**TITLE:** CLONAL DIVERSITY IN MULTIDRUG RESISTANT *Escherichia coli* CARRIED BY FLIES

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**ABSTRACT:**

Environmental routes of antimicrobial resistance spread generally include direct contact with animals, food, and effluents. However, due to the contact that some fly species have with the domestic animals' feces, associated with their wandering behavior, provide that flies can also participate in such epidemiological routes. In this context, we aimed to investigate the dissemination of commensal multidrug resistant *Escherichia coli*, isolated from flies collected in two dairy farms in Botucatu, SP, Brazil, 35 km apart. The strains were analyzed for antimicrobials susceptibility by disc diffusion test. The *bla*$_{\text{TEM}}$, *bla*$_{\text{CTX-M}}$, *tetA* and *ampC* genes, as well as *E. coli* phylogroups were determined by PCR. Multidrug-resistant strains (MDR, resistant to ≥ 3 antimicrobial classes) were selected for clonal profile analysis by Pulsed-Field Gel Electrophoresis (PFGE). From the external surface of 96 flies, 62/198 MDR *E. coli* were isolated, of which 43 were selected for the PFGE analysis, generating 36 pulsetypes. Our results showed that one *E. coli* strain from one farm was 100% similar to three strains from the other farm, but with distinct phenotypic and genotypic resistance profiles among to the four strains. In addition, *E. coli* isolated from the same fly displayed less than 80% of similarity, with the same resistance genes pattern, but different antimicrobial resistance profiles. All strains were assigned to phylogenetic group B1, except one attributed to group E. The resistance genes more frequent was *bla*$_{\text{TEM}}$ (58.1%; 25/43), followed by *tetA* (32.6; 14/43), *bla*$_{\text{CTX-M}}$ (27.9%; 12/43) and *ampC* (13.9%; 6/43). Around one-quarter (25.6%) did not contain any genes tested. In view of results, we can say that flies can carry *E. coli* strains, similar or not, with different antimicrobial resistance profiles. Thus, flies seem to play a role in the antimicrobial resistance spread and flies should be considered in the environmental dissemination route.

**Keywords:** PFGE, dairy farm, phylogroups
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