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Investigation of House Flies as a Potential Reservoir of Multidrug Resistant Bacteria in Selected Locations within Port Harcourt, Rivers State, Nigeria

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Abstract
The common houseflies are known vectors capable of transmitting organisms from human to human. At present most studies have focused simply on pathogen detection rather than frequency while a few have described antibiogram patterns and multidrug resistance (MDR). This study aimed at analyzing house flies caught in two poultry farms and close-by residential areas using standard microbiological techniques. The house flies were identified by their physical characteristics using standard keys. Of the total bacteria isolated from 80 flies, Salmonella spp. were the most commonly occurring and together with Escherichia coli, Proteus spp. and Klebsiella spp made up 81.4% of total bacteria. Most isolates were resistant to amoxicillin (82.9%) and co-trimoxazole (81.4%), while the least resistance was against ciprofloxacin (6.2%). In total 58 different antibiograms were detected, though nearly 50% of these (29/59) were observed in only a single isolate. This study noted a high degree of MDR (74.4%) among bacterial isolates. In addition to the ability of houseflies to serve as reservoirs for possible bacteria pathogens, this study clearly describes its ability to serve as reservoirs for MDR also. The diversity of bacteria identified points at a possible major public health issue due to the potential difficulty in controlling acquisition of bacteria by flies. Control efforts might therefore need to be more suited to reducing access of these flies to human food and water.

Keywords: *Houseflies, reservoirs, antibiogram, MDR, Nigeria*

1. Introduction

Musca domestica more commonly referred to as the popular housefly, is the most prevalent species of the domestic flies generally associated with humans (Hemmatinezhad *et al.,* 2015). They are known vectors transmitting pathogenic organisms from one human to another often by contaminating food or water, due to their feeding and reproductive habits with a high level of attraction to dumpsters, excrements, decaying matter, human food sources and the ability to travel very long distances (Butler *et al.,* 2010). Flies have been said to be able to contaminate clean surfaces with approximately 0.1 mg of food per landing (Bryce *et al.,* 2004). These insects can carry pathogens from areas where they pose little risk to humans to areas of high risk. Several studies from around the globe have reported the association of possible pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* sp, *Bacillus* sp, *Salmonella* sp and *Shigella* sp, with flies (Ahmed *et al.,* 2013, Liu *et al.,* 2013, Ogbalu and Douglas 2015, Nseobong *et al.,* 2017, Adeleke *et al.,* 2017, Onwugamba *et al.,* 2018). A number of these studies have focused on flies in and around dumpsters, while some others have looked at flies from general locations, airports and in hospital environments. Most of these studies simply reported on the presence rather than frequency of occurrence of the pathogens, and others focused on the detection of specific pathogens. Additionally, while a number of studies carried out susceptibility testing, few of these described multidrug resistance or provided individual antibiograms which provide a clue to relatedness of isolates. Multidrug resistance is a growing problem worldwide, resulting in the evolution of the so-called superbugs which are resistant to most antibiotics and resulting in therapy failures. One key step in preventing the development of drug resistance involves a knowledge of possible reservoirs of this resistance. This study was aimed at investigating the role houseflies play as reservoirs of multidrug resistance.

2. Materials and Methods

2.1. Sample Collection

A total of 80 house flies were captured using sterile nets (as described by Davari *et al*.,2012) from 2 poultry farms and close-by residential areas 400 and 600 m around the poultry houses at choba in obio akpor local government area of Rivers State. The flies were attracted using ripened mango as bait and preserved each in separate sterile universal bottles to prevent cross contamination. The house flies were identified by their physical characteristics using standard keys. House flies were also captured from an area were poultry farm was not located which served as control.

2.2. Bacterial Isolation and Identification

Following the processing of the flies involving a 30 min incubation in 9 ml of normal saline at room temperature, bacteria were isolated using the spread plate method by culturing on Nutrient agar, MacConkey agar and Salmonella-Shigella agar. Distinct colonies were then purified and their identities determined using standard biochemical tests (Cheesbrough 2000, Cowan and Steel 1985).

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of isolates was carried out using the Kirby Bauer diffusion test (disc diffusion method) as described by Michal *et al.* (2019). A standard inoculum of each isolate corresponding to 0.5 McFarland standard was inoculated to the surface of a Mueller Hinton plate using a sterile swab stick. Following a 15 min pre-incubation at room temperature, the standard commercial antibiotic disc (Abtek UK) was introduced to the plate and the whole set up incubated at 37°C for 24 hours. Zones of inhibition were determined following incubation and the organism classed as resistant or susceptible based on the CLSI standard (NCCLS 2000).

