

Enterotoxigenic strain of *Staphylococcus aureus* causing food-borne outbreak in a private context

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Abstract

In the last European Food Safety Authority (EFSA) report on zoonoses a total of 5262 food-borne outbreaks (FBOs) have been reported in Europe in 2010. Staphylococcal FBOs are caused by consuming food contaminated with one or more preformed enterotoxins and are characterised by rapid onset of symptoms. In May 2012, an Italian family made up of five people was involved in a FBO: food sample of *arancini* (fried rice balls) were analysed and resulted positive for coagulase positive staphylococci (CPS) (>100,000 cfu/g) and for staphylococcal enterotoxins (SE) (types A and C). Laboratory analyses also led to the isolation of *Staphylococcus aureus* strain carrying the gene encoding for enterotoxin type A and belonging to the human biotype. The FBO described in this paper should be included in the next official FBO report as a strong evidence case: food and toxins responsible for symptoms and enterotoxigenic *S. aureus* strain were identified and the clinical symptoms matched with the final diagnosis.

Introduction

Foodborne illnesses are syndromes caused by the ingestion of food contaminated by chemical substances or microorganisms and/or their toxins. To date, more than 250 different food-related syndromes have been described, including nausea, vomiting, abdominal cramps and diarrhoea associated or not with fever.

The last European Food Safety Authority

(EFSA) report (EFSA, 2012) shows that in 2010, 5262 food-borne outbreaks (FBOs) were reported in Europe: out of these, 698 were classified as outbreaks with strong causal evidence, with the involvement of 12,409 people; other 4564 FBOs were considered weak outbreaks and involved a total of 31,064 people. When a FBO is suspected, the State Health System needs to co-ordinate activities of different professional figures in order to establish the degree of relationship between anamnesis, suspected foods and laboratory results. If medical, epidemiological and laboratory data are consistent, a FBO investigation can be considered well conducted and strong causal evidence can be highlighted.

Referring to the causative agent of FBOs occurred in Europe in 2010, pathogens responsible for the majority of foodborne illnesses and hospitalisations were classified as follows: *Salmonella* spp., viruses, *Campylobacter* spp., bacterial toxins, *Escherichia coli*, Shiga toxin-producing *E. coli* (STEC), and parasites.

In the same year, 225 weak causal evidence FBOs were reported in Italy, involving 1205 people; in particular, in Piedmont region (North-Western Italy) 35 strong causal evidence outbreaks involving 327 patients and other 25 suspected outbreaks involving 217 people were reported (Ferrari *et al.*, 2011).

Among different food-borne illnesses, FBOs caused by *Staphylococcus aureus* [staphylococcal food poisoning (SFP)] are related to the consumption of food contaminated with one or more preformed enterotoxins (Argudin *et al.*, 2010). Symptoms appear rapidly (2-8 h after ingestion) and include nausea, vomiting, abdominal cramps with or without diarrhea (Murray, 2005). Staphylococcal food poisoning is usually self-limiting within 24-48 h: young or debilitated patients may sometimes require hospitalisation for dehydration. In 2010, staphylococcal enterotoxins (SE) were responsible in Europe for 274 FBOs: 38 were characterised by strong causal evidence and 236 were weakly correlated. A total of 2796 people were involved (EFSA, 2012).

In Piedmont region from 2002 to 2010 (Ferrari *et al.*, 2011) a total of 181 people have been involved in outbreaks caused by *Staphylococcus aureus* and its toxins; however, the true incidence is very difficult to assess due to under-reporting which characterises food poisoning.

The present paper reports the epidemiological and analytical investigations carried out during a FBO which occurred in a private context and involved a family composed by 5 members. During investigations, surveillance system of the National Health Service and Official Food Laboratory activities were co-ordinated and led to the solution of the case.

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Materials and Methods

On 23 May 2012 at about 11 p.m., four people went to the emergency room of Tortona Hospital (Northern Italy) with gastrointestinal symptoms such as nausea, vomiting, diarrhoea and abdominal cramps. Patients were part of the same family and were identified as follows: father (patient A), mother (patient B), 16-year-old son (patient C), 12-year-old son (patient D), and a third son aged 10 (child 3) showing no symptoms at the time of arrival at the emergency room.

