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# Tracing and growth inhibition of *Staphylococcus aureus* in barbecue cheese production after product recall

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## ABSTRACT

Staphylococcal food poisoning is one of the most prevalent causes of foodborne intoxication worldwide. It is caused by ingestion of enterotoxins formed by *Staphylococcus aureus* during growth in the food matrix. Following a recall of barbecue cheese due to the detection of staphylococcal enterotoxins in Switzerland in July 2015, we analyzed the production process of the respective dairy. Although most cheese-making processes involve acidification to inhibit the growth of pathogenic bacteria, barbecue cheese has to maintain a pH >6.0 to prevent undesired melting of the cheese. In addition, the dairy decided to retain the traditional manual production process of the barbecue cheese. In this study, therefore, we aimed to (1) trace *Staph. aureus* along the barbecue cheese production process, and (2) develop a sustainable strategy to inhibit growth of *Staph. aureus* and decrease the risk of staphylococcal food poisoning without changing the traditional production process. To this end, we traced *Staph. aureus* in a step-wise blinded process analysis on 4 different production days using *spa* (*Staphylococcus* protein A gene) typing, DNA microarray profiling, and pulsed-field gel electrophoresis analysis. We subsequently selected a new starter culture and used a model cheese production including a challenge test assay to assess its antagonistic effect on *Staph. aureus* growth, as well as its sensory and technological implications. We detected *Staph. aureus* in 30% (37/124) of the collected samples taken from the barbecue cheese production at the dairy. This included detection of *Staph. aureus* in the final product on all 4 production days, either after enrichment or using quantitative detection. We traced 2 enterotoxigenic *Staph. aureus* strains (t073/CC45 and t282/CC45) colonizing the nasal cavity and the forearms of the cheesemakers to the final product. In the challenge test assay, we were able to show that the new starter culture inhibited growth of *Staph. aureus* while meeting the sensory and technological requirements of barbecue cheese production.

**Key words:** *Staphylococcus aureus*, contamination routes, process analysis, barbecue cheese, starter culture

## INTRODUCTION

In July 2015, the Swiss Federal Food Safety and Veterinary Office (Bern) issued a warning concerning the consumption of barbecue cheese, in which *Staphylococcus aureus* and staphylococcal enterotoxins (**SE**) had been detected. The dairy producing the barbecue cheese issued a recall, followed by a comprehensive external process analysis to trace *Staph. aureus* in the cheese-making process.

*Staphylococcus aureus* can cause staphylococcal food poisoning (**SFP**), the most prevalent foodborne intoxication worldwide. Ingestion of major or newly described SE (Hennekinne et al., 2010; Johler et al., 2015) formed during growth of the organism in food leads to symptoms of acute gastroenteritis and violent emesis (Hu and Nakane, 2014). Although symptoms usually subside within 24 h, SFP can, in rare cases, be fatal for children and the elderly. The Centers for Disease Control and Prevention (Atlanta, GA) estimates 240,000 cases per year in the United States, resulting in 1,000 hospitalizations and 6 deaths (Scallan et al., 2011).

As SE are heat-stable and will not be inactivated during the cooking process, preventive measures focus on inhibiting growth of *Staph. aureus* in the food matrix (Le Loir et al., 2003). To this end, starter cultures are used in the production of a wide range of foods including cheese. A suitable starter culture will outcompete the organism, thus effectively preventing *Staph. aureus* growth and SE formation. However, starter cultures need to meet several criteria to be suitable for the production of barbecue cheese. Although the antagonistic effect of many starter cultures is due to acidification of the food matrix, the pH of barbecue cheese cannot be lowered to values <6.0, as this would result in melting of the cheese when it is exposed to high temperatures during preparation by the customer. In addition, suitable starter cultures must have no negative sensory implications.

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In this study, we aimed to (1) trace *Staph. aureus* in the barbecue cheese production process, and (2) develop a sustainable strategy to inhibit growth of *Staph. aureus* and decrease the risk of SFP without changing the traditional production process.

