Check for updates

Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Contamination sources, biofilm-forming ability and biocide resistance of *Staphylococcus aureus* in tilapia-processing facilities

Daniel Vázquez-Sánchez, Juliana A Galvão and Marília Oetterer

Abstract

The major contamination sources, biofilm-forming ability and biocide resistance of *Staphylococcus aureus* in tilapia-processing plants were evaluated. Twenty-five processing control points were analysed twice in two factories, including whole tilapias, frozen fillets, water and food-contact surfaces. No final product was contaminated with *S. aureus*. However, high concentrations of *S. aureus* carrying enterotoxin (*se*) genes were found in several processing points of both factories due to the application of inadequate hygienic and handling procedures, which generate a high risk of cross-contamination of the tilapia fillets with staphylococcal enterotoxins. Nine *S. aureus* strains were characterized by RAPD-PCR using primers AP-7, ERIC-2 and S. A wide diversity of *se* gene profiles was detected, most strains being multi-*se*-carriers. All *S. aureus* strains showed high biofilm-forming ability on stainless steel and polystyrene. Biofilm-forming ability was correlated with the presence of *fliC H7* and the type of origin surface (metallic or plastic). A marked resistance of *S. aureus* to peracetic acid and sodium hypochlorite was also observed, required doses being higher than those recommended by manufacturers to be eradicated. Case-by-case approaches are thus recommended to determine the sources and degree of contamination present in each factory, which would allow applying precise responses that avoid, or at least reduce, the presence of bacterial pathogens and the emergence of antimicrobial resistance.

Keywords

Staphylococcus aureus, tilapia-processing facilities, enterotoxin genes, biofilm formation, biocide resistance

Date received: 29 June 2017; accepted: 25 October 2017

INTRODUCTION

Oreochromis niloticus tilapias are one of the most produced fish species in freshwater aquacultures due to their high international demand (FAO, 2016). In particular, Brazil is a major producer and exporter of tilapia, southeast being the large production region (IBGE, 2015). Good hygienic practices are applied to guarantee the safety of products, using frequently chlorine-based biocides and peroxides to kill microorganisms. However, novel trends in food production such as minimal processing, mass production and globalization, among others, have introduced new factors and conditions that can favour the presence of bacterial pathogens (Abee and Wouters, 1999; Havelaar et al., 2010; Rendueles et al., 2011). Moreover, sub-lethal doses of disinfectants and unsuitable exposure times are often applied, which can cause the emergence of antimicrobial resistance and

Food Science and Technology International 24(3) 209–222
© The Author(s) 2017 Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/1082013217742753
journals.sagepub.com/home/fst
SAGE

Department of Agri-Food Industry, Food and Nutrition, Laboratory of Freshwater Fish and Seafood Technology, 'Luiz de Queiroz' College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, Brazil

Corresponding author:
Daniel Vázquez-Sánchez, Departamento de Agroindústria, Alimentos e Nutrição, Laboratório de Qualidade e Inovação Tecnológica de Pescado, Escola Superior de Agricultura 'Luiz de Queiroz' (ESALQ), Universidade de São Paulo (USP), Av. Pádua Dias 11, Piracicaba/SP CEP 13418-900, Brazil.
Email: danielvazquezsanchez@gmail.com

a long-term presence (even persistence) of some pathogens (Bagge-Ravn et al., 2003; Langsrud et al., 2003; Sheridan et al., 2012; Vázquez-Sánchez et al., 2014).

Staphylococcus aureus is an opportunistic pathogen repeatedly detected in fishery products (Novotny et al., 2004; Papadopoulou et al., 2007; Vázquez-Sánchez et al., 2012). Food handlers are considered the main vector of spread of *S. aureus* during food processing, due to the presence of this pathogen in the microbiome of most humans (DeVita et al., 2007; Sattar et al., 2001; Simon and Sanjeev, 2007; Wertheim et al., 2005). Nevertheless, *S. aureus* is also able to develop biofilms on food-contact surfaces, which highly increase the stress tolerance and, thus, the persistence in food-related environments (Bridier et al., 2015; Gutiérrez et al., 2012; Van-Houdt and Michiels, 2010; Vázquez-Sánchez et al., 2014).

The ingestion of food containing staphylococcal enterotoxins (SEs) is one of the major causes of foodborne intoxications in humans worldwide (EFSA, 2016; Hennekinne et al., 2012; Kadariya et al., 2014). SEs can be produced at a wide range of temperatures, pH, water activity and salt content without affecting the sensory characteristics of the contaminated food (Hennekinne et al., 2012; Schelin et al., 2011). Moreover, the heat stability and the resistance to proteolytic degradation allow SEs to retain their emetic activity after food processing and consumption (Landgraf and Destro, 2013; Omoe et al., 2005).

