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Main topic : Antimicrobial resistance: A worldwide concern

Experimental infection of *Galleria mellonella* larvae with *Campylobacter* spp. Isolated from Chickens: An Alternative Animal Model to Determine Antimicrobial Efficacy

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Introduction: *Campylobacter* spp. is the world's leading cause of gastrointestinal disorders in humans. These bacteria are associated with consuming contaminated poultry products since *Campylobacter* is considered a commensal microorganism in the gut of broilers. In Peru, chickens from traditional markets showed *Campylobacter coli* (97.5%) with tetracyclines and macrolides resistance. For this reason, the need to evaluate antibiotics in vitro and in vivo has increased; it is in the latter that complications arise due to ethical and economic issues. Alternative animal models such as the *Galleria mellonella* larval (GML) are being used in investigations of antibacterial efficacy in pathogens for their easy implementation, low cost, and not require authorization from ethics committees. Our objective is to determine if it is possible to carry out the experimental infection of the GML with strains of *C. coli* isolated from carcass chickens from markets in Lima, Peru. **Methods:** *C. coli* strains (five from chicken samples and one ATCC-33559) were reactivated in tubes with Preston blood broth (5%) at 37°C/24h in microaerophile, centrifuged at 1000g/5 min, and the pellet was washed three times with PBS. An initial inoculum of approximately 10⁷ CFU/mL (OD600) was serially diluted to obtain eight 10-fold dilutions. GML (n=1000) with 250-300mg weight, 2-3 cm long, and creamy white coloration were selected. After disinfection, the inoculum (10 µL) was placed between the last left pro-legs. Control groups (without inoculum, needle trauma, and PBS) and challenged groups (10⁸ – 10¹ CFU/mL) for each *C. coli* strain were considered. Larvae were kept without food in Petri dishes at 37°C/4 days, evaluating mortality, survival, and appearance (leg movement, return to ventral decubitus position, cocoon formation, color change). Each group had ten larvae, and all groups repeated twice. **Results:** The results showed that groups without inoculum, PBS, Trauma, and the inoculum of 10¹ and 10² CFU/mL did not present mortality throughout the four days. Inoculum of 10⁸, 10⁷, 10⁶ CFU/mL showed a mortality of 100%, 78%. and 56%, respectively, at 24 hours, and a total mortality of 100%, 84%, and 60%, respectively, at four days. The 10⁵ CFU/mL inoculum only presented a mortality of 39% on the first day. The 10⁴ and 10³ dilutions showed a 14% and 13% mortality, respectively, on the first day and a total mortality of 16% and 15%, respectively. **Conclusions:** These results indicate that it is possible to develop *C.coli* infection in GML with doses between 10⁵ and 10⁷ CFU/mL and that mortality occurs 24 hours after injection. These data are helpful to be able to develop assays to evaluate conventional antibiotics and natural products in vivo.

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