

Housefly (*Musca domestica* L.)-borne multidrug resistant enteric bacteria in Nigerian urban abattoirs and their spread in surrounding built environment

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ABSTRACT

Houseflies (*Musca domestica* L.) are known carriers of infectious agents which may include multidrug-resistant (MDR) pathogenic bacteria. Thus the prevalence and antibiotic resistance profiles of housefly-borne enteric bacteria in 16 Nigerian urban-based abattoirs and their dispersal in built environment surrounding the abattoirs were investigated. The insect-baiting technique was used to capture 1600 houseflies in the abattoirs and surrounding residential/commercial buildings' outdoor environments. Enteric bacteria were isolated from the flies using selective and differential media, and they were identified by cultural and biochemical characteristics. Antibiotics resistance of these bacteria was ascertained by agar diffusion technique using 15 antibiotics. The identified isolates were *Escherichia coli*, *Salmonella enterica*, *Shigella dysenteriae* and *Klebsiella pneumoniae* with prevalence variations of 4.3-7.3% and 4.5-7.4% in abattoirs and buildings' outdoor environment, respectively. The enteric bacteria populations from abattoirs to buildings did not correlate with distance ($r=0.03-0.10$; $P>0.05$); and no significant inter-bacterial correlation in dispersals was found except between *S. enterica* and *S. dysenteriae* ($r=0.65$; $P=0.006$).

All the isolates were susceptible to ceftriaxone and gentamycin, while varied resistance and susceptibility was observed with respect to other antibiotics. The isolates were mostly MDR with multiple antibiotics resistant index of 0.50-0.78 without significant difference between isolates from the abattoir and buildings' outdoor houseflies. Compared to building outdoors, MDR isolates from abattoir houseflies were significantly more in number ($P=0.000-0.015$) in over 65% of the abattoir locations. These findings can be an impetus for the intervention of public health agencies in abattoir operations to control the dispersal of MDR bacteria in built environment.

Keywords: Abattoir, houseflies, enteric bacteria, multidrug resistance, built environment

1.0 Introduction

Buildings and other structures where animals are slaughtered and prepared for human consumption are generally known as abattoirs [1] and they have consistently been a source of major concern to public health professionals and environmentalists because of the wastes generated. Abattoirs have been recognized as a critical link in spreading pathogenic bacteria to the environment [2, 3]; and this includes enteric bacteria which may be multidrug-resistant (MDR). Indeed, MDR enteric bacteria such as *E. coli*, *Salmonella*, and *Shigella* spp. have been isolated in abattoir settings [4].

Wastes generated by abattoirs usually attract houseflies, which feed, breed, and lay numerous eggs on the animal wastes and nearby rotten organic matter [5, 6]. Houseflies (*Musca domestica* L.) are generally known to be associated with man and his activities [7]. Eateries, markets, kitchens, hospital environment, and poultries are typical examples of places where flies are found in abundance and can potentially become vectors of diseases [8]. Houseflies hop from one substrate to another in the course of feeding and the substrates may contain pathogenic bacteria thereby making them vectors of pathogenic bacteria [9, 10]. It is therefore obvious that the presence of

houseflies in abattoirs can portend danger in the context of public health management; and this should be worrisome. [11-14]. Several reports have shown that infectious microorganisms have been transmitted to humans by houseflies from various sources. The type of infectious agent is dependent on the source of the carrier-housefly hence flies captured from sites where the use of antibiotics is common (e.g. hospital and agricultural settings) bear antimicrobial-resistant bacteria and fungi [11, 15-19]. In addition, there are indications that nosocomial infections are transmissible by houseflies present in the hospital environment [15, 20] while housefly-borne strains of virulent microorganisms and antibiotic-resistant genes can also be dispersed in the same environment [21, 22]. The potential risk of the spread of housefly-borne MDR bacteria from agricultural, clinical and domestic settings should be of public health concern. Abattoirs generate enormous effluents which can become potential "hot zones" for gene pools that may ultimately culminate in the emergence of MDR organisms [23]. Reports have shown that antibiotic-resistant bacteria abound in abattoir environments [24-26], with *Salmonella* [25], enterohemorrhagic *E. coli* (EHEC) 0157:H7 [27] and methicillin-resistant *S. aureus* [28] as typical examples among others.

