



# Houseflies (*Musca domestica*) as vectors of multidrug-resistant, ESBL-producing *Escherichia coli* in broiler poultry farms of North India: implications for antibiotic resistance transmission

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## Abstract

The transmission of antibiotic resistance (AR) from farm animals to healthy human communities, beyond the food chain, is often facilitated by biological vectors, notably houseflies (*Musca domestica*). This study aimed to evaluate the role of *M. domestica* collected from commercial broiler chicken farms as a carrier of multidrug-resistant (MDR), extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli*. *E. coli* were isolated separately from the housefly's external surface (ES) and internal homogenate (IH) to determine the primary AR transmission route within houseflies. Remarkably, 68.6% houseflies harboured *E. coli*. Isolated *E. coli* were evaluated for susceptibility to clinically relevant antibiotics and screened for the presence of 22 plasmid-borne AR genes (ARGs) using PCR. Results revealed significant resistance to key antibiotics, with > 70% of isolates resistant to ampicillin and > 50% resistant to tetracycline and nalidixic acid in both ES- and IH-derived *E. coli*. Notably, a significant prevalence of resistance was observed to third-generation cephalosporins. Additionally, > 80% of *E. coli* isolates were MDR. A statistically significant difference (unpaired *t*-test,  $p < 0.05$ ) was observed in the presence of ESBL-producing *E. coli* between the houseflies' ES (28.14%) and IH (38.14%). ARGs such as, *ampC*, *tetA*, *qnrS*, *strA*, *strB*, and *sul3* were frequently detected in both ES- and IH-derived *E. coli* isolates. Among the ESBL-producing genes, *bla*<sub>CTX-M</sub> was the most abundant. Pearson's correlation analysis predicted the ARGs responsible for phenotypic resistance to specific antibiotics. Farm-derived flies harboured a significantly higher number of MDR *E. coli* (unpaired *t*-test,  $p < 0.05$ ) than the ones isolated from flies housing a distant non-farm environment. Conclusively, this study illustrates the role of houseflies as vectors for AR transmission from AR hotspots to human communities.

**Keywords** Housefly (*Musca domestica*) · Antibiotic resistance · ESBL-production · AR vectors · AR dissemination · One-health

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## Introduction

Food animal industry has emerged as an antibiotic resistance (AR) hotspot, harbouring AR bacteria (ARB) carrying AR determinants (ARDs) (AR genes (ARG) + mobile genetic elements (MGEs)) (Diarrassouba et al. 2007; Rawat et al. 2022; Yu et al. 2024). For instance, multidrug-resistant (MDR), extended-spectrum  $\beta$ -lactamase (ESBL)-producing and colistin-resistant Enterobacteriaceae, harbouring *bla*<sub>CTX-M</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>SHV</sub>, and *mcr-1* are frequently detected in farm animals (Brower et al. 2017; Islam et al. 2020; van den Bogaard et al. 2001). Food-chain-mediated AR transmissions to human communities are well-reported (Aarestrup et al. 2008; Bengtsson-Palme 2017). However, other routes of AR dissemination from farm animals are also envisaged, where the role of small synanthropic