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Preliminary Quantitative Microbial Risk Assessment for Staphylococcus enterotoxins in fresh Minas cheese, a popular food in Brazil

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ABSTRACT

The main objective of this work was to assess the risk associated with staphylococcal enterotoxins (SE) intoxication after the consumption of fresh *Minas* cheese by the Brazilian population. Coagulase-positive staphylococci data from 350 samples were obtained from monitoring programs, and were used as a proxy for *S. aureus* contamination, considering that 73% of the strains were toxigenic. The Combined Database for Predictive Microbiology (ComBase) and the Pathogen Modeling Program (PMP) models were used to predict *S. aureus* growth rate and lag-phase in fresh *Minas* cheese at different pH, salt concentration and storage temperature in a household refrigerator, up to 7 days before consumption. Change in storage temperature had the largest impact on the growth rate and lag-phase obtained from both models. Cumulative probability of SE intake events equal to or higher than the toxigenic dose of 100 ng were calculated using Monte Carlo simulations performed by the @Risk software. The toxic dose was exceeded at the 99.95th percentile of exposure in the ComBase model (upper bound) for the adult population, the lowest percentile identified in the study. The *S. aureus* initial concentration was the parameter that most impacted the output obtained by @risk, indicating the importance of good manufacturing practices for fresh *Minas* cheese production, and proper storage conditions at the point of sale. This preliminary assessment indicated that the risk of staphylococcal intoxications from the consumption of fresh Minas cheese by the Brazilian population is probably low. The study identified many data gaps that needs to be addressed to improve the risk assessment.

1. Introduction

Staphylococcus aureus is the most prevalent and economically significant pathogen causing intramammary infections in dairy ruminants (Nader Filho, Ferreira, Amaral, Rossi, & Oliveira, 2007; Peles et al., 2007; Wang et al., 2009). It can contaminate milk either by direct excretion from udders with staphylococcal mastitis or during handling and processing of raw milk (André et al., 2008; Wang et al., 2009). *S. aureus* is a facultative anaerobe Gram-positive, catalase and coagulase positive coccus, which can grow in a wide range of pH (4.5–9.3) and temperature (7–47.8 °C), and at water activity (a_w) as low as 0.83 (FDA, 2012). *S. aureus* strains are also highly tolerant to salts and sugar (FDA, 2012).

S. aureus produces a wide variety of toxins, including the classic staphylococcal enterotoxins (SE) with demonstrated emetic activity (SEA to SEE, SEG to SEI, SER to SET) (Argudim, Mendoza, & Rodicio, 2010; Le Loir, Baron, & Gautier, 2003). More recently, new proteins (SE-like toxin, SElK to SElQ) with similar amino acid sequences were demonstrated to also have emetic activities in a primate model (Omoe et al., 2013). Ono et al. (2015) identified a novel staphylococcal toxin (SElY), which exhibited emetic activity in house musk shrews. SE are single-chain proteins with molecular weights of 24,000 to 29,000, resistant to proteolytic enzymes, which allows them to transit intact through the digestive tract, and are resistant to temperatures that would destroy the bacilli (FDA, 2012; Le Loir et al., 2003). Temperature control below 10 °C is required to inhibit SE production (Tutsuura & Murata, 2013). Other factors that affect SE production include the *S. aureus* strain, storage conditions, and type of milk (Janštová, Necidová, Janštová, & Vorlová, 2012).

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The intoxication dose of SE is less than 1000 ng, a level that is reached when *S. aureus* populations exceeds 100,000 organisms/g in food, indicative of unsanitary conditions. In highly sensitive people, ingestion of 100–200 ng of enterotoxin can cause symptoms of staphylococcal food poisoning (FDA, 2012). The symptoms include nausea, vomiting, abdominal cramps and diarrhea (Carmo et al., 2004; FDA, 2012). Although severe dehydration may occur, the illness is usually self-limiting, and recovery occurs within 24–48 h with proper supportive care (FDA, 2012; Kérouanton et al., 2007; Le Loir et al., 2003). Scallan et al. (2011) estimated that 241,188 illnesses due to *S. aureus* occur each year in the United States occurs, with 1,064 hospitalizations, and six deaths annually. In Brazil, 10,666 foodborne outbreaks were notified to the Ministry of Health from 2000 to 2014, mainly from food consumed in the household (MS, 2015). About 42% of the outbreaks had the agent identified, of which 18.5% involved *S. aureus;* milk and milk products were involved in 7% of the outbreaks with the food identified. In the investigation of two outbreaks that occurred in the state of Minas Gerais, Brazil, *S. aureus* strains showing to be producers of SEA, SEB and SEC were isolated from *Minas* cheese and raw milk samples (Carmo et al., 2002).

Various studies in Brazil have shown that *Minas* cheese presented the highest prevalence of coagulase-positive *Staphylococci* among dairy products (Carvalho, Viotto, & Kuaye, 2007; Ferreira et al., 2011; Moraes, Viçosa, Yamazi, Ortolani, & Nero, 2009; Rodrigues et al., 2011). Arcuri et al. (2010) showed that over 70% of the *S. aureus* strains isolated from fresh *Minas* cheese were enterotoxigenic. Typically Brazilian, Minas cheese is the most consumed cheese in the country (about 30 g/person/day; IBGE, 2011). The fresh type (*Minas frescal*) has high humidity (up to 45.9% water content), a pH between 5 and 6 (Rocha, Buriti, & Saad, 2006), and a maximum shelf life of 9 days under refrigeration (Sangaletti et al., 2009). A previous study highlighted the inadequate hygienic-sanitary conditions of the Minas cheese available for consumption in Brazil and the need to further investigate the potential risk of consumers (Nunes, Mota, & Caldas, 2013).

