Intestinal carriage of ampicillin-
and vancomycin-resistant
*Enterococcus faecium* in humans
and pets in the Dutch general
population
Transparency Declaration

• None
**Vancomycin-resistant & Ampicillin-resistant Enterococcus faecium**

VRE: Vancomycin-resistant *Enterococcus faecium*

ARE: Ampicillin-resistant *Enterococcus faecium*

- *Enterococcus faecium* exposure to antibiotics:
  - changes in the gut microbiota
  - facilitate colonization of the GI tract by VRE/ARE (Bonten M.J.M., 2001)

## Background

### Hospitals
- **2012-2015**: 44 VRE outbreaks in Dutch hospitals
- **2015**: incidence of VRE in Dutch hospitals ~1% (Nethmap, 2016)

### Community
- **1996**: study to VRE in turkey farmers and area residents
  - 14% area residents (van den Boogaard, 1997)
- **2002**: VRE not observed in dogs in the Netherlands (Wagenvoort J., et al., 2002)
- **2006-2007**: ARE in healthy dogs
  - 23% in UK
  - 76% in Denmark (Damborg P., et al., 2009)

→ What is the current status of VRE/ARE in the community?
**Aims**

Determine:

1. the prevalence of ARE and VRE in humans, dogs and cats
2. risk factors for ARE and VRE carriage in humans, dogs and cats
3. the co-carriage of ARE and VRE between human-pet pairs
4. The genetic relatedness of non-hospitalized humans and pets
Study design

Cross-sectional

• ESBLAT population study
• from October 2014 to October 2015
• Monthly-repeated
• Online questionnaires
• Random faecal samples of ~2000 Dutch inhabitants
  – and their dog or cat
Laboratory methods

- Plated on Enterococcus plates:
  - Amoxicillin (16 mg/L)
  - Vancomycin (4 mg/L)
- Susceptibility testing with Roscetablet diffusion method.
- PCR: *vanA* and *vanB* (vancomycin)
Invitation & response

Humans

- 25,365 Invited
- 4,721 (18.6%) completed questionnaire
- 1,992 (42.2%) Faecal samples submitted

Pets

- 395 (277 dogs & 118 cats) submitted
Prevalence of ARE/VRE in humans, dogs and cats

**Humans**
- **VRE:**
  - 1 case
  - VanA gene.
  - prevalence: $1/1992 = 0.05\%$
- **ARE:** 29 cases
  - Prevalence:
    $29/1992 = 1.5\%$ (95% CI: 1.0-2.1)

**Dogs**
- **VRE:**
  - 2 dogs
  - VanA genes.
  - Prevalence: $2/277 = 0.7\%$
- **ARE:** 66 dogs
  - Prevalence dogs:
    $71/277 = 25.6\%$ (95% CI: 20.8-31.1%)

**Cats**
- Prevalence cats:
  $6/126 = 4.8\%$ (95% CI: 2.2%-10.0%)
Geographical distribution of ARE
Risk factors for ARE and VRE carriage in humans, dogs and cats

• Multivariable logistic regression models:
  – for humans
  – for dogs
Potential risk factors ARE for human

- Age
- Level of education
- Gender
- Country of birth is the Netherlands
- Use of antibiotics
- Use of proton pump inhibitors
- Use of other drugs
- Hospitalization
- GI complaints
- Travel
- Animals in/around home
- Dogs/cats in households
- Direct contact
- Ate raw or undercooked meat
## Risk factors for ARE in humans

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate OR (95% CI)</th>
<th>Multivariate OR (95% CI)</th>
<th>Bootstrapped multivariate OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>age</strong></td>
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<tr>
<td>&lt;18</td>
<td>Reference</td>
<td>reference</td>
<td>reference</td>
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<tr>
<td>≥18</td>
<td>0.6 (0.2-1.4)</td>
<td>0.4 (0.2-1.1)</td>
<td>0.5 (0.2-1.3)</td>
</tr>
<tr>
<td>use of AB in past 8 wk</td>
<td>4.7 (1.9-11.2)</td>
<td>4.6 (1.9-11.5)</td>
<td>4.2 (1.7-11.2)</td>
</tr>
<tr>
<td>use of proton pump inhibitors in past 6</td>
<td>2.7 (1.2-5.8)</td>
<td>2.7 (1.2-6.2)</td>
<td>2.7 (1.1-6.3)</td>
</tr>
<tr>
<td>travel abroad in past 12 m</td>
<td>0.6 (0.3-1.2)</td>
<td>0.4 (0.2-1.1)</td>
<td></td>
</tr>
</tbody>
</table>
Potential risk factors ARE for dogs

- Age
- Gender
- Hospital
- AB use
- GI symptoms
- Travel
- Contact other animals
- Catch prey
- Coprophagy (eating of stool)
- Eat raw meat
- Eat wet food
- Eat dry food
## Risk factors for ARE in dogs

