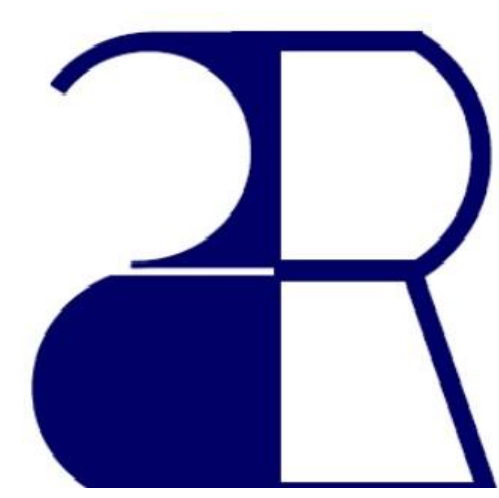




Antimicrobial Resistance Profiles of Emergent *Enterococcus cecorum* causing Systemic Disease in Chickens

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Introduction

The emergence of *Enterococcus cecorum* (EC), responsible for severe systemic disease in chickens two weeks and older, prompts the elucidation of the genetic factors contributing to the phenotypic characteristics differing from those of the EC strains causing classical vertebral osteoarthritis in older chickens. The present study focused on characterizing EC causing systemic infection by Whole Genome Sequencing (WGS) to enhance understanding of emergent phenotypes and offering insights into addressing antimicrobial resistance (AMR) under the One Health approach.