The present study therefore aimed to evaluate the major contamination sources, biofilm-forming ability and biocide resistance of putative enterotoxigenic *S. aureus* in tilapia-processing plants.

MATERIAL AND METHODS

Sampling

Two tilapia-processing plants (Factory A and B) located in the Southeast of Brazil were inspected twice at approximately two-month interval. Factory A processes about 40 t of tilapia per day, whereas Factory B processes 4.2 t of tilapia per day approximately. Samples from 25 processing control points were collected in Factory A (Table 1) and B (Table 2), following APHA (2013). Briefly, freshly caught whole *O. niloticus* tilapias from aquaculture were aseptically placed in sterile bags. Areas of 100 cm^2 of contact surfaces and gloves were sampled by using two sterile swabs moistened with D/E neutralizing broth (Neogen Corporation, Brazil) added with 3% (v/v) polysorbate 80 (Labsynth, Brazil), which were subsequently immersed in 10 ml of peptone water (10 g/l triptone (Neogen Corporation) and 5 g/l sodium chloride (Labsynth)). Samples of gloves on the hand of workers were taken after the working shift, whereas surface samples were collected after daily cleaning and sanitizing to assess the validity of procedures applied. Samples of 1 l of fresh tap water (and ice) were aseptically placed in sterile bottles. All these samples, as well as packaged frozen tilapia fillets, were placed into isothermal containers and immediately shipped to the laboratory under chilled conditions.

Enumeration, isolation and identification of S. aureus

Samples of 25 g of tilapia were mixed with 225 ml of peptone water and homogenized for 1 min in a stomacher masticator (ITR Instrumentos para Laboratórios Ltd, Brazil). Water samples were vacuum filtered through 0.45 µm nitrocellulose membrane filters (F. Maia Indústria e Comércio Ltd, Brazil), which were aseptically placed in sterile bags with 10 ml of peptone water and homogenized for 1 min in a stomacher masticator. Swabs were vigorously vortexed for 1 min to resuspend cells adhered. After homogenization, samples were 10-fold serially diluted in peptone water and aliquots of 0.25 ml of appropriate dilutions (1:10, 1:100 and 1:1000) were spread on Baird Parker agar plates (Becton Dickinson, Brazil) supplemented with egg yolk tellurite emulsion (Laborclin Ltd, Brazil) (BP-EY). Plates were then incubated for 48 h at 37 °C. Afterwards, typical colonies of *S. aureus* (grey-black colonies surrounded by an opaque halo) were counted to calculate the number of CFU/g (products), CFU/cm^2 (surfaces) and CFU/l (water samples). Between one and five colonies from each contaminated point were sub-cultured twice on BP-EY agar for isolation of single colonies (isolates). Production of catalase and coagulase was then assessed using cultures grown in Brain Heart Infusion (BHI) broth (Neogen Corporation) for 24 h at 37 °C. Catalase production was tested by exposing 100 µl of bacterial culture to 100 µl of 3% (v/v) hydrogen peroxide (F. Maia Indústria e Comércio Ltd) and visualizing the formation of bubbles. Meanwhile, coagulase production was assessed by adding 100 µl of culture in 300 µl of rabbit plasma with EDTA (Laborclin Ltd) and incubating for 6 h at 37 °C to check the clotting of plasma.

Isolates able to produce catalase and coagulase were confirmed to be *S. aureus* by species-specific *23S rDNA* gene PCR. Bacterial DNA was extracted from 24-h-old cultures in BHI by using an InstaGene™ Matrix kit (Bio-Rad Ltd, USA), and it was quantified by assuming that an absorbance value at 260 nm of 0.100 corresponds to 5 µg/ml of DNA. Primers staur4 (5′-ACGGAGTTACAAAGGACGAC-3′) and staur6 (5′-AGCTCAGCCTTAACGAGTAC-3′) were used (Straub et al., 1999). Expected size of PCR products was 1250 bp. Each reaction mixture consisted of

Table 1. Processing control points examined in Factory A, indicating the number of samples (n) collected and the concentration of *Staphylococcus aureus* detected

