Surveillance system of staphylococcal food-borne poisoning outbreaks in France

Florence GUILLIER (florence.guillier@anses.fr)
Maisons-Alfort Laboratory for Food Safety
Unit Characterisation of Toxins, Team Bacterial Toxins

V workshop del Laboratorio Nazionale di Riferimento (NRL) per gli stafilococchi coagulasi positivi compreso S. aureus

TORINO, 14-15 giugno 2012
Plan

I. Background on staphylococci and SFPO
II. Surveillance system of foodborne illness in France and Europe
III. Epidemiological data (2010) from EFSA and France
IV. Some examples of SFPO in France
I. Background on staphylococci and SFPO
Conditions needed for a Staphylococcal Food-Poisoning

- **Source of *Staphylococcus***
  - Enterotoxigenic strains

- **Transfer of *Staphylococcus* in food**
  - *animal, operator, equipment...*

- **Staphylococcus development in food**
  - Favorable conditions (*pH, temperature, salt concentrations...*)

- **Enterotoxins production**

- **Ingestion of the contaminated food**
  - *in sufficient amount to induce symptoms*

- **Staphylococcal Food Poisoning Outbreak**
Conditions needed for a Staphylococcal Food Poisoning

Source of *Staphylococcus*

Transfer of *Staphylococcus* in food

*Staphylococcus* development in food

Enterotoxins production

Ingestion of the contaminated food

Staphylococcal Food Poisoning Outbreak
Characteristics of the genus **Staphylococcus**

- **Staphylococcaceae family**
- **Staphylococcus** → staphylê: bunch of grapes  
  → kokkos: grain or berry
- **Present in all type of natural environments** (air, water, dust...), on animals and human people (healthy carriers)
- **Grows in food proteic matrices** such as cheeses, ready-to-eat meals...
- **Some species** are useful (enzymatic degradation, ferments), some are responsible of alteration (lipolyse), and some are **pathogenic species** (toxinogenic strains) → responsible for SFPO
Physiology of staphylococci

- Facultative anaerobic Gram positive coccus
- Immobile and non sporulated
- Catalase +, oxydase –
- Coagulase positive (*S.aureus*) or negative
- Needs vitamins and amino-acids
- Mesophilic bacteria
- Inhibition of growth by competitive flora
- Allows high NaCl concentration and low Aw (0.86)
- Survives into frozen or freeze-dried foods
- Heat sensitive bacteria (contrary to enterotoxins)
Research and count of *S. aureus* in food

Direct count of colonies on selective media

- **Baird-Parker medium (ISO 6888-1 standard)**
- **Rabbit plasma fibrinogen medium (RPF) (ISO 6888-2 standard)**
Conditions needed for a Staphylococcal Food-Poisoning Outbreak

Source of *Staphylococcus*

Transfer of *Staphylococcus* in food

*Staphylococcus* development in food

Enterotoxins production

Ingestion of the contaminated food

Staphylococcal Food Poisoning Outbreak
**S. aureus** growth and enterotoxigenic factors

<table>
<thead>
<tr>
<th>Factors</th>
<th>Growth</th>
<th>SE production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>6 – 46 ° C</td>
<td>10 – 45 ° C</td>
</tr>
<tr>
<td>Optimum temp.</td>
<td><strong>37 ° C</strong></td>
<td><strong>40 ° C</strong></td>
</tr>
<tr>
<td>pH</td>
<td>4 – 9.8</td>
<td>5 – 8</td>
</tr>
<tr>
<td>[NaCl]</td>
<td>0 – 20 %</td>
<td>0 – 10 %</td>
</tr>
<tr>
<td>Aw</td>
<td>0.83 – 0.99</td>
<td>0.86 – 0.99</td>
</tr>
</tbody>
</table>

Toxigenic conditions are more restrictive than growth conditions.
Enterotoxins (SE) characteristics

- 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

- 21 SE described (SEA → SE\(\omega\))

  **but only** 5 are detectable with commercial kits : SEA to SEE

  - SE\(\omega\)
  - SE\(\omega\)O
  - SE\(\omega\)N
  - SE\(\omega\)L
  - SE\(\omega\)M
  - SE\(\omega\)U
  - SE\(\omega\)P
  - SET
  - SEA
  - SEB
  - SEC\(_{1, 2, 3}\)
  - SEH
  - SEG
  - SEF (⇒ TSST)


