Prolonged carriage of extend-spectrum β-lactamase and AmpC β-lactamase-producing \textit{Escherichia coli} and \textit{Klebsiella pneumoniae} in humans: molecular characteristics and risk factors

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Transparency Declaration

- The authors have no conflicts of interest to declare
Introduction

● Infections with ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-E/K) are increasing worldwide

● Often preceded by asymptomatic carriage

● Prevalence of carriage in the general population in the Netherlands:
  – 5-10%
  – To date mainly cross-sectional studies
  – Some longitudinal studies on specific groups: travelers & patients
  – Little is known about persistent carriage in humans in the general population
Objective of the longitudinal study

- To determine duration of carriage as well as the characteristics of and risk factors for persistent carriage of extended-spectrum and plasmidic AmpC β-lactamase-producing (ESBL/pAmpC) *E. coli* and *K. pneumoniae* (ESBL-E/K) in predominantly healthy adults in a livestock dense area in the Netherland

- How long do ESBL-E/K positive individuals remain positive?
- If tested positive repeatedly is it with the same ESBL-producing *E. coli* or *K. pneumoniae* (gene, plasmid, bacterium)?
- Do negative individuals remain negative?
- What is the relevance of single testing?
Material & Methods

Part of the Dutch Farming and Neighbouring Residents’ Health (VGO) Study

To be eligible persons had to:

● be between 18 – 70 years
● live in the eastern part of North Brabant or the northern part of Limburg of the Netherlands
● not live or work on a farm

Participants were asked to provide:

● One faecal sample:  cross-sectional part
● 5 faecal samples with one month interval:  longitudinal part
● fill in questionnaires (6 times)
Material & Methods

ESBL-E/K were isolated and characterized using:

- selective enrichment and culture
- 5 colonies/person/sample moment were tested
- β-Lactamase genes by PCR and sequencing
- *E. coli* and *K. pneumoniae* by MLST
- Plasmid typing by PCR-based replicon typing and pMLST from selection of isolates from persistent carriers

Logistic regression was used to identify risk factors for prolonged carriage and odds ratios (OR) and 95% confidence intervals (95% CI) were calculated.
Material & Methods – Study population

Cross-sectional study

- Positive: 4.5% (n=109)
- Negative: 96% (n=2323)
- Total: 2432

Selection longitudinal study

- Positive: 23% (n=78)
- Negative: 77% (n=255)
- Total: 333

Wielders et al., 2016. Extended-spectrum β-lactamase- and pAmpC-producing Enterobacteriaceae among the general population in a livestock dense area, *Clinical Microbiology and Infection* 2017 Feb;23(2):120.e1-120.e8.
Longitudinal study

- 333 Participants
  - 255 cross-sectional ESBL-E/K negative individuals
  - 78 cross-sectional ESBL-E/K positive individuals
- Cross-sectional study (T0)
- Monthly samples for 5 consecutive months (T1, T2, T3, T4, T5)
- Time between T0 and T1 on average 4 months and between T0 and T5 8 months
- An individual was considered positive for ESBL-E/K if the presence of an ESBL/pAmpC-gene was confirmed
- 8 persons were excluded because less than 4 samples were submitted
325 participants included

76 ESBL+
- n=25 (33%) ESBL+ in all samples provided
- n=24 with the same gene
- n=17 with same E/K
  **Persistent carriers**
- n=51 (67%) ESBL- at least once
- 31 negative 5x (41%)

249 ESBL-
- n=32 (13%) ESBL+ at least once
- n=2 (0.8%) positive 5x
- n=217 (87%) ESBL- in all samples provided
  **Persistent negatives**

n=83 **Intermittent carriers**
Percentage ESBL-carriage of initially ESBL-positives

- T0: 100%
- T1: 50%
- T2: 40%
- T3: 40%
- T4: 40%
- T5: 40%

% ESBL positives
Predominant ESBL genes found

- **Cross-sectional**
  - Total: n=2432

- **T0**
  - n=325

- **Persistent carriers**
  - n=25

The graph shows the distribution of different ESBL genes:
- **CTX-M-1**
- **CTX-M-14**
- **CTX-M-15**
- **CTX-M-27**

The bar chart indicates the prevalence of these genes across different samples.
Number of participants with identical isolates in all samples provided

<table>
<thead>
<tr>
<th>Isolate Description</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> ST 131 / CTX-M-15</td>
<td>3</td>
</tr>
<tr>
<td><em>E. coli</em> ST 131 / CTX-M-27</td>
<td>2</td>
</tr>
<tr>
<td><em>E. coli</em> ST 38 / CTX-M-14</td>
<td>2</td>
</tr>
<tr>
<td><em>E. coli</em> ST 131 / CTX-M-14</td>
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</tr>
<tr>
<td><em>E. coli</em> ST 648 / CTX-M-14</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em> ST 95 / CTX-M-15</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em> ST 405 / CTX-M-15</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em> ST 501 / CTX-M-15</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em> ST 701 / CTX-M-15</td>
<td>1</td>
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<tr>
<td><em>E. coli</em> ST 224 / TEM 52</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em> ST 2076 / CTX-M-15</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em> ST 3727 / CMY-2</td>
<td>1</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> ST 902 / CTX-M-15</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>17</strong></td>
</tr>
</tbody>
</table>
Results

- *E. coli* predominated, *K. pneumoniae* was rarely found
- No significant risk factors could be identified for persistent carriage
## One example

<table>
<thead>
<tr>
<th>Sample</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1122</td>
<td><strong>1122 / CTX-M-15 incI1 (ST31)</strong></td>
<td><strong>1122 / CTX-M-15</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>1276 / CTX-M-15</strong></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>1308 / CTX-M-15 incI1 (ST31)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

- 33% (n=25) of the initially ESBL-positives (n=76), remained positive in all subsequent samples (persistent carriers) (8-9 month)
- 41% (n=31) of the initially ESBL-positives (n=76) tested ESBL-negative 5 times, 42% (n=32) were positive at the end of the study
- Nearly all persistent carriers remained positive with the same gene and plasmid, but not always in the same strain, indicative of horizontal transmission
- CTX-M-15, CTX-M-14, CTX-M-27 predominated (mainly on incF plasmids)
- *E. coli* ST 131 seems to colonize well, irrespective of the gene
- 87% of the ESBL-negatives remained negative in all consecutive samples
- 0.8% (n=2) initially ESBL-negative participants (n=249) tested ESBL-positive throughout the longitudinal study
- Single ESBL-positive test result provides no accurate prediction for persistent carriage
Acknowledgements

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