Room	Point	Sample	Material	n	Concentration of *S. aureus*
Aquaculture farm	A1	Freshly caught whole tilapias[a]	Biotic	10	
Slaughter	A2	Cutting boards[b]	Polyethylene	4	2.41 ± 0.57
	A3	Helix[b]	Stainless steel	4	
	A4	Glove[b]	Cotton	5	
	A5	Disposable glove[b]	Nitrile	10	3.13 ± 0.69
	A6	Water[c]	Water	4	
Desquamation	A7	Desquamation machine IS069[b] (Baader ehf, Iceland)	Stainless steel	4	
	A8	Disposable glove[b]	Nitrile	10	4.14 ± 0.45
Fileting	A9	Cutting boards[b]	Polyethylene	4	
	A10	Carrier ramps[b]	Polyethylene	4	2.49 ± 0.49
	A11	Skinner machine 9000S[b] (Townsend M/C Inc., USA)	Stainless steel	4	
	A12	Disposable glove[b]	Nitrile	10	4.92 ± 0.57
	A13	Glove[b]	Stainless steel	5	2.91 ± 0.96
	A14	Water[c]	Water	4	
Inspection	A15	Cutting boards[b]	Polyethylene	4	
	A16	Carrier ramps[b]	Polyethylene	4	
	A17	Disposable glove[b]	Nitrile	10	
	A18	Water[c]	Water	4	
Packaging	A19	Balance 2096-H/1[b] (Toledo Ltd, Brazil)	Stainless steel	4	
	A20	Packaging machine R105[b] (MULTIVAC, Brazil)	Stainless steel	4	
	A21	Disposable glove[b]	Nitrile	10	
General	A22	Knifes[b]	Stainless steel	10	
	A23	Crates[b]	Polyethylene	6	
	A24	Ice[c]	Water	4	
Freeze storage warehouse	A25	Frozen tilapia fillets[a]	Biotic	10	

Results were expressed as follows:
[a]Log CFU/g of product.
[b]Log CFU/cm^2.
[c]Log CFU/l.

200 ng DNA, 0.4 µm of each primer (Thermo Fisher Scientific, Brazil), 1 × Green GoTaq Flexi Buffer added with 2 mm $MgCl_2$, 0.2 mm dNTPs, 1.25 U GoTaq Hot Start Polymerase (Promega Corporation, USA), and sterile Milli-Q water up to a final volume of 50 µl. PCR was performed in a thermocycler C1000^{TM} (Bio-Rad) using the conditions proposed by Vautor et al. (2008). Amplicons were subjected to electrophoresis on 1.5% agarose gel containing ethidium bromide for 2 h at 75 V and 100 mAmp. A DNA ladder of 100–1517 bp (100 bp DNA ladder, New England Biolabs, Brazil) was included as a molecular size marker. Gels were visualized in a Gel Doc XR + system (Bio-Rad) using the ImageLab^{TM} software (Bio-Rad).

S. aureus ATCC 6538 (CECT, Spain) was used as positive control in all confirmatory tests. Stock cultures of *S. aureus* isolates were maintained at −80 °C in tryptic soy broth (TSB) (Kasvi, Brazil) containing 50% glycerol (v/v).

Genotyping of S. aureus isolates

S. aureus isolates were genotypically characterized by RAPD-PCR. DNA was extracted and quantified

Table 2. Processing control points examined in Factory B, indicating the number of samples (n) collected and the concentration of *Staphylococcus aureus* detected

Room	Point	Sample	Material	n	Concentration of *S. aureus*
Aquaculture farm	B1	Freshly caught whole tilapias[a]	Biotic	10	1.73 ± 1.08
Slaughter	B2	Bleeding tank[b]	Ceramic	4	
	B3	Water	Water	4	
Desquamation	B4	Access ramp[b]	Stainless steel	4	
	B5	Desquamation machine EG100[b] (Engemac, Brazil)	Stainless steel	4	
	B6	Catch tray[b]	Stainless steel	4	
	B7	Water[c]	Water	4	
Fileting	B8	Cutting boards[b]	Polyethylene	4	
	B9	Ramp[b]	Stainless steel	4	
	B10	Table[b]	Stainless steel	4	
	B11	Skinner machine 460VL[b] (Cretel N.V., Belgium)	Stainless steel	4	
	B12	Gloves[b]	Cotton	5	
	B13	Disposable gloves[b]	Vinyl	10	
	B14	Water[c]	Water	4	
Inspection	B15	Cutting boards[b]	Polyethylene	4	3.65 ± 0.64
	B16	Table[b]	Stainless steel	4	
	B17	Disposable gloves[b]	Vinyl	10	
	B18	Water[c]	Water	4	
Packaging	B19	Trays[b]	Stainless steel	4	
	B20	Balance 2096-H/1[b] (Toledo Ltd, Brazil)	Stainless steel	4	
	B21	Disposable gloves[b]	Vinyl	10	
General	B22	Knifes[b]	Stainless steel	10	
	B23	Crates[b]	Polyethylene	6	
	B24	Ice[c]	Water	4	
Freeze storage warehouse	B25	Frozen tilapia fillets[a]	Biotic	10	

Results were expressed as follows:
[a]Log CFU/g of product.
[b]Log CFU/cm^2.
[c]Log CFU/l.