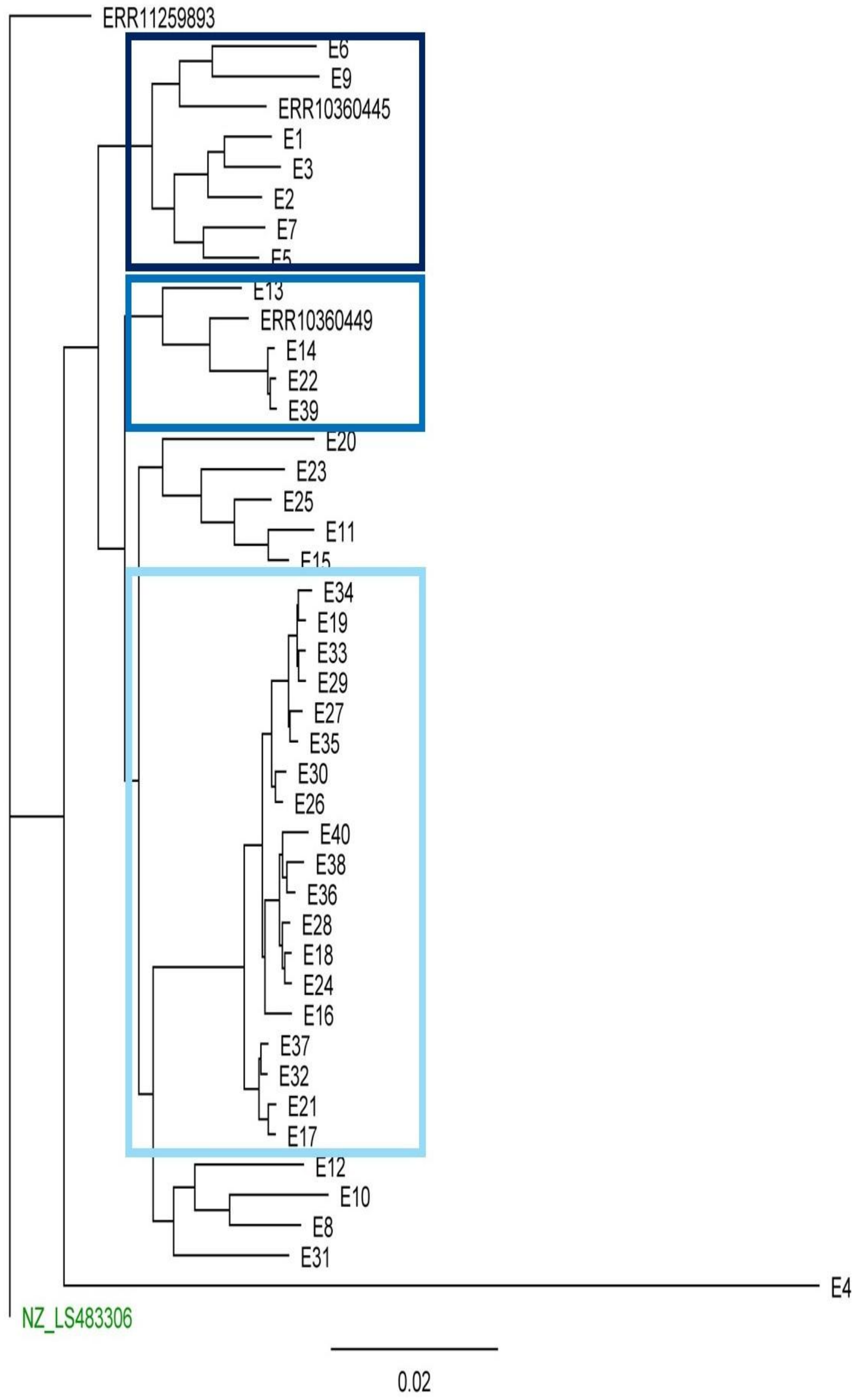
Materials and Methods

A total of 40 EC isolated in 2023 from chicken breeders and broilers at the Poultry Research and Diagnostic Laboratory of Mississippi State University were analyzed to identify the presence of genes predicting antimicrobial resistance. Sequencing was conducted on a MiSeq system (Illumina Inc., San Diego, CA) with a paired-end read length of 2x250 bp. Sequencing reads were assembled using SPADes and the assemblies mapped to the EC classical strain NCTC12421. A RAxML phylogenetic tree was built using the EC SA3 strain linked to enterococcal spondylitis in chickens, the EC non-pathogenic CE1 strain, and *E. faecalis* V583. FASTA files of assembled genomes were uploaded to Rapid Annotation using Subsystems Technology server (RAST), and the SEED viewer was utilized to categorize and compare annotated proteins at <50% identity threshold. Acquired antimicrobial resistance gene identification was performed using the ResFinder (v2.4) database for 90% gene identity.