Abattoirs in many urban areas of Nigeria are often located close to residential and commercial buildings or human settlements grow to meet the abattoirs. While it has been established that abattoirs' effluents contain pathogenic and MDR bacteria, there is a paucity of information on the role of flies in spreading pathogens from abattoirs in built environments in urban settings. Thus the study was designed to: 1, determine the presence of MDR enteric bacteria on houseflies in abattoirs located in urban built environment; and 2, ascertain the spread of MDR enteric bacteria to surrounding buildings by the houseflies. The outcome of the investigation may prove useful for public health control measures.

2.0 Materials and Methods

2.1 Abattoir location and sample collection

Abattoirs and environments located in four urban areas (Warri, Abraka, Obiaruku and Kwale towns) in Delta State, Nigeria were selected for the investigation. Abattoirs close to residential and commercial areas were investigated and 16 at 4 per town were selected for the study.

Adult houseflies were captured from the selected abattoirs and surrounding buildings' outdoors (0-100m) using insect-baited trap [29]. The flies were collected over a period of six months at 100 per location bringing it to a total of 1,600. They were subsequently placed in zip-locked polythene bags and taken to the laboratory and killed by storage in refrigerator at -20 °C [30]. Thereafter, they were singularly stored in 2 mL sterile NaCl (0.85% w/v) in tubes at 4°C till needed for subsequent tests.

2.2 Isolation and identification of housefly-borne enteric bacteria

Houseflies weighing 1 g was placed in 9 ml of sterile phosphate-buffered saline (PBS) and shaken manually to dislodge the bacteria on the flies. Aliquots of the dilutions were used to inoculate MacConkey agar, *Salmonella-Shigella* agar, and Eosine Methylene Blue agar all Oxoid, Basingstoke). Incubation of plates was at 37°C for 24-48h and distinct colonies were sub-cultured and stored for identification. Representative colonies (5/plate) were selected and subjected to biochemical tests for confirmation of their identities. The biochemical tests carried out include indole, methyl red, catalase, Voges-Proskauer, citrate utilization, triple sugar iron agar (TSI), motility, urease, L-lysine decarboxylase, L-ornithine decarboxylase, sodium acetate and Christensen's citrate test. Fermentation tests using dulcitol, glucose, lactose, mannitol, raffinose, salicin, sorbitol, sucrose and xylose, (Oxoid, UK), were also carried out. The Analytical Profile Index (API) system (Liofilchem, Italy) was used to confirm the outcome of the cultural and biochemical identification tests.

2.3 Antibiotics susceptibility test

Antibiotic susceptibility test was conducted by the disk diffusion method using Mueller-Hinton agar (Oxoid, UK) following CLSI [31] standards. The antibiotics used were: Cefotaxime, 30µg; Tetracycline, 30 µg; Ceftriaxone, 30µg; Piperacillin, 20µg; Ampicillin, 20µg; Cefoxitin, 30 µg; Cotrimazole, 30 µg; Gentamycin, 30µg; Ciprofloxacin, 30µg; Levofloxacin, 30µg; Ofloxacin, 30µg; Chloramphenicol, 20µg; Azythromycin, 20µg; Imempenem, 20µg; and Vancomycin, 20µg. The plates were incubated for 18-24h at 37°C before the zones of clearance (inhibition) were measured.

2.4 Data analysis

Determination of multiple antibiotics resistance (MAR) index was by dividing the number of antibiotics resisted by the total number of antibiotics used. Bacteria isolates with MAR index ≥ 0.3 was by convention taken as multidrug-resistant (MDR).. The difference between the MAR index of the enteric bacteria isolated from houseflies in abattoirs and surrounding buildings was analyzed by *t*-test. A similar comparative analysis was carried out for MDR bacterial population after \log_{10} transformation. Pearson correlation statistics was used to test the relationship between distances from abattoir and the population of housefly-borne enteric bacteria in the surrounding buildings after \log_{10} transformation. Correlations within the enteric bacteria were similarly analyzed.