as aforementioned from two different cultures of each isolate to check reproducibility of banding profiles. Primers AP-7 (5′-GTGGATGCGA-3′), ERIC-2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) (Van-Belkum et al., 1995), and S (5′-TCACGA TGCA-3′) (Martín et al., 2004) were used in single reactions with each isolate. Each PCR mixture contained 200 ng DNA, 4 µm primer, 1 × Green GoTaq Flexi Buffer added with 2.5 mm $MgCl_2$, 0.2 mM dNTPs, 2 U GoTaq Hot Start Polymerase and sterile Milli-Q water up to 50 µl. DNA from *S. aureus* ATCC 6538, ATCC 12600, ATCC 13565, ATCC 19095, ATCC 14458 and ATCC 27664 (CECT) was also included to assess possible similarities with the isolates. RAPD was performed following conditions proposed by Van-Belkum et al. (1995) for primers AP-7 and ERIC-2, and Martín et al. (2004) for primer S. Amplicons were separated and visualized as previously described.

The exponential relationship between molecular size and mobility in each gel ($r > 0.99$) was used to determine the molecular size of DNA bands. Variations in at least one band resulted in distinct RAPD patterns. Differences in band intensity were not considered. Bands too weak to be reproduced were discarded. RAPD profiles were coded with a binary value (0 or 1) meaning absence or presence of each band. Similarity analysis calculating the Dice coefficients (Dice, 1945) was executed by IBM SPSS 19.0, whereas cluster

analysis by UPGMA (Sneath and Sokal, 1973) and dendrograms were performed by StatistiXL 1.11. The discriminatory power of the analysis was also calculated for patterns obtained with each primer and for the combined patterns, using the Hunter–Gaston index (Hunter and Gaston, 1988)

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j (n_j - 1)$$

where D is the index of discriminatory power; N is the number of unrelated strains tested; S is the number of different patterns and n_j is the number of strains belonging to the jth pattern, assuming that strains will be classified into mutually exclusive categories.

Detection of enterotoxin (se) genes

Two series of multiplex PCR detecting *sea*-*see* and *seg*-*sei* genes were performed for each isolate, following the method proposed by Omoe et al. (2002) with slight modifications. DNA previously extracted and quantified was used. Each reaction mixture contained 200 ng DNA, 1 × Green GoTaq Flexi Buffer added with 2 mm $MgCl_2$, 0.2 mm dNTPs, 2 U GoTaq Hot Start Polymerase, the set of primers at concentrations indicated in Table 3, and sterile Milli-Q water up to 50 µl. All PCRs included a negative control (*S. aureus* ATCC 12600) and positive controls for *sea* and *sed* (*S. aureus* ATCC 13565), *sec*, *seg*, *seh* and *sei* (*S. aureus* ATCC 19095), *seb* (*S. aureus* ATCC 14458) and *see* (*S. aureus* ATCC 27664). PCRs were performed following previously optimized conditions (Vázquez-Sánchez et al., 2012). Amplicons were subjected to electrophoresis and visualized as aforementioned.

Biofilm formation and quantification

The flat bottom of 1.93 cm^2 of each well of 24-well polystyrene microtiter plates (Kasvi) and stainless steel coupons (AISI 304, 2B finish) (Botam Oxicorte, Brazil) of 1 cm^2 (and 0.8 mm thickness) were used as experimental surfaces. Stainless steel coupons were previously soaked in 2 M NaOH to remove residues, rinsed several times with distilled water and dry-heat sterilized for 1 h at 170 °C. Stainless steel coupons were then placed into wells of sterile 24-well microplates, other than those used as experimental surface of polystyrene.

Overnight cultures were adjusted to an absorbance value of 0.100 ± 0.01 at 700 nm with phosphate buffer saline (PBS, composed by 7.6 g/l NaCl, 0.2 g/l KCl, 0.245 g/l Na_2HPO_4 and 0.71 g/l K_2HPO_4 (Labsynth)), corresponding to 10^8 CFU/ml approximately. Adjusted cultures were then serially diluted in TSB, and aliquots of 700 µl (containing approximately 7×10^5 CFU) were added into each well. Inoculum size was checked by plating on tryptic soy agar (TSA)

