Travellers and food items: Transmission of pathogens and antimicrobial resistances

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PROMISE = Protection of consumers by mitigation of segregation of expertise
PROMISE studied neglected exogenous routes of FBP transmission

...contamination status of food carried by travellers

...contamination rate of food items carried away across ground borders and seaports located at the Balkan countries

...contamination rate of food items traded across the Moldavian-Romanian border

...contamination rate of food items traded at market selling mainly homegrown food in Turkey

...contamination rate of food items imported to Greece
PROMISE selection of airports

Frankfurt (Schönefeld): global hub serving all continents

Vienna: Focus Near East, Asia (China) in total passengers from 34 countries

Bilbao: Focus South America
Procedures

Harmonization of methods and sampling

Salmonella spp.
- Isolation (all labs)
  - Microarray (Germany)
  - Virulotyping (Austria)

Listeria monocytogenes
- Isolation (all labs)

Escherichia coli
- Isolation E. coli (Austria)
- Isolation VTEC (all labs)
- AMR genes (VTEC)
  - Virulotyping AMR (Austria, Croatia)

Campylobacter jejuni/coli
- Isolation (all labs)
- AMR (Spain)

Staph. aureus
- Isolation (Austria)
- AMR (Spain)

FB viruses
- Detection (Spain)
How much food has been confiscated: example Vienna airport

- Vienna International Airport
- August 2012 – March 2013
- Around 14.7 million arrivals on app. 70,000 flights
- From 61,355 travellers, 1,473 products of animal origin were confiscated
- The food confiscates totalled to an amount of 6,229 kg
Experiences from Vienna airport: identifiable food items

Yerevan, Armenia

St. Petersurg, Russia

Delhi, India

Seoul, south Korea

Cairo, Egypt
Experiences from Vienna and Bilbao airport: exotic food items

- Duck tongues, China
- Egg (1000 years old)
- Squirrel (courtesy UoB)
- Rat (courtesy UoB)
Experiences from Vienna airport: unidentifiable origin

Bush meat, Nigeria
Bush meat, Ethiopia
Kudu biltong, South Africa
96.2% meat and milk products

Milk & products (n=315)
- 83.2% hard cheese
- 11.4% semihard
- 5.4% fresh

Meat & products (n=262)
- 17.2% raw meat
- 47% cooked meat
- 35.8% sausages

Fish & products (n=6)
- 7

Bush meat (n=6)
- 5

Funded by the 7th Framework Programme of the European Union
FP7 – Knowledge-based Bio-Economy (KBBE)
Non-compliance and bacterial pathogens

Samples non compliant with EU 2073/2005 (%)

N=600, all confiscates recovered from Vienna airport
Non-compliance and regions

Samples non compliant (%) with EU 2073/2005

N=600, all confiscates recovered from Vienna airport

Total  Near East  Asia  Africa  Other

%  15  20  25  30
Isolate characterization
Salmonella spp.

• n=1474 samples were included (mainly airport Frankfurt, Vienna, Bilbao)

• 20 samples positive for Salmonella spp. (total prevalence 1.35%; Frankfurt/Schöneberg 0.14%; Vienna 1.16%; Bilbao 5.5%)

• 60% meat samples; 30% dairy samples; 10% poultry

• S. enterica serovar Anatum (n=4; same virulotype); S. enterica serovar 4,12:d- (n=3); S. enterica serovar Enteritidis (n=2); S. enterica serovar Montevideo (n=2).

• AMR against one AB in 40% (n=8).
Strain characterization: Virulence markers in *L. monocytogenes*

- n=1474 samples were included (airport Frankfurt, Vienna, Bilbao)

- 57 samples positive for *L. monocytogenes* (total prevalence 3.78%; Frankfurt 1.5%; Vienna 2.5%; Bilbao 10%; Rychli et al., 2018)

- ST9 and ST 121 overrepresented (food-associated clonal types)

- Multiple mutations in *inlA* (20 variants), *actA* (4 variants) and *hly* (8 variants) virulence genes
Virulence and AMR in pathogenic *E. coli*

- n=1526 samples (600 samples from airport Vienna, 823 samples from airport Frankfurt, 103 samples from Ljubljana groundborder)

- 15 samples positive for shigatoxin-producing *E. coli* (1,0%), mostly hard cheese from Turkey (12 out of 15 positive samples)

- 113 samples positive for *E. coli* (11%), 14 isolates multidrug resistant (0,9%)\(^a\)

- MDR *E. coli* showed resistance to up to 14 AB \(^b\)

\(^a\) Tested against ampicillin (AMP), amoxicillin/clavulanic acid (AMC), cefotaxime (CTX), ciprofloxacin (CIP), gentamicin (GEN), and tetracycline (TET)

