Staphylococcal Enterotoxins
Detection in Food Matrices

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• Introduction

• Staphylococcal enterotoxins (SEs) and their detection in food matrices

• How to investigate SFPO in EU-MSs?

• SFPO examples

• Conclusion
1- Anses: Missions

ANSES contributes to ensuring:
- human health and safety in the fields of environment, work and food as well as
- protecting animal health and welfare
- protecting plant health

Recommend public health measures
Assess nutritional and health risks and benefits
Conduct public health monitoring missions
Conduct, coordinate and initiate research projects
Conduct laboratory reference missions
Provide training and information and contribute to public debate
Authorise marketing of veterinary medicinal products and plant protection and fertilizers

Collaborate with European and international agencies (EFSA, ECHA, EEA, EU-OSHA, ECDC and EMA)
Anses – The Laboratory for Food Safety, Maisons-Alfort: acts on biological and chemical hazards that affect food safety

ANSES appointed as NRL and EURL for Coagulase Positive Staphylococcal (CPS),

Reference missions of EURL CPS:
(i) Select, develop and transfer analytical methods to NRLs,
(ii) Evaluate their ability to use the official method through ILPT (in order to comply with EU regulation towards official controls).
(iii) Support to NRLs (training and SFPO investigation)

Reference activities on both:
- Staphylococcal bacteria
- Staphylococcal enterotoxins

ILPT: Inter-Laboratory Proficiency Testing Trials

* NRL: National Reference Laboratory; ** EURL: European Union Reference Laboratory (EC N° 776/2006).
Introduction

- **2010-2015: Foodborne outbreaks due to bacterial toxins: third causative agent**

  - Unknown
  - Viruses
  - Salmonella
  - Bacterial toxins
  - Campylobacter
  - Other causative agents
  - E. coli, pathogenic (including VTEC)
  - Other bacterial agents
  - Parasites
  - Yersinia

  ![Graph showing number of outbreaks by causative agent over years.](image)

  - **Number of outbreaks**
  - **% reported outbreaks in EU**

  - 2011: 13%
  - 2012: 15%
  - 2013: 16%
  - 2014: 16%
  - 2015: 20%

  - **49%**
Exoproteins produced by toxinogenic strains of coagulase positive staphylococci (S. aureus)

A strain can produce one or several SE type(s), in variable quantities (1 ng to more than 1 µg/ml; 10 to 20 ng/ml = low level)

Preformed in food (with high protein content)

Molecular weight: 22 to 30 kDa

SEs are highly stable exoproteins, soluble in water and in saline solutions. They are resistant to:
- High temperature (121°C for 30 minutes)
- Large range of pHs 3 – 9
- Irradiation
- Proteolytic enzymes
SEs assumed to be a threat to public health: Commission Regulation (EC) No 1441/2007 defines microbiological criteria for food stuffs; for SEs in cheeses, milk products ➔ No detection of SE in a 25 g test portion using European Screening Method

detectable with commercial kits

SE → emetic action; SEℓ (SE-like) → emetic activity not tested
SEs assumed to be a threat to public health: Commission Regulation (EC) No 1441/2007 defines microbiological criteria for food stuffs; for SEs in cheeses, milk products ➔ No detection of SE in a 25 g test portion using European Screening Method.
## EFSA data 2010 - 2014

Distribution of food vehicles in strong-evidence outbreaks caused by staphylococcal toxins (excluding strong-evidence water-borne outbreaks) in the EU, 2010 - 2014

<table>
<thead>
<tr>
<th></th>
<th>Meat and meat products</th>
<th>Mixed food</th>
<th>Cheese and Dairy products</th>
<th>Bakery products</th>
<th>Other foods and food stuff</th>
<th>fish and fish products</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>32%</td>
<td>29%</td>
<td>18%</td>
<td>11%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>9%</td>
<td>40%</td>
<td>14%</td>
<td>11%</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>14%</td>
<td>31%</td>
<td>20%</td>
<td></td>
<td>34%</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>23%</td>
<td>19%</td>
<td>6%</td>
<td>5%</td>
<td>39%</td>
<td>6%</td>
</tr>
<tr>
<td>2014</td>
<td>26%</td>
<td>29%</td>
<td>13%</td>
<td>7%</td>
<td>19%</td>
<td>7%</td>
</tr>
<tr>
<td>Mean</td>
<td>21%</td>
<td>30%</td>
<td>14%</td>
<td>8%</td>
<td>26%</td>
<td>7%</td>
</tr>
</tbody>
</table>

Regulation concerns only cheese and milk products

Sources: EFSA Journal 2010 - 2015
How to characterize food poisoning outbreaks?

Aim: characterize FBO and as reference lab we have to provide support to decision makers.

