Enterotoxin production by *Staphylococcus aureus*: An outbreak at a Barcelona sports club in July 2011

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**ABSTRACT**

An outbreak of acute gastroenteritis due to staphylococcal food poisoning occurred in July 2011 at a summer school held by a sports club in Barcelona (Catalonia, Spain). Of the 42 cases involved, 20 were hospitalised. To identify the outbreak source, a retrospective cohort study was performed on the group at risk, which included 73 summer school students and 18 staff members. Food exposure at the sports club restaurant was identified as the most relevant common link among the study cohort. Although the preliminary microbiological investigation suggested that enterotoxigenic *Staphylococcus aureus* (*S. aureus*) infections were the possible source, enterotoxin types A and D were identified, quantified and confirmed in the different biological samples collected. A descriptive, in-depth epidemiological and clinical investigation subsequently pointed to food intoxication rather than bacterial infection as being the cause of the outbreak. Molecular investigation of the strain isolates, using pulsed-field gel electrophoresis typing, revealed that all eight strains of *S. aureus* had the same profile and spa type (t008). Samples of the incriminated foods, i.e., boiled macaroni, tuna and fresh tomatoes, specimens of vomit of those affected, and bilateral fingernail scrapings and nasal swabs of food handlers were shown to be the common source of transmission of the contamination. Following the outbreak, appropriate hygiene and control measures could be implemented to prevent any recurrence.

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1. Introduction

*Staphylococcus aureus* (*S. aureus*) is a gram-positive micro-organism that is often involved in food poisoning, due to heat-stable enterotoxins being produced in foodstuffs, including dairy products (such as ewe’s milk cheese, and cream), meat and fish pies, in which they eliminate competing micro-organisms unable to support high temperatures, high osmotic pressures and relatively low humidity (Wieneke, Roberts, & Gilbert, 1993).

This, in turn, tends to give rise to outbreaks of food-borne infections via a toxigenic mechanism. The resulting toxins, which are thermostable and resistant to digestive enzymes, are produced in the food and ingested preformed, thus causing sudden vomiting, diarrhoea, nausea, malaise, abdominal cramps, pain and, sometimes, prostration, in which case hospital admission may become necessary after a short incubation period of one to 7 h (Bone, Bogie, & Morgan-Jones, 1989).

*S. aureus* enterotoxins are globular proteins consisting of a single-chain polypeptide, and have the property of breaking the helical structure of DNA between purine or pyrimidine bases and a phosphoric acid chain composed of simple, unbranched, relatively large amounts of lysine, tyrosine, aspartic acid and glutamic acid. The molecules, with weights ranging from 28,000 to 35,000 Da, are soluble in water and possess high thermostability. Enterotoxin production by strains of *S. aureus* is affected by substrate nutrient quality and pH, temperature, atmosphere, sodium chloride, chemicals and other competing micro-organisms (Bécquer A, 1996).

The aim of this paper was twofold: firstly, to describe the epidemiological and laboratory investigation of an outbreak of food poisoning, which led to confirmation of the hypothesis of food
poisoning due to consumption of a menu prepared at a summer school; and secondly, to assess the outbreak control measures which were implemented to prevent any future recurrence.

1.1. Background epidemiology

On 11 July 2011, the news media reported the occurrence of a cluster of gastroenteritis cases at a summer school being held by a sports club in Barcelona (Catalonia, Spain).

At 17:30 h on the same day, the Epidemiology Department of the Public Health Agency of Barcelona (PHAB) was notified by the Catalan Subdirectorate-General for Epidemiological Surveillance and Emergency Response (Subdirecció General de Vigilància i Resposta a Emergències de Salut Pública) of a possible outbreak of food poisoning which had affected a group of children (age range 3–12 years) attending the club’s summer school. Over 20 of the children were reported to have been affected. Most of the cases presented with symptoms of acute gastroenteritis (AG). The PHAB immediately initiated an investigation to assess the extent of the outbreak, identify the mode and vehicle of transmission, and implement appropriate control measures.

