

Family Intoxication Associated to an Enterotoxigenic *Staphylococcus aureus* Present in a Cream Filled Pastry

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Abstract: The aim of this study was to determine the origin and nature of the aetiological agent responsible of a gastroenteritis outbreak in a family party located in Majorca on June 2014. For this purpose, an epidemiological questionnaire was given to each assistant and a case control study was carried out. Microbiological analysis of suspicious food was performed by international recognized standard methods. The study included toxin production and detection in the food. The subsequent investigation revealed a total of 8 cases out of 11 attendees (73%) with onset of symptoms between 4-6 h after consumption of a party dinner. Symptoms included vomits, abdominal pain, diarrhoea and/or fever. The case control analysis demonstrated a strong epidemiological evidence of the role of a cream filled pastry. Microbiological analyses demonstrated the presence of an enterotoxin producing *Staphylococcus aureus* in the pastry. Moreover, the immunological analysis detected the existence of enterotoxins directly from the food. Altogether, these results demonstrated the outbreak was caused by an enterotoxigenic *Staphylococcus aureus* strain present in a cream-filled pastry.

Keywords: Enterotoxin, gastroenteritis, intoxication, food poisoning, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus is among the most frequent pathogens associated to food poisoning [1, 2].

In contrast to most other gastrointestinal illnesses, the onset of *Staphylococcus aureus* mediated food poisoning (SFP) symptoms is very fast, within a few hours after ingestion of the contaminated food. This is because the foodborne illness caused by this gram positive bacterium is based on intoxication mechanisms rather than infection. SFP is usually a self-limiting illness with a short incubation period: the median incubation period is estimated to be four hours, with a range from one to seven hours [1,3]. Emesis is the most commonly observed symptom, but other common symptoms include nausea, diarrhoea, abdominal cramps and headaches. Fever is infrequently reported [4]. The median duration of illness is 15 hours, ranging from 4 to 60 hours [3].

Staphylococcus aureus produces different enterotoxins (SE) which are responsible for intoxication episodes worldwide [1,5]. More than 20 SEs possessing

superantigenic activity have been described: SEA to SEIV. Among these, only half of them have emetic activity and represents a potential hazard for consumers. Besides, 11 SEs (i.e., SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEP, SER) are suspected to cause SFP outbreaks [6–8]. These gastroenteritis outbreaks results from the ingestion of enterotoxins preformed in food and produced by enterotoxigenic strains of staphylococci. These toxins are highly stable and heat-resistant. Then, although the bacteria may be killed by the heat treatment applied to the food prior to consumption, the highly stable SE performed by *S. aureus* in the food may still be emetically active [9].

National surveillance systems for SFP are active in several countries. However, it is not an officially reportable disease, and is thought to be under-reported. This is mainly because of the short duration of symptoms, and is especially frequent in outbreaks

occurring at home. Different estimations indicates less than 10% of SFP patients visit a hospital [1,4]. Even if patients seek medical care, the microbiological analyses often do not include testing for the presence of enterotoxigenic *S. aureus* [10]. In our country, based on the Spanish foodborne disease surveillance system, *Staphylococcus aureus* has ranked as the third cause of foodborne outbreaks in the last years: 137 (4.9%) of the 2342 reported foodborne outbreaks were caused by this pathogen [11].

A possible gastroenteritis outbreak affecting the members of a single family was reported on 24th June 2014 in Mallorca, Spain. The aim of this study was to determine the origin and nature of the aetiological agent responsible for the outbreak.

MATERIAL AND METHODS

Epidemiological investigation

The epidemiological investigation was performed within one week from the dinner. Attendants were kindly asked to complete an epidemiological questionnaire related to the dinner. The questionnaire included questions about amount and type of drink and food consumed that night, as well as typical symptoms related to gastroenteritis episodes. A case was defined as any person with acute onset of diarrhoea, vomiting and/or abdominal cramps in less than 24 h after the family dinner on 23rd June 2014. All the diners fulfilled the questionnaires completely.