  SE emetic; SE-like non emetic; SE-like non tested
Enterotoxins (SE) properties

- Soluble in water and saline solutions

- Resistant to:
  - Heat treatment (high temperature for long period (for ex. 121°C for 30 minutes)
  - Low and high pHs (3 to 9)
  - Irradiation
  - Proteolytic enzymes

Stability depends on SE type, amount of SE, type of matrix
S. aureus \[?\] enterotoxins

**Presence of S. aureus / absence of SEs**
- All the staphylococcus strains are not enterotoxigenic
- Toxigenic conditions are more restrictive than growth conditions

**Absence of S. aureus / presence of SEs**
- S. aureus is pH and heat sensitive
- SEs are very resistant

SE not eliminated during process (ripening, pasteurization) and properties not modified in the gastrointestinal tract => HAZARD
Conditions needed for a Staphylococcal Food-Poisoning

- Source of *Staphylococcus*
- Transfer of *Staphylococcus* in food
- *Staphylococcus* development in food
- Enterotoxins production
- Ingestion of the contaminated food
- Staphylococcal Food Poisoning Outbreak
CPS in food: Symptomatology

**SFP - Symptomatology**

- 30 minutes to 8 hours after ingestion
- of 20 to 40 ng of SEs in food... (for the most sensitive people)

---

**Emetic action**

- Nausea
- Violent vomiting

**Superantigenic action**

- Abdominal cramps
- Profuse diarrhea

---

**Complication**

- Severe dehydration
- Low blood pressure
- General weakness
- Hospitalization

⇒ Impressive symptomatology but unusual deaths (0.4‰)
⇒ Fast recovery (generally 24 to 48 h)
**Emetic dose ED\textsubscript{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)

**Emetic dose ED\textsubscript{50} (aerian route)**

- Human: 0.4 ng SEB per kg of body weight

Some references:
II. Surveillance system of foodborne illness in France and Europe
Surveillance system of foodborne illness in France

- Based on several systems:
  - Mandatory notification
  - National Reference Centres (NRC)
  - Laboratory and physician based networks

- FBO
- Anthrax
- Botulism
- Brucellosis
- Cholera
- Hepatitis A
- Typhoid and paratyphoid fevers
- Listeriosis
- Tularaemia
- Creutzfeldt-Jakob disease and other human transmissible spongiform encephalopathies
FBO notification / french level

MANDATORY NOTIFICATION

- Laboratory
- Establishment responsible
- Health professional
- Consumer
- other: poison control centre...

ARS (Health regional agency)
DDI (Interministerial Department Directorate)

DGS / Health Ministry
DGAL / Health Emergency Mission / Ministry of Food, Agriculture and Fishery
INVESTIGATIONS

Epidemiological inquiry

ARS

Organisation of sampling

ARS (ills and water)

DDI (food)

Inquiry on food business

DDI

Laboratory

Samples

Laboratory

Samples

NRC

Samples and/or associated strains (clinical)

NRL

Samples and/or associated strains (food)
RESULTS OF INVESTIGATIONS

Identification of the food vehicle
Identification of the pathogen
Identification of predisposing circumstances
Identification of batches or commercial dispatching

Measures on product(s) and/or establishment(s)

Health Ministry
DGAL / Ministry of Food, Agriculture and Fishery

Measure(s) of recall
FR and/or EU level
RASFF (rapid alert system for food and feed) EU level
EU notification every 3 months or annually
Annual epidemiological report: Reporting on 2009 surveillance data and 2010 epidemic intelligence data

Annual report: EU summary report on zoonoses, zoonotic agents and food-borne outbreaks 2010
The competent authority of each MS must provide the Commission with a summary report of the results of the investigations of food-borne outbreaks, which is sent to the European Food Safety Authority (EFSA).

The European Union (EU) system for the monitoring and collection of information on zoonoses is based on the Zoonoses Directive 2003/99/EC, which obligates EU Member States (MSs) to collect relevant and, where applicable, comparable data on zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks. In addition, MSs shall assess trends and sources of these agents as well as outbreaks in their territory, transmitting an annual report to the European Commission (EC), covering the data collected. The European Food Safety Authority (EFSA) is assigned the tasks of examining these data and publishing the EU Summary Report.

