



## 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

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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 Catalanian 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: Oxoid 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 *Sma*I 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).

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