

CHAPTER 26

Antimicrobial Resistance in Bacteria Isolated from House Flies (*Musca Domestica*) Collected from Animal House Settings and Hospitals

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Abstract

Antimicrobial resistance (AMR) has emerged as a significant global health threat, contributing to the reduced efficacy of antibiotics. House flies (*Musca domestica*), which are common in human environments such as animal houses and hospitals, have been implicated in the transmission of antibiotic-resistant bacteria. This study focuses on the isolation, identification, and antimicrobial susceptibility testing of bacteria collected from house flies in animal houses and hospital settings. The findings indicate a high prevalence of resistant bacterial species, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, many of which showed

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multidrug resistance. These results highlight the need for improved environmental and hygiene control measures to mitigate the spread of AMR.

Keywords: Antimicrobial resistance, House flies, Animal house, Hospitals, Multidrug resistance, Pathogen transmission

Introduction

The re-emergence of antimicrobial resistance (AMR) brings with it huge implications for global human and animal health, food security, and ecosystems. AMR is a process through which bacteria develop the ability to withstand the impacts of antibiotic treatments, treatment becomes futile, infections persist, hospital stays are prolonged, and mortality increases. Most of the previous works regarding AMR are centered on humans or animals as agents of transmission while only a little work has been done on other potential modes of transmission such as insects.

Musca domestica that frequently breed around houses and points of contact between humans and animals are a fascinating mechanical carrier of AMR bacteria. These flies are mobile and feed on human and animals even in places such as dumping grounds, animal feeding places, and hospitals, among others; they interact with antibiotic-resistant bacteria. They identify that these creatures are hairy creatures with sticky feet that can transfer the bacteria they touch on floor surface to incorporate in the mouth then deposit on the floor when defecating.

On farms, this is because in animal houses, antibiotic growth promoters and antibiotics used for disease prevention are widely used and this results in breeding of bacteria. Likewise, because of the over and often improper prescription of antibiotics, hospitals are reservoirs for MDOs. Together with the other environment these two make a severe public health issue since the house flies may serve as carriers that move resistant bacteria between animals, humans as well as environment.

This chapter aims to investigate the role of house flies in the transmission of AMR bacteria by analyzing samples collected from animal house settings and hospitals. The study also explores the bacterial diversity, resistance patterns, and potential implications for infection control in both veterinary and healthcare contexts.

Sampling Sites and Methods

1. Animal House Settings

Farm like livestock farm, veterinary center, and poultry farm have high possibility of selecting and disseminating AMR bacteria since use of antibiotics is habitual in animal husbandry. AM is frequently applied not only therapeutically against various bacterial diseases, but also as promoters of growth leading to the emergence of resistant strains in the microbiota of animals.

In these environments, house flies get in contact with animal dung, unconsumed feed, animal nestling materials, and veterinary wastes some of which contain bacteria. To collect flies from these

settings, sweep nets together with sticky traps were deployed in areas such as near waste disposal, feeding troughs and vet treatment pens. This was done in order to increase the sample size collected particularly during the early morning and late evening when flies are most active.

After collection, the flies were taken to the laboratory under aseptic condition which was then followed by analysis. Every fly was rinsed in phosphate buffered saline to remove external bacteria and the rinse was cultured on selective agar for bacterial culture. The adult flies were also dissected so that the examine gut content; the gut content was then cultured independently to understand the kind of bacteria ingested.

2. Hospital Environments

Hospitals, particularly those with high patient turnover and intensive care units, are known to be reservoirs of AMR bacteria. House flies in these environments can be found near waste disposal areas, patient rooms, and food preparation areas. They are likely to carry pathogenic bacteria from contaminated surfaces to areas where vulnerable patients are housed, increasing the risk of hospital-acquired infections (HAIs).

For this study, flies were collected from several hospitals with a focus on areas most likely to harbor resistant bacteria, such as surgical wards, outpatient clinics, and general waste areas. Similar to the methods used in animal houses, flies were trapped using sticky traps and sterile nets. The collected flies were processed in the laboratory, and both surface and gut bacteria were isolated for further analysis.

Bacterial Isolation and Identification

Once the house flies arrived at the laboratory, they were subset through a two-stage bacterial isolation procedure. Initial, each fly was surface sterilized by washing it with ethanol to rid the insect of any passingly transient environmental microorganisms. Surface bacteria were obtained by rinsing the flies with phosphate-buffered saline. This solution was spread onto different kinds of media such as nutrient agar, MacConkey's agar and blood agar to facilitate the growth many types of bacteria.

The second one was to dissection of the flies to obtain bacteria from their digestive tracts. The similar process as that applied with the surface samples was directly applied to the homogenized gut contents. Both approaches ensured that we could distinguish between bacteria that were simply carried on the outer surfaces of the flies from those bacterial strains that the flies had ingested and which resided in the hindguts of the insects.