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<thead>
<tr>
<th>Variables</th>
<th>Univariate OR (95% CI)</th>
<th>Multivariate OR (95% CI)</th>
<th>Bootstrapped multivariate OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of AB in past 6m</td>
<td>2.2 (1.1-4.4)</td>
<td>2.3 (1.1-4.8)</td>
<td>2.3 (1.1-4.6)</td>
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<tr>
<td>Dog eat raw meat</td>
<td>3.2 (1.6-6.7)</td>
<td>3.2 (1.5-6.7)</td>
<td>3.2 (1.4-6.6)</td>
</tr>
<tr>
<td>Dog eat stool</td>
<td>1.5 (0.2-2.7)</td>
<td>1.5 (0.8-2.9)</td>
<td>1.5 (0.8-3.0)</td>
</tr>
</tbody>
</table>

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**Background**

**Methods**

**Results**

**Discussion**

**Conclusion**
Co-carriage of ARE in human-pet pairs

- Is there a relation between humans and pets regarding ARE prevalence?
  - 384 human-pet pairs belonging to the same household

No co-colonization observed
Is there a genetic relatedness between humans and pets regarding ARE prevalence?

• Phylogenetic analysis based on core genome multilocus sequence typing (cgMLST)

Is there a genetic relatedness between humans and pets regarding ARE prevalence?
Is there a genetic relatedness between humans and pets regarding ARE prevalence?

Background

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Discussion

• Is there a genetic relatedness between humans and pets regarding ARE prevalence?
  – Based on only epidemiological data: NO
    • No co-colonization within 1 household
    • Having a dog in the same household is not observed as a risk factor
  – Based on molecular cgMLST data: probably YES
    • Dogs and human ARE are co-located in the same phylogenetic lineages
Conclusion

• VRE only observed in 1 human and 2 dogs
• What about ARE?
  – Prevalence in human relatively low (1.5%)
  – High prevalence in dogs (25.6%)
  – Antibiotic use and proton pump inhibitors are risk factors for ARE in humans
  – Antibiotic use and eating raw meat are risk factors for ARE in dogs
  – No co-carriage in human-pet pairs
  – Human and pet isolates are molecular closely related
Acknowledgment

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  – Ellen Brouwer
  – Janetta Top

• UU
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  – Mirlin Spaninks
  – Arjen Timmermans
Thank you!
Epidemiological data collection:

- Dutch general population
- Invitation and link to questionnaire. Participation?
  - Yes: Wants to provide faecal sample?
    - Yes: Also for dog or cat?
      - Yes: Human and pet sample & All epidemiological relevant data available
      - No: faecal sample human + additional (brief) questionnaire
    - No: Information from population registry
  - No: Additional information from questionnaire
Risk factors for ARE and VRE carriage in humans, dogs and cats

