*Bulgarian Journal of Agricultural Science, 23 (No 1) 2017, 159–164*
*Agricultural Academy*

# CASE STUDY OF STAPHYLOCOCCAL ENTEROTOXIN POISONING AFTER CONSUMPTION OF READY-TO-EAT ROASTED CHICKEN PRODUCTS

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## Abstract

Ivanova, T. N., E. M. Gyurova and H. Y. Daskalov, 2017. Case study of staphylococcal enterotoxin poisoning after consumption of ready-to-eat roasted chicken products, *Bulg. J. Agric. Sci.,* 23 (1): 159–164

*Staphylococcus aureus* is a food-borne pathogen, capable to produce several enterotoxins (SEs) that cause intoxication with varying intensity in humans after ingestion of contaminated food. The present paper reports the results of an outbreak, provoked by Coagulase Positive Staphylococci (CPS) and enterotoxins in ready-to-eat chicken products, occurred in city of Yambol (Bulgaria) in January 2015. An old woman and a child were hospitalized after consumption of ready-to-eat roasted chicken legs. A total of 5 samples were examined and one of them was found to contain high level of CPS. The level of *Staphylococcus aureus* contamination in the positive sample was 6.92 log CFU/g, which was a significant reason to suspect the production of toxin. The sample with high level of contamination was analyzed according to the European Screening Method v5 using mini VIDAS SET2. The results showed presence of staphylococcal enterotoxin (TV 1.16), which was the cause for the ensuing food intoxication. EURL for coagulase positive staphylococci confirmed presence of SEA at a level of 0.033 ng/g applying quantitative indirect sandwich-type ELISA. Two CPS isolates were confirmed as *S. aureus* by a species-specific 23S rRNA targeted PCR test. Both isolates carried the *sea* gene only.

*Key words: Staphylococcus aureus*; Staphylococcal enterotoxin; staphylococcal poisoning; RTE poultry products
*Abbreviations: CPS:* Coagulase Positive Staphylococci; SE*:* staphylococcal enterotoxins; EURL-CPS*:* European Union Reference Laboratory for Coagulase Positive Staphylococci; ELISA*:* enzyme-linked immunosorbent assay

## Introduction

*Staphylococcus aureus* is a gram-positive microorganism that is often involved in food poisoning outbreaks, due to the production of heat-stable enterotoxins in different foodstuffs, including dairy products (such as ewe's milk cheese, and cream), meat and fish pies, in which they eliminate competing microorganisms unable to support high temperatures, high osmotic pressures and relatively low humidity (Wieneke et. al.,1993).

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases worldwide (Hennekinne et. al., 2009) due to the ingestion of staphylococcal enterotoxins, produced in food by enterotoxigenic strains of coagulase-positive staphylococci, mainly *S. aureus*. As staphylococcal enterotoxins are heat stable, they can be presented in food even when *S. aureus* is absent (Balaban and Rasooly, 2000). Moreover, not all strains of *S. aureus* are enterotoxigenic. Therefore, a conclusive staphylococcal food poisoning diagnosis is mainly based on the detection of staphylococcal enterotoxins in food. To date, 21 staphylococcal enterotoxins (SEs) have been described: from SEA to SelV, all possessing superantigenic activity, whereas only a few of them (SEA to SEI, SER, SES and SET) have been proven to be emetic (Ono et. al., 2008). These toxins are produced by enterotoxigenic strains of coagulasepositive staphylococci (mainly *S.*

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*aureus*) in food with high protein content. Enterotoxin production of strains of *S. aureus* is affected by the substrate nutrient quality and pH, temperature, atmosphere, sodium chloride, chemicals and other competing micro-organisms (Lombard, 1996).

A tendency of increasing the number of food-borne outbreaks via toxigenic mechanism is observed. The 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 1 to 7 h (Bone et. al*.,* 1989). Symptoms of SFP include rapid onset (within 1–6 h) of nausea, vomiting, diarrhea and abdominal cramps (Jorgensen et al*.,* 2005b). These symptoms subside within 24–48 h but the illness may remain for 7–10 days.

The aim of this article was to describe the laboratory study of a food poisoning outbreak, due to the consumption of ready-to-eat chicken products in January 2015 in city of Yambol, Southeast Bulgaria.