1 - Need to develop reliable methods using tool box:
   - Microbiology
   - Molecular biology: PCR, RT PCR, PFGE, MLVA, MLST, WGS
   - Immunoassays: RPLA, ELFA, ELISA, WB
   - Toxicological assay
   - Physicochemical tool: Mass spectrometry…

2 - Use of epidemiological data
Symptoms depend on the ingested pathogen(s)
   - Short delay (from 30 minutes to 8 hours) = preformed toxic component (SEs)

   nausea  vomiting  abdominal cramps  diarrhea  fever

Emetic dose $ED_{50}$ (oral route):
monkey: 5 µg (SEA, SEB, SEC) to 20 µg (SED); piglet: 20 µg; shrew: 200 ng; human: 1 to 10 µg (20 to 40 ng for the most sensitive people)

How to investigate SFPO in EU-MSs?
How to manage CPS/SEs analysis in the reference laboratory

Food samples → SEA to SEE detection

Food samples → CPS enumeration

Food samples → CPS Characterization

Positive

EUROPEAN SCREENING METHOD

END

GARPOX (Ab labelled with peroxydase)

Ab anti-SE

SE extract

Capture Ab

Double sandwich

ELISA quantitative

ABTS + H₂O₂

ABTS oxidized + 2H₂O

ISO 6888

CFU / g

PCR

Real-time PCR

PFGE

Comparison of strains

Source

11 genes (sea, seb, sec, sed, see, seg, seh, sei, sej, sep, ser)

se genes
First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009

A Ostyn (a.ostyn@afssa.fr), M L De Buyser, F Guillier, J Grouli, B Félix, S Salah, G Delmas, J A Hennekinne

1. AFSSA-LERGAP (French Food Safety Agency, Food Quality and Food Processes Research Laboratory), European Union Reference Laboratory for Coagulase Positive Staphylococci including Staphylococcus aureus, Maisons-Alfort, France
3. InVS, Infectious diseases department, national institute for public health surveillance, Saint Maurice, France

October-November 2009

- **Incriminated food:** Soft cheese made from unpasteurized cow milk
- **Number of cases:** 23 from 6 districts

<table>
<thead>
<tr>
<th>Steps / analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS</td>
<td>&gt; $1.5 \times 10^5$ cfu/g</td>
</tr>
<tr>
<td>Official method (Ridascreen SET Total and Vidas SET2)</td>
<td>positive</td>
</tr>
<tr>
<td>Quantitative ELISA method se genes (PCR)</td>
<td>SEE = 0.36 to 1.14 ng/g</td>
</tr>
</tbody>
</table>

Citation style for this article:

www.eurosurveillance.org
## SFPO example 2: Prison in Italy

**August 2014 (Terni - Italy):**

- Important prisons in terms of type of prisoners
- Nb of cases: 80 cases
- Symptoms: nausea, abdominal cramps, vomit and diarrhea
- Time of onset: few hours from the breakfast.

### Steps / analysis

<table>
<thead>
<tr>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS</td>
<td>$3.6 \times 10^8$ cfu/g</td>
</tr>
<tr>
<td>Official method (Vidas SET2)</td>
<td>positive</td>
</tr>
<tr>
<td>Biotyping / Spa - type</td>
<td>Human / $t104$</td>
</tr>
<tr>
<td>Quantitative ELISA method</td>
<td>SEA = 2.2 ng/g and SED = 9.6 ng/g</td>
</tr>
<tr>
<td>se genes (PCR)</td>
<td>sea, sed, sej and ser</td>
</tr>
</tbody>
</table>

**Conclusion:**

- 😊 SEA and SED responsible for the SFPO
- 😞 Involvement of SEJ and SER ??
SFPO example 3: aircraft from Ireland to USA

October 2015: aircraft from Ireland to USA

Nb of cases: 44 cases (only 12 reported and 1 hospitalization)
Symptoms: abdominal cramps, vomiting, diarrhea and nausea. No fever
Time of onset: few hours

• Suspected food: strawberry crumble mousse

<table>
<thead>
<tr>
<th>Steps / analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS</td>
<td>&gt; $10^6$ cfu/g</td>
</tr>
<tr>
<td>Official method (Vidas SET 2)</td>
<td>positive (TV= 0.61 to 2.71)</td>
</tr>
<tr>
<td>Quantitative ELISA method</td>
<td>SEA = LOQ to 1.93 ng/g</td>
</tr>
<tr>
<td>se genes (PCR)</td>
<td>[sea, seh], [seg, sei], [seg, sei, sep]</td>
</tr>
<tr>
<td>PFGE</td>
<td>3 pulsotypes for 6 strains analyzed</td>
</tr>
</tbody>
</table>

Conclusion:

😊 SEA responsible for the SFPO
😊 Involvement of SEG, SEH, SEI ??
**4 – SFPO #3: France (canteen & baptism meal)**