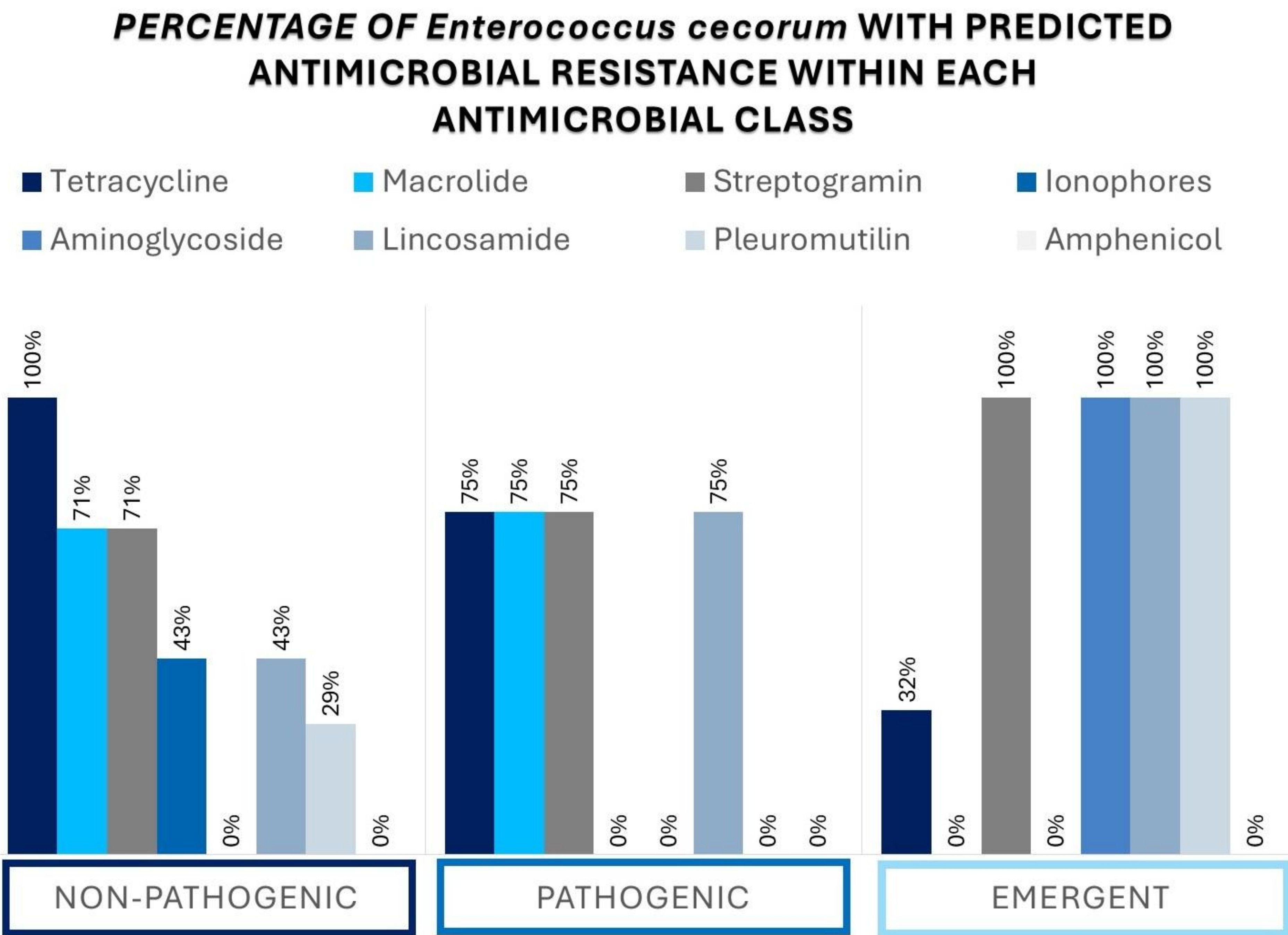
Results

The phylogenetic analysis showed that EC formed distinct clusters separating pathogenic from non-pathogenic strains. The non-pathogenic clade was formed by seven (07) strains, including CE1, with the highest genetic variability (98.3 to 99.2 % Identity). The pathogenic clade included four EC strains clustering with SA3 (98.7 to 99.9 % Identity). The EC emergent clade consisted of 19 isolates from 16 to 48-day-old broilers, forming a highly genetically similar clade (99.4 to 99.9% identity), indicating the greatest clonality among all isolates.

The EC in the emergent clade showed predicted resistance to the Streptogramin (Virginiamycin), Aminoglycosides (Streptomycin), Lincosamide (Lincomycin, Clindamycin), and Pleuromutilin (Tiamulin) classes. However, this clade showed reduced predicted resistance to Tetracycline and no resistance to Macrolides. Multidrug Resistance (MDR) was predicted in 75% and 100% of the EC in the pathogenic and emergent clades, respectively, and in 71% of the non-pathogenic clade. None of the EC strains had genes predicting resistance to Gentamicin or Chloramphenicol. In addition, predicted resistance to Ionophores, linked to the *narA* and *narB* genes, was detected in 7.5% of the total EC strains analyzed.



SNP Phylogenetic analysis: Reference sequence *E. cecorum* NCTC12421 (NZ_LS483306). *E. faecalis* V583 strain (ERR11259893), EC CE1 non-pathogenic strain (ERR10360445), EC SA3 pathogenic strain (ERR10360449)



Conclusions

- The analysis of predicted resistance to veterinary important antimicrobial agents for food-producing animals resulted in high rates of MDR detected in pathogenic EC.
- Predicted resistance to antibiotics that are commonly used (tetracycline) or were used in poultry was reduced.
- Predicted resistance to gentamicin or chloramphenicol was not detected.

These results indicate the emergence of EC capable of causing systemic disease in younger chickens and sharing specific AMR profiles, suggesting a niche adaptation.

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Acknowledgments

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