Tetracycline resistance \textit{tet}(A) plasmids transferring multiresistance in enterotoxigenic \textit{E. coli} strains from pigs

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MTA ATK
Állatorvos–tudományi Intézet
Antimicrobial resistance of porcine ETEC

- **ETEC** (enterotoxigenic *E. coli*): watery diarrhea in weaned pigs

- Antimicrobials used to treat and prevent diarrhea in young animals:
  
  1950... – **Tetracyclines** : feed additives
  - Sulfonamides

  2006... – **Aminopenicillins**
  - (Fluoro)quinolones

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<thead>
<tr>
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<td>8 (36)</td>
<td>25 (61)</td>
<td>43 (38)</td>
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<td>21 (95)</td>
<td>39 (95)</td>
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<td>1 (4)</td>
<td>2 (9)</td>
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<td>TMP</td>
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<td>SUL</td>
<td>22 (88)</td>
<td>24 (100)</td>
<td>22 (100)</td>
<td>34 (83)</td>
<td>102 (91)</td>
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</table>

- **Constant challenge** for the **therapy** and for the **bacteria**

Tetracycline resistance (Tet\(^R\)) plasmids

- **Conjugative resistance plasmids**: the most effective genetic vectors in the transfer of tetracycline resistance
  - \textit{tet}(A) or/and \textit{tet}(B) genes encoding efflux proteins

- **Hybrid plasmids**: resistance + virulence
  - represents further concern affecting therapy

\textbf{pTC plasmid}

representant of hybrid ETEC plasmids

- \textit{est}A, \textit{est}B: heat-stable enterotoxins
- \textit{tet}(B): \textit{Tn}10 – tetracyclin resistance

\textbf{What about \textit{tet}(A) plasmids?}

Fekete at al., 2012
Objectives

I. comparative description of antimicrobial resistance in porcine post-weaning ETEC strains (Hungary, Austria, Czech Republic, USA)

II. identification of tetracycline resistance \textit{tet} gene types and ETEC–specific \textit{virulence} genes

III. characterization of \textit{tet}(A) \textit{plasmids} and their role the (co)transfer of antimicrobial resistance (and virulence) genes
ETEC strains and methods

- ETEC strains – porcine post–weaning diarrhea = 87
  Hungary (16), Austria (n=34), Czech Republic (n=17), USA (20)

- Characterization of tetracycline resistant strains
  - resistance phenotype: disc diffusion – 17 relevant antimicrobials
  - typing of tet genes by – PCR
  - detection of ETEC–specific virulence genes: estA, estB, elt, f18, k88

- Detection and characterization of tet(A) plasmids
  - conjugational plasmid transfer: 8 tet(A)+ F18+ ETEC strains
  - antimicrobial resistance and virulence gene profiling – PCR
  - plasmid profile analysis: parental and transconjugant strains

- tet(A)–monoplasmidic derivatives (Hungary, Czech Republic)
  - PCR–based replicon typing (PBRT): – 21 Inc groups
  - cassette structure of class I integron: GenBank JQ313793
Results
Antimicrobial resistance of porcine ETEC

- **common multiresistance (MDR):**
  - sulfamethoxazole (SMX)
  - tetracycline (TET)
  - streptomycin (STR)

- **USA resistance > middle Europe**
Tetracycline resistance *tet* genes

- characteristically – one *tet* gene type / strain
- predominant *tet* genotypes: *tet*(B) and *tet*(A) genes
- *tet*(A): in majority of the Hungarian and Czech strains
### Conjugational transfer of *tet*(A) resistance

<table>
<thead>
<tr>
<th>Strains</th>
<th>Country</th>
<th>O type</th>
<th>ETEC-specific virulence</th>
<th>Plasmids (~kb)</th>
<th>Additional Information</th>
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</thead>
<tbody>
<tr>
<td>2185</td>
<td>Hungary</td>
<td>O141</td>
<td><em>estA, estB, f18</em></td>
<td></td>
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<td>2188</td>
<td>Hungary</td>
<td>O157</td>
<td><em>estA, estB, f18</em></td>
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<tr>
<td>9658</td>
<td>Czech Republic</td>
<td>O149</td>
<td><em>estB, elt</em></td>
<td></td>
<td>no transfer of tetracycline resistance</td>
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<tr>
<td>9877</td>
<td>Czech Republic</td>
<td>O35</td>
<td><em>estB, elt</em></td>
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<td>11112</td>
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<td>O149</td>
<td><em>estB, elt, f18</em></td>
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<tr>
<td>11674</td>
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<td>O108</td>
<td>-</td>
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<tr>
<td>2172</td>
<td>Hungary</td>
<td>O141</td>
<td><em>estA, estB, f18</em></td>
<td>174, 138, 38</td>
<td></td>
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<td>11732</td>
<td>Czech Republic</td>
<td>O141</td>
<td><em>estA, f18</em></td>
<td>138, 106, 60, 5, 4</td>
<td></td>
</tr>
</tbody>
</table>

- transfer of *tet*(A) genes in two F18+ ETEC strains
- two *tet*(A)-positive monoplasmidic transconjugants
  - Hungary (2172/11); Czech Republic (11732/71)

characterization of *tet*(A) plasmids
ETEC *tet*(A) plasmids mediating multiresistance

**Czech Republic**

<table>
<thead>
<tr>
<th>Transconjugant</th>
<th>Resistance</th>
<th>Virulence</th>
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<tbody>
<tr>
<td>11732/71</td>
<td><em>tet</em> (A), <em>aadA, strA, catA1</em></td>
<td><em>estA, f18</em></td>
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</table>

**Hungary**

<table>
<thead>
<tr>
<th>Transconjugant</th>
<th>Resistance</th>
<th>Integron type</th>
<th>Virulence</th>
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<tr>
<td>2172/11</td>
<td><em>tet</em> (A), <em>aadA1, strA</em></td>
<td><em>intI1</em></td>
<td><em>estA, estB, f18</em></td>
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</tbody>
</table>

**Novel class 1 integron**

- streptothricin (*estX*)
- aminoglycoside (*aadA1*)

**Plasmids**

- **Tn1721**
  - p11732/71: ~106 kb
    - Tet (A), aadA1, strA
    - catA1
    - estA
  - p2172/11: ~138 kb
    - Tet (A), aadA1, strA
    - catA1
    - estA

- **Integron type**: intI1

**Class 1 integron**

- 5' CS
- *estX*
- *aadA1*
- 3' CS
- qacEΔ1, sul1
Summary and conclusion

- Results indicated a relatively high proportion of tet(A) gene carrier strains, especially among Hungarian and Czech ETEC strains.

- The transfer of tet(A) genes was successful in case of one Hungarian and one Czech strain, on large multidrug resistance plasmids of IncI1 and IncF type.

- The tet(A) plasmid of the Hungarian strain carried a class 1 integron, which showed a novel gene cassette arrangement: estX–aadA1.
Thanks for Your attention!