Resistances in the Wilderness

Should we keep distance from dear deer?

ANTIMICROBIAL RESISTANCE IN E. COLI FROM WILD ANIMALS IN POLAND

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Department of Microbiology
National Veterinary Research Institute
Puławy, Poland
Antimicrobial resistance under One Health concept

- Safe food
- Resistance genes pool
- Healthy animals
- Healthy humans
- Antimicrobial consumption usage
- Healthy environment
- Antimicrobial selective pressure

One health
Resistance in Poland: humans

- **carbapenemases**
  - CTX-M-3: 80.6% (-15)
  - SHV: 17.5% (-2, -5, -12)
  - TEM: 0.7% (-19, -48)
  - CMY: *P. mirabilis* 20.5% (-12, -15)

- **NDM**

- **ESBLs**
Resistance in Poland: food animals

Antimicrobial resistance in commensal *Escherichia coli* isolated from animals at slaughter

Dariusz Wasył*, Andrzej Hoszowski, Magdalena Zająć and Krzysztof Szulowski

Resistance reflects antimicrobial usage policies and management practices different animal species

Cattle (N=707)

79,9%

Broilers (N=682)

5,1%

Turkey (N=693)

11,3%

Swine (N=710)

35,9%

Layers (N=638)

39,3%
Resistance in Poland: food animals

Antimicrobial resistance in commensal *Escherichia coli* isolated from animals at slaughter

*Dariusz Wasyly*, Andrzej Hoszowski, Magdalena Zając and Krzysztof Szulowski

Trends over 4-years period

- **stable resistance**
- **increasing %NWT**
- **increasing MICs**
- **decreasing %NWT**
- **decreasing MICs**

- broilers: Amp, Ctx
  - layers: Ctx, Ctz
  - turkeys: Amp, Ctz, Gen, Su
- cattle: Ctx, Ctz, Gen
- layers: Str
- layers: Chl, Flr, Tet
- cattle: Chl
Resistance in Poland: food animals

Prevalence and Characterization of Cephalosporin Resistance in Nonpathogenic *Escherichia coli* from Food-Producing Animals Slaughtered in Poland

Dariusz Wasyl, Andrzei Hoszowski, Magdalena Zając, and Magdalena Skarzyńska

Department of Microbiology, National Veterinary Research Institute, 24-100 Pulawy, Poland
wasyl@pvew.pulawy.pl

**TABLE 1. Resistance Mechanisms by Source of *Escherichia coli* Isolation**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Resistance mechanism</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL (n = 15)</td>
<td>CTX-M-1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CTX-M-1+TEM-1b</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SHV-12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Not identified</td>
<td>2</td>
</tr>
<tr>
<td>ampC (n = 33)</td>
<td>CMY-2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>CMY-2+ampC overexpression: C-85T+G-18A+C-1T+C58T</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C-73T+G-28A+C58T</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Not tested</td>
<td>2</td>
</tr>
</tbody>
</table>

*ARM, N=880* ESC selective screening (N=129)

**FIG. 1.** ESC prevalence in *Escherichia coli* isolated from food animals (digits in brackets stands for number of samples)

* huge burden of cephalosporin resistance in food animals
* genetic background different from humans
Resistance in Poland: food animals

Lalak et al., submitted

<table>
<thead>
<tr>
<th>Isolation source</th>
<th>Cephalosporin resistance genes (bla)</th>
<th>TEM-1</th>
<th>TEM-135</th>
<th>TEM-52/-92</th>
<th>SHV-12</th>
<th>CTX-M-1/61</th>
<th>CMY-2</th>
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</thead>
<tbody>
<tr>
<td>cattle</td>
<td>TEM</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CTX-M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SHV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>CMY-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TEM + CTX-M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>TEM + SHV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TEM + CMY-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>CTX-M + CMY-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TEM + CTX-M + SHV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TEM + CTX-M + CMY-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>not identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

Percentage and number of isolates:
Resistance in Poland: food animals

High-level fluoroquinolone resistant *Salmonella enterica* serovar Kentucky ST198 epidemic clone with IncA/C conjugative plasmid carrying *blaCTX-M-25* gene
Dariusz Wasyl*, Izabela Kern-Zdanowicz*, Katarzyna Domańska-Blicharz†, Magdalena Zając‡, Andrzej Hoszowski§

First isolation of ESBL-producing *Salmonella* and emergence of multiresistant *Salmonella* Kentucky in turkey in Poland
D. Wasyl*, A. Hoszowski

The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain

Occurrence and Characterization of Monophasic *Salmonella enterica* Serovar Typhimurium (1,4,[5],12:i:-) of Non-Human Origin in Poland
Dariusz Wasyl and Andrzej Hoszowski

Resistance in *Salmonella* – clonal spread
Resistance in Poland: food animals

Prevalence and characterisation of quinolone resistance mechanisms in *Salmonella* spp.