Patients declared they had consumed the last two meals (lunch and dinner on 23 May) at home. On the basis of anamnestic data reported in Table 1, food poisoning was suspected and the hospital activated surveillance system for FBOs through the National Health Service. This procedure includes the sampling of food and/or food waste at home and hygiene control at food business operator level. At the time of the inspection at home for sampling, only industrial made *arancini* (Italian traditional fried rice ball with meat) were found: they were collected, used to form the sample and delivered to the food control laboratory of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta. At the time of the inspection at the food factory, neither food connected to the outbreak, nor raw materials used for the production of the suspected food batch were found.

According to the rapid onset of symptoms, food sample was analysed for enumeration of *Bacillus cereus*, CPS and *Clostridium perfringens*; analyses for the detection of *B. cereus*

diarrheal toxin and SE type A, B, C, D and E were also performed. Furthermore, in order to investigate the hygienic level of the product, the following analyses were performed: total bacterial count, enumeration of β -glucuronidase *Escherichia coli* and enumeration of *Enterobacteriaceae*.

Methods used for analyses are reported in Table 2. All analyses performed are accredited according to the ISO/IEC 17025 standard by the Italian accreditation body (ACCREDIA, 2005).

Staphylococcal enterotoxins detection and quantification were performed by the European reference laboratory for CPS including *S. aureus* (Maisons-Alfort, Anses, France), using both qualitative and quantitative enzyme-immunoassay based methods (Ostyn *et al.*, 2011).

Coagulase positive staphylococci strains isolated from food matrix were analysed in order to detect genes encoding for staphylococcal enterotoxins by two different multiplex polymerase chain reaction (PCR) tests able to detect genes encoding for different SEs: SEA, SEB, SEC, SED, SEE and SER, and SEG, SEH, SEI, SEJ and SEP, respectively (De Buyser *et*

al., 2009a, 2009b).

Finally, CPS strains were biotyped according to the protocol described by Devrièse and colleagues (1984). In particular, tests to check the following reactions were performed: β -haemolysin production (H), bovine plasma coagulation (C), growth on crystal violet agar (CV), staphylokinase production (S).

Results

Results of laboratory investigations carried out on food are reported in Table 3.

Total viable count, enumeration of *Enterobacteriaceae* and enumeration of CPS resulted high, being 280,000,000 cfu/g, >150,000 cfu/g, and >100,000 cfu/g, respectively. Conversely, analyses for enumeration of *E. coli*, presumptive *Bacillus cereus* and *Cl. perfringens* gave negative results, as the total counts were lower than limit of detection of the methods (10 cfu/g).

Arancini sample resulted to be positive for the presence of SEs both with ELISA and ELFA method: this result was also confirmed by

quantitative ELISA test that revealed the presence of SEA (0.285 ng/g) and SEC (0.263 ng/g).

Table 4 shows results of laboratory tests performed on CPS strain isolated from *arancini*: the strain was identified as *S. aureus* and resulted to be positive for the presence of gene encoding SEA. Through biotyping, the strain resulted to be characterised by the formula -, C-, CVa+, S+ typical of strain of human origin.

Discussion

Arancini sample is considered responsible for the FBO herein described, since it resulted positive for the presence of two SEs (SEA and SEC). Furthermore, the parallel onset of symptoms in patients who ate *arancini* (patients A, B, C, D) and the absence of symptoms in the son who did not (child 3), also confirm the analytical results.

Arancini sample showed high bacterial contamination. The *arancini* manufacturing includes several handling steps in which the ingredients, namely boiled rice, cooked meat and tomato sauce are modeled in balls and fur-

Table 1. Anamnestic data reporting food consumed by the family in the last two meals, symptoms and time of the onset of food-borne outbreak.

Patients	23/05/2012		23/05/2012			Symptoms (time and day of onset)
	1:00 p.m. Pasta with meat sauce and mushrooms	Arancini	8:00 p.m. Prawns in pink sauce	Mixed fried fish	Crêpe with cheese	
A	+	+	+	+	-	Nausea and abdominal cramps (10.30 p.m. 23/5/12)
B	+	+	+	+	-	Nausea, abdominal cramps, vomit and diarrhea (10.30 p.m. 23/5/12)
C	+	+	+	+	-	Nausea, abdominal cramps, vomit and diarrhea (10.30 p.m. 23/5/12)
D	+	+	+	+	-	Nausea, abdominal cramps, vomit and diarrhea (10.30 p.m. 23/5/12)
Child 3	-	-	+	-	+	No symptoms

+, eaten; -, not eaten.