3.0 Results

Table 1 presents information on the features and state of the environment of the 16 abattoirs investigated across 4 towns in Delta State, Nigeria. The expected features of an abattoir were present in all the abattoirs although with some variations. Visual observation showed that the distances of wastewater discharge points, composting sites and bone dumpsites from the slaughter buildings varied (0-30m). The distances of the abattoirs from surrounding commercial and residential buildings also varied (Table 1)

The number of enteric bacteria isolated from houseflies located in the abattoirs and surrounding buildings' outdoor environments were 2008 and 1252, respectively. The isolates were *E. coli*, *S. enterica*, *S. dysenteriae* and *K. pneumonia* irrespective of locations and sites of abattoirs (Figure 1). Although not marked, there were variations in the prevalence of the isolates with ranges of: 6.0-6.9 (*E. coli*); 5.9-7.4, (*S. enterica*); 4.5-6.7 (*S. dysenteriae*); and 4.3-7.3 (*K. pneumonia*) as shown in Figure 1 based on the totality of the isolates from all abattoirs and surrounding buildings outdoor environment. Prevalence variations by locations (towns) and differences between abattoirs and surrounding buildings were not markedly different (Figure 1).

Table 1: Features of abattoir environment and distance from residential/commercial buildings

Abattoir location		Presence of:					Distance from buildings (m)
Town	Site	Slaughter slab	Cutting table	Wastewater discharge point	Composting site	Non-edible bone dumpsite	
Abraka	A	+	+	+	+	+	70
	B	+	+	+	+	+	25
	C	+	+	+	+	+	50
	D	+	+	+	+	+	28
Warri	A	+	+	+	+	+	50
	B	+	+	+	+	+	20
	C	+	+	+	+	+	35
	D	+	+	+	+	+	30
Obiaruku	A	+	+	+	+	+	5
	B	+	+	+	+	+	32
	C	+	+	+	+	+	31
	D	+	+	+	+	+	8
Kwale	A	+	+	+	+	+	35
	B	+	+	+	+	+	10
	C	+	+	+	+	+	12
	D	+	+	+	+	+	32

+, present"

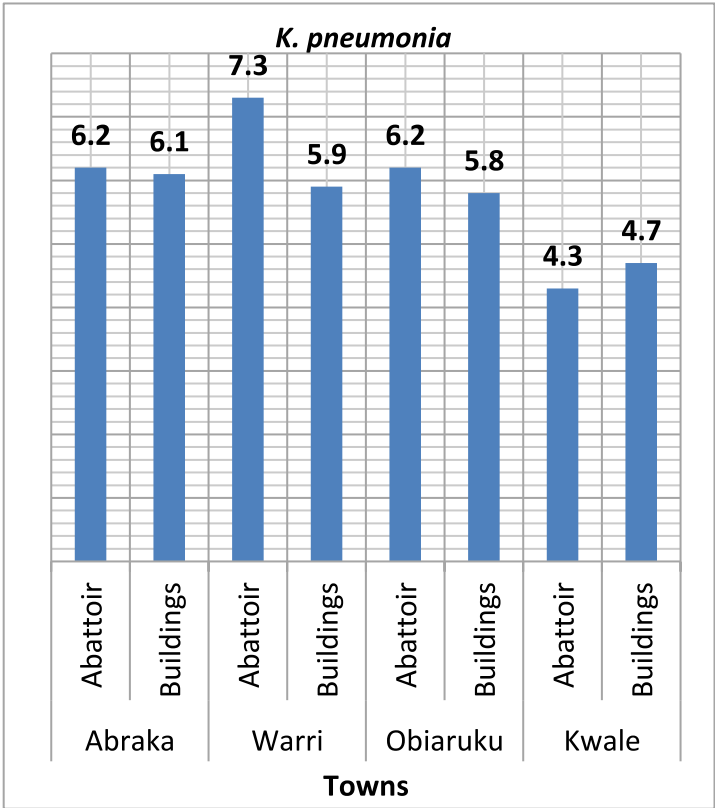
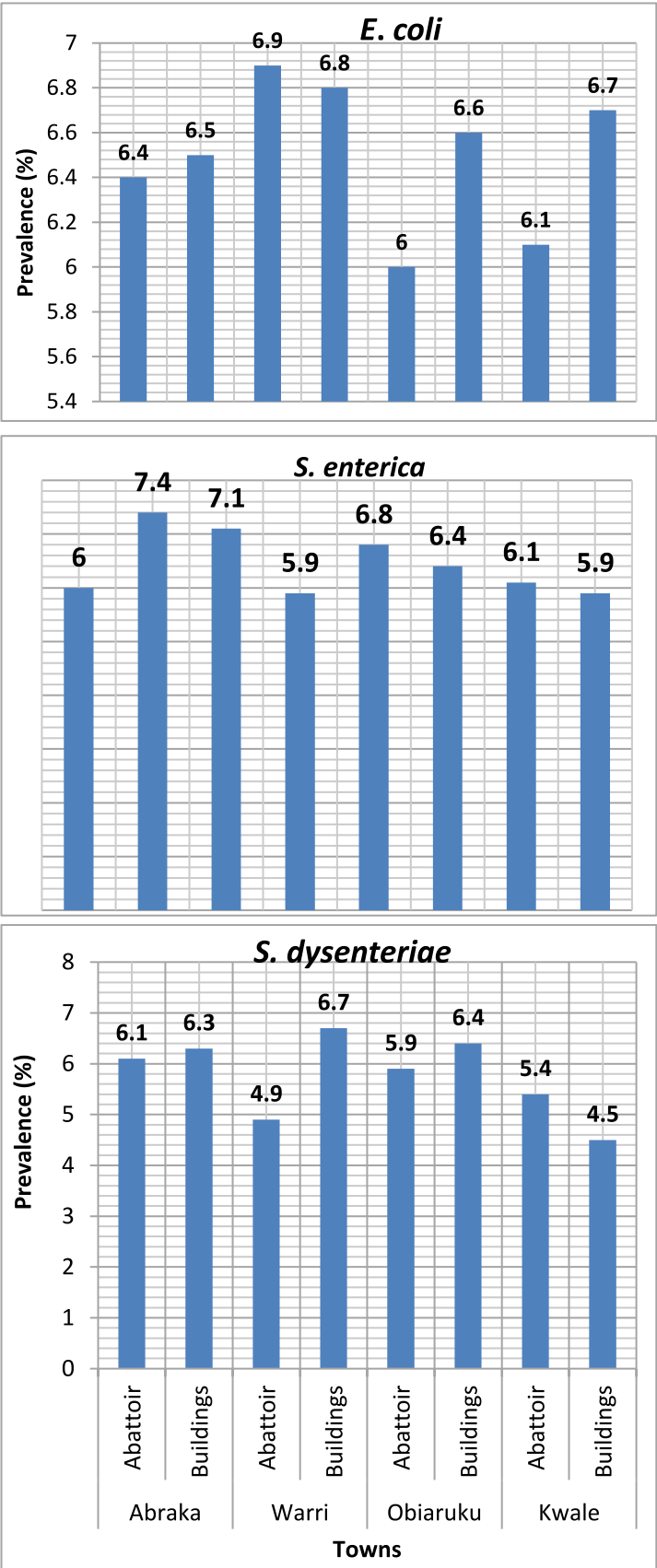