2. Patients and methods

2.1. Study hypothesis

It was postulated that the meal served at the club was the source of the outbreak of food poisoning, and that the clinical profile and symptoms were suggestive of *S. aureus* infection. A retrospective cohort study was designed to test this hypothesis.

2.2. Study population

All persons who had attended the sports club on 11 July 2011 and had eaten at the summer school dining room.

2.3. Case definition

Any person who had reported at least one of the following symptoms, i.e., vomiting, abdominal pain, nausea, fever or diarrhoea, after eating at the summer school on 11 July 2011.

2.4. Outbreak investigation

There were approximately 300 people at the summer school, including children, adolescents and staff (instructors, teachers). The kitchen was inspected and the entire process of food preparation and conservation was examined.

All health monitoring activities co-ordinated by the PHAB, namely, inspection, management and implementation of measures, were conducted from 11 to 12 July 2011.

A standardised questionnaire was administered to 97 people who had eaten at the summer school, to ascertain and assess the following information: demographic data; food consumption on 11 July; presence of symptoms; hospitalisation; type of treatment; and laboratory data. A cohort of 91 completed questionnaires (73 children, 11 teachers, 4 food handlers and 3 outworkers) formed the database for analysis purposes.

2.5. Microbiological analysis

Thirteen specimens of vomit from children and teachers, three nasal swabs and bilateral fingernail scrapings from three food handlers, and one sample of the “menu consumed” were collected and stored in a refrigerator at a temperature of 1–5 °C. Samples and strains were processed at the PHAB microbiology laboratory.

The methods applied to analyse the items of food consumed were those corresponding to coliforms, aerobic micro-organisms, *Bacillus cereus* and coagulase positive staphylococcal colony counts. In addition, specimens were also tested for *Salmonella* spp. and staphylococcal enterotoxin. To detect staphylococcal enterotoxin directly in the food, the European Community Reference Laboratory’s method for coagulase positive staphylococci was used.

In the coagulase positive staphylococcal colony count performed on samples of consumed food and vomit, and nasal and fingernail swabs, the presence of staphylococcal enterotoxin was determined by means of the Enzyme Linked Fluorescent Assay technique (VIDAS SET II, Biomérieux) and typed using reverse passive latex agglutination (SET-RPLA: Oxoide Limited, Basingstoke, England).

Seven *S. aureus* coagulase-positive strains, RPLA typed as enterotoxins A and D, were sent via the integral request and report programme to the National Microbiology Centre. The seven were isolated as follows: three from samples of vomit from affected patients; two from nasal exudate and fingernail scrapings of a food handler who was an asymptomatic carrier; and two from contaminated foods, namely, boiled macaroni and tuna.

The isolates were genotyped by pulsed-field gel electrophoresis (PFGE) after Smal digestion of chromosomal DNA, prepared by using a modification of the protocol described by (Cookson et al., 2007) and by spa typing (Turbeville, Cowan, & Greenfield, 2006).

2.6. Statistical analysis

A descriptive analysis was performed, with qualitative variables expressed as percentages, and relative risk (RR) used as the measure of association for the bivariate analysis. We used the SPSS computer software package (v18) to calculate the descriptive statistics, and the OpenEpi programme (v 2.3.1) for counts and calculation of exact confidence limits in the descriptive and analytical studies.

3. Results

Of the 300 persons who were at the sports club on the date of the outbreak, 97 (32.3%) belonged to the study cohort. The breakdown of the 91 (93.8%) cohort members surveyed was as follows: 73 (80.2%) were summer school students; 11 (12.1%) were teachers; 4 (4.4%) were food handlers; and 3 (3.3%) were outworkers.

The food was the same for all exposed subjects (children and adults).