Dariusz Wasyliński, Andrzej Hoszowski, Magdalena Zającz

Table 2: Prevalence and characterisation of quinolone resistance mechanisms in *Salmonella* spp.

**Table 2.** Prevalence of plasmid-mediated quinolone resistance phenotype by year and source of *Escherichia coli* isolated from slaughter animals in Poland, 2009–2012.

**Mechanisms**

Quinolone resistance diverse mechanisms
ANTIMICROBIAL RESISTANCE IN WILDLIFE

Study aims:
1. to evaluate antimicrobial resistance in indicator *E. coli* isolated from hunted wild boars and deer,
2. to assess the prevalence of cephalosporin resistance in wildlife,
3. to characterize cephalosporin and quinolone resistance mechanisms as well as their carrier plasmids.
Resistance in wildlife

Hunting seasons
- Oct 2012 ÷ Jan 2013
- Oct 2013 ÷ Jan 2014

Faecal samples
- red deer
- roe deer
- fallow deer
- European bison
- wild boars
Wild boar sampling

Number of samples (animals) tested
N = 332
42 hunts

Population density (per 1000 ha)
Red deer sampling

Number of samples (animals) tested
N = 225
42 hunts

Population density (per 1000 ha)
Roe deer (n=76), fallow deer (n=24), European bison (n=3) sampling
Isolation of *Escherichia coli*

**Indicator *E. coli***

- direct streak
- MacConkey Agar

**Cephalosporin-resistant *E. coli***

- cefotaxime 2 mg/L
- MacConkey Agar

**identification**

**PCR: uspA**

Antimicrobial resistance testing

• Minimal Inhibitory Concentrations: 14 antimicrobials/9 classes

• Interpretation: EUCAST epidemiological cut-off

microbiological resistance (NWT)
Antimicrobial resistance in *E. coli* from wild boars

*Wild boars (n=278)*

- Sulphonamides (5.5 ÷ 12.3%)
- 0.9 ÷ 1.9%
  - Ampicillin, aminoglycosides, trimetoprim, ciprofloxacin, tetracycline

Not significant differences

*Wild ruminants (n = 264)*
Antimicrobial resistance in *E. coli* from wild boars

### Wild Boars (n = 278)

- **AMP**: ≤8%
- **CAZ**: ≤8%
- **CTX**: ≤8%
- **GEN**: ≤8%
- **KAN**: ≤8%
- **STR**: ≤8%
- **NAL**: ≤8%
- **CIP**: ≤8%
- **SMX**: ≤8%
- **TMP**: ≤8%
- **COL**: ≤8%
- **CHL**: ≤8%
- **FLR**: ≤8%
- **T CY**: ≤8%

### Wild Rumminants (n = 264)

- **AMP**: ≤4%
- **CAZ**: ≤4%
- **CTX**: ≤4%
- **GEN**: ≤4%
- **KAN**: ≤4%
- **STR**: ≤4%
- **NAL**: ≤4%
- **CIP**: ≤4%
- **SMX**: ≤4%
- **TMP**: ≤4%
- **COL**: ≤4%
- **CHL**: ≤4%
- **FLR**: ≤4%
- **T CY**: ≤4%

### Swine (N = 710)

- **AMP**: ~40%
- **CAZ**: ~40%
- **CTX**: ~40%
- **GEN**: ~40%
- **KAN**: ~40%
- **STR**: ~40%
- **NAL**: ~40%
- **CIP**: ~40%
- **SMX**: ~40%
- **TMP**: ~40%
- **COL**: ~40%
- **CHL**: ~40%
- **FLR**: ~40%
- **T CY**: ~40%

### Cattle (N = 707)

- **AMP**: ~10%
- **CAZ**: ~10%
- **CTX**: ~10%
- **GEN**: ~10%
- **KAN**: ~10%
- **STR**: ~10%
- **NAL**: ~10%
- **CIP**: ~10%
- **SMX**: ~10%
- **TMP**: ~10%
- **COL**: ~10%
- **CHL**: ~10%
- **FLR**: ~10%
- **T CY**: ~10%
Resistance is present in the wildlife
How farm animals might contribute?