Figure 2 Prevalence of housefly-borne enteric bacteria in abattoir and surrounding buildi

Figure 1: Prevalence of housefly-borne enteric bacteria in abattoir and surrounding buildings' environment by overall assessment

There was no significant correlation between distance from abattoirs and the population of the housefly-borne enteric bacteria in the surrounding buildings' environment (Table 2). In the context of association within the four enteric bacteria, *S. enterica* and *S. dysenteriae* negatively highly ($r=-0.65$) correlated (Table 2). A further analysis of Table 2 showed that correlation coefficients were generally very low (≤ 0.10) as it concerns the association between distance and enteric bacterial population in buildings. Although the correlation coefficients were not significant, intra-enteric bacteria correlations were markedly higher (0.14-0.34) when compared to that of distance (Table 2).

Table 2: Correlation analyses of the associations within the housefly-borne enteric bacteria population and distance from abattoir to surrounding buildings

Enteric bacteria	Correlation coefficient (r)				
	Distance.	<i>E. coli</i>	<i>S. enterica</i>	<i>S. dysenteriae</i>	<i>K. pneumoniae</i>
Distance.	1.00				
<i>E. coli</i>	0.05	1.00	-	-	-
<i>S. enterica</i>	-0.10	0.14	1.00	-	
<i>S. dysenteriae</i>	-0.03	-0.19	-0.65*	1.00	
<i>K. pneumoniae</i>	0.08	0.27	0.34	-0.33	1.00

*P=0.006

All the isolates from flies in the abattoir and surrounding buildings' outdoor environment were susceptible to ceftriaxone and gentamycin, while varied resistance and susceptibility was observed amongst the other antibiotics used (Figures 2-5).

