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Evaluation of *Staphylococcus* spp. in food and kitchen premises of Campinas, Brazil

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ABSTRACT

The current study was aimed to evaluate the occurrence of *Staphylococcus* in institutional and domestic kitchens, as well as in meals served at child care centers and as desserts in local restaurants of Campinas, Brazil. Recovered isolates were analyzed by qPCR for Staphylococcal Enterotoxin (SE) expression and results compared to the traditional SE detection assay by immunoassay. Six different points (sink, refrigerator handle, stove burner knob, cutting board, cutting knives and dishcloth), were assessed in kitchens of 4 child care centers and 10 domestic kitchens. In addition, the hands and nostrils of the kitchen personnel were sampled. Meals served at the childcare centers (8 chicken meat and 8 chayotes) and desserts containing pastry cream from 10 restaurants were examined for the presence and levels present of *Staphylococcus* spp. Samples were collected during two distinct periods, and between them (1 month), a lecture regarding "Good practices in food handling" was given to all staff by the authors. Three hundred and twenty colonies (n = 320), typical and atypical belonging to *Staphylococcus* were recovered from all sampling points. Among 320 recovered colonies, fifty isolates were confirmed as *Staphylococcus* species (n = 47 from environmental samples and n = 3 from food samples). Also, *S. aureus* was responsible for the plurality of identified isolates (30%). The highest count for *Staphylococcus* was measured as 4.52 log10 CFU/50 cm^2 on the door handle of a refrigerator. Twelve percent of all tested strains (n = 50) likely expressed enterotoxins, and Staphylococcal Enterotoxin D (SED) was the SE most often (3 out of 6; or 50%) identified. The findings of the current study can be used is establishing guidelines for the improvement of cleanliness and food handling practices in the kitchens of Campinas, Brazil. Ultimately, reducing the occurrence of foodborne disease outbreaks and increased public health.

1. Introduction

Nowadays, contaminated food which can result in huge damages in direct and indirect ways can be considered as one most serious health issues among different societies. Globally, the number of diarrheal cases due to contaminated food are estimated to be 582 million cases per year, resulting in 25.2 million DALYs (Disability Adjusted Life Years) (Kirk et al., 2015). Of this total, approximately 700.000 children under five years old die each year (Fischer Walker et al., 2013). Data from the Secretariat of Health Surveillance of Brazil, ANVISA (Brasil, 2016), shows that from 2000 to 2015, 11.241 outbreaks of FBD (FoodBorne Diseases) were reported, involving more than 218.000 patients. Moreover, the educational institutions represent the third most common place of FBD outbreaks (8.7%), behind residences (38.4%) and restaurants (15.4%) in Brazil (Brasil, 2016). Additionally, *Staphylococcus* spp. coagulase-positive strains (~8%) is considered as the second most common identified etiological agent involved in confirmed outbreaks. However, due to the short duration of the disease and milder symptoms, the actual number of cases is probably should be higher than reported. In addition, ANVISA only recommends

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analysis of coagulase-positive *Staphylococcus* strains despite the fact that coagulase-negative strains also can be involved in the production of Enterotoxin (Oliveira, Padovani, Miya, Sant'Ana, & Pereira, 2010).

Evaluation of surface microbial species always has been the point of concern among the current investigations around the world. Pathogenic microorganisms, such as species of *Staphylococcus* spp., can be isolated from the environment, humans and other animals bodies (Gotz, Bannerman, & Schleifer, 2006). The cross contamination of *S. aureus, Salmonella,* and *Campylobacter* from raw meat onto the environment of Irish kitchens (sink drainers, taps, worktops, refrigerator handles) was confirmed by Kennedy and colleagues (Kennedy, Gibney, et al., 2011). Based on their results, the contamination can happen at all preparation stages and *S. aureus* was the most commonly detected microorganism.

Staphylococcal Enterotoxins (SE) that are related to FBD can be serologically classified into five main groups (SEA, SEB, SEC, SED, and SEE), with SEC being subdivided into three variants (Becker, Roth, & Peters, 1998). Most of the identified Staphylococcal outbreaks can be attributed to SEA activity (Asao et al., 2003; Fetsch et al., 2014; Schmitt, Schuler-Schmid, & Schmidt-Lorenz, 1990; Veras et al., 2008) although other SEs have also been detected.

Molecular and immunoassays methods, such as PCR and ELISA, respectively, can be considered as the routine analytical methods for SE detection. The regular PCR technique may be useful to detect enterotoxin-producing strains from contaminated food. However, this methodology has some disadvantages such as only providing information regarding the presence/absence of genes, without actual measurement of gene expression, bringing uncertainty to the SE production (Hennekinne, De Buyser, & Dragacci, 2012). Knowledge of SE gene expression gives supports to a better understanding of foodborne diseases etiology, allowing an improvement for microorganisms' control.