\(^b\) Tested against 19 AB; Nagy et al., 2015, IJFM
# Virulence in VTEC

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Serotypes</th>
<th>Food source</th>
<th>Country of origin</th>
<th>Isolation</th>
<th>Phylogroup</th>
<th>Sequence type (ST)*</th>
<th>Pulsotype (P)</th>
<th>Vero/CPE50</th>
<th>stx subtype</th>
<th>Virulence genes in functional groups</th>
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<tbody>
<tr>
<td>MRV13/01061</td>
<td>O178:H7</td>
<td>cheese</td>
<td>Turkey</td>
<td>Vienna</td>
<td>B1</td>
<td>ST 4505</td>
<td>P1</td>
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<td>Stx1c, Stx2b</td>
<td>hlyA, senB, lpfA, iha, espI, eaaA</td>
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<td>Vienna</td>
<td>A</td>
<td>ST 3519</td>
<td>P3</td>
<td>10</td>
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<td>hlyA, lpfA, iha, espI, epeA</td>
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<td>Vienna</td>
<td>A</td>
<td>ST 3249</td>
<td>P4</td>
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<td>Vienna</td>
<td>B1</td>
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<td>A</td>
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<tr>
<td>E001</td>
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<td>Turkey</td>
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<td>A</td>
<td>ST 13</td>
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</table>

* ST 4505, ST 4506 and ST 4507 have been newly established as a result of these studies.
<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Source</th>
<th>Sample type</th>
<th>Country of origin</th>
<th>Antimicrobial resistance</th>
<th>Virulence gene pattern*</th>
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<tbody>
<tr>
<td>P1/5</td>
<td>poultry</td>
<td>duck carcass</td>
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<td>P1/47</td>
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<td>raw breaded meat</td>
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<td>Amp Atm Chi Ctx Str Smx Tet</td>
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<tr>
<td>P1/105</td>
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<td>P1/217</td>
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<td>inners, raw</td>
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<td>P1/246</td>
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<td>whole fish without inners</td>
<td>Albania</td>
<td>Amp Chi Flo Str Smx Tet</td>
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<td>beef</td>
<td>raw meat sticks</td>
<td>Turkey</td>
<td>Amp Atm Chi Cip Ctx Flo Gen Kan Nat Str Smx Tet Tob Tmp</td>
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<td>P1/571</td>
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<td>Turkey</td>
<td>Amp Kan Str Smx Tet</td>
<td>&gt;64, &gt;1024, &gt;64, &gt;32</td>
</tr>
</tbody>
</table>

**Antimicrobial resistance**
- Amp: ampicillin
- Atm: aztreonam
- Caz: ceftazidime
- CH: chloramphenicol
- Cip: ciprofloxacin
- Ctx: cefotaxime
- Flo: florfenicol
- Gen: gentamicin
- Kan: kanamycin
- Nat: nalidixic acid
- Nor: norfloxacin
- Str: streptomycin
- Smx: sulphamethoxazole
- Tet: tetracycline
- Tob: tobramycin
- Tmp: trimethoprim

**Virulence gene pattern**
- iss
- cma
- cba
- bla
- sul2
- tet(B)
- intI1
- aadA2
- sul1
- aadA1
- tsh
- prfB
- astA
- ireA
- mchF
- tsh
- lpfA
- eae
- espAFJ
- nleB

Bold letters indicate antimicrobial resistance phenotype determined by MIC

* Genes are listed in descending order of their prevalence
Enterotoxinicity and AMR in *S. aureus*

- **n=868 samples** (600 samples from airport Vienna, 200 samples from airport Bilbao, 68 samples from Moldavian/Romanian border)
- **136 samples positive for *S. aureus* (15.6%)**
- **24 samples positive for MRSA (3.0%; dairy products of Bilbao samples 11% MRSA),**
- **69% of MRSA were multiresistant (3 or >3 AB)**
- **Majority of MRSA was also enterotoxigenic (19 out of 24 isolates)**
- **all MRSA *mecA* positive**
- **six isolates tested positive for luk-PVL genes (SCCmec IV subtypes IVc and IVe); all isolates non-enterotoxigenic**

Prevalence viral pathogens

Subset of samples (n=122 meat samples, Rodríguez-Lázaro et al., IJFM, 2015)
Pork and meat products 64.2% Hep E positive
Low range of contamination from 55 PDU to 90,000 PDU/g

PDU PCR- (MPN) detectable units
Conclusions

- Air travellers carry multiple foodstuffs for personal (souvenir), religious or ethnic reasons with them - efficient border control and consumer information is a demand.
- Prevalence of FBP might be double to what is observed in domestic food (e.g. *L. monocytogenes* in Austrian samples, Schoder *et al.*, 2014).
- Bilbao samples were contaminated at higher numbers (origin of flights mostly South America).
- Due to the small amount of food (up to 14kg) being carried, sporadic or family related disease is most likely.
- Of notice is the possibility, that novel virulence types or AMR traits are associated with incoming isolates.
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