## Materials and Methods

### Epidemiological information

On the 22nd of January 2015 in the laboratory of NDRVMI Sofia were received 5 pieces of RTE roasted chicken legs for enterotoxin detection and isolation of coagulase positive staphylococci (CPS). The samples were sent from the laboratory of the regional service of Bulgarian food safety agency in city of Sliven, Bulgaria. Samples were taken from a supermarket in city of Yambol, after a complaint about a food incident that had affected an old woman and a child, hospitalized with clinically appeared food poisoning.

### Methods

The five samples were subjected to a routine microbiological analyses for presence of coagulase positive staphylococci on Baird-Parker agar with the addition of egg yolk emulsion (Merck, Germany) incubated at 37°C for 24–48 h (ISO 6888-1:1999/Amd 1:2003). The colonies showing the typical appearance of coagulase-positive staphylococci were tested for coagulase activity with rabbit plasma fibrinogen (Merck, Germany), catalase and antibiotic susceptibility analysis by the minimal inhibitory concentrations (MICs (mg/mL).

#### *Isolation of CPS and identification of S. aureus*

Coagulase positive staphylococci were isolated as follows: 10 g of each sample were diluted with 90 ml of Buffered Pepton Water (Merck, Germany) and homogenized using Stomacher (400 Circulator, England). Then, of each sample 10-fold serial dilutions were prepared as 0.1 ml of the supernatant was spread in duplicate on Baird-Parker (BP) Agar (Merck, Germany) plates, following standard method for *S. aureus* and incubated under aerobic conditions at 37°C for 24 and 48 h. The samples producing typical colonies (grey-black, surrounded by a dull halo) were considered to contain CPS (Normanno et. al., 2005). The strains showing typical colonies of coagulase positive staphylococci were subjected to additional tests for catalase and coagulase activity. For coagulase test, rabbit plasma fibrinogen (Merck, Germany) and Heart Infusion Broth (HiMedia, India)-were used.

#### *Biochemical characterization*

Biochemical identification of the isolate was performed by MICRONAUT-RPO plate (Merlin, Germany) for Gram-positive bacteria. Principle of the test is addition of the bacterial suspension, in saline, to the wells. After incubation at 37° C for 24 hours, the plate was read using MICRONAUT Scan under the control of MICRONAUT software and evaluated. Forty four biochemical reactions were analyzed to calculate the identification profile.

#### *Antibiotic susceptibility testing*

The minimal inhibitory concentrations (MICs (mg/mL) for *S. aureus* strain were determined by the agar microdilution method according to CLSI (2012). After 24 h at 37°C, the inoculum was prepared of culture on CASO agar plates by suspension in sterile Mueller-Hinton broth in order to obtain turbidity equivalent to 0.5 McFarland standards. The plate that was used had a code GPALL1F, Sensititre for G-positive microorganisms. The antibiotics investigated were chloramphenicol, daptomycin, gentamicin, linezolid, rifampin, trimethoprim/sulfamethoxazole, quinupristin/dalfopristin, tetracycline, erythromycin, oxacillin plus 2% of NaCl, ampicillin, penicillin, vancomycin, levofloxacin, tigecycline, moxifloxacin, clindamycin, streptomycin, ciprofloxacin and nitrofurantoin. Reference strain used for quality control was *S. aureus* NBIMCC 3703 (LMG, Belgium-LMG 8224) = ATCC 25923.

#### *Staphylococcal enterotoxins (SEs) detection and quantification*

Extraction and detection of SEs using qualitative immunoassays were performed according to the European Screening Method v5. Briefly, the sample received for analysis was initially submitted to a protein extraction step, followed by dialysis concentration. Detection was performed from these extracts using the two validated kits: Vidas SET2 (bioMérieux®) and Ridascreen SET Total (R-biopharm®) SET2 de-

tection kit (bioMerieux, Marcy l'Étoile, France), which are able to detect SEA to SEE simultaneously in dairy products (European Screening Method).

When the ESM results proved to be positive, the extracts were subjected to in-house quantitative ELISA confirmatory method for SEA to SEE characterisation and quantification.

Quantification of SEs was performed by quantitative indirect sandwich-type ELISA. Single sandwich type was used for SEB, whereas double sandwich ELISA types were used for SEA, SEC and SED. LOQs where estimated, as follows: 0.038 ng/mL for SEA, 0.305 ng/mL for SEB, 0.054 ng/mL for SEC and 0.167 ng/mL for SED. This method was used in the frame of several studies on SEs detection in foods (Ostyn et al., 2009; Zeleny et al., 2015).