Thus, the current research aimed to investigate contamination of *Staphylococcus* spp. in institutional (childcare centers) and selected domestic facilities kitchens, and desserts with pastry cream. Also, the relative expressions of the primary SE-encoding genes (SEA, SEB, SEC and SED) from recovered isolates were evaluated.

2. Material and methods

2.1. Sample collection

2.1.1. Environmental (child care centers)

Four (n = 4) child care centers (named as "A, B, C and D", Campinas, Brazil) were selected for kitchen facilities and utensils sampling. The following points were aseptically sampled: sink, refrigerator handle, stove burner knob, cutting board. Also, four cutting knives randomly selected from the drawer and dishcloth. In addition, the hands and nostrils of the food handler in charge of preparing the child care centers' meals were analyzed. The accepted protocol by the Ethics Committee of the School of Medical Sciences (University of Campinas, Campinas, São Paulo, Brazil), has been followed in hands and nostrils' sampling. Sterile swabs (Dinâmica, Diadema, Brazil) moistened with 0.85% NaCl + peptone water 0.1% (Buffer solution, Difco, Sparks, USA) which laterally were placed in 9 mL of the same solution were used for sampling all points, except for the cutting board. In the case of cutting board sampling, wet sterile sponges were employed (3M, USA) and placed in sterile bags containing 90 mL of buffer solution. Also, the dishcloth samples were placed in sterile bags containing 90 mL of buffer solution.

2.1.2. Food samples

Twenty-five grams of cooked food (chicken and chayote, representing the menu of visiting days) were placed in empty sterile bags. This procedure was performed twice, contemplating two visits at childcare centers.

Twenty desserts containing pastry cream were also bought from local restaurants near to the university (Campinas, São Paulo, Brazil) in order to enumerate the presence of *Staphylococcus* spp. All samples were transferred to the Laboratory of Microbial Toxins (Department of Food Science, Faculty of Food Engineering, Unicamp) under refrigerated condition (4 °C) and analyzed within 30 min.

2.1.3. Environmental (residences)

The kitchen facilities of ten residences, belonging to Food Engineering students from University of Campinas (named as "Q to Z") were also sampled for the same above mentioned points, except for dishcloth. Table 1 summarizes the number of sampled points.

2.2. Enumeration/enrichment

Serial dilutions were carried out on Baird Parker agar (BP, Difco, Le Pont de Claix, France, plating on 10^{0}-10^{-3} surface), supplemented with egg yolk solution (Laborclin, Pinhais, Brazil) of 5% (w/v) and 1% of potassium tellurite solution (Laborclin, Pinhais, Brazil) at 1% (w/v) and then incubated at 37 °C for 48 h (Bennet & McClure, 1994). Brain Heart Infusion broth (225 mL, BHI, Difco, Le Pont de Claix, France), supplemented with NaCl 7.5% (Bennet, Hait, & Tallent, 2013) was used in order to perform enrichment of chicken meat and chayote samples (25 g of each). Afterward, samples were incubated for 48 h at 37 °C and then streaked onto BP slants for further confirmation tests (Gram, catalase, susceptible to furazolidone and DNAse)(Bennet et al., 2013). The final count was given as "CFU/g or 50 cm^{2}".

2.3. Identification of Staphylococcus

Following the incubation period of the plates (37 °C for 24–48 h), five colonies per plate were chosen for genus-diagnostic testing (Gram, catalase (Sigma-Aldrich, St. Louis, USA), coagulase (Thermo Fisher, Waltham, USA) and thermonuclease (Sigma-Aldrich, St. Louis, USA). Colonies retrieved from BP slants were also

Table 1
Summary of sample collection from kitchens and foods in Campinas Brazil Analyzed points in 4 childcare centers and 10 residences in Campinas, SP, Brazil and pastry cream desserts bought from local market.

Analyzed point	Childcare kitchens[a]	Residence kitchens[b]
Sink	8	10
Refrigerator door handle	8	10
Stove burner knob	8	10
Cutting board	8	8
Cutting knives	32	40
Dishcloth	8	
Hands	16	
Nostrils	16	
Food sample	**Number of samples**	**Grams per sample**
Chicken meat	8	25
Chayote	8	25
Pastry cream desserts	20	25

[a] Values represent number of samples of each type collected in study. Each point was sampled once per visit, e.g., 1 sink/per childcare center/visit (total n = 8). Hands and nostrils samples were taken only from one member of staff, during the first and second visit.

[b] Each residence was visited once, with one sampled point per local, e.g., 1 sink/residence (total n = 10). No cutting boards were available in two residences.