Microbiological investigation

Microbiological analysis of the suspicious food was performed following standard international recognized techniques, as previously described for outbreak investigation [12]. Twenty five grams of food were homogenised in 225 ml of peptone water (Sharlab, Barcelona, Spain) for 2 min in a BagMixer® 400 CC (Instescience, St Nom, France). Appropriate dilutions were prepared and plated onto different media: plate count agar (PCA) (Sharlab, Barcelona, Spain) for total aerobic count, violet red bile agar with lactose (VRBL) (Sharlab, Barcelona, Spain) for coliforms, COLI ID agar (Biomerièux, Madrid, Spain) for *Escherichia coli* and Baird-Parker with rabbit plasma fibrinogen (BP-RPF) (Sharlab, Barcelona, Spain) for *Staphylococcus aureus*. For enumeration of *Bacillus cereus* Bolton broth and *Bacillus cereus* agar (Oxoid, Madrid, Spain) were used.

For the investigation of additional pathogens individual 25 g portions were homogenised in 225 ml of specific broth, enriching for 24 h and plating onto the appropriate specific media. In the case of *Listeria monocytogenes*, semi-Fraser broth was used, and an additional enrichment in Fraser broth was achieved before plating onto Otaviani-Agosti agar (Sharlab, Barcelona, Spain). *Salmonella* investigation was

performed as follows: after the initial enrichment using ONE Broth-Salmonella (Oxoid, Madrid, Spain), samples were cultured onto xylose lysine deoxycholate agar (XLD) and Brilliance Salmonella Agar (Oxoid, Madrid, Spain). All incubations were carried out at 37 °C except for PCA, incubated at 31 °C, RVS at 41.5 °C and COLI ID, at 44 °C. Catalase and oxidase tests (Oxoid, Madrid, Spain) were performed when needed.

Detection of staphylococcal enterotoxins

The analysis for the detection of staphylococcal enterotoxin was achieved by using the VIDAS Staph Enterotoxin II (SET2) ELFA test [13] (Biomerièux, Madrid, Spain), the ELISA-like method with greater specificity and sensitivity [14]. Enterotoxin expression of *Staphylococcus aureus* isolates was performed as follows: one colony was resuspend in 10 mL of tryptic soy broth (TSB) (Sharlab, Barcelona, Spain) and growth at 37°C overnight on an orbital shaker. Culture was centrifuge for 5 minutes 3500 × g, and supernatant was recover and filtered through a 0.22 µm membrane. These culture supernatants were considered as the extracts. For the detection on enterotoxins in food samples, 25 g of food were homogenized in 25 mL of reconstituted extraction buffer, and incubated 15 min at room temperature. Sample was then centrifuge for 15 min at 5000 × g. Supernatant liquid was collected and pump through moistened absorbent cotton placed in a syringe, using the plunger. A 500 µL aliquot of the extracts was then added to the reagent strip. Standards and controls were analyzed in the same run. Extracts were read in a mini-Vidas® automated immunoassay system (Biomerièux, Madrid, Spain).

RESULTS AND DISCUSSION

Several members of the same family felt sick after a dinner celebrated at home on 24th June 2014 in Mallorca, Spain. The epidemiological investigation revealed a total of 8 cases (73%) with onset of symptoms from 23th to 24th of June. The dinner consumed by the family members on 23rd was suspected as the source of the pathogen responsible for the outbreak.

Eight of the eleven guests were affected by gastroenteritis symptoms: 5 adults and 3 children (attack rates were 71.4% and 75%, respectively). The most frequent symptoms were vomiting (75%), abdominal pain (75%), diarrhoea (25%) and fever (25%). Questionnaire's results, which included all drinks and foods consumed that night, pointed to a food poisoning caused by a cream filled pastry, as it was consumed by all the affected people, while no symptoms were presented among the guests who haven't eaten it. The case control analysis confirmed this hypothesis, as symptoms presence was significantly associated only to the consumption of the pastry (Table 1). These results are considered an strong

epidemiological evidence by the European Food Safety Authority [15]. The onset of symptoms ranged from 4 to 6 hours after pastry consumption. Illness lasted for 1-2 days approximately and came to an end without any

specific treatment but hydration support and diet, especially in the case of children. No hospitalisation was necessary.