- Missing data $\rightarrow$ imputation
- Logistic regression:
  - Model for humans
  - Model for dogs
- $P \leq 0.25$ selected for multivariate models
- Multivariate analyses: forward stepwise regression analyses, model selection based on Akaike information criteria
- Covariates considered as risk factor if $P$ value $< 0.05$
- Model checked for confounders and interactions.
- Internal validation $\rightarrow$ bootstrapping
<table>
<thead>
<tr>
<th>Variables</th>
<th>ARE+ (n=29)</th>
<th>ARE- (n=1963)</th>
</tr>
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<tbody>
<tr>
<td><strong>Variables</strong></td>
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<tr>
<td><strong>age</strong></td>
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<tr>
<td>&lt;18</td>
<td>6/29 (20.7)</td>
<td>259/1962 (13.2)</td>
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<tr>
<td>≥18</td>
<td>23/29 (79.3)</td>
<td>1703/1962 (86.8)</td>
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<tr>
<td><strong>level of education</strong></td>
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<td></td>
</tr>
<tr>
<td>Intermediate/lower educated (degree below BSc.)</td>
<td>16/29 (55.2)</td>
<td>728/1938 (37.6)</td>
</tr>
<tr>
<td>High educated (BSc., MSc., PhD degree)</td>
<td>13/29 (44.8)</td>
<td>1210/1938 (62.4)</td>
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<tr>
<td><strong>gender (male)</strong></td>
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<td></td>
<td>12/29 (41.4)</td>
<td>878/1962 (44.8)</td>
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<tr>
<td><strong>country of birth is the Netherlands</strong></td>
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<tr>
<td></td>
<td>28/29 (96.6)</td>
<td>1870/1937 (96.5)</td>
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<tr>
<td><strong>use of AB in past 8 wk</strong></td>
<td>7/27 (25.9)</td>
<td>128/1829 (7.0)</td>
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<tr>
<td><strong>use of AB in past 6 m</strong></td>
<td>9/29 (31.0)</td>
<td>322/1932 (16.7)</td>
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<td><strong>use of proton pump inhibitors in past 6 m</strong></td>
<td>10/29 (34.5)</td>
<td>318/1932 (16.5)</td>
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<tr>
<td><strong>use of blood pressure lowering drugs in past 6 m</strong></td>
<td>8/29 (27.6)</td>
<td>379/1932 (19.6)</td>
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<tr>
<td><strong>use of tranquilizers in past 6 m</strong></td>
<td>1/29 (3.4)</td>
<td>72/1932 (3.7)</td>
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<td><strong>use of cholesterol lowering drugs in past 6 m</strong></td>
<td>5/29 (17.2)</td>
<td>287/1932 (14.9)</td>
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<tr>
<td><strong>use of antidiabetic drugs in past 6 m</strong></td>
<td>2/29 (6.9)</td>
<td>68/1932 (3.5)</td>
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<td><strong>hospitalization in past 12 m</strong></td>
<td>1/29 (3.4)</td>
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<td>14/1824 (0.8)</td>
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<td><strong>hospitalization household member in past 12 m</strong></td>
<td>5/29 (17.2)</td>
<td>196/1731 (11.3)</td>
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<tr>
<td><strong>any complaints in past 6 m</strong></td>
<td>17/29 (58.6)</td>
<td>1210/1897 (63.8)</td>
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<tr>
<td><strong>travel abroad in past 12 m</strong></td>
<td>16/29 (55.2)</td>
<td>1330/1950 (68.2)</td>
</tr>
<tr>
<td><strong>travel abroad in past 4 wk</strong></td>
<td>3/27 (11.1)</td>
<td>387/1824 (21.2)</td>
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<tr>
<td><strong>animals in/around home</strong></td>
<td>14/29 (48.3)</td>
<td>949/1943 (48.8)</td>
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<tr>
<td><strong>dog in household</strong></td>
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<td>450/1943 (23.2)</td>
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<tr>
<td><strong>cats in household</strong></td>
<td>6/29 (20.7)</td>
<td>410/1943 (21.1)</td>
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<td><strong>direct contact animals in past 4 wk</strong></td>
<td>13/27 (48.1)</td>
<td>1068/1807 (58.9)</td>
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<tr>
<td><strong>ate raw or undercooked meat in past week</strong></td>
<td>9/26 (34.6)</td>
<td>532/1818 (29.3)</td>
</tr>
<tr>
<td>Variables</td>
<td>Dogs ARE+ (n=71)</td>
<td>Dogs ARE- (n=199)</td>
</tr>
<tr>
<td>-----------------------------------</td>
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<tr>
<td></td>
<td>n (%)</td>
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<tr>
<td>age</td>
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</tr>
<tr>
<td>&lt;6</td>
<td>34/70 (48.6)</td>
<td>91/198 (46.0)</td>
</tr>
<tr>
<td>≥6</td>
<td>36/70 (51.4)</td>
<td>107/198 (54.0)</td>
</tr>
<tr>
<td>gender (male)</td>
<td>34/70 (48.6)</td>
<td>96/198 (48.5)</td>
</tr>
<tr>
<td>hospitalized or consult</td>
<td>14/62 (22.6)</td>
<td>36/189 (19.0)</td>
</tr>
<tr>
<td>AB in past 6 wk</td>
<td>4/61 (6.6)</td>
<td>14/189 (7.4)</td>
</tr>
<tr>
<td>AB in past 8 wk</td>
<td>17/69 (24.6)</td>
<td>25/199 (12.6)</td>
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<tr>
<td>kennel in past 4 wk</td>
<td>5/62 (8.1)</td>
<td>16/189 (8.5)</td>
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<tr>
<td>any complaints in past 4 wk</td>
<td>18/59 (30.5)</td>
<td>56/186 (30.1)</td>
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<td>abroad in past 12 m</td>
<td>14/70 (20.0)</td>
<td>31/200 (16.1)</td>
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<td>contact other animals</td>
<td>33/69 (47.8)</td>
<td>90/193 (46.6)</td>
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<tr>
<td>catch prey</td>
<td>11/68 (16.2)</td>
<td>41/191 (21.5)</td>
</tr>
<tr>
<td>coprophagy</td>
<td>24/70 (34.3)</td>
<td>50/198 (25.3)</td>
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<tr>
<td>eat raw meat</td>
<td>18/70 (25.7)</td>
<td>17/199 (8.5)</td>
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<tr>
<td>eat wet food</td>
<td>15/70 (21.4)</td>
<td>37/199 (18.6)</td>
</tr>
<tr>
<td>eat dry food</td>
<td>63/70 (90.0)</td>
<td>188/199 (94.5)</td>
</tr>
</tbody>
</table>
Discussion

• ARE from healthy humans do not cluster among *E. faecium* susceptible for ampicillin from the community

• No direct transmission (no co-carriage)
  – but close phylogenetic linkage suggests epidemiological linkage

• AB use is a risk factor for ARE in humans and dogs:
  – ARE already present -> Selection due to AB use -> detection?
  – In humans: continues exposure of ARE (due to dogs?) -> using AB -> colonization -> detection?

Limitations

• Relatively low number of carriers of ARE and VRE in humans and cats