Quantitative Microbial Risk Assessment (QMRA) framework is a useful tool to evaluate the risk of consuming contaminated food and prevent foodborne diseases. Predictive models for microbial growth and survival under particular environmental conditions have been used for risk assessment of food-borne microorganisms (Ding et al., 2016; Fujikawa and Morozumi, 2006; Heidinger, Winter, & Cullor, 2009; Kim, Griffiths, Fazil, & Lammerding, 2009; Rho & Schaffner, 2007; Schelin et al., 2011). The extent of microbial growth is a function of the time the population is exposed to combinations of intrinsic food properties (e.g., salt concentration and acidity), and extrinsic storage conditions (e.g., temperature, relative humidity, and gaseous atmosphere) (McMeekin et al., 1997). Predictive models such as ComBase and PMP have been used by other authors to estimate the growth rate and lag-phase for QMRA studies of *S. aureus* (Heidinger et al., 2009; Lindqvist et al.; 2002; Yoon et al., 2011).

The main objective of this work was to estimate the risk associated with SE exposure from the consumption of fresh *Minas* cheese in Brazil purchased at retail stores. Microbiological data were obtained from monitoring programs around the country and the ComBase and PMP growth models were used to simulate the contamination levels at the time of consumption.

2. Materials and methods

The QMRA process includes the hazard identification, hazard characterization (dose-response), exposure assessment and risk characterization steps. In this study the hazard was identified as the staphylococcal enterotoxins (SE), for which 100 ng was considered the dose to cause symptoms of staphylococcal food poisoning (FDA, 2012; hazard characterization). Exposure assessment and risk characterization were conducted using the data and the models explained in the next sections.

2.1. Microbiological data on fresh Minas cheese at the time of purchase

Brazilian food legislation includes analysis of coagulase-positive staphylococci (CPS; maximum of 10^3 CFU/g for fresh Minas cheese), which is conducted under state sanitary surveillance programs. *S. aureus* and staphylococcal enterotoxin investigations are only performed in food samples suspected to be involved in foodborne outbreaks.

In this work, CPS data on fresh *Minas* cheese were obtained from the National Sanitary Surveillance Agency (ANVISA), which was a compilation of data from nine state laboratories on samples analyzed from 2010 to 2012. Additionally, data were obtained directly from the Central Laboratory of the Federal District (LACEN-DF) on samples analyzed between 2000 and 2014. The samples were analyzed using standard protocols (APHA, 2001).

The CPS data include results reported as zero or absent, as censored data (<3, <10 or < 100 CFU/g) and as finite enumeration. In this study, two levels of exposure were estimated: 1) the lower bound, where results reported as below 3, 10 or 100 CFU/g, zero, or absent, were assigned as 1 CFU/g, and 2) the upper bound, where levels reported as <3 CFU/g were assigned as 3 CFU/g, <10 CFU/g as 10 CFU/g, <100 CFU/g as 100 CFU/g, and those reported zero or absent as 1 CFU/g.

2.2. Fresh Minas cheese consumption

Consumption data for fresh Minas cheese were obtained from the 2008/2009 Brazilian Household Budget Survey (*Pesquisa de Orçamento Familiar*; IBGE, 2011) in which 34,003 individuals 10 years or older from all 26 Brazilian states and the Federal District completed a two non-consecutive daydietary reports. The data (portion, in g) also include information on fresh Minas cheese consumption in sandwiches, and the age of the consumer. Mean consumptions were 85.2, 88.8 and 72.3 g for teenagers, adults and seniors, respectively. In the exposure model used in this study, the variable (P) represents the cheese portion size. The histogram distribution was used to model the portion size for teenagers (10–19 years), adults (20–59 years) and elderly persons (60 years or older).

2.3. Household storage temperature, pH and % NaCl (w/w)

Bacterial growth was simulated from the time of purchase at the selling point to immediate consumption or after storage (t) in a domestic refrigerator, ranging from 1 to 168 h (7 days). A uniform distribution (continuous) was used to model the time of storage. Daily temperature records of a domestic refrigerator during a 16 months period (n = 734) were used to simulate the storage temperature (T) in the household. The values ranged from −0.9 to 17 °C, with a mean of 4.3 °C, median of 4.0 °C, and mode (most likely value) of 7.8 °C. The histogram distribution was used to model the temperature.

pH values of fresh Minas cheese were kindly provided by Prof. Susana Saad, from the University of São Paulo, Brazil, and concerned 76 samples obtained in the local São Paulo market. The pH values ranged from 4.9 to 6.5, with a mean and median of 5.8, and mode of 5.5. Salt concentrations of fresh Minas cheese, in % NaCl (w/w), were reported for 40 samples analyzed by the state laboratories and the LACEN-DF. Values ranged from 0.64 to 4.6% (mean of 1.28%, median of 1.14%, and mode of 1.46%).