Antibiotic resistance: from farm to the fork

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The antibiotic timeline

Changes in the food sector

Intensive Production
Trade globalization of animals and food products
Antibiotic Use and Resistance

Use of antibiotics

VRE, MRSA, GN-ESBL/MBL, MDR/XDR-TB.....

Philips et al., JAC. 2004; Philips, IJAA, 2007; Goossens et al., Lancet. 2005
Antibiotic Use and Resistance in Animal Production

Antimicrobial Consumption

- Few reliable data outside EU on antibiotic (AB) consumption in animals
- In the most populated parts of the world the antibiotic consumption is unknown
- Some countries, e.g. USA, also used AB as growth promoters (all forbidden since 2006 in EU)
- Irrational drug use, availability of counterfeit and substandard drugs

### Netherlands
- 16.5 million people consume 40,000 Kg/year
- 15.0 million cattle and pigs and 100 million poultry consume 500,000 Kg/year
- 12 Xs AB used in veterinary

(Maran 2008, Gossens et al., 2005)

### Denmark
- 5.475 million people consume 48,500 Kg/year
- 103,000 Kg in pigs and 15,000 Kg in cattle;
- 645 Kg in poultry
- 2,200 Kg in Companion animals
- 2.4 Xs more in animals

(DANMAP, 2009)
Antibiotic Use and Resistance in Animal Production

Food-borne pathogens

*Salmonella* non-Typhi

*Campylobacter* spp.

Increase likelihood for infection in pathogens

Infections with resistant isolates

> Morbidity
> Mortality

Molbak K. *Clin Infect Dis* 2005; 41: 1613-20

Commensal Bacteria

*E. coli*

*Enterococci*

Source of transferable resistance genes to pathogenic human bacteria

MRSA

Direct contact with animals
– Farmers
– Slaughterhouse workers/meat industry
– Veterinary personnel

• Risk via food – Low
Antibiotic Use and Resistance in Animal Production

**Foodborne pathogens**

- Salmonella non-Typhi
- Campylobacter spp.

**Indicator Bacteria**

- Escherichia coli
- Enterococcus

**Resistance**

(specie, serotype, clone, animals specie and country variability)

- Sulphonamides, trimethoprim, tetracycline, streptomycin, ampicillin, cephalosporins and fluorquinolones
- Tetracyclines, macrolides and fluorquinolones
- Source of transferable resistance genes to pathogenic human bacteria

EFSA Journal 2010; 8:1658; MARAN, 2008; DANMAP, 2009; Freitas et al., IJAA.2011;
Antibiotic Resistance Dispersion

VRE and ESBL producers in wild animals (Radhouani H et al., 2010; Gonçalves A et al., 2010; Simões et al., 2010; Quinteira et al., ECCMID, 2011)

MDR Salmonella ifood handlers (Gomes Neves et al., ECCMID, 2011; Campo et al., 2001)

NDM producers in drinking water and rivulets (Walsh et al., Lancet. 2011)

MDR enterococci in insects (Ahmad et al., 2011)

MDR E. coli and VRE feed mills (da Costa et al., 2007)


S. Typhimurium DT 204b; ESBL producer (Crook, et al., CMI, 2003; Campos et al. ECCMID. 2011)

Clinical VRE, VIM and ESBL producers in rivers, marine waters (Novais et al., 2005; Quinteira et al., 2006; Machado et al. 2009)
Evidence of correlation

Quinolone-resistant MDR Salmonella Typhimurium DT104, United Kingdom, 1992-97

Evidence of correlation


*Dutil et al., EID. 2009.16:48-54*
Antibiotic Use and Resistance in Animal Production

Use of antibiotics is considered the main factor in the development of bacterial resistance. Use of biocides (including disinfectants, antiseptics, preservatives, sterilants) may also make some contribution.

EFSA Journal 2009; 7(11):1372

‘Show us the science that use of antibiotics in animal production is causing this antibiotic resistance’
Dave Warner of the National Pork Council, USA. Washington Post. 2010
Uncertainties

**The extent to which antibiotic use on the farm contributes to increases antibiotic resistance among bacteria that cause illnesses in people**

**The contribution of foodborne antibiotic resistant bacteria (AbR), and namely of commensals, to the overall problem of antibiotic resistance in Humans**
## Molecular Multilayer Studies

<table>
<thead>
<tr>
<th>Level</th>
<th>Component</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>Clone</td>
<td>Identification of clones/clonal lineages (Pulsotypes, STs and CCs)</td>
</tr>
<tr>
<td>Level 2</td>
<td>Plasmid</td>
<td>Conj. / Non-Conj. Identification (Inc)</td>
</tr>
<tr>
<td>Level 3</td>
<td>Transposon</td>
<td>Type, characterization, variability</td>
</tr>
<tr>
<td>Level 4</td>
<td>Integron</td>
<td>Type, Cassette gene</td>
</tr>
<tr>
<td>Level 5</td>
<td>Resistance Gene</td>
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<tr>
<td></td>
<td>Virulence Gene</td>
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</tbody>
</table>
Contribution of ESC-resistant *E. coli* from animal production and food products:

Potential source of ESBL genes for *Salmonella spp.* or other pathogens?