The reporting of investigated food-borne outbreaks has been mandatory for EU MSs since 2005. Starting from 2007, harmonised specifications on the reporting of these outbreaks at the EU level have been applied. However, it is important to note that the food-borne outbreak investigation systems at the national level are not harmonised among MSs. Therefore, the differences in the numbers and types of reported outbreaks, as well as the causative agents, may not necessarily reflect the levels of food safety situations among MSs; rather they may be indicative of the differences in the efficiency and sensitivity of the national systems for identifying and investigating food-borne outbreaks.

Annual report: *EU summary report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010*
TECHNICAL REPORT


European Food Safety Authority

European Food Safety Authority (EFSA), Parma, Italy

TECHNICAL REPORT


European Food Safety Authority

European Food Safety Authority (EFSA), Parma, Italy
9. Reporting on other pathogenic microbiological agents in foodstuffs

9.1. Staphylococcal enterotoxins in foodstuffs

Relevant food categories to be reported

Food categories for which staphylococcal enterotoxins food safety criterion is laid down in Regulation (EC) No 2073/2005:

- cheeses made from raw milk or milk that has undergone a lower heat treatment than pasteurisation;
- ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment;
- unripened soft cheeses (fresh cheeses) made from milk or whey that has undergone pasteurisation or a stronger heat treatment;
- milk powder and whey powder not intended for further processing in the food industry.

Case definition/definition of a positive sample

Positive sample - a sample in which staphylococcal enterotoxins have been detected. It is recommended to indicate the weight of the sample tested.

Positive batch - a batch where staphylococcal enterotoxins have been detected in at least one of the samples in the batch. It is recommended to indicate the weight of the sample tested. When using quantitative analysis, it is also recommended to indicate the limit of detection of the method used.

Diagnostic/analytical methods typically used

The recommended method is the European screening method of the EU - RL for Staphylococci (ANSES - Lerqap, Maison-Alfort) in accordance with Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.
III. Epidemiological data (2010)
FBO due to bacterial toxins / Europe

Figure OUT1. Distribution of food-borne outbreaks (weak and strong evidence - excluding strong evidence waterborne outbreaks) per causative agent in the EU, 2010

FBO due to bacterial toxins / Europe

2010: 5,262 FBO (weak + strong evidence) reported by the 24 MSs (comparable to 2009: 5,550 outbreaks reported by 24 MSs) 43,473 human cases, 4,695 hospitalisations and 25 deaths (case fatalities)

Figure OUT2. Total number of food-borne outbreaks (weak and strong evidence - excluding strong evidence waterborne outbreaks) in the EU, 2008-2010

Different notification systems in MSs ⇒ under-reporting?

Figure OUT3. Distribution of food-borne outbreaks (weak and strong evidence - excluding strong evidence waterborne outbreaks) in Member States and non-Member States, 2010

### FBO due to bacterial toxins / Europe

**Table OUT4. Causative agents in all food-borne outbreaks (weak and strong evidence - excluding strong-evidence waterborne outbreaks) in the EU, 2008-2010**

<table>
<thead>
<tr>
<th>Causative agent</th>
<th>2010</th>
<th>2009</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td>1,604</td>
<td>30.5</td>
<td>1,722</td>
</tr>
<tr>
<td></td>
<td>341</td>
<td></td>
<td>324</td>
</tr>
<tr>
<td></td>
<td>1,263</td>
<td></td>
<td>1,398</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td>790</td>
<td>15.0</td>
<td>1,043</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>703</td>
<td></td>
<td>973</td>
</tr>
<tr>
<td><strong>Campylobacter</strong></td>
<td>470</td>
<td>8.9</td>
<td>333</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>443</td>
<td></td>
<td>317</td>
</tr>
<tr>
<td><strong>Bacterial toxins</strong></td>
<td>461</td>
<td>8.8</td>
<td>558</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td></td>
<td>218</td>
</tr>
<tr>
<td></td>
<td>374</td>
<td></td>
<td>340</td>
</tr>
<tr>
<td><strong>Other causative agents</strong></td>
<td>229</td>
<td>4.4</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td></td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td></td>
<td>159</td>
</tr>
<tr>
<td><strong>Other bacterial agents</strong></td>
<td>64</td>
<td>1.2</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td><strong>Escherichia coli, pathogenic</strong></td>
<td>31</td>
<td>0.6</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td></td>
<td>57</td>
</tr>
<tr>
<td><strong>Parasites</strong></td>
<td>30</td>
<td>0.6</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td><strong>Yersinia</strong></td>
<td>-</td>
<td>-</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>1,583</td>
<td>30.1</td>
<td>1,502</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td></td>
<td>218</td>
</tr>
<tr>
<td></td>
<td>1,524</td>
<td></td>
<td>1,264</td>
</tr>
<tr>
<td><strong>EU Total</strong></td>
<td>5,262</td>
<td>100</td>
<td>5,550</td>
</tr>
<tr>
<td></td>
<td>698</td>
<td></td>
<td>977</td>
</tr>
<tr>
<td></td>
<td>4,564</td>
<td></td>
<td>4,573</td>
</tr>
<tr>
<td></td>
<td>5,332</td>
<td></td>
<td>4,442</td>
</tr>
</tbody>
</table>

