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Prolonged carriage of extended-spectrum beta-lactamase and pAmpC beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in humans: molecular characteristics and risk factors

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Background: So far, little is known about the duration and dynamics of carriage of Extended-spectrum and/or plasmid mediated AmpC β-lactamase-producing (ESBL/pAmpC) *Escherichia coli* and *Klebsiella pneumoniae (*ESBL-E/K*)*. As part of the Livestock Farming and Neighbouring Residents' Health (VGO) Study, a longitudinal study was initiated aiming to determine duration, characteristics of and risk factors for prolonged carriage of ESBL-E/K*,* in predominantly healthy adults living in a livestock-dense area in the Netherlands.

Material/methods: Following a cross-sectional study among 2,432 adults, with an ESBL-E/K prevalence of 4.5%, a subset of ESBL-E/K positive (n=78) and negative individuals (n=255) were selected for follow-up for 5 months with monthly faecal samples and questionnaires about potential risk factors. 76/78 and 249/255 participants were included in the analysis. ESBL-E/K were cultured using selective enrichment and culture. ESBL/pAmpC genes were analysed using PCR and sequencing and multilocus sequence types (MLST) were determined. Per positive sample, 5 colonies were analysed. Risk factors were analysed using logistic regression and odds ratios (ORs) with 95% confidence intervals (95%CI) were calculated.

Results: Of the 76 initially ESBL-E/K positive participants 27 (35.5%) were prolonged carriers (i.e. testing positive for ESBL-E/K with the same gene, for at least 5 sample moments), of which 20 (26.3%) also carried the same E/K MLST for all positive samples. 46/76 (60.5%) tested ESBL-E/K negative at some time point during the follow-up of which 31/76 (40.8%) stayed negative throughout the study.

Of the 249 initially ESBL-E/K negative participants, the majority (n=217, 87.1%) tested continuously negative. Overall, 32/249 (12.9%) participants acquired an ESBL-E/K during the course of this study.

Participants that were nor prolonged carriers for the same gene, nor continuously negative were categorized as intermediate/dynamic carriers (n=81; 49/76 plus 32/249).

For the prolonged carriers, the predominant genes found were *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-27}}$ and the predominant MLST found was ST131. Testing multiple colonies per positive sample revealed within host diversity for 20 participants: 43 of their 116 samples contained multiple isolates.

Statistically significant risk factors for prolonged carriage in multivariate logistic regression analysis were: living within 1,000 meters of at least one mink farm (OR 7.48, 95%CI 1.11-50.35) and antibiotic use during last six months (OR 3.75, 95%CI 1.06-13.34). Comorbidity was borderline statistically significant (OR 2.98, 95%CI 0.91-9.81).

Conclusions: Preliminary results of this longitudinal study show that ESBL-E/K carriage is often not persistent and easily acquired and lost. Further analysis will provide more insight on risk factors of acquisition and prolonged carriage of ESBL/E-K.

Our results also imply that a single test result provides no accurate prediction for prolonged carriage and repeated testing may be needed to assess if someone is a prolonged carrier. This could help inform hospital policy.