Table-1: Results of the case-control study

Type of drink or food consumed	Cases (n=8)		Controls (n=3)		OR ¹	95% CI ²	P value
	n	%	n	%			
Boiled ham	6	75%	2	67%	1.50	0.84 - 26.86	0.782
Bread	7	88%	2	67%	3.50	0.15 - 84.74	0.425
Cheese	4	50%	1	33%	2.00	0.13 - 31.98	0.621
Chips	7	88%	3	100%	1.43	0.95 - 2.14	0.521
Chorizo	2	25%	1	33%	0.67	0.04 - 11.94	0.782
Cream-filled pastry	8	100%	1	33%	9.00	1.42 - 57.12	0.011
Cured ham	6	75%	2	67%	1.50	0.84 - 26.86	0.782
Loaf	1	13%	1	33%	0.29	0.12 - 6.91	0.425
Olives	7	88%	2	67%	3.50	0.15 - 84.74	0.425
Orange juice	5	63%	1	33%	3.33	0.20 - 54.53	0.387
Snacks	7	88%	3	100%	1.43	0.95 - 2.14	0.521
Water	5	63%	3	100%	1.64	0.94 - 2.74	0.214
Wine	4	50%	0	0%	0.57	0.30 - 1.09	0.125

¹ OR: Odds ratio. ² CI: Confidence interval

The analysis of the suspicious food showed unacceptable sanitary conditions for consumption. The total aerobic count was 3.8×10^6 UFC/g, while the levels of enterobacteria were 9.7×10^3 UFC/g. These results indicated inappropriate cooking, handling and/or storage procedures. More important, up to 1.7×10^5 UFC/g *Staphylococcus aureus* were detected, suggesting the contamination of the food was directly introduced by the food handlers. These levels of *S. aureus* have been related to food poisoning episodes in the literature (1). No other pathogens, including *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus* and *Salmonella* spp., were present in the pastry. The staphylococcal strain was isolated from food and analysed for enterotoxin production, with positive results. Moreover, investigation of enterotoxin presence in the suspicious food was also performed, and the ELFA test demonstrated that enterotoxin was present as well. Detection of staphylococcal enterotoxins in a food epidemiologically associated to a gastroenteritis outbreak is considered conclusive, and demonstrates their etiological role. Outbreaks caused by *S. aureus* have been associated to cream, good conditions for growth and enterotoxin production [1,16]. This seems to be the case presented in our investigation.

Staphylococcus aureus food poisoning is usually self-limiting; therefore the incentive to report cases is lower than that for other foodborne illnesses. There are national surveillance systems for this illness in most western countries, but it is not an officially reportable disease, and isolated cases occurring in the home are not habitually reported [1,17]. In our community,

outbreaks caused by the pathogen are clearly underreported: only 12 outbreaks has been declared to the public health authorities in the last three decades [18]. As our economy is based on tourism, food safety is a major objective and different efforts are made to avoid food poisoning episodes. Therefore, health officers mainly focus their hygiene audits on hotels and related facilities. Besides, the cream filled pastry responsible for the outbreak described here was prepared in a bakery. Consequently, health authorities should increase their efforts to diversify the variety of audited facilities, particularly including in the inspection schedules facilities from small businesses, street vendors, etc.

CONCLUSION

In summary, the present investigation describes a gastroenteritis outbreak caused by an enter toxigenic strain of *Staphylococcus aureus*, associated to a cream filled pastry. This case reflects the need of an increase of health authorities controls, especially in the inspection of facilities from small businesses, street vendors, etc.

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