## MATERIALS AND METHODS

### Sampling Along the Production Process and Isolation of Coagulase-Positive Staphylococci

To identify potential sources for contamination of the barbecue cheese at the dairy, 4 production cycles (see Figure 1) of the barbecue cheese were screened for coagulase-positive staphylococci in a blinded setup. To avoid bias, the dairy and the cheesemakers were only informed of the results upon completion of the study. Samples were taken from cheesemakers and at each step of the cheese-making process on August 24 ($\mathbf{T_1}$), August 27 ($\mathbf{T_2}$), September 1 ($\mathbf{T_3}$), and September 3 ($\mathbf{T_4}$), 2015 (see Table 1). Before the start of cheese production, swabs from the anterior nares and forearms of the cheesemakers were taken, as well as a swab from the inner and outer side of the end of the milk hose after pasteurization. All swabs were moistened using 0.85% NaCl. During cheese production, 10-mL samples of milk, whey, and starter cultures, and 30-g samples of curd and cheese were taken. Samples were screened for coagulase-positive staphylococci (**CPS**) using enrichment in Mueller-Hinton broth with 6.5% NaCl and plating on rabbit plasma fibrinogen (RPF) agar (Oxoid, Pratteln, Switzerland) and quantitatively, following the EN ISO 6888-2 protocol (ISO, 1999). Species identification of *Staph. aureus* was achieved through subsequent *spa* typing and the detection of species-specific markers by Staphytype DNA microarray profiling (Alere, Jena, Germany).

### Cell Lysis and DNA Extraction

For cell lysis and DNA extraction, reagents of the Staphytype genotyping kit 2.0 and the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) were used according to the manufacturers' instructions. The concentration of nucleic acids was measured using a Nanodrop ND-1000 UV/Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE).

### spa Typing

The polymorphic X region of *spa* was amplified as previously described (Wattinger et al., 2012). Each PCR product was subsequently purified using the GenElute PCR Clean-Up Kit (Sigma-Aldrich, Buchs, Switzerland) and sequencing was outsourced (Microsynth, Balgach, Switzerland). Subsequently, *spa* types were determined using the *spa* server (http://spa.ridom.de/; Harmsen et al., 2003).

### DNA Microarray-Based Genotyping

The Staphytype genotyping kit 2.0 was used to detect the presence or absence of over 300 virulence and resistance genes and their allelic variants in *Staph. aureus* strains that were traced from the cheesemakers to the final product. Detection included genes encoding the major SE (*sea*, *seb*, *sec*, *sed*, *see*), as well as genes coding for newly described SE and enterotoxin-like superantigens (*seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *selm*, *seln*, *selo*, *seq*, *ser*, *selu*). Microarray profiles also allowed for assignment of the strains to *agr* types, as well as clonal complexes (**CC**; Monecke et al., 2008).

### Pulsed-Field Gel Electrophoresis Analysis

Preparation of chromosomal DNA and pulsed-field gel electrophoresis (**PFGE**) analysis of *Sma*I-digested fragments was performed as previously described (Bannerman et al., 1995). Electrophoresis was carried out in a Bio-Rad CHEF-DR III electrophoresis cell (Bio-Rad, Hercules, CA). *Salmonella enterica* serovar Braenderup strain H9812 digested with 50 U of *Xba*I (12 h, 37°C) was used as a molecular size standard. Gels were analyzed with Gel Compar II software (Applied Maths, Sint-Martens-Latem, Belgium) using the dice coefficient and were represented by unweighted pair grouping by mathematical averaging (UPGMA) with an optimization of 0.5% and position tolerance of 1%.

### Model Cheese Production Including Challenge Test Assay Using New Starter Culture

A new starter culture, consisting of CNS, was chosen (START Crudo 500, Christl Gewürze GmbH, Moosdorf, Austria). Species identification of CNS was performed by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS). To evaluate the suitability of the new starter culture for the barbecue cheese production, the cheesemakers produced 4 batches of model cheese (batches A to D) in a laboratory setting.

To assess growth of the starter culture during the cheese-making process, we determined CNS counts in 2 barbecue cheese production batches (batches A and B) at 3 time points. The first sample was taken from the curd directly after coagulation, the second sample was obtained from the cheese after pressing (same day), and the third sample was taken in the morning of the