2.4. Determination of Multi Antibiotic Resistance (MAR) Index and Multidrug Resistant Status

Based on the results of the susceptibility testing, the MAR index and MDR status of isolates were determined by the method as described by Cookey and Otokunefor 2016. MAR index is usually determined as a/b, where a is sum of antibiotics to which an isolate is resistant to and b is total number of antibiotics to which the isolate is exposed to. MDR is described as resistance to one or more antibiotics in at least 3 classes of antibiotics.

3. Results

3.1. Bacterial Identification

A total of 80 flies were collected; 6 from each of the poultry farms and 64 from the residential areas close to the poultry farms (400 m – and 600 m away from the poultry farms) and 4 from an area not close to a poultry farm which served as a control. Microbiological analysis of these flies yielded 159 individual bacterial isolates which were found to belong to 8 different bacterial groups (Fig 1). *Salmonella* sp was the most commonly occurring organism and one of the 4 organisms which made up 81.4% of all organisms isolated.

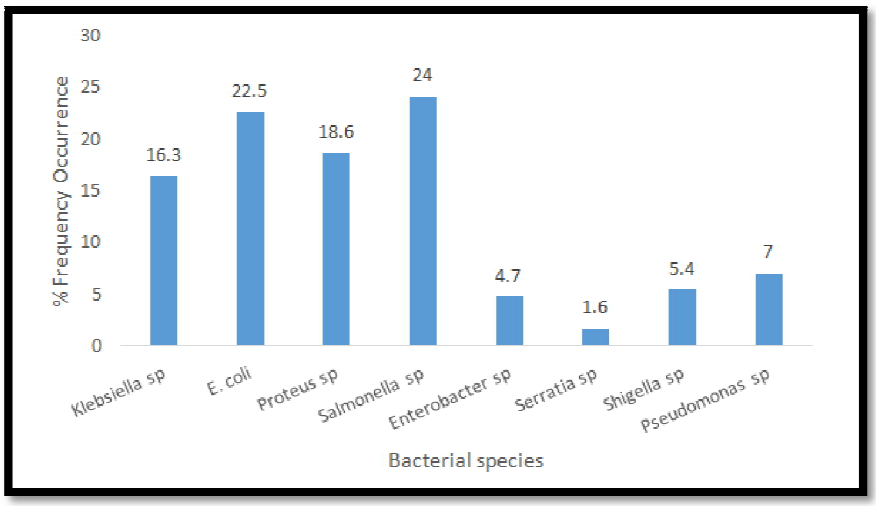

Figure 1: Occurrence of Bacterial Isolates

The organisms isolated showed a wide range of resistance to the tested antibiotics (Fig. 2). Most isolates (82.9% and 81.4% respectively) were resistant to amoxillin (AM) and septrin (SXT), while the least level of resistance was against ciprofloxacin (CPX).

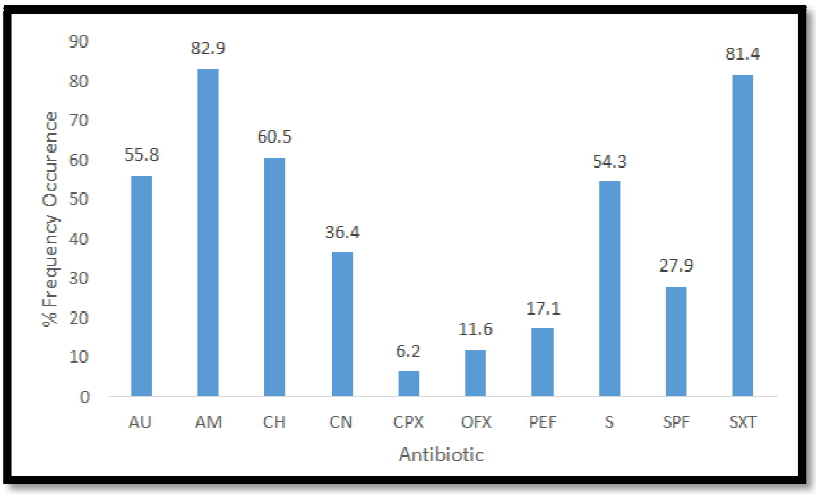

Figure 2: Bacterial Resistance to Antibiotics

(Am: Amoxicillin, Au: Augmentin, CH: Chloramphenicol, CN: Gentamycin, CPX: Ciprofloxacin, OFX: Ofloxacin, PEF: Perfloxacin, S: Streptomycin, SPF: Sparofloxacin, SXT: Co-trimoxazole)

In total, 59 different antibiograms were observed in this study (Table 1). The AM-AU-S-SXT-CH-CN-SPF antibiogram was the most commonly occurring, noted in 8 cases (13.5%), followed by the AM-AU-S-SXT-CH antibiogram (7/59, 11.7%). Nearly 50% of antibiograms (29/59) were noted in only a single isolate.