In addition, *E. coli* isolates were susceptible to cefoxitin while strains from houseflies in buildings tended to be more highly resistant to greater number of antibiotics than those from abattoirs (Figure 2). In addition to ceftriaxone and gentamycin, *S. enterica* from both sources (abattoirs and buildings) were also susceptible to ciprofloxacin (Figure 3). Again, the trend of more high antibiotic resistance from building strains was repeated (Figure 3). Compared to *E. coli* and *S. enterica*, there was susceptibility to more antibiotics by *S. dysenteriae* (Figure 4). In contrast to the trends shown in Figures 2 and 3, *S. dysenteriae* strains from abattoirs were more highly resistant to greater number of antibiotics than those from buildings (Figure 4). Isolates of *K. pneumonia* were generally less resistant to antibiotics with building strains showing greater antibiotic susceptibility (Figure 5)

The MAR index of the enteric bacteria isolates is presented in Table 3. It ranged from 0.5 to 0.78 making them highly multidrug resistant (≥ 0.3). Although differences between isolates from abattoir and surrounding buildings can be seen, statistical analysis by *t* test did not reveal any significant differences (Table 3).

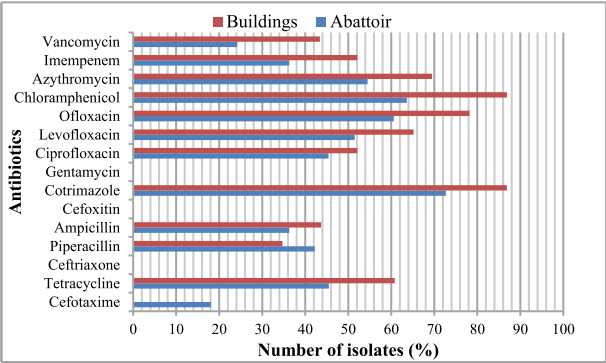


Figure 2: Antibiotics resistance status of housefly-borne *E. coli* in abattoirs and surrounding buildings (abattoirs, n= 33; buildings, n=23)

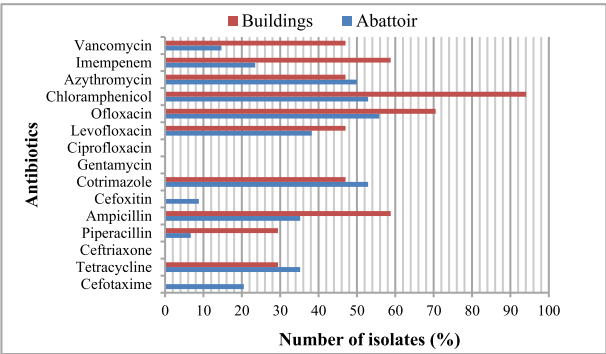


Figure 3: Antibiotics resistance status of housefly-borne *S. enterica* in abattoirs and surrounding buildings (abattoirs, n= 34; buildings, n=17)

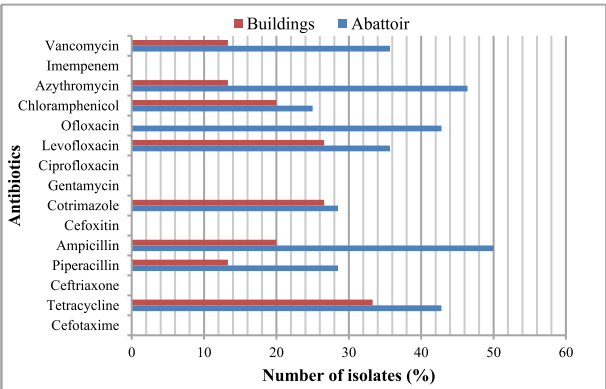


Figure 4: Antibiotics resistance status of housefly-borne *S. dysenteriae* in abattoirs and surrounding buildings (abattoirs, n= 28; buildings, n=15.)

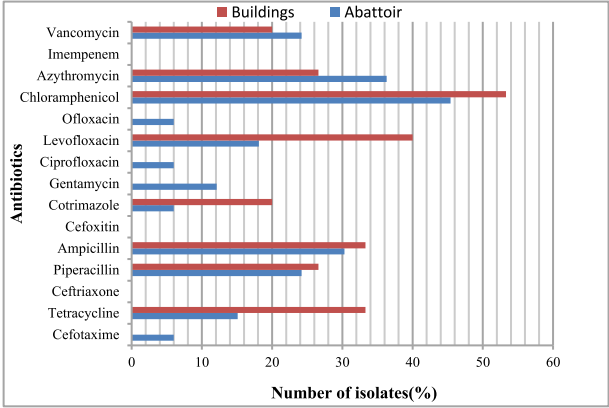


Figure 5: Antibiotics resistance status of housefly-borne *K. pneumonia* in abattoirs and surrounding buildings (abattoirs, n= 33; buildings, n=15.)