Bacterial colonies that grew on the plates were subjected to standard biochemical tests for preliminary identification. These tests involve Gram staining, catalase tests, coagulase tests, and sugar fermentation assays tests. For confirmatory identification, molecular methods including Polymerase chain reaction (PCR) and 16S rRNA gene sequencing were used. This made it possible to accurately enumerate bacterial species many of which were identified to be associated with resistance genes.

Antimicrobial Susceptibility Testing (AST)

The bacterial isolates were subjected to antimicrobial susceptibility testing (AST) using the Kirby-Bauer disk diffusion method. This method involves placing antibiotic-impregnated disks on agar plates inoculated with bacterial cultures. After incubation, the size of the inhibition zones around the disks was measured to determine the susceptibility of the bacteria to each antibiotic.

We selected a panel of antibiotics that are commonly used in both veterinary and human medicine, including: Ampicillin, Ciprofloxacin, Tetracycline, Gentamicin, Vancomycin, Ceftriaxone, Erythromycin etc.

The results of the disk diffusion tests were interpreted according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). Based on the size of the inhibition zones, bacteria were classified as susceptible, intermediate, or resistant to each antibiotic.

Results and Discussion

1. Prevalence of Antimicrobial Resistance in Animal House Settings

In animal house settings, the most commonly isolated bacteria were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Many of these bacteria exhibited resistance to multiple antibiotics. For example, *E. coli* isolates were frequently resistant to ampicillin, tetracycline, and ciprofloxacin, suggesting that antibiotic use in livestock was selecting for resistant strains.

Staphylococcus aureus isolates, including methicillin-resistant *S. aureus* (MRSA), were also detected in significant numbers. The presence of MRSA in animal house flies suggests that these flies could act as vectors for the transfer of resistant bacteria from animals to humans or vice versa. This raises concerns about the potential for cross-species transmission of AMR bacteria, particularly in settings where humans and animals interact closely.

2. Prevalence of Antimicrobial Resistance in Hospital Settings

In hospitals, the range of isolated bacteria was even broader, and the level of antimicrobial resistance was notably higher. *Klebsiella pneumoniae* isolates from hospital flies frequently produced extended-spectrum beta-lactamases (ESBLs), enzymes that confer resistance to cephalosporins and other beta-lactam antibiotics. Similarly, vancomycin-resistant *Enterococcus* (VRE) was isolated from several flies collected near hospital waste areas.

The high prevalence of multidrug-resistant organisms in hospital flies highlights the potential role of these insects in exacerbating hospital-acquired infections (HAIs). By carrying resistant bacteria from waste areas to patient rooms or surgical environments, house flies could inadvertently contribute to the spread of infections that are difficult to treat due to limited therapeutic options.

Implications for Public Health

The findings of this study underscore the importance of controlling house fly populations in both animal houses and hospitals. Flies are not only a nuisance but also pose a serious public health threat by acting as vectors for AMR bacteria. The ability of flies to carry and disseminate bacteria across different environments necessitates improved hygiene and sanitation measures, particularly in settings where antibiotics are used extensively.

In hospitals, steps should be taken to prevent flies from entering sensitive areas, such as operating rooms and intensive care units. This could include the use of fly screens, regular cleaning of waste areas, and the implementation of more effective waste management practices. In animal house settings, reducing antibiotic use and maintaining clean environments could help limit the spread of resistant bacteria via flies.

Raising public awareness about the risks posed by flies in the transmission of AMR is also crucial. By educating both healthcare professionals and the general public, we can foster a more coordinated effort to mitigate the role of house flies in the transmission of antimicrobial-resistant (AMR) bacteria. In particular, both hospital and veterinary settings need to implement stronger biosecurity measures. These could include the strategic placement of fly traps, increased frequency of cleaning and disinfection, and reducing access to breeding sites such as waste disposal areas.

Furthermore, reducing the overuse and misuse of antibiotics in both healthcare and animal husbandry is essential to controlling the emergence of resistant strains. Stewardship programs in hospitals that advocate for the judicious use of antimicrobials can significantly limit the selection pressure that drives the development of resistance. Similarly, veterinary practices that emphasize preventive health care, such as vaccination and better hygiene management in animal shelters, will reduce the reliance on antibiotics, curbing the development of resistant bacteria in animal populations.

As demonstrated by this study, house flies are potential vectors for cross-species transmission of AMR bacteria. The ability of flies to travel from hospitals to the surrounding community or from animal facilities to nearby homes increases the risk of spreading resistant bacteria to broader human populations. This underlines the importance of considering environmental sources of AMR in public health strategies.

