High frequency of multi-drug resistant Enterococcus spp. isolated from conventional and antibiotic-free chicken meat production

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Conventional chicken meat production relies on antibiotics for growth promotion and disease prevention, contributing to the development and spread of antibiotic-resistant *Enterococcus*. On the other hand, antibiotic-free production systems aim to minimize the risk of antibiotic resistance avoiding the use of antibiotics. However, these systems face unique challenges in managing bacterial infections without these treatments. The aim of this study was to evaluate the antibiotic resistance profile of *Enterococcus* spp. isolates from conventional and antibiotic-free chicken meat production chains.Chicken and farm environmental samples were collected from eight chicken producers (four from conventional system and four from antibiotic-free system). For each farm, the production cycle was monitored, with samples collected before the housing and during the chicken’s production, as well as during the batch slaughter. A total of 1,079 samples were collected, including water, feed, insects, drag swabs from inside and outside poultry houses, cloacal swabs, surface and utensil swabs at the slaughterhouse, carcasses and cecum during slaughter. Isolation was performed on BD Enterococcosel™ agar, followed by molecular confirmation (gene *Tuf*). *Enterococcus* spp. isolates (n=527; n=231 from convention and n=296 from antibiotic-free) were submitted to the disk diffusion testing for antibiotic resistance, following CLSI standards. Isolates resistant to three or more antibiotic classes were classified as multidrug-resistant (MDR). Chi-square test was used to assess the statistical significance of the observed differences.Out of 527 isolates, 99.80% were classified as multidrug-resistant. The results showed that a higher frequency of MDR *Enterococcus* spp. were found in the antibiotic-free system (99.90%) compared to the conventional system (99.1%). However, the difference between the systems was not statistically significant (X^2 0.04; $p > 0.05$) suggesting that the presence of MDR *Enterococcus* spp. is not significantly influenced by the type of system. High resistance rates were observed for clindamycin (99.62%), erythromycin (98.86%), vancomycin (94.68%), teicoplanin (94.11%), and penicillin (93%), severely limiting therapeutic options, particularly against glycopeptides. Lower resistance percentages were found for ciprofloxacin (51.21%), streptomycin (45.92%), chloramphenicol (43.64%), and gentamicin (21.25%), still remaining a significant concern. The results indicate high frequency of MDR *Enterococcus* spp. in both conventional and antibiotic-free production systems, with most isolates demonstrating significant resistance to multiple antibiotics, including clindamycin, erythromycin, vancomycin, teicoplanin, and penicillin. This widespread resistance

severely compromises available therapeutic options, especially against infections treated with glycopeptides. Despite variations in resistance rates among the antibiotics tested, the levels observed for ciprofloxacin, streptomycin, chloramphenicol, and gentamicin are still alarming. No significant differences were found between conventional and antibiotic-free systems, suggesting potential horizontal spread of resistance genes.

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