S/No	Antibiograms	Freq
1.	Nil	7
2.	AM	4
3.	CH	1
4.	SXT	4
5.	AM-SXT	5
6.	AM-CN	1
7.	AU-CH	2
8.	SXT-CH	2
9.	SXT-CN	1
10.	AM-AU-S	2
11.	AM-AU-CH	2
12.	AM-AU-CN	1
13.	AM-S-CN	1
14.	AM-S-SXT	5
15.	AM-SXT-CH	5
16.	AU-S-PEF	1
17.	S-SXT-CH	5
18.	AM-AU-S-CH	1
19.	AM-AU-S-SXT	5
20.	AM-AU-SXT-CH	2
21.	AM-AU-SXT-CN	1
22.	AM-AU-SXT-SPF	1
23.	AM-S-SXT-CN	1
24.	AM-SXT-CH-CN	2
25.	AM-SXT-CH-SPF	2
26.	AU-S-SXT-CH	3
27.	AM-AU-S-SXT-CH	7
28.	AM-AU-S-SXT-CN	3
29.	AM-AU-S-SXT-OFX	1
30.	AM-AU-S-CH-CN	2
31.	AM-AU-SXT-CH-CN	1
32.	AM-AU-SXT-CH-SPF	2
33.	AM-S-SXT-CH-CN	2
34.	AM-S-SXT-CH-SPF	1
35.	AM-S-SXT-CN-PEF	1
36.	AM-AU-S-SXT-CH-CN	3
37.	AM-AU-S-SXT-CH-SPF	4

S/No	Antibiograms	Freq
38.	AM-AU-SXT-CH-CN-SPF	1
39.	AM-AU-SXT-CH-CN-PEF	1
40.	AM-AU-SXT-CH-SPF-PEF	1
41.	AM-S-SXT-CH-CN-SPF	1
42.	S-SXT-CH-CN-SPF-PEF	1
43.	AM-AU-S-SXT-CH-CN-SPF	8
44.	AM-AU-S-SXT-CH-CN-PEF	1
45.	AM-AU-S-SXT-CH-CN-OFX	2
46.	AM-AU-S-SXT-CN-PEF-OFX	1
47.	AM-AU-SXT-CH-SPF-CPX-PEF	1
48.	AM-AU-SXT-SPF-CPX-PEF-OFX	2
49.	AM-S-SXT-CH-CN-SPF-PEF	2
50.	AM-AU-S-SXT-CH-CN-SPF-PEF	2
51.	AM-AU-S-SXT-CH-CN-PEF-OFX	1
52.	AM-AU-SXT-CH-CN-SPF-PEF-OFX	1
53.	AM-AU-S-SXT-CH-CN-CPX-PEF-OFX	1
54.	AM-AU-S-SXT-CH-CN-SPF-CPX-PEF	2
55.	AM-AU-S-SXT-CH-CN-SPF-PEF-OFX	1
56.	AM-AU-S-SXT-CH-SPF-CPX-PEF-OFX	1
57.	AM-AU-S-SXT-CH-SPF-CPX-PEF-OFX	1
58.	AM-AU-S-SXT-CN-SPF-CPX-PEF-OFX	1
59.	AM-AU-S-SXT-CH-CN-SPF-CPX-PEF-OFX	2

Table 1: Antibiogram Patterns and Frequency Occurrence Of Bacterial Isolates

3.2. MAR Index

The antibiograms show that the organisms differed widely in their degree of resistance as indicated by the MAR index (Fig 3). The majority of isolates (84.5%) had a MAR index greater than 0.2, indicating that isolates originated from a region of high antibiotic use.

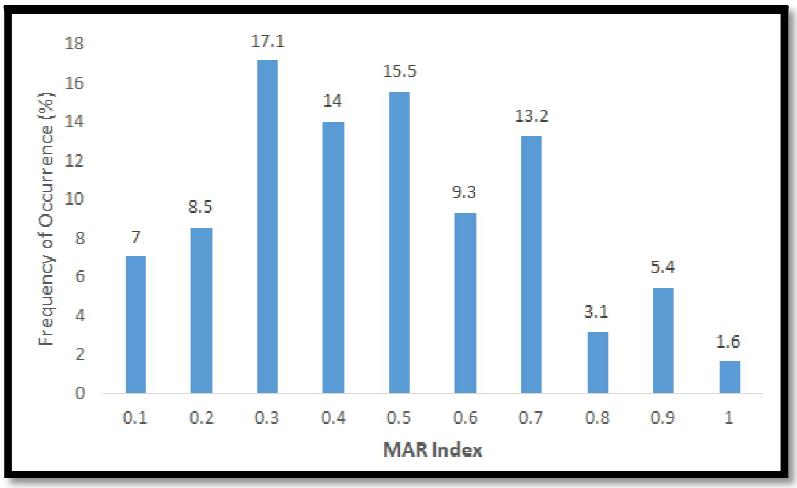

Figure 3: MAR Index of Test Isolates

3.3. MDR in Test Isolates

Results of this study showed that the majority of isolates (96/129, 74.4%) were MDR. There was a similar distribution of MDR isolates with regards to the exact number of drug classes each was resistant to. As 35.4% of MDR isolates were resistant to 3 classes, 30.2% resistant to 4 classes and 34.4% resistant to all 5 drug classes.

