Caecal microbiota composition of broiler chickens colonised and non-colonised with ESBL-*Escherichia coli*.

Ingrid Cardenas Rey[1,4] • Teresita Bello Gonzalez[1] • Kees Veldman[1] • Daniela Ceccarelli[1*] • Stephanie Jurburg[2] • Jeanet van der Goot[1] • Gerwin Bouwhuis[3] • Arjan de Visser[4] • Michael Brouwer[1]

1. Dept. of Bacteriology, Host Pathogen Interaction and Diagnostics Development, Wageningen Bioveterinary Research, The Netherlands
2. German Centre for Integrative Biodiversity Research, Halle-Jena-Leipzig, Germany • 3. Gezondheidscentrum voor Pluimvee, Emmen, The Netherlands • 4. Laboratory of Genetics, Wageningen University and Research, Wageningen, The Netherlands. * Current Affiliation: Research Executive Agency, European Commission, Brussels, Belgium.

Escherichia coli strains expressing Extended-Spectrum ß-lactamases (ESBL-Ec) have emerged globally in livestock with a high prevalence in poultry production. The chicken's caeca harbours complex and dynamic microbial communities. Among them, *E. coli* is a ubiquitous early coloniser and a potential reservoir for ESBL-plasmid dissemination. This study aims to understand the successional dynamics of the broilers caecal microbiome of ESBL-colonised (ESBL-Co-B) and non-colonised broiler chickens (ESBL-Non-Co-B) and identify any existing microbial composition differences therein.

Methods

Caecal samples from 216 commercial broiler chickens were collected from day 0 to 35 after hatching, daily for the first week of life and weekly thereafter. Culture-based methods (selective isolation) were applied to caecal samples to discriminate between ESBL-Co-B and ESBL-Non-Co-B. A subset (n =89) of caecal samples from day 3 to 28 were sequenced (16S rRNA genes) targeting the V3-V4 region. Microbiota and statistical analyses were performed using R 3.6.1 and the DADA2, Phyloseq and Vegan packages.

Results

ESBL-Ec was detected from day 2 in caecal samples with an increasing prevalence from 0.11, 95% CI [0.01; 0.34] to 1.00, 95% CI [0.81; 1.00] on day 35 (Fig. 1). Microbiota analysis showed no differences in evenness and richness between ESBL-Co-B and ESBL-Non-Co-B, except on day 3 (Wilcoxon rank-sum test, $p = 0.015$) (Fig. 2a). Relative abundance of top 10 genera showed distinct patterns for ESBL-Co-B and ESBL-Non-Co-B overtime (Fig. 3). Bray-Curtis principal coordinate analysis (BC-PCoA) showed significant clustering of samples according to age (Adonis, $p < 0.001$) (Fig. 4a). Age explained 14 % of the ceacal microbiota variation (BC-dbRDA: F = 6.47, $p = 0.001$), while no variability was explained by ESBL (BC-dbRDA: F = 0.73, $p = 0.918$) (Fig. 4b).
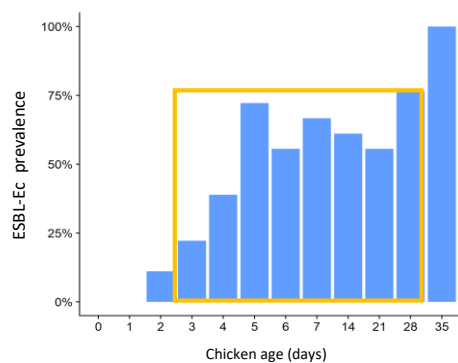

Figure 1. ESBL-Ec prevalence over time. Orange box represents the time points selected for microbiota analyses.
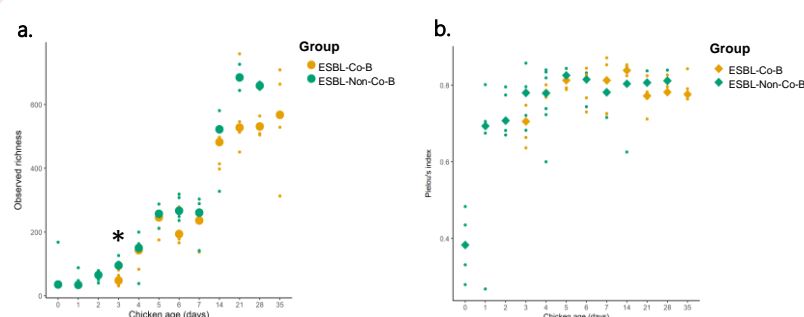

Figure 2 Comparison of microbial richness and evenness between ECB and ENCB. a. Observed microbial richness over time b. Microbial evenness overtime. The asterisk denotes a $p<0.05$ for Wilcoxon rank-sum test.
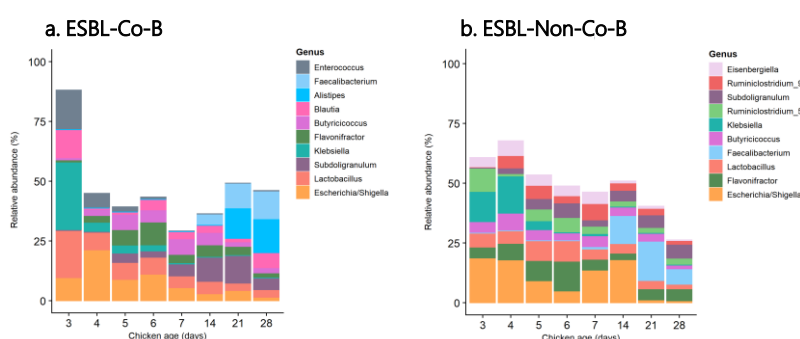

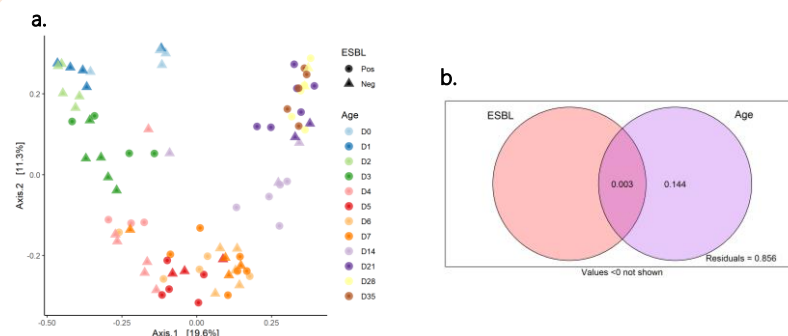Figure 3. Relative abundance of the top ten genera of ESBL-Co-B and ESBL-Non-Co-B.

Figure 4. Microbial composition analyses. a. Changes in caecal microbial community composition over time b. Venn diagram (multivariate dbRDA analysis); variability explained by Age and ESBL.

Understanding the chicken's microbial successional dynamics is essential for the development of intervention strategies. Although our results suggested differences between ESBL-Co-B and ESBL-Non-Co-B, causality can't be explained due the nature of our study. Supplemental research will include additional farms to further elucidate the relation between the broiler caecal microbial composition and ESBL-Ec colonisation.

References
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Contact: Ingrid.cardenasrey@wur.nl