Note: 2010 data on outbreaks were based on strength of evidence (strong or weak) rather than in previous years in which outbreaks were defined as verified or suspected.

Note: Food-borne viruses include calicivirus, flavivirus, rotavirus, hepatitis A virus and other unspecified food-borne viruses. Bacterial toxins include toxins produced by Bacillus, Clostridium and Staphylococcus. Other causative agents include mushroom toxins, marine bictoxins, histamine, mycotoxins, wax esters and other unspecified agents. Parasites include primarily Trichinella, but also Anisakis, Giardia and Cryptosporidium. Other bacterial agents include Brucella, Listeria, Shigella and Yersinia.

- **2010 : 5 262 FBO notified (19.7% FR)**
- **461 FBO (8.8%) caused by bacterial toxins**

## FBO due to bacterial toxins / Europe

- **S. aureus** = 1\(^{\text{st}}\) cause of FBO for bacterial toxins (data 2010)

|                     | S. aureus | B. cereus | Clostridium (including Cl. perfringens, botulinum…)
|---------------------|-----------|-----------|---------------------------------------------------
| **Outbreaks**       | 274       | 99        | 88                                                 |
| (38 S + 236 W)      | (26 S + 73 W) |          | (23 S + 65 W)                                     |
| **Cases (ills)**    | 2796      | 1242      | 2046                                              |
| (941 S + 1855 S)    | (561 S + 681 W) |          | (795 S + 1251 W)                                 |
| **Hospitalisations**| 357       | 54        | 43                                                 |
| **Deaths**          | 0         | 0         | 1 (Cl. botulinum)                                 |

S : strong evidence  /  W : weak evidence

FBO due to bacterial toxins / Europe

Figure OUT21. Distribution of food vehicles in strong evidence outbreaks caused by staphylococcal toxins in the EU, 2010

Note: Data from 38 outbreaks are included: Belgium (2), France (8), Germany (2), Poland (7), Portugal (2), Romania (6), Slovakia (1), Slovenia (1) and Spain (9).

Données Tiac 2010 – InVS:
http://www.invs.sante.fr/content/download/36247/175238/version/2/file/tiac_donnees_2010.pdf
FBO due to bacterial toxins / France

Number of FBO notified in France between 1990 and 2010

Sources: Données Tiac 2010 – InVS
(http://www.invs.sante.fr/content/download/36247/175238/version/2/file/tiac_donnees_2010.pdf)
FBO due to bacterial toxins / France

1032 FBO notified in 2010, 331 FBO (32%) due to bacterial toxins

Sources: Données Tiac 2010 – InVS (http://www.invs.sante.fr/content/download/36247/175238/version/2/file/tiac_donnees_2010.pdf)
FBO due to bacterial toxins / France

Evolution of foodborne outbreaks since 2006

Sources: Données Tiac 2010 – InVS
(http://www.invs.sante.fr/content/download/36247/175238/version/2/file/tiac_donnees_2010.pdf)
## FBO due to bacterial toxins / France

<table>
<thead>
<tr>
<th>Number of</th>
<th>S. aureus</th>
<th>B. cereus</th>
<th>Cl. perfringens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outbreaks</td>
<td>225 (21.8%) 21 C + 204 S</td>
<td>61 (5.9%) 10 C + 51 S</td>
<td>45 (4.4%) 8 C + 37 S</td>
</tr>
<tr>
<td>1st cause (before Salmonella (13.7%))</td>
<td>2nd cause</td>
<td>3rd cause</td>
<td></td>
</tr>
<tr>
<td>Cases (ills)</td>
<td>2027 (406 C + 1621 S)</td>
<td>703 (213 C + 490 S)</td>
<td>883 (209 C + 674 S)</td>
</tr>
<tr>
<td>Hospitalisations</td>
<td>283</td>
<td>51</td>
<td>9</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