Role as an opportunistic pathogen?
Resistance to ESC in *E. coli*

ESC are critical antibiotics (cefotaxime, ceftriaxone, ceftazidime, cefepime, and cefpirome).

Resistance due to β-lactamase production:

- **ESBLs** (TEM, SHV, and **CTX-M**)
- AmpC-type beta-lactamase more frequent: CMY-2
- OXA-type: OXA-1

Encoded on large plasmids

Multidrug resistance

Human Clinical Isolates

Remarkable increase in several EU countries

![Map showing percentage resistance across EU countries.](image-url)
Resistance to ESC in Animal and Foodborne *E. coli*

Cefotaxime resistance in broilers, Netherlands. 1998-2009

Ceftiofur and cefquinome used in animal production

Cross resistance with other 3\(^{\text{rd}}\) and 4\(^{\text{th}}\) cefalosporins
Cefotaxime resistance in broilers, 6 EU MS. 2008

<table>
<thead>
<tr>
<th>Country</th>
<th>% Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>0</td>
</tr>
<tr>
<td>Finland</td>
<td>1</td>
</tr>
<tr>
<td>Austria</td>
<td>3</td>
</tr>
<tr>
<td>France</td>
<td>6</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>15</td>
</tr>
<tr>
<td>Spain</td>
<td>30</td>
</tr>
</tbody>
</table>

EFSA Journal 2010; 8:1658
Resistance to ESC in Animal and Foodborne *E. coli*

- 2003, all poultry farms tested in Spain were positive for ESBL-*E. coli*. Blanc et al., Vet. Microb. 2006.

- 2005, 60% of chicken meat in Portuguese butcheries and 50% poultry from farms with ESBL-bacteria. Machado et al., JAC 2008.

- 2008, reported 60% of Belgium poultry farms with ESBL-*E. coli*. Smet et al., AAC 2008

- 2009 two of four flocks with *E. coli* ESBL. Bortolaia et al., AAC 2010°

- 2010, nearly all chicken (94%) in Dutch and at poultry farms (2006) with ESBL-*E. coli*. Leverstein-van Hall et al., 2011.

Also reported in Czech Republic, France, Greece, Great Britain, Italy, Ireland, Canada, Japan, Korea, China, Tunisia, Senegal, USA .... Kojima et al., AAC 2005. Girlich et al., AEM 2007, Bortolaia et al., AAC 2010, Li et al. FBPD 2010, Kolar et al., VM 2010, Chiaretto et al., VM 2008, Randall et al., JAC 2011

**Imported meat**

- ESBL/AmpC producers in 88% of chicken meat imported in Sweden from Southern American; 36% imported in Denmark

(Borjesson S. et al., O353 – ECCMID-ICC.2011); Bergholz et al, 2009)
Resistance to ESC in Animal and Foodborne *E. coli*

- Variable frequency in pigs and pork - England and Wales, Germany, Hong-Kong, Portugal, Spain, Tunisia

- 8 of 10 farms with ESBL-*E. coli* (2003) and 25% of pork samples (2009) in Spain

- 25% of pigs tested in Portugal (2007).

  *Blanc 2006; Doi et al., 2009; Tian et al., 2009; Escudero et al., 2010; Goncalves et al., 2010; Duan et al., 2006; Li et al., 2010*

- Low frequency – Denmark, France, Japan, Tunisia and Egypt

- Higher frequency in UK (outbreak) and USA (88,5%)

  *Liebana et al., 2006, Madec et al., 2008, Jouini et al., 2007, Shiraki et al., 2004, Ben Slama et al., 2009, Duan 2006, Jensen 2006, Hammad, 2009, Donaldson et al., 2006*
Resistance to ESC in Animal and Foodborne *E. coli*

**ESBL detected frequently in EU**

. **TEM-52** (Spain, Portugal, Belgium, Netherlands, Ireland, Denmark and Germany); TEM-20 (Netherlands, Ireland and Germany), TEM-106 (Belgium) and TEM-126 (France)

. **SHV-2** (Spain, Portugal, and Netherlands), SHV-5 (Spain) and **SHV-12** (Spain, France, Italy, Netherlands, Denmark, Ireland and Czech Republic).