***Staphylococcal enterotoxins genes (se) characterization***

Coagulase-positive staphylococci (CPS) in the suspected sample were counted using the standard method ISO 6888-1:1999/Amd 1:2003 part 1 as described in the relevant European Union (EU) legislation (Commission Regulation 1441/2007). CPS isolates were tested for enterotoxin genes by PCR targeting the *S. aureus* 23S rRNA gene and biotyped as described by Kerouanton *et al.* (2007). The isolates were tested for *sea-e*, *seg-j*, *ser* and *sep* genes using two multiplex PCR assays according to the procedures of the EU Reference Laboratory for CPS. The isolates were also typed by pulsed-field gel electrophoresis (PFGE) according to Kerouanton et al. (2007).

## Results

Detection and enumeration of CPS in ready to eat chicken products was done. Coagulase positive staphylococci were detected in only one of all five samples. More than $8.4x10^6$ colony-forming units (CFU) of CPS/g were enumerated in the sample involved in the outbreak. Two CPS isolates were further analysed and characterised. These isolates were confirmed to be *S. aureus* by a species-specific 23S rRNA targeted PCR test. All were found to carry the *sea* gene only. The other four RTE poultry products were free of CPS (below the detection limit ($<10^2$ CFU/g)). The *S. aureus* isolate was positive for catalase and coagulase, oxidase negative, nonmotile cocci with well appeared β-hemolysis on Blood agar (Merck, Germany).

Using of MICRONAUT-RPO plate (Merlin, Germany) (Figure 1), some specific characteristics of *Staphylococcus aureus* were confirmed (presented in Table 1).

The mini VIDAS SET2 test for enterotoxin production was performed in National Reference Laboratory for CPS for all poultry samples, but only one of them was positive for staphylococcal enterotoxin (TV 1.16).

**Table 1**
**Biochemical characteristics of *Staphylococcus aureus***

| MICRONAUT-RPO plate | |
|---|---|
| Biochemical reactions | Tested *Staphylococcus aureus* strain |
| β- Glucosidase | + |
| p- Nitrophenil-β- galactosidase | – |
| p- Nitrophenil-β- glucuronidase | – |
| Trehalose | + |
| Maltose | + |
| Sucrose | + |
| Lactose | + |
| Ribose | – |
| Turanose | + |
| Urease | + |
| β-Fucosidase | – |
| Raffinose | – |
| Xylose | – |

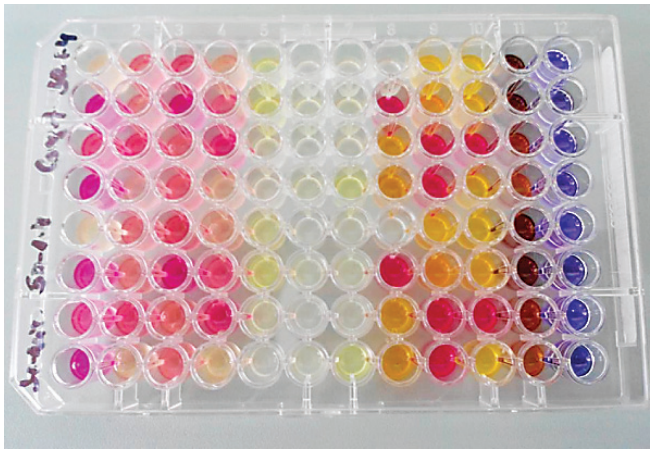

**Fig. 1 MICRONAUT-RPO plate of tested *S.aureus* isolate**

European Union Reference Laboratory – Coagulase Positive Staphylococci (EURL-CPS) applying the EU-RL screening method (ESM) using the qualitative and combined Vidas SET2 and Ridascreen SET Total tests for the detection of SEA to SEE resulted in an positive result for the sample involved in the outbreaks. EURL-CPS applied quantitative ELISA method and proved presence of SEA at low level (0.002 ng/g) in the same sample of the product. In the extract sent from our NRL-CPS for confirmation was detected the same SEA enterotoxin, quantified at 0.033 ng/g. The same isolate was analyzed for detection of staphylococcal enterotoxin (se) genes. The results showed that the isolate was positive for 23S rDNA gene of *S. aureus* and *sea* gene (Yacine NIA & Frédéric AUVRAY, EURL-CPS).

