp.	S	R	R	S	S	R	S	R	S	0.44

GEN: Gentamicin, **CS**: Colistin, **CB**: Imipenem, **M**: Metronidazole, **AG**: Augmentin, **E**: Erythromycin, **CD**: Clindamycin, **TE**: Tetracycline, **CIP**: Ciprofloxacin, **S**: Susceptible, **R**: Resistant, **MAR**= Multiple antibiotics resistance

The fact that flies can navigate relatively long distances or have a wide flight range gives more credence to the relevance of our findings to public health. For instance, *M. domestica* has been reported to fly up to a distance of 5 km (Service, 2012). Therefore, due to the proximity of the dumpsite to food vendors, other traders who may have their food exposed or even residents within the access range of the flies that visits these exposed refuse waste sites, there is an increased risk of transmitting all incriminated microbes and potentially encountering additional ones (even though they were not found in this study). This situation poses a serious public health concern.

Elevated coliform counts in *L. cuprina* samples may indicate heightened fecal contamination, further underscoring the potential public health risk (Tomberlin *et al*., 2017). Pathogens like *Enterobacter* and *Escherichia* are capable of causing foodborne and waterborne illnesses. These blowflies, which may come into contact with food, food preparation surfaces, or food-related equipment, can serve as vectors for microorganisms that pose a tangible threat to human health (Tomberlin *et al*., 2017). All isolated bacteria from both species of blowflies and the houseflies in our study demonstrated that they are multi-drug resistant isolates. When bacteria are resistant to several antibiotics, it becomes a serious challenge in that, treatment of infection can become very expensive and unaffordable. It will be necessary to carry out further screening and investigation to evaluate microbial burden of these flies and also take necessary steps towards good sanitation and hygiene practices.

4.0. Conclusion

Fly species including *M. domestica, L. cuprina* and *L. sericata*, collected from Uselu Market dumpsite were found to habour antibiotic-resistant bacteria species. The proximity and plausible association of these flies near food, fruit vendors, other traders and nearby residents raises significant public health concerns. This is important because bacteria can potentially be transmitted through cooked food and other contact means. Therefore, it is imperative that the siting of dumpsites close to human dwellings and areas of human activity be done with great caution. If it is necessary to have such dumpsites, they should always be covered, and all other standard precautions and practices should be consistently eansured. Government agencies should work towards the complete elimination of waste dumpsites around markets

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