Table 2. Analyses performed on suspected food and methods used.

Pathogen targets	Methods used
Presumptive <i>Bacillus cereus</i>	ISO 7932:2004 (ISO, 2004a)
CPS	ISO 6888-2:1999/Amd 1:2003 (ISO, 2003a)
<i>Clostridium perfringens</i>	ISO 7937:2005 (ISO, 2005)
Aerobic mesophilic bacteria at 30°C	ISO 4833:2003 (ISO, 2003b)
β -glucuronidase positive <i>Escherichia coli</i> <i>Enterobacteriaceae</i>	ISO 16649-2:2001 (ISO, 2001) ISO 21528-2:2004 (ISO, 2004b)
SE A, B, C, D, E (ELFA)	European screening method of EU-RL for CPS VER 5 Sept 2010 (European Union, 2010)
SE A, B, C, D, E (ELISA)	European screening method of EU-RL for CPS VER 5 Sept 2010 European Union, 2010)
<i>Bacillus</i> spp. diarrhoeic toxin (ELISA)	MI 10CA085 rev 0/1 2011

CPS, coagulase positive staphylococci; SE, staphylococcal enterotoxin.

thermore cooked in boiling vegetal oil: this deep-frying step usually reduces the microbial viable count. In this sample, high bacterial count in the final product could be due to i) a post-cooking contamination or to ii) a cooking temperature that did not allow to reduce bacterial contamination.

On the other hand, due to their thermal resistance, the detection of SEs was not useful to discriminate which of two events occurred: however, their presence in the sample would suggest that bacterial contamination occurred during food preparation, since *S. aureus* strains required several hours to grow at a level expected to produce SE. Furthermore, human biotyping supported a possible contamination during preparation due to operator handling.

This notwithstanding, a discrepancy could

be noticed between toxins characterisation (SEA and SEC detected in food sample) and the genes encoding toxins in the isolated strains (SEA encoding gene); this result confirms the importance to perform PCR analyses in more than one isolated strain whenever possible: in this specific case, at least two different clones of enterotoxigenic strains were supposed to be present in the sample, carrying different SEs encoding genes.

Conclusions

In conclusion, the reported FBO evidenced as the symptomatology corresponded to a SFP outbreak and can be classified as a strong causal evidence. In this one, symptomatology

corresponded to a SFP outbreak and both toxigenic CPS strain and SEs have been recovered from the food waste.

The epidemiological survey performed by interviews indicated three different dishes (pasta, *arancini* and fish) as the potential food vehicle: among these ones, none of them have been eaten by child 3, which was in accordance with the lack of symptoms. Also, among the tested suspected foods, only *arancini* were contaminated by pathogens leading to the demonstration that the rice fried balls were FBO responsible.

Management of this FBO was efficient, both from the analytical and the inquiry points of view and demonstrated the importance of an integrated collaboration between the different operators of National Health System involved in the investigations of a suspected foodborne illness' outbreak.

Table 3. Results of microbial analyses performed on food.

Pathogen targets	Result (cfu/g)
Presumptive <i>Bacillus cereus</i>	<10
<i>Clostridium perfringens</i>	<10
Aerobic mesophilic bacteria at 30°C	280,000,000
β -glucuronidase positive <i>Escherichia coli</i>	<10
<i>Enterobacteriaceae</i>	>150,000
<i>Listeria monocytogenes</i>	Absence in 25 g
<i>Salmonella</i> spp.	Absence in 25 g
CPS	>100,000
SE A, B, C, D, E (ELFA)	Detected in 25 g
SE A, B, C, D, E (ELISA)	Detected in 25 g
<i>Bacillus</i> spp. diarrhoeic toxin (ELISA)	Possible presence in 10 g

CPS, coagulase positive staphylococci; SE, staphylococcal enterotoxin.

Table 4. Results of analyses performed on the isolated coagulase positive staphylococci strain.

Analyses	Results
Multiplex PCR 1	
Coding genes for SE A, B, C, D, E and R	SEA + SEB - SEC - SED - SEE - SER -
Multiplex PCR 2	
Coding genes for SE G,H, I, J and P	SEG - SEH - SEI - SEJ - SEP -
Biotyping	β - C - CV + Specific host + Human biotype +

PCR, polymerase chain reaction; SE, *staphylococcal enterotoxins*; +, present; -, absent; β , β -haemolysin production; C, bovine plasma coagulation; CV, growth on crystal violet agar.

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