The minimal inhibitory concentrations (MICs (mg/mL) for *S. aureus* strain were tested and no resistance to main antimicrobials was detected (Table 2).

## Discussion

European screening method of the EURL for CPS including *S. aureus*, Version 5, September 2010 is known to be rapid and easy to perform but detects only the expression of staphylococcal enterotoxins SEA–SEE and cannot detect the SEs that have been recently described by several authors (Kerouanton et al., 2007; Lawrynowicz-Paciorek et al., 2007; Chiang et al., 2008). In addition, this methodology only gives a positive or negative result concerning the expression of the SE toxins A–E and does not differentiate them. According to Chiang *et al.* (2008) PCR detection using primers specific for all SEs or SAgs, including toxic shock syndrome toxin I (TSST-1) is applicable in laboratory practice. Our case study proved it by a species-specific 23S rRNA targeted PCR test. Solano et al. (2013) reported 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). The preliminary microbiological investigation suggested that enterotoxigenic *S. aureus* infections were the possible source, and enterotoxin types A and D were identified, quantified and confirmed in the different biological samples collected. In our case study, we found a single contaminated vacuum packed chicken product. The probable reason in our registered outbreak is secondary contamination of particular pieces of RTE chicken legs. Staphylococci are commonly found in a wide variety of mammals and birds, and transfer of *S. aureus* to food has two main sources – one of them is human carriage during food processing according to Hennekinne, De Buyser and Dragacci (2012). Our data showed that the only possible way for contamination of the examined RTE chicken legs was secondary contamination during some technological operations (vacuum packing). In the sample with proved presence of SEs (SEA) a great number of *S. aureus* cells was found, what is an additional reason to confirm this way of contamination. Hennekinne, De Buyser and Dragacci (2012) pointed that prevention of staphylococcal food-borne poisoning is based on hygiene measures to avoid or reduce contamination of food by *S. aureus*. These procedures must include control of raw materials, proper handling, cleaning and disinfection of equipment from farm to fork. Hennekinne et al. (2010) recommended using of an integrated gene-to-protein approach for characterizing staphylococcal food poisoning, such as the identification of *S. aureus* biovars, PCR and RT-PCR methods to identify the *se* genes involved, immunodetection of specific SEs and absolute quantification by mass spectrometry.

**Table 2**
**Antimicrobial resistance of *S.aureus* strain using EUCAST MIC Distribution (μg/ml) epidemiological cut-off as a reference**

| Antimicrobial agent | EUCAST MIC distribution (μg/mL) Epidemiological cut-off (ECOFF)(R is >) | Tested *S.aureus* strain | |
|---|---|---|---|
| | | Sensitive | Resistant |
| Chloramphenicol | 16 | 8 | – |
| Daptomycin | 1 | ≤ 0.5 | – |
| Gentamicin | 2 | ≤ 2 | – |
| Linezolid | 4 | 4 | – |
| Trimethoprim/Sulfamethoxazole | 0.5 | ≤ 0.5 | – |
| Quinupristin/dalfopristin | 1 | 1 | – |
| Tetracycline | 1 | ≤ 2 | – |
| Erythromycin | 1 | ≤ 0.25 | – |
| Oxacillin | 2 | 1 | – |
| Ampicillin | ND | – | 8 |
| Vancomycin | 2 | 1 | – |
| Levofloxacin | 1 | ≤ 0.25 | – |
| Tigecycline | 0.5 | 0.5 | – |
| Moxifloxacin | 0.25 | ≤ 0.25 | – |
| Clindamycin | 0.25 | ≤ 0.5 | – |
| Streptomycin | 16 | ≤ 1000 | – |
| Ciprofloxacin | 1 | ≤ 1 | – |
| Nitrofurantoin | 32 | – | 64 |
| Cefoxitin | 4 | ≤ 6 | – |

Food is an important factor for the transfer of resistant strains of *S. aureus*. Such transfer can occur by means of antibiotic residues in food, through the transfer of resistant food-borne pathogens or through the ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms (Khan et al., 2000; Pesavento et al., 2007). *S. aureus* strains are known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier and because of their location within micro-abscesses, which limit the action of drugs (Gundogan et al., 2006). Chakraborty, Mahapatra and Roy (2011) reported moderate resistance of *S. aureus* clinical isolates to kanamycin and oxacillin. Pereira et al. (2009) found resistance to b-lactams, ampicillin and penicillin in isolates from different foods, most of them enterotoxigenic (SEs). Our isolates showed resistance against nitrofurantoin (64 μg/mL) and ampicillin (8 μg/mL), no methicillin resistance was found.