C : confirmed / S : suspected

**S. aureus:** the most incriminated/suspected agent in FBO since > 5 years, 2nd place (10.0%) for confirmed agent ; 1st place (42.4%) for suspected agent
FBO due to bacterial toxins / France

Type of incriminated food for SFPO (2010)

- 37% autres aliments
- 16% viandes
- 18% lait et PL
- 7% ovoproduits
- 7% charcuterie
- 6% poissons et crustacés
- 4% volailles
- 4% autres aliments
- 1% aliments non retrouvés

Sources: Données Tiac 2010 – InVS
(http://www.invs.sante.fr/content/download/36247/175238/version/2/file/tiac_donnees_2010.pdf)
FBO due to bacterial toxins / France

Distribution of SFPO in 2010 per commonly reported setting

Commercial restaurants 28%

School 18%

Medical and social institute 3%

Canteen or workplace catering 4%

Others (banquet, prisons...) 9%

Unspecified 1%

Household/domestic kitchen 37%

Sources : Données Tiac 2010 – InVS
(http://www.invs.sante.fr/content/download/36247/175238/version/2/file/tiac_donnees_2010.pdf)
Health and economical issues

• Health issues
  – 1\textsuperscript{st} cause of bacterial origin FBO in France since 2006
  – 1\textsuperscript{st} cause of FBO involving \textit{milk and milk products}:
    FR $\rightarrow$ SED: most frequently found toxin
    other MSs $\rightarrow$ SEC

• Other issues
  – Economical issues: withdrawing and/or destruction of incriminated batches, sales decreasing, establishments closures, unemployment…
  – Media impact
IV. Some examples of SFPO in France
Involving emerging toxins
Example 1 : France, 2009 (SEE)

**RAPID COMMUNICATIONS**

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

A Ostyn (a.ostyn@afssa.fr), M L De Buysert, F Guillier, J Groult, 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


This article has been published on 1 April 2010

www.eurosurveillance.org

- **Type of incriminated food:**
  Soft cheese made from unpasteurized cow milk

- **Period:** October-November 2009

- **Number of cases:** 23 in 6 départements

- **CPS > 1.5 \(10^5\) cfu/g and presence of see gene

- **[SEE] = 0.36 to 1.14 ng/g**
Examples 2 : France, 2011 (seg, sei)

1\textsuperscript{st} case (May 2011):
- Number of cases: 40 with 6 hospitalizations
- Food incriminated: cream made with raw milk (served with apple pie)
- Symptoms: vomiting – 1 h after ingestion
- Analyses’ results:
  → by Vidas SET2: negative
  → by quantitative method: SEA to SED < LD
  → genes seg et sei identified

2\textsuperscript{nd} case (June 2011):
- Number of cases: 56 (6 outbreaks) with 3 hospitalizations
- Food incriminated: curd cottage cheese
- Symptoms: vomiting, nausea, diarrheas,
  abdominal cramps – 3 to 8 h after ingestion
- Analyses’ results:
  → by Vidas SET2: negative
  → by quantitative method: SEA quantified or SEA à SED < LD
  → genes seg et sei identified (upon curd cottage cheese and 1 milk used)
Conclusions

- *S. aureus* = 1st rank among bacterial agents responsible of FBO
- Bacteria the most involved in FBO due to milk and milk products (SED)
- France: MS reporting the largest number of outbreaks in 2009 and in 2010 (20% of all reported outbreaks)
- Notification system through the mandatory notification
- Strong collaboration between Ministry for Food, Agriculture and Fishery and Health Ministry → investigations, measures of recall, epidemiology…
- According to EFSA, French surveillance system of foodborne illness is considered as efficient and relevant for identifying and investigating food-borne outbreaks (example of SEE SFPO), but some problems could occur !!!
Thanks for your attention

Characterisation of Toxins unit (CAT), Bacterial Toxins team (BAC)

J-A. HENNEKINNE
I. MUTEL
A. OSTYN
A-L. PRUFE
EURL for CPS

S. HERBIN
S. MESSIO
S. PAIRAUD
F. GUILLIER

Contact:
French Agency for Food, Environmental and Occupational Health Safety (Anses)
Maisons-Alfort Food Safety Laboratory (LSAl)
Characterisation of Toxins unit (CAT), Bacterial Toxins team (BAC)
23 avenue du Général de Gaulle – pôle HQSA
F-94700 MAISONS-ALFORT