No resistance in \textit{E. coli} from fallow, red deers and bisons

Selection pressure of antimicrobial usage in the agriculture?
Plasmid-mediated quinolone resistance in *E. coli* isolated from wild boars

Wild boars (n = 278)

PCR: *qnrS*
Sequencing: *qnrS1/S3*
Prevalence of cephalosporin resistance in *E. coli* from wildlife

<table>
<thead>
<tr>
<th>Source (No of samples)</th>
<th><em>E. coli</em></th>
<th>Cephalosporin-resistant <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>isolation rate</td>
</tr>
<tr>
<td>fallow deer</td>
<td>24</td>
<td>87,5%</td>
</tr>
<tr>
<td><strong>wild boar</strong></td>
<td>332</td>
<td>83,7%</td>
</tr>
<tr>
<td>red deer</td>
<td>225</td>
<td>78,2%</td>
</tr>
<tr>
<td>roe deer</td>
<td>76</td>
<td>84,2%</td>
</tr>
<tr>
<td>European bison</td>
<td>3</td>
<td>100,0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>660</td>
<td>82,1%</td>
</tr>
</tbody>
</table>
Cephalosporin-resistant pathogenic *Enterobacteriaceae* in humans are multidrug-resistant. What about wildlife isolates?

Identification of cephalosporin resistance mechanisms (PCR):

- \( \text{ampC} \rightarrow \text{blaCMY-2} \) (\( N = 8 \))
- ESBL \( \rightarrow \text{blaCTX-M} \) (\( N = 3 \))

![Graph showing resistance in cephalosporin-resistant E. coli](image)

- PMQR
- Few other resistances in wildlife isolates
Cephalosporin resistance background in *E. coli* from wild boars

<table>
<thead>
<tr>
<th>ID</th>
<th>R-profile</th>
<th>bla</th>
<th>Plasmids (kb; S1+B&amp;D)</th>
<th>Inc groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>039B</td>
<td>AmpCtxCazGenKanNalCip</td>
<td>Tem-1, Cmy-2</td>
<td>104; 40; 10,0; 2,5</td>
<td>?</td>
</tr>
<tr>
<td>364B</td>
<td>AmpCtxCazNalCip</td>
<td>Tem-161, Cmy-2</td>
<td>90; 7,0; 5,2; 3,1</td>
<td>IncI1-alpha, IncY</td>
</tr>
<tr>
<td>479B</td>
<td>AmpCtxCazCip</td>
<td>Tem-1, Cmy-2</td>
<td>104; 90; 54</td>
<td>IncX1, IncI1-alfa, IncFII</td>
</tr>
<tr>
<td>480B</td>
<td>AmpCtxCaz</td>
<td>Cmy-2</td>
<td>90; 80; 60; ?</td>
<td>IncI1-alpha, IncFIC, IncFII</td>
</tr>
<tr>
<td>481B</td>
<td>AmpCtxCaz</td>
<td>Ctx-M1/61</td>
<td>140; 85; ?</td>
<td>IncI1-alpha, IncFII</td>
</tr>
<tr>
<td>482B</td>
<td>AmpCtxCazTet</td>
<td>Tem-1, Cmy-2</td>
<td>140; 85; ?; 4,8</td>
<td>IncI1-alpha, IncHI1, IncF (FIB), IncFII</td>
</tr>
<tr>
<td>483B</td>
<td>AmpCtxCaz</td>
<td>Ctx-M1/61</td>
<td>140; 85; 3,5</td>
<td>?</td>
</tr>
<tr>
<td>486B</td>
<td>AmpCtxCaz</td>
<td>Tem-1, Cmy-2</td>
<td>200;100; ?</td>
<td>IncI1-alfa, IncF (FIB), IncFII, IncB/O</td>
</tr>
</tbody>
</table>

Humans: *blaCTX-M3* Gołębiewski et al. AAC, 2007, 51(11): 3789-95
## Transferability of cephalosporin resistance