Table 3. Primers used to detect enterotoxin *se* genes

se genes	Primers[a]	Set	Concentration per PCR mix (µM)	Nucleotide sequences (5′→3′)	Amplicons size (bp)
sea	SEA-3	A	0.5	CCTTTGGAAACGGTTAAAACG	127
	SEA-4			TCTGAACCTTCCCATCAAAAAC	
seb	SEB-1		1.5	TCGCATCAAACTGACAAACG	477
	SEB-4			GCAGGTACTCTATAAGTGCCTGC	
sec	SEC-3		1	CTCAAGAACTAGACATAAAAGCTAGG	271
	SEC-4			TCAAAATCGGATTAACATTATCC	
sed	SED-3		0.5	CTAGTTTGGTAATATCTCCTTTAAACG	319
	SED-4			TTAATGCTATATCTTATAGGGTAAACATC	
see	SEE-3		0.5	CAGTACCTATAGATAAAGTTAAAACAAGC	178
	SEE-2			TAACTTACCGTGGACCCTTC	
seg	SEG-1	B	0.5	AAGTAGACATTTTTGGCGTTCC	287
	SEG-2			AGAACCATCAAACTCGTATAGC	
seh	SEH-1		0.5	GTCTATATGGAGGTACAACACT	213
	SEH-2			GACCTTTACTTATTTCGCTGTC	
sei	SEI-1		0.5	GGTGATATTGGTGTAGGTAAC	454
	SEI-2			ATCCATATTCTTTGCCTTTACCAG	

SE: staphylococcal enterotoxin.
[a]Primers SEA to SEE were designed by Becker et al. (1998), whereas primers SEG to SEI were designed by Omoe et al. (2002).

(Kasvi). A negative control with no inoculum was included in all assays. Microplates were incubated at 25 °C under static conditions until analysis. The number of cells adhered of each strain to the experimental surfaces was quantified in triplicate after 5, 24 and 48 h. After each incubation time, non-adhered cells were discarded and surfaces washed with 1 ml of PBS. Biofilm cells were collected by thoroughly rubbing the surfaces with two sterile swabs (Cremer Diagnostica), which were immersed in 10 ml of peptone water. Cells were then resuspended by vortexing for 1 min and aliquots of 0.1 ml of appropriate dilutions in peptone water were spread in triplicate on TSA plates, which were incubated for 24 h at 37 °C. Afterwards, the number of CFU/cm^2 of each strain adhered to the experimental surfaces was calculated. Assays were repeated twice using independent bacterial cultures.

Biocide resistance assays

Sodium hypochlorite and 39% (v/v) peracetic acid solution (Sigma-Aldrich, Brazil) were tested against *S. aureus* strains with the highest biofilm-forming ability on each surface. Disinfectants were diluted in ultrapure water to working concentrations prior to each assay.

Minimum bactericidal concentration (MBC). The resistance of planktonic cells was assessed in terms of MBC (i.e. the lowest concentration at which no viable cells were detected under experimental conditions). An optimized method based on Mann and Markham (1998) was used. Aliquots of 75 µl of bacterial cultures (containing 7.5×10^4 CFU approximately) were exposed to 75 µl of each disinfectant concentration in sterile 96-well U-bottom microplates (Kasvi). Planktonic cells were exposed to final concentrations of 10, 25, 50, 75, 100, 500, 1000, 2000, 3000 and 4000 mg/l of each disinfectant, being tested in triplicate in two independent experiments. Positive controls with no disinfectant, negative controls with no inoculum and blanks with medium only were also included. After 24 h at 37 °C, wells were stained with 10 µl of 0.01% (w/v) resazurin sodium salt solution (Sigma-Aldrich) and incubation continued for further 2 h. Colour change from blue to pink indicated the presence of viable cells in cultures. Visually undetectable growth was also checked by plating 0.1 ml of cultures on TSA.

Logarithmic reduction (LR) of viable biofilm cells. As both factories apply sanitizing treatments once a day, the resistance of 24-h-old biofilms formed on stainless steel and polystyrene by the strain with the highest biofilm-forming ability on each surface was determined in terms of LR. After biofilm formation, non-adhered cells were removed by washing surfaces with 1 ml of PBS. Biofilms were then exposed to 0.5 ml of disinfectant for 30 min at 25 °C, simulating conditions observed in both factories to sanitize a whole processing area. Concentrations of 100, 500, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750 and 3000 mg/l of each disinfectant were tested in triplicate in two independent assays. Biofilms exposed to sterile ultrapure water were also included as negative disinfection controls. Treatments were neutralized with 2.5 ml of D/E neutralizing broth for 10 min at 25 °C. Surviving biofilm cells on the experimental surfaces were collected and quantified as aforementioned. Also, counts were added up with the number of viable biofilm cells washed out during neutralization, which were quantified in TSA plates spread with 0.1 ml of appropriate dilutions of neutralizing broth after 24 h at 37 °C. LR caused by each disinfectant concentration was calculated as the difference between the logarithm of the total number of viable cells in non-disinfectant-exposed biofilms (negative disinfection controls) and the logarithm of the number of surviving viable cells in disinfectant-exposed biofilms.