4. Discussion

In addition to their association with humans, the houseflies are one of two main fly species noted for their involvement in the dissemination of bacteria of medical importance. Their notable possible long flight range of 5 to 7 km (Onwugamba *et al.,* 2018) and propensity to feed on decaying matter makes them potential important agents in bacteria spread. Flies in this study were found associated with various bacteria of medical importance. The species isolated were generally similar to reports of other previous studies (Ogbalu and Douglas 2015, Adeleke *et al.,* 2017, Parvez *et al.,* 2016, Ahmed *et al.,* 2013). It however differed slightly from the Ogbalu and Douglas study carried out in Rivers State, Nigeria which made no mention of the isolation of *Serratia* sp. Of the 4 predominant bacteria groups isolated in this study, 3 (*E. coli*, *Proteus* sp and *Klebsiella* sp) were previously described as predominant in a number of other studies too (Chiawong *et al.,* 2014, Nazari *et al.,* 2017, Nseobong *et al.,* 2017). *Salmonella* sp, which was the most frequently occurring bacteria

isolated in this study (24%) was isolated at lower levels of 7% and 17.3% in other studies (Pava-Ripoli *et al.,* 2015, Chiawong *et al.*, 2014). Some studies totally failed to isolate any *Salmonella* sp from house flies (Kassiri *et al.,* 2012, Nazari *et al.,* 2017). The higher levels of *Salmonella* sp detected in this study may have arisen from ecological differences or could simply be because this study employed the use of a media selective for *Salmonella* (*Salmonella*-Shigella agar). The results of this study however contrasted sharply with a previous report from Nigeria which noted mainly the presence of Gram positive bacteria from the house flies assayed (Banjo *et al.,* 2005).

This study notes an association between flies and bacteria with high levels of resistance against a variety of antibiotics in and around the poultry farms. Resistance levels of 50% and above were observed against half of the antibiotics assayed. The highest rate of resistance in this study was against amoxicillin and this has similarly been reported by several other studies. A 2016 report on antibiotic resistance in bacteria from flies notes a 95% amoxicillin resistance (Parvez *et al.,* 2016), while a China study noted a 100% amoxicillin resistance (Liu *et al.,* 2013). Mohammed and colleagues also reported a 100% resistance to penicillin (Mohammed *et al.,* 2016). Additionally, this study reported a low level of resistance to the fluoroquinolone group of antibiotics ranging from 6.2% to 27.9%. More recent years have seen an increase in the use of fluoroquinolones due to an increase in resistance to more commonly used antibiotics and a reduction in cost of the fluoroquinolones. The lower rate of resistance against this group of organisms in comparison to others might be linked to the fact that prior to the start of the 21st century this group of antibiotics was not widely used in Nigeria due to cost limitations.

House flies caught in the control area were also resistant to several antibiotics albeit they had low MAR index.

The bacteria isolated in this study showed a high level of diversity, with 58 different antibiograms observed. This level of diversity is quite significant as it indicates that the flies acquire the organisms from multiple sources rather than a common source. This is probably a reflection of the distances flies can travel and potential bacterial sources they are exposed to and points at a possible difficulty then in controlling bacterial acquisition by flies thereby placing a greater demand on controlling the fly population in densely human populated areas to reduce possible transmission events.

Multidrug resistance is a growing problem worldwide, and much focus has been on determining reservoirs of this problem in a bid to curb further spread. Results of this study show a high association between the flies sampled and multidrug resistance. This study noted a 74.4% level of MDR which is quite high compared to the 55% reported by the 2016 Parvez study (Parvez *et al.,* 2016). Though few other papers clearly noted the level of MDR observed in their studies, this level of MDR is more similar to that obtained from clinical environments which have the added pressure of antibiotic use rather than non-clinical isolates.

5. Conclusion

Houseflies, in addition to playing an important role in public health as carriers of bacteria of medical importance, now also have a more problematic role as reservoirs of MDR. Considering the diversity of the isolates obtained, the possible public health issues which could result from this could have widespread and far-reaching consequences to residents living around poultry farms. This phenomenon would need to be further explored to provide a better understanding of the problem and put adequate prevention measures in place.

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