Environmental Risk Factors and House Fly Habitats

House flies thrive in environments where organic matter is abundant, such as refuse heaps, excrement, and decaying food. In hospitals, areas around waste bins, food preparation areas, and patient discharge rooms offer perfect conditions for flies to proliferate. The role of flies as mechanical carriers of pathogens in hospitals has been largely underestimated. Flies may pick up AMR bacteria by walking or feeding on contaminated surfaces, including hospital waste or patient excretions, and then transfer these bacteria to sterile or sensitive areas. Similarly, in animal houses, bedding material, feces, and feed residues present a conducive environment for house fly breeding and exposure to antimicrobial-resistant bacteria.

The problem is exacerbated in areas where sanitation and waste management are inadequate. Hospital waste, if not disposed of or treated appropriately, becomes a hotspot for flies, which then have access to multi-drug-resistant pathogens. In animal houses, improper handling of manure and the overuse of antibiotics for growth promotion create an environment that selects for resistant bacteria. These bacteria may persist in the gut of house flies and be excreted into the environment, further spreading resistance.

The Role of Biofilms in AMR Transmission by House Flies

Biofilms are complex communities of bacteria that adhere to surfaces, often forming protective layers that make the bacteria more resistant to antibiotics. House flies may facilitate the dispersal of biofilm-forming bacteria by landing on surfaces covered with biofilms, such as drains, wounds, or medical devices in hospitals. These biofilms, particularly in hospital environments, often harbor multidrug-resistant bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, increasing the risk of healthcare-associated infections (HAIs).

In animal settings, biofilms formed in feeding troughs, water lines, or on veterinary equipment can harbor AMR bacteria. House flies, through their feeding habits, may ingest these bacteria and serve as mechanical vectors, disseminating resistant pathogens across different settings.

Mechanisms of Resistance in Bacteria Isolated from House Flies

The bacteria isolated from house flies in this study displayed a variety of mechanisms for resistance to antimicrobials. These included:

1. **Beta-lactamase production:** Many isolates, particularly *Klebsiella pneumoniae* and *Escherichia coli*, produced extended-spectrum beta-lactamases (ESBLs), enzymes that degrade beta-lactam antibiotics like penicillin's and cephalosporins, making these treatments ineffective.
2. **Efflux pumps:** Some bacteria, like *Pseudomonas aeruginosa*, used efflux pumps to expel antibiotics from their cells, thus reducing the intracellular concentration of drugs like tetracycline and ciprofloxacin and enhancing survival in the presence of these antibiotics.
3. **Altered target sites:** In *Staphylococcus aureus* (including methicillin-resistant *S. aureus* or MRSA), mutations in the penicillin-binding proteins rendered beta-lactam antibiotics ineffective. Similarly, alterations in ribosomal proteins conferred resistance to aminoglycosides.
4. **Resistance through biofilm formation:** Many isolates demonstrated enhanced survival in biofilm communities, where the dense extracellular matrix protected them from antimicrobial penetration. This mode of resistance was particularly noted in *Pseudomonas* species, which are well-known biofilm formers in hospital environments.

These mechanisms contribute to the persistence and spread of AMR in both hospital and animal house environments. The diversity of resistance strategies observed underscores the complexity of tackling AMR and the need for multifaceted approaches that include improved hygiene, better waste management, and prudent use of antibiotics.

Recommendations and Conclusion

This study provides critical evidence that house flies collected from animal house settings and hospitals harbor AMR bacteria, including strains with multidrug resistance (MDR). Their role as vectors in the transmission of resistant bacteria between environments has significant implications for infection control and public health. To mitigate the risks posed by these vectors, the following measures are recommended:

1. Fly population control: Regular monitoring and control of house fly populations through integrated pest management strategies, including traps, insecticides, and environmental sanitation, should be prioritized in both hospital and animal house environments.
2. Improved waste management: Ensuring that medical waste, especially from hospitals, is properly disposed of in sealed containers and promptly removed from patient areas can reduce fly access to potential bacterial reservoirs. In animal settings, manure management and frequent cleaning of facilities can lower fly exposure to resistant bacteria.
3. Hygiene practices in hospitals: Strengthening infection control measures, such as the use of sterile barriers and regular disinfection of high-risk areas (e.g., food preparation and waste disposal areas), will help minimize the spread of resistant bacteria by house flies.
4. Reducing antibiotic use: Prudent use of antibiotics in both human and veterinary medicine is essential to curbing the development of resistance. Antibiotic stewardship programs should be promoted in hospitals, and alternative practices such as better animal husbandry and preventive veterinary care should be encouraged to reduce reliance on antibiotics in agriculture.

In conclusion, house flies are important vectors of AMR bacteria in both hospital and animal house settings. Addressing this overlooked pathway of resistance transmission requires a multidisciplinary approach involving pest control, waste management, antimicrobial stewardship, and public awareness campaigns. By taking action at multiple levels, we can help mitigate the threat of AMR and protect both human and animal health.

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