**Date:** September 2015

**Place:** Loire; 2 outbreaks: middle school canteen & meal after baptism celebration

**Nb of cases:** ~ 50 cases (0 hospitalization)

**Symptoms:** nausea, vomiting, abdominal cramps, diarrhoea. No fever

**Time of onset:** ~ 5 h after consumption

**Suspected food:** *cheese (Vachard), toasts with salmon*

**Analysis of cheese & *S. aureus* strains**

<table>
<thead>
<tr>
<th>Steps / analysis</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS</td>
<td>9.3 x 10^6 cfu/g</td>
</tr>
<tr>
<td>Official method (Vidas SET2)</td>
<td><strong>negative</strong> (TV = 0.01)</td>
</tr>
<tr>
<td>Quantitative ELISA method</td>
<td><strong>not done</strong></td>
</tr>
<tr>
<td>se genes (PCR)</td>
<td><em>[seg, sei]</em> (4/5), none* (1/5)</td>
</tr>
</tbody>
</table>

*: negative for the 11 genes (sea to see, seg to sej, sep and ser)

**Analysis of *S. aureus* strains by PFGE**

- Raw cow’s milk
- Raw goat’s milk
- Cheese Fourme d’Ambert
- Cheese Bourgogne
- Vennin

=> same Smal pulsotype (#20) for strains from the cheese (Vachard) collected during the SFPO and from other cheeses and raw cow’s milk from the producer.

**Conclusion:** Involvement of **SEG** and **SEI** in SFPO ?
SEs detection in food matrices from SFPOs

More than 30 SFPOs investigated from 2015 to March 2017 (EURL/NRL network)

<table>
<thead>
<tr>
<th>Outbreaks</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 outbreaks</td>
<td>SE type and se genes detected in full agreement (SEA / sea)</td>
</tr>
</tbody>
</table>
| 10 outbreaks | SE type and se genes in partial agreement
SEA-SEE detected and genes detected (e.g. sea, seb, sed)
Other genes also detected (e.g. seg, sei, seh, sej, ser, sep) |
| 13 outbreaks | SEA-SEE not detected / se genes detected
(genes detected: seg, seh, sei >> sej, ser, sep) |
| 5 outbreaks | SEA, SEC and SED detected but no information on se (or analysis in progress) |

SE emetic; SE-like (not tested)

Need of tools allowing detection of SE other than SEA to SEE
Research programs

1- Anses / Biomerieux:

2- CBRNe (chemical, biological, radiological, nuclear and explosives) dedicated to toxins including SEB, SEG, SEH and SEI

3- EJP – Tox-DETECT dedicated to *S. aureus*, *B. cereus* and *C. perfringens* and their toxins/virulence factors
Anses / Biomerieux:

Detection of SEG, SEH and SEI

2016

Feasibility study with a pilot VIDAS kit (Screening method, qualitative)

- Evaluation of specificity with culture supernatants
- Evaluation of sensitivity with diluted pure solutions of SEG, SEH and SEI
- Evaluation with artificially contaminated food samples
- Evaluation with « naturally contaminated food »
CBRNe dedicated to toxins including SEG, SEH and SEI

Collaboration with CEA (French Alternative Energies and Atomic Energy Commission)

**Project:** CBRNe dedicated to toxins including SEG, SEH and SEI

- Developpement of ELISA based method for caracterization of toxins SEG, SEH and SEI
- rSEG, H and I produced from well charaterized SFPO CPS strains
- MAb produced in mice: transferd to Anses end 2017
- Anses: devloppment of specific ELISA based test
  - test on culture
  - test on artificially spiked samples
  - test on naturally contaminated samples
EJP TOX-Detect


- 2.6 M €

- Coordination: Anses (JA Hennekinne and Y NIA)

- Duration: 3 years (01/18 → 12/20)

- Partners: n=6 (Anses, WIV-ISP (Belgium), BfR (Germany), INRA (France), Institut Pasteur (France), NIV (Norvège))
Main objectives

- Establishment of an EU-wide network focusing on the detection and identification of *S. aureus*, *B. cereus* and *C. perfringens*,

- Evaluation of different diagnostic approaches (e.g. spectrometric, immunological, functional approaches) to characterize *S. aureus*, *B. cereus* and *C. perfringens*,

- Generation and characterization of a reference collection of bacterial strains,

- Implementation and development of methods to identify bacteria and associated toxins and/or virulence factors,

- PT organization
Conclusions and Future works

Staphylococcal enterotoxins (SEs) are a major cause of FPO due to bacterial toxins (49%).

Official method (screening) and ELISA quantitative method: allowing investigation of many SFPOs

Large part of weak evidence versus strong evidence outbreaks …

In the littérature: seh, seg, sei, sem, sen, seo genes have been associated with food poisoning (S Jolher, 2015 and M-A Argudin 2010)

However, genes encoding novel SEs as well as SE/s with untested emetic activity are widely represented in S. aureus, and their role in pathogenesis may be underestimated. ➔ Need of development of new assays (eg SEG, SEH and SEI) and confirmatory method based on another principle than ELISA (eg mass spectrometry)
Acknowledgements

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A. CAUQUIL, P BOUCHEZ, N VINGADASSALON,

Head of Unit SBCL
JA. HENNEKINNE

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