Statistical analysis

Experimental results were statistically analysed with the software package IBM SPSS 19.0. Significance of the data was assessed using a one-way ANOVA. Homogeneity of variances was examined by a post hoc least significant difference test. Otherwise, a Dunnett's T3 test was performed. An independent samples Student's t-test was also done to compare variables in pairs. Bivariate correlations were analysed using the Pearson correlation coefficient. Statistical significance was accepted at a confidence level greater than 95% ($P < 0.05$).

RESULTS

Contamination level of tilapia-processing plants

Typical colonies on BP-EY were detected in 42 out of 50 processing control points. A total of 336 colonies were picked up, isolated and subjected to catalase and coagulase tests, being 44 positive for both assessments. All of them were definitely confirmed as *S. aureus* by species-specific *23S rDNA* PCR. Thirty-five isolates were obtained in six points of Factory A (A2, A5, A8, A10, A12 and A13), whereas nine isolates were obtained in two points of Factory B (B1 and B15). The same points were affected by *S. aureus* in both samplings, which indicated the long-term presence of this pathogen in these points. Higher contamination

level was thus detected in Factory A (34% of points) than in Factory B (8%).

None of final products collected in both factories were contaminated with *S. aureus* (Tables 1 and 2), being thus in concordance with legal limits in force (10^2–10^3 CFU/g of product) (Brazil, 2001; European Commission, 2005; FDA, 2011). However, tilapias of Factory A have been exposed to high concentrations of *S. aureus* during the processing. In particular, most gloves of handlers (A5, A8, A12 and A13) were highly affected by *S. aureus* (3–5 logs CFU/cm^2). Moreover, the cutting boards of the slaughter room (A2) and carrier ramps of the filleting room (A10) showed deficient hygienic conditions, reaching ~2.5 log CFU/cm^2. In contrast, control of aquaculture conditions and sanitizing procedures must be improved in Factory B, due to the relevant contamination of whole tilapia (B1) and the cutting boards of the inspection room (B15).

Similarity of S. aureus isolates

All *S. aureus* isolates (n = 44) were genotypically characterized by RAPD-PCR. Ten banding patterns were obtained with primers AP-7 and ERIC-2, whereas primer S yielded eight patterns (Figure 1). Primer AP-7 generated a total of 14 bands with sizes between 168 and 1880 bp. Primer ERIC-2 produced 17 bands ranging from 134 to 1438 bp, whereas primer S amplified 11 bands with sizes between 267 and 2547 bp. Amplification of DNA from different cultures of each isolate showed a good reproducibility of RAPD patterns.

The discriminatory power of the analysis was increased from 0.924 (AP-7), 0.943 (ERIC-2) and 0.905 (S) to 0.991 by the combination of patterns obtained for each primer. Fourteen combined patterns were thus created, being described by a three-digit code that represents the patterns obtained for each isolate with primers AP-7, ERIC-2 and S, respectively (Figure 2). All *S. aureus* isolated from the same point showed the same combined pattern, except isolates from A8 that had different patterns (6.8.1 and 5.7.1). No cluster reached a similarity of 0.75 or higher, so each combined pattern was considered to belong to a single bacterial clone or strain. Factory A was thus affected by seven strains (S2, S3, S4, S5, S6, S7 and S8), whereas two strains (S9 and S10) were isolated from Factory B. Interestingly, S8 strain isolated from stainless steel gloves (A13) was genotypically similar than *S. aureus* ATCC 14458. A relevant similarity was also observed between strains S4 (A8) and *S. aureus* ATCC 12600, S6 (A10) and *S. aureus* ATCC 13565, and S2 (A2) and *S. aureus* ATCC 27664. In contrast, high genotypical differences were detected between strains from Factory A and B, particularly with strain S9 that came from a natural environment (B1). These results were confirmed by the high value of hierarchical F-measure for combined banding patterns (0.679) obtained with the validity assessment of cluster analyses.

Presence of enterotoxin genes

All isolates belonging to the same combined RAPD pattern showed the same enterotoxin (*se*) gene profile, which validated the genotyping. Nevertheless, diverse profiles were detected (Figure 2). Five strains (S2, S3, S6, S7 and S10) were multi-*se*-carriers, whereas strains S4 and S5 only carried *seh*. Strain S8 carried *seb* gene as well as *S. aureus* ATCC 14458, which supports that they could be bacterial clones. However, no cluster grouped strains by their *se* gene profile, neither in clusters of single-*se*-carrying strains nor in clusters of multi-*se*-carrying strains. Moreover, no relationship was found between *se* genes carried and the type of origin surface.

