A comprehensive study on ESBL-producing *E. coli* in Sweden from food producing animals, food, and humans

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Project 2012-2014

- Collaborative effort
  - Public Health Agency of Sweden
  - National Food Agency
  - National Veterinary Institute

- Funded by the Swedish Civil Contingencies Agency
Questions asked

• What is the prevalence of ESBL-producing *E. coli* (EPE) in food, food producing animals, healthy volunteers and in the environment in Sweden?

• Is food a possible vector for transmission of ESBL-producing bacteria to humans?
ESBL-producing *E. coli* (EPE) in Sweden

ESBL and pAmpC is included in EPE
Project strategy

- All sampling within a reasonable time-frame (2010-2013)
- Sampling in the same geographic area (Sweden)
- Similar (almost identical) methods used for isolation
- Identical methodology for further characterization of isolates
Sampling

Healthy humans
n=2130

Bloodstream infections
n=387

Gulls
n=273

Food producing animals
n=1182

Food
n=1448

Sewage
n=12

Raw surface water
n=98
# Sampling

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Samples</th>
<th>Year</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leafy greens (domestic and imported)</td>
<td>630</td>
<td>2012-2013</td>
<td>Egervärn et al, in preparation</td>
</tr>
<tr>
<td>Food producing animals (domestic)</td>
<td>1182</td>
<td>2010 - 2012</td>
<td>SVARM 2013, Duse et al 2014, Börjesson et al 2013</td>
</tr>
<tr>
<td>Raw surface water</td>
<td>98</td>
<td>2012</td>
<td>Egervärn et al, in preparation</td>
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<td>Gulls</td>
<td>273</td>
<td>2013</td>
<td>Bonnedahl et al, in preparation</td>
</tr>
<tr>
<td>Sewage</td>
<td>12</td>
<td>2012-2013</td>
<td>Kwak et al, 2014</td>
</tr>
<tr>
<td>Healthy humans</td>
<td>2130</td>
<td>2012-2013</td>
<td>Ny et al, in preparation</td>
</tr>
</tbody>
</table>
Methods

Pre-enrichment
1 mg/L cefotaxime (food)

Direct plating
agar 1 mg/L cefotaxime (animal, water) or 3 mg/L cefpodoxime (human, sewage)

Chromogenic agar

ESBL production phenotypically confirmed
Double-disk diffusion/E-tests
Occurrence of EPE in food-producing animals in Sweden

Turkeys (n= 55)
Calves (n= 742)
Slaughter pigs (n=184)
Layer hens (n= 69)
Broilers (n= 100)

0%
1%
2%
13%
40%

Vertical transmission in the broiler production pyramid

Pedigree
Great grandparents
Grandparents
Parents
Broilers

Imported to Sweden

Nilsson et al. 2014 J Antimicrob Chemoter, 69, 1497-1500
Occurrence on meat in Sweden

<table>
<thead>
<tr>
<th>Type</th>
<th>Sample Size (n)</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork (n=219)</td>
<td>7%*</td>
<td></td>
</tr>
<tr>
<td>Beef (n=178)</td>
<td>0%*</td>
<td>6%*</td>
</tr>
<tr>
<td>Chicken (n=311)</td>
<td>95%*</td>
<td>38%*</td>
</tr>
</tbody>
</table>

*Egervärn et al. 2014 Int J Food Microbiol, 171, 8-14  
**Börjesson et al. 2013 Appl Environ Microbiol, 79, 2463-2466  
***SVARM 2011
Leafy greens

Leafy greens
(n=630)

0%

0.5%

0%

0%

Sid
The environment

- Raw surface water (n=98) (n=98)
  - 28%
- Gulls (n=273)
  - 11%
- Sewage (n=12 24h samples)
  - 100% (1,8%)

Henriksdal sewage plant

**Incoming water**
- 250,000,000 liter
- 50,000,000,000,000 E. coli
- 1.8% ESBL-producing

**Purification 99.93% reduction**
- 35,000,000,000,000 E. coli
- >1,000,000,000,000 ESBL-producing

Thus, every second 12 million ESBL-producing E. coli are released into the Baltic Sea
**Data collection**