Table 3: MAR index of enteric bacteria isolated from houseflies in abattoirs and surrounding buildings

Isolate	Mean MAR index \pm SD	
	Abattoir	Surrounding buildings
<i>E. coli</i>	0.70 \pm 0.09	0.50 \pm 0.04
<i>S. enterica</i>	0.78 \pm 0.04	0.57 \pm 0.09
<i>S. dysenteriae</i>	0.76 \pm 0.05	0.58 \pm 0.07
<i>K. pneumonia</i>	0.73 \pm 0.08	0.56 \pm 0.07

A comparison of the population of the housefly-borne MDR isolates in abattoirs and surrounding buildings outdoor environment is presented in Table 4. Except in few cases, the MDR isolates from abattoir houseflies were significantly greater in number than those from surrounding buildings irrespective of the bacteria species.

Table 4: Comparison of the population of the housefly-borne MDR isolates in abattoirs and surrounding buildings

Enteric bacteria	Abattoir locations*	Mean log cfu/g \pm SD		Sig. diff. (P)
		Abattoir	Surrounding buildings	
<i>E. coli</i>	A	1.54 \pm 0.02	1.50 \pm 0.72	0.740
	B	1.57 \pm 0.01	1.37 \pm 0.01	0.741
	C	1.48 \pm 0.01	1.54 \pm 0.00	0.000
	D	1.48 \pm 0.01	1.38 \pm 0.00	0.000
<i>S. enteric</i>	A	1.50 \pm 0.01	1.32 \pm 0.01	0.000
	B	1.59 \pm 0.01	1.17 \pm 0.007	0.000
	C	1.55 \pm 0.01	1.23 \pm 0.011	0.000
	D	1.47 \pm 0.01	1.16 \pm 0.008	0.000
<i>S. dysenteriae</i>	A	1.48 \pm 0.00	1.20 \pm 0.040	0.000
	B	1.54 \pm 0.01	1.17 \pm 0.018	0.000
	C	1.47 \pm 0.00	1.16 \pm 0.008	0.173
	D	1.48 \pm 0.00	1.12 \pm 0.053	0.206
<i>K. pneumonia</i>	A	1.51 \pm 0.00	1.27 \pm 0.037	0.010
	B	1.73 \pm 0.15	1.20 \pm 0.023	0.029
	C	1.49 \pm 0.00	1.15 \pm 0.013	0.015
	D	1.50 \pm 0.00	1.14 \pm 0.042	0.014

*Towns: A, Abraka; B, Warri; C, Obiaruku; Kwale

4.0 Discussion

An abattoir or slaughterhouse is a premise for slaughtering, processing and practical preservation of animals for human consumption that is usually registered with the appropriate public health agency [32]. Meat cutting table, slaughter slabs, wastewater discharge points, and composting sites are common features of abattoirs that also characterized the abattoirs investigated in this study; and they are potential sources of pathogenic bacteria arising from faecal matter of slaughtered animals [33]. In this study, enteric bacteria were present on the bodies of houseflies captured in all abattoirs as well as the outdoor environments of the surrounding buildings investigated; and they were found to be MDR. Indeed, MDR pathogenic bacteria have been isolated from abattoirs especially the waste water [33]. Houseflies become contaminated by bacteria in the course of feeding in the abattoirs and their hopping behavior spreads the bacteria [9, 10].

Houseflies regurgitate food and deposit them on solid food before ingestion in what is known as bubbling; and this regurgitation and their faecal matter is associated with transmission of pathogens [34]. Given this scenario, the presence of enteric bacteria-contaminated houseflies in abattoirs and the surrounding environment would not have been a surprise.