. **CTX-M-1** in most of the European countries; CTX-M-14 and CTX-M-32 in Mediterranean and Southern European countries, also in UK and Belgium; CTX-M-2 Central and Northern European countries, including UK and Ireland

. CTX-M-15, incidentally reported in *E. coli* (France from diseased cattle, in Belgium from healthy poultry, and in UK in broiler chickens and turkey)

Rare in USA- CTX-M-1 group

**AmpC type frequent in USA**

EU: Mostly CMY-2; ACC-1 sporadic (Netherlands)
Resistance to ESC in Animal and Foodborne *E. coli*

High diversity in imported Southern American chicken meat

*(Borjesson S. et al., O353 – ECCMID-ICC.2011)*

- TEM-19, TEM-52, SHV-12, CMY-2, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-11, CTX-M-25
Main Issue

_E. coli_ resistant to Extended Spectrum Cephalosporin (ESC) in Humans, animal production and food products

Contribution of ESC-resistant _E. coli_ from animal production and food products:

Potential source of ESBL genes for _Salmonella spp._ or other pathogens?

Role as an opportunistic pathogen?
### Common genetic elements encoding ESBL/AmpC in *E. coli* and *Salmonella* different settings

<table>
<thead>
<tr>
<th>Gen. Env.</th>
<th>Gene R</th>
<th>Plasmid</th>
<th>Specie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn3</td>
<td>tnpA</td>
<td>blatem-52</td>
<td>Incl1</td>
</tr>
<tr>
<td></td>
<td>tnpR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IScep1</td>
<td>blactx-M-1</td>
<td>Incl1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IncN</td>
<td></td>
</tr>
<tr>
<td>IScep1</td>
<td>blacmy-2</td>
<td>blc</td>
<td>IncA/C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sugE</td>
<td>Inc2</td>
</tr>
<tr>
<td>ISCR1</td>
<td>blactx-M-2/9</td>
<td>Tn21</td>
<td>E/S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IncH2</td>
<td></td>
</tr>
<tr>
<td>IScep1</td>
<td>blactx-M-14</td>
<td>IncK</td>
<td>E</td>
</tr>
<tr>
<td>ISCR1</td>
<td>blactx-M-14</td>
<td>IncK</td>
<td>E</td>
</tr>
<tr>
<td>IS26</td>
<td>blashv-12</td>
<td>Incl1</td>
<td>E/K</td>
</tr>
</tbody>
</table>

**Specie:**
- E/S: *E. coli; K. pneumoniae; S- Salmonella*
- E/S/A
- E/S
- E
- E/K

**E/K/S:** *E. coli; K. pneumoniae; S- Salmonella*

Coque 2008; Carattoli 2009; Cloackaert 2007; Girlich 2007; Moodley 2010; McIntosh 2008; Hasman 2009; Bortolaia 2010; Valverde 2009; Novais 2007
Contribution of ESC-resistant \textit{E. coli} from animal production and food products: 

Potential source of ESBL genes for \textit{Salmonella spp.} or other pathogens?

Role as an opportunistic pathogen?
<table>
<thead>
<tr>
<th>PhG</th>
<th>Sequence Type (ST)</th>
<th>ESBL / AmpC</th>
<th>Others</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B2</strong></td>
<td>ST131</td>
<td>CTX-M-1, -9, -14, -15, SHV-12, CMY-2, TEM-4, -24</td>
<td>Hospital residual water</td>
<td>worldwide</td>
</tr>
<tr>
<td><strong>B2</strong></td>
<td>ST95</td>
<td></td>
<td>Honeydew melon</td>
<td></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>ST648</td>
<td>CTX-M-15, -32</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>ST69</td>
<td>CMY-2, CTX-M-14</td>
<td>RTE-Salads</td>
<td></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>ST117</td>
<td>CTX-M-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>ST10</td>
<td>TEM-52, CTX-M-14, CMY-2, SHV-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>ST23</td>
<td>TEM-52, CTX-M-14, DHA-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>ST410</td>
<td>CTX-M-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B1</strong></td>
<td>ST101</td>
<td>CTX-M-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B1</strong></td>
<td>ST155</td>
<td>TEM-52, CMY-2</td>
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</tr>
</tbody>
</table>

C=Chicken; P=Pork; B=Beef
Conclusions

i) Resistance to different antibiotics is widespread among both animals and humans

ii) Extended Spectrum Cephalosporin resistance is increasingly observed in animal production and food products, mostly in broiler chickens and poultry products, probably associated with antibiotic consumption.

iii) The frequent occurrence of *E. coli* resistant to ESC and the presence of common genetic elements supports its the potential role to act as a source of genes coferring resistance to these β-lactams

iv) Scientific evidence is amounting that resistant *E. coli* involved in human infections might be spread via the food chain

iv) Current control options for preventing the spread of antimicrobial resistant bacteria and/or resistance genes from animals and food products should be revisited
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