## Conclusion

In conclusion, this is the first report in Bulgaria of a food poisoning outbreak due to staphylococcal enterotoxins in RTE chicken products. Positive for 23S rDNA gene and *sea* gene *S. aureus* was found in RTE roasted chicken legs and displayed almost in distinguishable pheno- and genotypes with regard to the methods applied in this study. Suitable hygienic measurements, including personal hygiene when handling food, are needed at all processing steps to reduce the possibility of introduction of a severe hazard into the food chain. In case of a future food-borne outbreak, investigations should include analysis of nasal samples taken from all people handling the food products, as well as packing materials and involved in the process of packing. Subsequent in-depth analyses of strains are needed as the results allow the identification of epidemiological correlations of strains. Hence, an interdisciplinary experts from both, the human and veterinary health side, epidemiologists, microbiologists and food hygiene experts, all of them working together hand in hand is a crucial prerequisite for successful outbreak investigation. This case study showed the impact of good business practices related to thermal processing, secondary contamination, packing, proper cooling and storage of RTE products.

***Acknowledgements***

The authors express their gratitude to Yacine NIA *and* Frédéric AUVRAY from EURL-CPS for their help in determining the type of staphylococcal enterotoxin trough quantitative ELISA method and for the detection of staphylococcal enterotoxin (SE) genes.

## References

**Balaban N and A. Rasooly**, 2000. Staphylococcal enterotoxins. *Int J. Food Microbiol*, **61** (1): 1-10.

**Bone, F. J., D. Bogie and C. S. Morgan-Jones**, 1989. Staphylococcal food poisoning from sheep milk cheese. *Epidemiology and Infection,* 103: 449-458.

**Chakraborty, S. P., S. K. Mahapatra and S. Roy**, 2011. Biochemical characters and antibiotic susceptibility of *Staphylococcus aureus* isolates. *Asian Pac. J. Trop. Biomed.*, **1** (3): 212-216.

**Chiang, Y.-Ch., W.-W. Liao, C.-M. Fan, W.-Y. Pai, Ch.-S. Chiou, H.-Y.Tsen,** 2008. PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in Staphylococcus aureus isolates from food-poisoning cases in Taiwan. *International J. of Food Microbiol.*, 121: 66-73.

**Clinical and Laboratory Standards Institute (CLSI**), 2007. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI document M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.

**Clinical and Laboratory Standards Institute (CLSI**), 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard – Ninth Edition. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.

**European Commission**, 2007. Commission Regulation 1441/2007 of 5 December 2007. Official journal of the European Unuion. 2007;L322:12-29. http://eur-lex.europa.eu/ LexUriServ/LexUriServ.do?uri=OJ:L:2007:322:0012:0029:EN:PDF

**European Screening Method of the EURL**, 2010. Coagulase positive staphylococci, including Staphylococcus aureus, version 5, September 2010, Detection of staphylococcal enterotoxins types SEA to SEE in all types of food matrices. https://sites.anses.fr/en/system/files/CPS-Cr-201017M.pdf

**Gundogan, N., S. Citak and E. Turan**, 2006. Slime production, DNAse activity and antibiotic resistance of *Staphylococcus aureus* isolated from raw milk, pasteurized milk and ice cream samples. *Food Control,* **17**: 389-392

**Hennekinne J. A., V. Brun, M. L. De Buyser, A. Dupuis, A. Ostyn and S. Dragacci**, 2009. Innovative contribution of mass spectrometry to characterise staphylococcal enterotoxins involved in food outbreaks. *Appl Environ Microbiol*,**75** (3): 882-884.

**Hennekinne, J.-A., M.-L. De Buyser and S. Dragacci**, 2012. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol Rev.,* 36: 815-836.

**Hennekinne, J.-A., A. Ostyn, F. Guillier, S. Herbin, A.-L. Prufer and S. Dragacci**, 2010. How Should Staphylococcal Food Poisoning Outbreaks Be Characterized? *Toxins,* 2: 2106-2116.