The finding that there was no marked difference between abattoirs and surrounding buildings in the prevalence of housefly-borne enteric bacteria indicated that the flies migrated from abattoirs. Although houseflies tend to remain in their feeding and breeding localities, they are also known to travel long distances and disperse to residential areas, schools, and business premises [35, 36]. Indeed, dispersal of houseflies at distances greater than 12km has been reported [34]. The dispersal of houseflies may not be unidirectional like a swarm of bees or follow a pattern of reduced population density with distance. Dispersal and distances tend to be influenced by location of feeding sites and deflected wind in built environment hence it may therefore be circuitous [37]. This observation is supported by the absence of a significant correlation between the density of housefly-borne enteric bacteria and distance from abattoirs. The significant negative correlation between *S. enterica* and *S. dysenteriae* both with the same intestinal ecological niche can be explained by the higher survival rate of *Salmonella enterica* in the environment. This explanation is supported by the observation that the prevalence of *S. enterica* in the outdoor environment of buildings was higher than that of *S. dysenteriae* (6.4 vs 5.9%)

The movement of the flies from abattoirs to the buildings is further indicated by the non-significant difference between the MAR index of the housefly-borne enteric bacteria in abattoirs and surrounding buildings outdoor environment. Similarity in antibiotic resistant profile was indicated by the common susceptibility to gentamycin and ceftriaxone and resistance to five of the antibiotics used. The finding that the number of housefly-borne MDR enteric bacteria in abattoirs exceeded those in buildings does not contradict the dispersal trend. The reason is that flies can remain longer in their abattoir feeding sites and may aggregate in other sites where they find food in the course of migration [34]. It was observed that *E. coli* and *S. enterica* on houseflies in the outdoor environment of surrounding buildings exhibited greater antibiotic-resistance when compared to isolates from abattoir-based flies while it was the opposite for *S. dysenteriae* and *K. Pneumonia*. This seemingly inconsistency can be accounted for by the organisms' population differences upon which the percentage resistance was based. The non-marked differences in the overall prevalence of enteric bacteria, and the absence of significant differences in MAR indexes, already indicated abattoir as the source of the housefly-borne enteric bacteria.

A major objective of the investigation was ascertaining the occurrence of MDR enteric microorganisms in abattoirs located in urban built environments. The results revealed the presence of MDR *E. coli*, *S. enterica*, *S. dysenteriae* and *K. pneumonia* with MAR index >0.5 in the abattoirs and the surrounding environment. Although the occurrence of MDR bacteria in abattoirs located in Nigeria and other countries have been reported [4, 24-26, 32, 38, 39], this level of MDR bacteria can be seen as not just substantiating these previous reports, but an update in the global trend of antibiotics resistance. This is in line with the need for constant monitoring of trends in antibiotics resistance for the knowledge of public health agencies.

This is important in sub-Sahara Africa where antibiotics resistance is increasing but under-reported [40].

Implication of findings

The implication of this study outcome is that flies originating from abattoirs can gain access to residential kitchens and deposit MDR bacteria on foods and kitchen utensils which can then spread by cross-contamination. Ill-health arising from MDR bacteria-contaminated food would be difficult to cure because of limited therapeutic options. The marginal differences in the prevalence of housefly-borne enteric bacteria amongst the abattoirs suggest similar compromised hygienic practices across the abattoirs investigated. This is worrisome hence the need for constant monitoring of hygienic practices in abattoirs. Insistence on the constant use of pesticides for the control of flies by public health agencies can limit the spread of housefly-borne pathogens from abattoirs.

Study limitation and future studies

The indoor houseflies were not investigated due to refusal of residents to give their consents. The non-investigation of housefly "nesting" sites near buildings (waste dumpsites, open soak away and septic tanks) for authenticating the source of the flies is a limitation that can be addressed in future studies. The application of social practice theory for interventions in abattoir hygienic practices may be a useful control measure can be explored as indicated in the findings concerning the outdoor environment of makeshift eateries and safety of pushcart foods [41, 42]

5.0 Conclusion

Houseflies in the urban-based abattoirs were found to be carriers of *E. coli*, *S. enterica*, *S. dysenteriae*, and *K. pneumonia*. These enteric bacteria were isolated from houseflies within the abattoirs and outside at the surrounding buildings' outdoor environment with a total number of 2008 and 1252 isolates, respectively. The overall prevalence of each enteric bacterial species did not exceed 7.5%. They were all MDR without significant differences in MAR index between those borne on houseflies within and outside the abattoirs. The inference is that the houseflies in the outdoor environment of the buildings emanated from the abattoirs. The spread of MDR enteric bacteria from abattoirs to residential/commercial areas in usually highly populated urban settings should be of concern to public health authorities. These MDR organisms are well known to be associated with gastro-intestinal disease that is challenging to treat. In conclusion, it is important that public health agencies in Nigeria enforce measures for the control of flies in abattoirs and ensure compliance with hygiene standards and regulations.

Conflict of interest

There is no conflict of interest.

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