Feces: Swedish population
2011-2013

- ~11400 questionnaires sent out
- ~2500 questionnaire in return
- 2130 fecal samples
- 101 identified ESBL carriers
- Swedish carriage rate: 4.7%

BSI isolates: Swedish hospital
2011-2012

- 715 EPE reported
- 387 EPE collected

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Risk factors

• Travel abroad
  – Swedish studies have shown that ~24% of people travelling abroad return as ESBL-carriers (Tham et al 2010 och Tängden et al 2010)

• Contact with health care
  – Difference in carriage rate between people that were hospitalized compared to people visiting primary care facilities (6.8% vs. 3%) (Strömdahl et al 2011)

• Previous antibiotic treatment
  – Evidence inconclusive in literature

• Direct contact with animals
  – People working in chicken industry more often colonized with ESBL (bl.a. Dierikz et al 2012)
  – Owning multiple animals a risk factor in Dutch study (Huijbers et al 2013)

• Food habits?
Risk factors associated with community carriage

- No difference seen for
  - age, sex, antibiotic treatment

- Risk factors detected in univariate analysis
  - Travel to countries in the Asian and African region
  - Seeking healthcare outside Sweden
  - A diet without pork
  - No daily contact with animals

- Risk factors remaining after multivariable analysis
  - Travel to countries in the Asian and African region
  - A diet without pork
Travel outside of the Nordic countries a risk factor

Carriage rate and travel previous six months

North America: 6% (31)
South America: 0% (13)
Europe: 4% (919)
Asia: 13% (66)
North America: 0% (13)
Multiple countries: 6% (304)
Summary

• Prevalence in food, food producing animals and the environment was studied:
  – Low in food with Swedish origin except for chicken meat
  – Low in food producing animals in Sweden except for in broilers and laying hens
  – Varying in imported food, low on leafy greens
  – High in anthropogenic waters

• Carriage in Sweden in healthy population 4.7 %
Genetic and phenotypic characterization

- Gene – sequencing of ESBL enzyme
- Plasmid – transformation and plasmid replicon typing
- Chromosomal similarities – multilocus typing of core genes (MLST)
- Antibiotic susceptibility testing
Distribution of ESBL genotypes

- **tem-52**
- **ctx-m-55**
- **shv-12**
- **ctx-m-12**
- **ctx-m-8**
- **ctx-m-2**
- **ctx-m-14**
- **ctx-m-1**
- **ctx-m-15**
- **cmy-2**
- **Other genes**

- Food (imported), n=103
- Food (domestic), n=74
- Food producing animals, n=53
- Community carriers (n=1,141)
- Bloodstream infections, n=387
- Environment (e.g., raw water, soil)
- Sewage
Overlap on gene level

![Bar chart showing overlap on gene level across different categories.]

- **Food (imported)**: n=103
- **Food (domestic)**: n=74
- **Food producing animals**: n=33
- **Community carriers**: n=101
- **Bloodstream infections**: n=387

- **tem-52**
- **ctx-m-55**
- **shv-12**
- **ctx-m-2**
- **ctx-m-14**
- **ctx-m-1**
- **ctx-m-15**

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Folkhälso MYNDIGHETEN
PUBLIC HEALTH AGENCY OF SWEDEN
Plasmid replicon typing

ESBL/pAmpC genes

Transformation and plasmid replicon typing
Plasmid-gene combinations

All combinations above 5 isolates

- Imported foods (n=89)
- Swedish foods (n=74)
- Food producing animals (n=50)
- Community carriers (n=69)
- Severely ill patients (n=230)
- Environment (n=31)
- Sewage water (n=18)