A total of 42 AG cases were detected, 25 (53.2%) females and 17 (38.6%) males. Of this total, 30 (71.4%) were summer school students, 9 (21.4%) teachers and 3 (7.1%) outworkers. The overall attack rate was 46.2%. The median age of those affected was: 9 years in the case of summer school students (interquartile range 7–12 years); minimum 3, maximum 14 years); and 24 years in the case of adults (interquartile range 22–25 years; minimum 18, maximum 59 years).

When stratified by age group, the attack rate was highest in the 6- to 14-year age group among the students, and proved even higher among the adults (Table 1). The most frequent symptoms were abdominal pain 40 (95.2%), vomiting 37 (88.1%), diarrhoea 33 (82.5%), and nausea, 33 (78.6%). In addition, 15 (35.7%) subjects had fever but there was no thermometer to establish their precise temperature (Table 2). Of those affected, a total of 20 (33.3%), 14 females and 6 males, were hospitalised, with the median duration of hospitalisation being one day (range 1–3 days). The epidemic curve depicting the temporal distribution of cases according to symptom onset after eating, ranged from one to 3 h (Fig. 1).
The bivariate analysis of foods consumed as possible disease risk factors showed that, of the 91 persons who had dined at the sports club on 11 July, 42 were and 49 were not cases. Analysis of the food items served on the date of the outbreak revealed the highest relative risk for macaroni (RR 3.9; 95% CI 0.62–25.04), with an increased risk almost four times higher for affected versus unaffected subjects (Table 3). Consumption of fish nuggets (hake) was associated with an RR of 1.02 (95% CI 0.47–2.21) and consumption of yogurt with an RR of 1.89 (95% CI 0.68–5.18). A total of 84 persons had eaten macaroni (86.5%), 15 had eaten tomatoes (16.5%) and, among those who had been exposed, 66 (72.5%) could not recall whether or not they had eaten tomatoes.

Of the thirteen (30.9%) vomit samples collected from affected subjects, eight (19.04%) were found to contain enterotoxins in the strains of coagulase positive S. aureus culture obtained. These were typed as enterotoxins A and D. We analysed the bacterial strains of coagulase positive S. aureus culture obtained from five (11.9%) samples of vomit taken from 5 (25%) hospitalised patients.

The nasal swabs and bilateral fingernail scrapings taken from the three food handlers showed that one was an asymptomatic carrier, with enterotoxin A and D production by coagulase positive S. aureus strains being present in both samples.

S. aureus enterotoxins A and D were detected both in culture and directly in two food components featured on the menu (in the boiled macaroni and tuna, and in the fresh tomatoes that accompanied the fish nuggets). Counts were as follows: in the case of boiled macaroni and tuna, the micro-organism count at 30 °C was >3.0 x 10^5 colony-forming units per gram analysed (CFU/g), the coliform count was >1.5 x 10^6 CFU/g, and the coagulase-positive staphylococcus count was 3.2 x 10^6 CFU/g; in the case of fresh tomatoes, the micro-organism count at 30 °C was 2.5 x 10^6 CFU/g, the coliform count was 2.9 x 10^4 CFU/g, the coagulase-positive staphylococcus count was 2.9 x 10^4 CFU/g, and that of B. cereus was 3.5 x 10^4 CFU/g. None of the food analysed showed any trace of Salmonella spp.

In the molecular analysis of the seven coagulase positive S. aureus strains, PFGE genotyping showed that all seven isolates had the same profile and belonged to spa type t008 (Fig. 2).

None of the samples tested positive for Campylobacter, Yersinia, B. cereus, Clostridium perfringens and enterohaemorrhagic Escherichia coli. No laboratory analysis for gastroenteritis virus producers was required because the clinical profile was suggestive of bacterial infection.

