Detection of VIM-1 carbapenemase encoding *E. coli* in German pig production

Alexandra Irrgang, Jennie Fischer, Silvia Schmoger, Bernd-Alois Tenhagen, Mirjam Grobbel, Jens Hammerl, Annemarie Käsbohrer
Background

RESET 1 IP3 (FU Berlin):
longitudinal studies of 7 pig farms (S1-S7) and 7 chicken farms (G1-G7)

IP1 (BfR)
molecular characterization of isolates

Detection of VIM-1 carbapenemase producing *E. coli* and *Salmonella* in samples of four different farms

- *Salmonella* Infantis R3
  - pooled dust
  - G1

- *Salmonella* Infantis R25
  - boot swaps (outside)
  - S1

- *Salmonella* Infantis V363
  - feacal sample
  - S3

- *Salmonella* Infantis R27
  - feacal sample
  - E. coli R29, R178
  - pooled feacal sample, boot swap

Alexandra Irrgang
Background

Salmonella Infantis R27 feecal sample
E. coli R29, R178 pooled feecal sample, boot swap

retrospective study

Further 35 E. coli isolates obtained from dust, faeces, boot swaps, flies, liquid manure
Task 6: Screening for new carbapenemases producing Enterobactericeae in samples from animal, food and environment

IP1 → National Reference Laboratory for Antimicrobial Resistance (NRL-AR)

Monitoring on:
- i) commensal *E. coli*
- ii) ESBL producing *E. coli*
- iii) carbapenemases producing *E. coli*

Screening for carbapenem resistant isolates

MIC

24 isolates in 2015

- MERO $\geq 0.12$ mg/L
- ETP $\geq 0.12$ mg/L
- IMI $\geq 1$ mg/L
Results of the Screening for Carbapenemases March 2016

1/24 isolates positive for $bla_{VIM}$

PCR/sequencing: **VIM-1**

**R1176:**

obtained from swine colon content

December 2015 (ESBL monitoring)

**MIC:**
- MERO: 0.5 / 0.25 mg/L
- ETP: 0.12 mg/L
- IMI: 2 mg/L
Molecular Characteristics of the *E. coli* Isolate R1176

**XbaI**

- S2/2011
- clonal

**S1 nuclease**

- VIM-1 plasmid

<table>
<thead>
<tr>
<th></th>
<th>R1176</th>
<th>R29/ R178</th>
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<tr>
<td><strong>bla genes</strong></td>
<td>VIM-1</td>
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<td>ACC-1</td>
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- known VIM-plasmid missing
- negative by southern blot hybridization
- chromosomally located
Unique finding?

slaughter house

additional colon content samples from the same producer

6 samples send to BfR

enrichment selective/non-selective

plating on selective culture media
-CTX 1mg/L
-MEM 0.125 mg/L
-MEM 0.06 mg/L

direct plating
-CTX 1mg/L
-MEM 0.125 mg/L
-MEM 0.06 mg/L

single colonies picked → PCR
Characteristics of the slaughter house Isolate

→ 1 sample (4 isolates) positive for \( \text{bla}_{\text{VIM}} \)

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**Characteristics of the slaughter house Isolate**

**S1 PFGE**

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## Characteristics of the slaughter house Isolate

**Recurrent detection of VIM-1-producing *Escherichia coli* clone in German pig production**

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Persistence at farm vs. contamination at slaughter??

**Sampling:**

1) pooled faeces of piglets arriving at the farm

   6 samples send to BfR April 2016

2) pooled faeces from fattening pigs and their environment

   sampling in May 2016
2. Sampling on the pig farm

fattening farm:

- 6 barns
- 8-14 pens each
- faeces of two pens pooled (n=33)
- + boot swaps, wipe sample of barn environment…

in total: ~70 samples
2. Isolation method

**Recommended protocol by EFSA (meat and caeca):**

Unselective pre-enrichment in Buffered Peptone water (1:10)

37° C / o.n.

selective agar plates

37° C / o.n.

subcultivation of presumptive colonies

faecal samples

over-growing by *Pseudomonas aeruginosa*
2. Isolation procedure

- **unselective pre-enrichment in BPW**
  - 1:10 diluted
  - MC+CTX
  - MC+CTX
  - ChromID Carba

- **selective pre-enrichment in LB+CTX 1 mg/L**
  - -80°C
  - one pinkish single colony detected out of 40 samples
  - MC+CTX
  - ChromID Carba

Did we miss something?
2. Isolation procedure – PCR screening

- **Unselective pre-enrichment in BPW**
  - 1:10 diluted
  - MC+CTX
  - ChromID Carba

- **Selective pre-enrichment in LBL+CTX 1 mg/L**
  - -80°C
  - MC+CTX
  - ChromID Carba

- **Isolates from two samples**

- **Real-time PCR**
  - LBL+CTX
  - 3 additional positive samples

- **Single colonies in 96 well format in LBL+MEM 0.125 mg/L**
  - 1:10 diluted
  - MC+CTX
  - ChromID Carba

- **Real-time PCR**

- **Additional positive samples**
2. Isolation procedure

- Unselective pre-enrichment in BPW
- Selective pre-enrichment in LBL+CTX 1 mg/L

1:10 diluted

- MC+CTX
- MC+CTX
- ChromID Carba

-80°C

- LBL+CTX 44°C/24h

- MC+CTX
- ChromID Carba

Isolates from the 4th sample
Investigation fattening farm - Isolates

→ 4 positive feacal samples:

<table>
<thead>
<tr>
<th>Barn</th>
<th>No. of positive samples</th>
<th>No. of isolates</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3 / 18</td>
</tr>
<tr>
<td>Σ</td>
<td>3</td>
<td>4</td>
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<td></td>
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Investigation fattening farm - characterization of the isolates

→ high similarities
Investigation fattening farm - characterization of the isolates

→ variability of the plasmids

→ two samples → bla\textsubscript{VIM-1} chromosomally located
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Characterization of the isolates - sequencing

→ 2015/2016: Σ 37 Isolates

**PacBio**
- R1176 (chromosomal)
- R1184 (plasmid)
- „reference genomes“

**Miseq (Illumina)**
- R1176 (chromosomal; at slaughter)
- R1180 (plasmid, slaughter)
- R1182 (plasmid, farm, barn 6, sample 3)
- R1184 (plasmid, farm, barn 6, sample 3)
- R1191 (chromosomal, farm, barn 6, sample 6)
- R1203 (plasmid, farm, barn 5)
- R1207 (plasmid, farm, barn 5)
- R1208 (plasmid, farm, barn 5)
- R1209 (chromosomal, farm, barn 1)

Figure: https://research.medicine.umich.edu