All *S. aureus* isolated from Factory A (S2, S3, S4, S5, S6, S7 and S8) carried enterotoxin genes. Therefore, 34% of processing control points of Factory A was affected by high concentrations of *S. aureus* with ability to produce enterotoxins. In contrast, whole tilapias of Factory B (B1) were affected by a non-*se*-carrying *S. aureus* (S9), whereas the cutting boards of the inspection room (B15) were highly contaminated with a multi-*se*-carrying strain (S10).

Biofilm-forming ability of S. aureus

The biofilm-forming ability of *S. aureus* strains from tilapia-processing facilities was compared with that of *S. aureus* ATCC 6538 (S1), the reference Gram-positive strain in United States and European bactericidal standard tests. All strains showed a continuous (and significant, $P < 0.01$) biofilm formation on polystyrene and stainless steel for 48 h at 25 °C (Figure 3). Interestingly, biofilm formation on experimental surfaces was found to be correlated with the type of origin surface (metallic or plastic) (Table 4). Thus, strains isolated from plastic surfaces showed a higher biofilm-forming ability on polystyrene, whereas strains from metallic surfaces had a higher biofilm formation on stainless steel. In particular, strains S8 (a *seb*-carrier from A13, i.e. stainless steel gloves) and S10 (a multi-*se*-carrier from B15, i.e. polyethylene cutting boards) showed the highest biofilm-forming ability on stainless steel and polystyrene, respectively. In contrast, biofilm formation of *S. aureus* ATCC 6538 (S1) and the strain S9 isolated from whole tilapias was rather similar on both abiotic surfaces. Therefore, biofilms formed by