### Table 1
Attack rate stratified by age group.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Affected</th>
<th>Unaffected</th>
<th>Attack rate (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–5</td>
<td>4</td>
<td>12</td>
<td>26.6</td>
<td>15</td>
</tr>
<tr>
<td>6–8</td>
<td>10</td>
<td>18</td>
<td>35.7</td>
<td>28</td>
</tr>
<tr>
<td>9–11</td>
<td>8</td>
<td>8</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>12–14</td>
<td>8</td>
<td>5</td>
<td>61.5</td>
<td>13</td>
</tr>
<tr>
<td>Subtotal</td>
<td>30</td>
<td>43</td>
<td>41.1</td>
<td>73</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 a 27</td>
<td>12</td>
<td>6</td>
<td>66.6</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>49</td>
<td>46.2</td>
<td>91</td>
</tr>
</tbody>
</table>

### Table 2
Frequency of symptoms.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>40</td>
<td>95.2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>37</td>
<td>88.1</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>33</td>
<td>82.5</td>
</tr>
<tr>
<td>Sickness</td>
<td>33</td>
<td>78.6</td>
</tr>
<tr>
<td>Fever</td>
<td>15</td>
<td>35.7</td>
</tr>
</tbody>
</table>

### Table 3
Bivariate analysis of food-related outbreak.

<table>
<thead>
<tr>
<th>Food</th>
<th>RR</th>
<th>IC95% Lower</th>
<th>IC95% Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaroni</td>
<td>3.95</td>
<td>0.62</td>
<td>25.04</td>
</tr>
<tr>
<td>Tomato</td>
<td>1.66</td>
<td>0.72</td>
<td>3.85</td>
</tr>
<tr>
<td>Nuguets</td>
<td>1.02</td>
<td>0.47</td>
<td>2.21</td>
</tr>
<tr>
<td>Yogurt</td>
<td>1.89</td>
<td>0.68</td>
<td>5.18</td>
</tr>
</tbody>
</table>

### 3.1. Results of inspection of the sports club

The inspection of the sport club revealed deficiencies in the handling and storage of prepared foods, and in the sanitary conditions of the kitchen, bar, and food- and beverage-storage facilities.

### 3.2. Intervention and control measures implemented

After receiving the results of the laboratory tests, the food handler who had proved to be an asymptomatic carrier was contacted by telephone. The preventive and hygienic measures to be put into practice were explained, including the need: to wash both hands consecutively and scrupulously, scrubbing between the fingers and under the nails, with abundant soap and water; to wear gloves, a nose/mouth mask and a hair net; to refrain from handling food whilst receiving antibiotic treatment; and, in the event of continuing to work, to disinfect all work surfaces and areas.

Similarly, we telephoned the asymptomatic food handler’s general practitioner, to request that he prescribe the necessary medical treatment (in this case, cloxacillin 500 mg/8 h for 10 days) and arrange for his patient to be given sick leave until the results of the post-treatment laboratory tests were negative.

### 4. Discussion

We were notified of an outbreak of food poisoning due to S. aureus, which affected 42 persons, and in which the clinical
characteristics, incubation period, duration of case symptoms, laboratory test results, and microbiological and molecular biological analysis of the strains confirmed the aetiology of the infection.

*S. aureus* enterotoxins A and D were detected in biological specimens of the patients affected and in samples of the foods (macaroni and fresh tomato) which had acted as the vehicle of the infection. The source of transmission was a food handler who proved to be an asymptomatic carrier.

Furthermore, deficiencies in food-handling and processing were found to be the cause of this outbreak.

Although food poisoning episodes caused by *S. aureus* tend to be sporadic, there can be person-to-person transmission which generally manifests itself in the form of outbreaks at collective institutions. (de Jong et al., 2004; Schmid et al., 2009) which, in this particular case, was a summer school run by a sports club.

The aetiology of such outbreaks can usually be readily identified if timely epidemiological investigation, intervention and control are implemented, such as the measures taken from the time this outbreak was reported to the Epidemiology and Inspection Department at the PHAB.