<table>
<thead>
<tr>
<th>ID</th>
<th>R-profile</th>
<th>bla</th>
<th>Plasmids (kb; S1+B&amp;D)</th>
<th>Inc groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>.......</td>
<td></td>
<td></td>
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<tr>
<td>482B</td>
<td>AmpCtxCazTet</td>
<td>Tem-1, Cmy-2</td>
<td>140; 85; ?, 4,8</td>
<td>IncI1-alpha, IncHI1, IncF (FIB), IncFII</td>
</tr>
<tr>
<td>Trans 482B</td>
<td>Amp Ctx Caz</td>
<td>Cmy-2</td>
<td>85</td>
<td>IncI1-alpha</td>
</tr>
</tbody>
</table>

Table showing the transferability of cephalosporin resistance with specific IDs, R-profiles, bla genes, plasmid sizes, and Inc groups.
PMQR in cephalosporin resistant *E. coli*

<table>
<thead>
<tr>
<th>ID</th>
<th>R-profile</th>
<th>bla</th>
</tr>
</thead>
<tbody>
<tr>
<td>039B</td>
<td>AmpCtxCazGenKanNalCip</td>
<td>Tem-1, Cmy-2</td>
</tr>
<tr>
<td>358B</td>
<td>AmpCtxCazSmxTetTmp<em>Cip</em></td>
<td>Ctx-M-15</td>
</tr>
<tr>
<td>364B</td>
<td>AmpCtxCazNalCip</td>
<td>Tem-161, Cmy-2</td>
</tr>
<tr>
<td>479B</td>
<td>AmpCtxCaz<em>Cip</em></td>
<td>Tem-1, Cmy-2</td>
</tr>
<tr>
<td>480B</td>
<td>AmpCtxCaz</td>
<td>Cmy-2</td>
</tr>
<tr>
<td>481B</td>
<td>AmpCtxCaz</td>
<td>Ctx-M1/61</td>
</tr>
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<td>AmpCtxCazTet</td>
<td>Tem-1, Cmy-2</td>
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<tr>
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</tr>
<tr>
<td>486B</td>
<td>AmpCtxCaz</td>
<td>Tem-1, Cmy-2</td>
</tr>
</tbody>
</table>

*qnrS1/3*
Pathogenicity of cephalosporin resistant *E. coli*

<table>
<thead>
<tr>
<th>ID</th>
<th>R-profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>039B</td>
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<td>483B</td>
<td>AmpCtxCaz</td>
</tr>
<tr>
<td>486B</td>
<td>AmpCtxCaz</td>
</tr>
</tbody>
</table>

**PCR: pathogenicity markers**
- STEC(*stx1/stx2*)
- EPEC (*eae, bfpA*)
- EIEC (*ipaH*)
- ETEC (*elt, est*)
- EAEC (*aggR*)


all negative
Cephalosporin resistant *E. coli* from wild boars

Two different mechanisms in 8 of 11 cephalosporin resistant *E. coli* isolated from, respectively 2 and 6 boars hunted in 13 Nov and 3 Dec 2013, approximately 100 km from each other.
Antimicrobial resistance in other wildlife

Invasive turtle species as a source and vector of animal and human pathogens (NCN project No. 2013/11/B/NZ7/01690)

no *Escherichia coli*,

but

numerous isolates of:

• *Acinetobacter* spp.,
• *Citrobacter* spp.,
• *Pseudomonas* spp.,
• *Shewanella* spp.,
• *Aeromonas* spp.
• ...

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Antimicrobial resistance in wildlife

*Salmonella* from

- invasive alien turtles
- European grass snakes (*Natrix natrix*)

resistance is sparse (mainly streptomycin)
Conclusions

• *E. coli* in wild animals (boars) might be considered a reservoir and vector (transferable) of resistance determinants (environmental gene pool), but not the pathogen themselves (no pathogenicity markers found)

• Resistance to cephalosporins and quinolones (PMQR) are presumably of environmental origin – the prevalence might be enhanced by selective pressure of antimicrobial usage in agriculture (i.e. manure/slurry)

• ...

Is it safe to hug a boar?
Funding and acknowledgements

The studies were supported by governmental founding of


- National Science Centre Grants
  - No. NR12-0126-10/2011 (wildlife) and
  - No. 2013/11/B/NZ7/01690 (invasive alien turtles).

Colleagues from National Reference Laboratory, publications co-authors and project co-investigators are acknowledged for their valuable contribution to the presented results.