- Other combinations
- bla-ctx-m-14, nt
- bla-ctx-m-15, IncFIA, FIB
- bla-ctx-m-2, IncHI2, P
- bla-ctx-m-14, IncFIA, FIB, FII
- bla-ctx-m-15, IncFII
- bla-ctx-m-14, IncFII
- bla-ctx-m-8, IncI1
- bla-ctx-m-15, IncFIA, FII
- bla-ctx-m-15, IncI1
- bla-cmy-2, IncI1
- bla-ctx-m-27, IncFIA, FIB, FII
- bla-ctx-m-1, IncI1
- bla-ctx-m-15, IncFIA, FIB, FII
- bla-ctx-m-15, nt
- bla-cmy-2, IncK
## Overlap on plasmid+gene level

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imported meat</td>
<td>89</td>
</tr>
<tr>
<td>Swedish meat</td>
<td>74</td>
</tr>
<tr>
<td>Food-producing animals</td>
<td>50</td>
</tr>
<tr>
<td>Community carriers</td>
<td>69</td>
</tr>
<tr>
<td>Blood stream infections</td>
<td>230</td>
</tr>
</tbody>
</table>

**Gene Distribution**

- tem-52, Inc-I1
- ctx-m-8, Inc-I1
- ctx-m-1, Inc-N
- ctx-m-1, Inc-I1
- ctx-m-1, Inc-FII
- ctx-m-15, Inc-I1
- ctx-m-14, Inc-FII
- cmy-2, Inc-K
- cmy-2, Inc-I1
Multiple Locus Sequence Typing (MLST)

- **ESBL/pAmpC genes**
- **Transformation and plasmid replicon typing**
- **Susceptibility to 14 antibiotics by broth microdilution**
Multi-resistance

Share MDR (%)

- Imported meats: 71%
- Swedish meats: 28%
- Food producing animals: 34%
- Community carriers: 79%
- Blood stream infections: 90%
- Environment: 74%
- Sewage: 83%

Börjesson, Ny et al, manuscript
Antibiotics to humans and animals
(approximation)
Identical in all assays (gene, plasmid, MLST)

<table>
<thead>
<tr>
<th></th>
<th>Imported foods</th>
<th>Food-producing animals</th>
<th>Community carriers</th>
<th>BSI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
<td>% (n)</td>
</tr>
<tr>
<td>bla-ctx-m-15, IncI1, ST10</td>
<td>2 (2)</td>
<td>1 (2)</td>
<td>3 (2)</td>
<td>0.5 (1)</td>
</tr>
<tr>
<td>bla-cmy-2, IncK, ST57</td>
<td>2 (2)</td>
<td>3 (6)</td>
<td>2 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>bla-ctx-m-1, IncI1, ST155</td>
<td>1 (1)</td>
<td>3 (6)</td>
<td>2 (1)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

5 isolates of 1448

6 isolates of 488

The clonal overlap thus very small
Summary

• ESBL-producing bacteria are spread in the food sector, environment and among humans in Sweden

• Food on the Swedish is likely not a major source for ESBL-producing *E. coli* seen in the health care system

• Results are a snapshot of the situation right now
More..

• Report in Swedish (English summary)
  – http://www.livsmedelsverket.se/globalassets/rapporter/2014/esbl-bildande-e-
    coli---livsmedel-som-spridningsvag.pdf

• 2 oral presentations at ECCMID 2015
  – Sofia Ny, Saturday @ 4.40 PM hall D
    – Circulation of ESBL-producing *E. coli* in the Swedish population; a comparison of
      isolates from community carriers, non-invasive- and invasive infections
  – Stefan Börjesson, Sunday @ 12.05 PM hall G
    – Distribution of ESBL and pAmpC-producing *E. coli* in different sectors in Sweden, with
      a specific focus on food as a dissemination route to humans

• Look out for more scientific publications!
Thank you!

Jonas Bonnedahl, Linneus University, Kalmar

Inger Kuhn and Roland Möllby, Karolinska Institutet

Clinical microbiology laboratories

Swedish municipalities

Volunteers in community carriage study

Personnel at water works and sewage plant

Swedish Civil Contingencies Agency