Prompt and speedy action by and collaboration among all the professionals implicated in the different stages of this investigation was vital to its success.

The clinical and epidemiological data initially collected led us to think that the initial hypothesis pointed to food poisoning, with the suspected vehicle being macaroni and fresh tomatoes as a result of deficiencies in the process of handling and preparing these items. Other studies published have reported the same staphylococcal enterotoxins being detected in similar foods (Petrushina, 1976; Strommenger et al., 2008).

Although the statistical analysis showed no association or risk for ingestion of food items featured on the menu, the laboratory results nevertheless confirmed that it was consumption of macaroni and fresh tomatoes that had given rise to food poisoning and the appearance of the outbreak.

The presence of the same bacterial enterotoxins in the fingernail and nose swabs taken from one of the food handlers and in the foods ingested led us to think of the existence of a human reservoir as the possible principal source of the outbreak. This pointed to cross-contamination having taken place, something that supported our results and did not refute our initial hypothesis.

The macaroni constituted an increased risk that was almost four times higher in affected versus unafflicted persons. Although this difference was not significant, no foods were found that were significantly associated with the disease.

In terms of the temporal distribution of cases related with the symptom onset after eating, an increase in affected cases was observed two or 3 h after meal time (Bone et al., 1989).

The difference in rates between children aged 3–5 years and those who were older might indicate that children who had eaten at the first seating were less affected than those who had eaten at the second because fewer food items had been left at ambient temperature and were thus less contaminated, indicating that both bacterial burden and proliferation increased with the passage of time.

Of the 42 persons affected, 20 were hospitalised (14 females and 6 males).

As is characteristic in these types of outbreaks, the most frequent symptoms were abdominal pain, vomiting and diarrhoea, a finding in line with other studies published (Ostyn et al., 2010; Thein, Trinidad, & Pavlin, 2010).

The combination of *S. aureus* enterotoxins, albeit rare, is very common in food poisoning outbreaks. In our study, PFGE showed that all the isolates had the same profile and spa type (t008).

Thanks to the sensitivity of the phenotypic and genotypic characterisation technique, the source of contamination of both the contaminated foods and those affected by the outbreak was shown to be the asymptomatic food handler, since the strains displayed the same biochemical characterisation. Molecular biology confirmed the contamination of the foods by showing that they had the same clonal origin.

It is widely known that PFGE is a highly discriminatory technique and is valuable for *S. aureus* typing, and spa typing is twice as accurate as *S. aureus* typing (Francois et al., 2005; Ross, Merz, Farkosh, & Carroll, 2005).

This technique is reproducible, easy to use and enjoys access to a central database (http://spa.ridom.de), which enables comparisons to be made with data obtained by laboratories based in different countries. A number of studies have shown that spa typing is equally applicable to epidemiological investigations, at both a local and an international level (Mellmann et al., 2008).

The type of food poisoning outbreak described here has a great impact on society and highlights the fragility of food control, and, to a lesser extent, of disease control and prevention systems.

In most food poisoning outbreaks it is difficult to trace the source of infection of patients affected, and even more difficult to trace the source of contamination of the foods involved, particularly since there is a good likelihood in these types of outbreaks that the persons affected will be unable to recall the foods ingested and/or that samples of the foods implicated will not have been kept.

These are some of the reasons why these types of outbreaks go unnoticed, thereby leading to considerable underreporting of cases. On other occasions, and in large-scale outbreaks in particular, intervention measures are often adopted without identifying the causes that have given rise to the outbreak (Mellmann et al., 2008).

It can be concluded here however that coagulase positive *S. aureus* was positively identified as the causative agent of the food poisoning outbreak.

PFGE showed that the same *S. aureus* clone was present in two of the food items ingested, samples of vomit of 8 persons affected, and nasal and fingernail swabs taken from one food handler, thereby proving that the food handler in question had been in contact with the food after it had undergone heat treatment.
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References


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