**ISO 6888-1:1999/Amd 1:2003**, 2003. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium - Amendment 1: Inclusion of precision data.

**Jorgensen, H. J., T. Mork, R. H. Hogasen and M. L. Rorvik**, 2005. Enterotoxigenic Staphylococcus aureus in bulk milk in Norway. *Journal of Applied Microbiology,* 99: 158-166.

**Kérouanton, A., J. A. Hennekinne, C. Letertre, L. Petit, O. Chesneau, A. Brisabois and M. L. De Buyser**, 2007. Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. *Int J Food Microbiol.*, **115** (3), 369-375.

**Khan, A. S., S. M. Nawaz, A. A. Khan and E.C. Cerniglia,** 2000. Transfer of erythromycin resistance from poultry to human clinical strains of *Staphylococcus aureus*. *J. Clin. Microbiol*., **38**: 1832-1838.

**Lawrynowicz-Paciorek, M., M. Kochman, A. Grochowska and B. Windyga**, 2007. The distribution of enterotoxin and enterotoxin-like genes in Staphylococcus aureus strains isolated from nasal carriers and food samples. *Int. J. Food Microbiol*, 117: 319-323.

**Lombard, A. B., L. M. de la Garza, C. L. Ortiz**, 1996. Importancia de la detección de enterotoxinas estafilocócicas. *Revista Cubana Aliment Nutr.,*10 (2). http://bvs.sld.cu/revistas/ali/vol10_2_96/ali12296.htm

**Normanno G., A. Firinu, S. Virgilio, G. Mula, A. Dambrosio, A. Poggiu, L. Decastelli, R. Mionid, S. Scuota, G. Bolzoni, E. Di Giannatale, A. P. Salinetti, G. La Salandra, M. Bartoli, F. Zuccon, T. Pirino, S. Sias, A. Parisi, N. C. Quaglia and G. V. Celano**, 2005. Coagulase-positive Staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *International Journal of Food Microbiology,* **98** (1), 73-79.

**Ono, H. K., K. Omoe, K. Imanishi , Y. Iwakabe , D. L. Hu , H. Kato , N. Saito , A. Nakane , T. Uchiyama and K. Shinagawa**, 2008. Identification and characterization of two novel staphylococcal enterotoxins types S and T. *Infect Immun*, **76** (11): 4999-5005.

**Ostyn A, De Buyser ML, Guillier F, Groult J, Félix B, Salah S, Delmas G, Hennekinne JA.,** 2009-2010. First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009. 2010*., Euro Surveill.,***15** (13): pii=19528. www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19528

**Ostyn, A., M. L. De Buyser, F. Guillier, S. Krys and J.-A. Hennekinne**, 2012. Benefits of the combined use of immunological- and PCR-based methods for determination of staphylococcal enterotoxin food safety criteria in cheeses. *Food Analytical Methods*, **5** (2): 173-178.

**Pereira, V., C. Lopes, A. Castro, J. Silva, P. Gibbs and P. Teixeira**, 2009. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. *Food Microbiology,* **26:** 278-282.

**Pesavento, G., B. Ducci, N. Comodo and A. L. Nostro**, 2007. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: a research for methicillin resistant *Staphylococcus aureus* (MRSA). *Food Control,* 18: 196-200.

**Solano, R., S. Lafuente, S. Sabate, C. Tortajada, P. G. de Olalla, A. V. Hernando and J. Caylà**, 2013. Enterotoxin production by *Staphylococcus aureus*: An outbreak at a Barcelona sports club in July 2011. *Food Control*, **33**: 114-118.

**Wieneke, A. A., D. Roberts and J. R. Gilbert**, 1993. Staphylococcal food poisoning in the United Kingdom, 1969-90. *Epidemiology and Infection*, 110: 519-531.

**Zeleny, R., H. Emteborg, J. Charoud-Got, H. Schimmel, Y. Nia, I. Mutel, A. Ostyn, S. Herbin and J. A. Hennekinne**, 2015. Development of a reference material for Staphylococcus aureus enterotoxin A in cheese: Feasibility study, processing, homogeneity and stability assessment. *Food Chemistry,* 168: 241-246.

*Received November, 7, 2016; accepted for printing January, 9, 2017*