

1 Prevalence and antimicrobial resistance of *Enterococcus* spp. isolated from animal feed in Japan

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The rising prevalence of antimicrobial resistance (AMR) of bacteria is a global health problem at the human, animal, and environmental interfaces, which necessitates the “One Health” approach. AMR of bacteria in animal feed are a potential cause of the prevalence in livestock; however, the role remains unclear. To date, there is limited research on AMR of bacteria in animal feed in Japan. In this study, a total of 57 complete feed samples and 275 feed ingredient samples were collected between 2018 and 2020. *Enterococcus* spp. were present in 82.5% of complete feed (47/57 samples), 76.5% of soybean meal (62/81), 49.6% of fish meal (55/111), 33.3% of poultry meal (22/66), and 47.1% of meat and bone meal (8/17) samples. Of 295 isolates, *E. faecium* (33.2% of total isolates) was the dominant *Enterococcus* spp., followed by *E. faecalis* (14.2%), *E. hirae* (6.4%), *E. durans* (2.7%), *E. casseliflavus* (2.4%), and *E. gallinarum* (1.0%). Of 134 isolates which were tested for antimicrobial susceptibility, resistance to kanamycin was the highest (26.1%), followed by erythromycin (24.6%), tetracycline (6.0%), lincomycin (2.2%), tylosin (1.5%), gentamicin (0.8%), and ciprofloxacin (0.8%). All *Enterococcus* spp. exhibited susceptibility to ampicillin, vancomycin, and chloramphenicol. Of 33 erythromycin-resistant isolates, only two showed a high minimum inhibitory concentration value (>128 µg/mL) and possessed *ermB*. These results revealed that overall resistance to antimicrobials is relatively low; however, animal feed is a source of *Enterococcus* spp. It is essential to elucidate the causative factors related to the prevalence of AMR in animal feed.

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2 Occurrence Evaluation of Aflatoxigenic *Aspergilli* in Thai Corn Using Dichlorvos-ammonia and Whole-agar Extraction Methods

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Aflatoxins and aflatoxigenic fungi are hazardous to food security and safety since mycotoxins and related fungi in cereals significantly affect animal and human health. The relatively high frequency of aflatoxigenic *Aspergilli* isolates in corn samples remains a concern. Accordingly, we randomly collected corn samples from 10 farms in northern and central Thailand (TM1-TM10) and aimed to detect aflatoxigenic fungi using our recently developed methods: dichlorvos-ammonia (DV-AM) and whole-agar extraction methods. When we placed 100 grains from each sample on 20 agar dish cultures (five grains per dish) to monitor the emergence of fungal colonies, the presence of *Aspergillus niger* and *A. flavus*, with an emergence frequency of 1-8 and 1-7 per 100 grains, respectively, was detected. Some isolates of *A. flavus* produced aflatoxin B₁ and B₂ in the culture media, indicating typical features of aflatoxigenic *A. flavus*, whereas the non-aflatoxin-producing isolates produced kojic acid, thereby suggesting that they belong to *Aspergillus* section *Flavi*. Chemical analysis revealed aflatoxin B₁ and B₂ contamination in some grains and sporadic contamination with fumonisin B₁. Therefore, continuous monitoring and surveillance are required owing to the prevalence of mycotoxigenic fungi in corn.

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