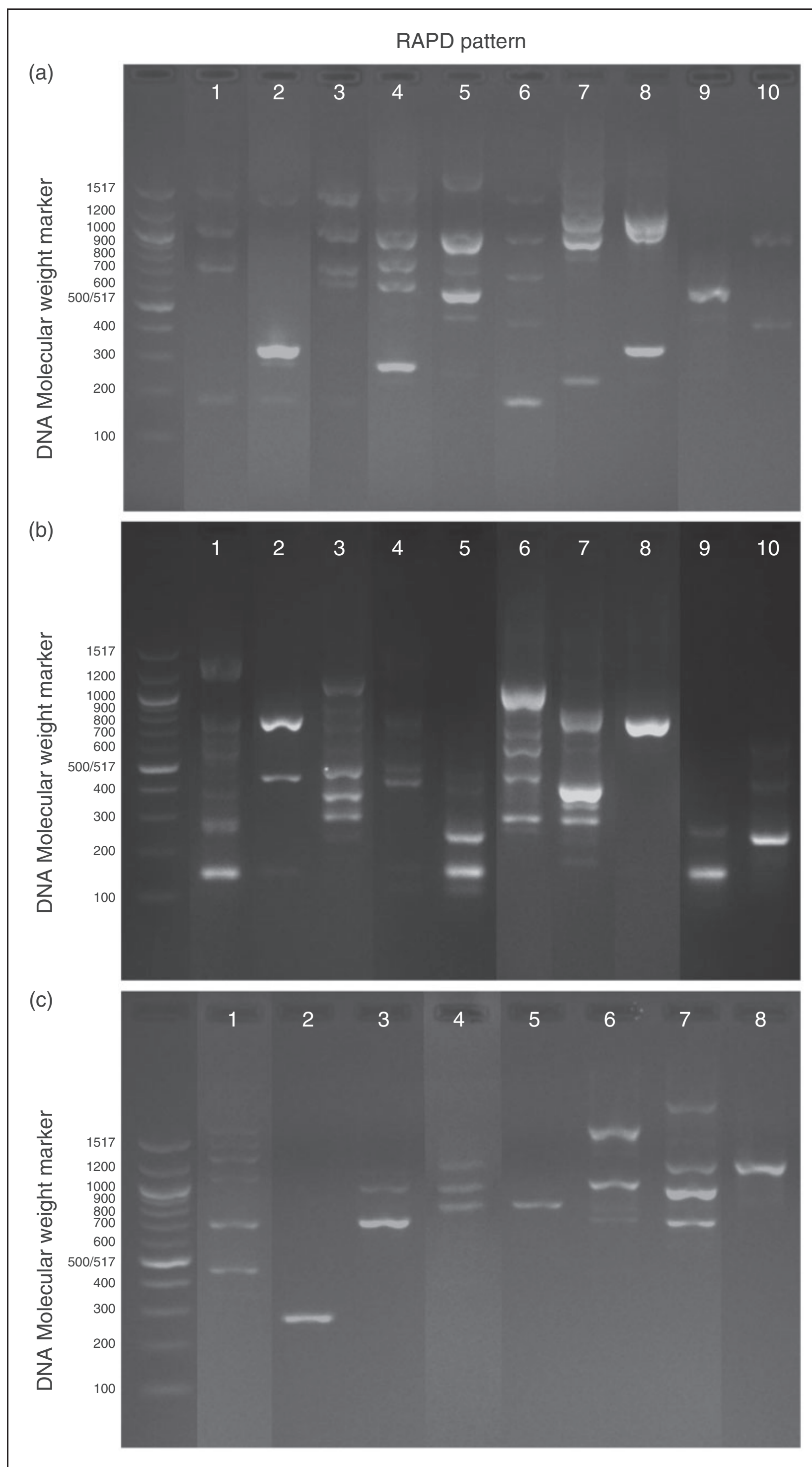

Figure 1. Agarose gels showing RAPD patterns of coagulase-positive *S. aureus* for primers AP-7 (a), ERIC-2 (b) and S (c).

strains S8 and S10 were considered the most appropriate to simulate the worst-case scenarios on stainless steel and polystyrene surfaces under conditions present in tilapia-processing facilities (i.e. 25 °C and frequency of sanitizing of 24 h).

Efficacy of sodium hypochlorite and peracetic acid against S. aureus

Peracetic acid was five-fold more effective against planktonic cells of *S. aureus* strains S8 and S10

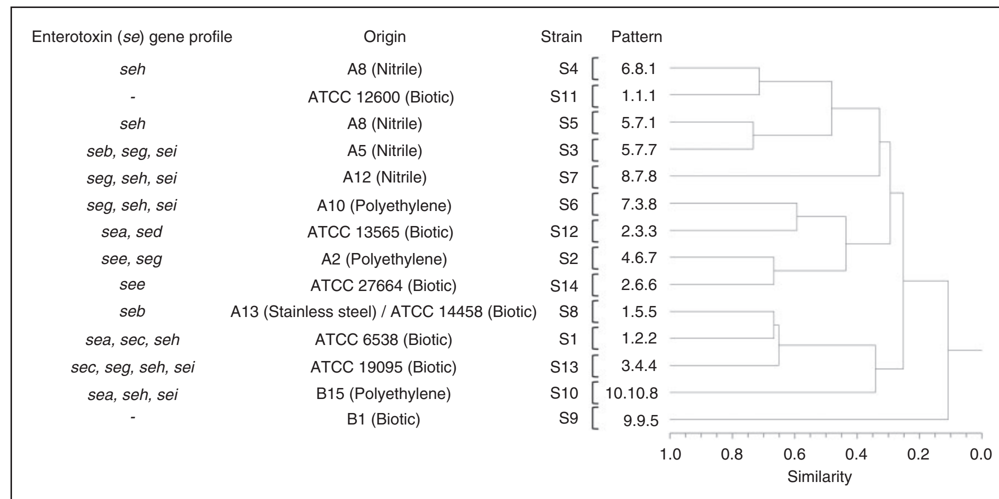

Figure 2. Dendrogram from cluster analysis of combined banding patterns of *S. aureus* from tilapia-processing facilities and reference strains, obtained with primers AP-7, ERIC-2 and S, indicating the enterotoxin (*se*) gene profile and the origin of each strain.

(MBC = 100 mg/l) than sodium hypochlorite (MBC = 500 mg/l). LR caused by peracetic acid in 24 h-old biofilms formed by S8 and S10 on stainless steel and polystyrene, respectively, was also significantly ($P < 0.01$) higher than that of sodium hypochlorite for all concentrations tested (Figure 4). However, none of the disinfectants were able to eradicate completely biofilms formed on experimental surfaces in this range of concentrations. The effect of both disinfectants against biofilms was positively correlated with the increase of concentrations, but peracetic acid showed a higher effect ($r = 0.990$ for S8, $r = 0.982$ for S10, with $P < 0.01$) than sodium hypochlorite ($r = 0.925$ for S8, $r = 0.911$ for S10, with $P < 0.01$). In general, the antimicrobial resistance of biofilms formed by S8 was significantly ($P < 0.05$) higher than that of S10 biofilms.

DISCUSSION

O. niloticus tilapias are highly cultured worldwide due to the high international demand, with Brazil being one of the major producers (FAO, 2016). The presence of coagulase-positive *S. aureus* in cultivation water and tilapia fillets produced in Brazil was previously evaluated (Bartolomeu et al., 2011; Boari et al., 2008; Carbonera et al., 2011; Junior et al., 2014). However, factors involved in the long-term presence of *S. aureus* in tilapia-processing facilities were first determined in the present study, which provide more relevant data to optimize accurately the manufacturing and sanitizing procedures and, consequently, increase the safety of tilapia products.

No final product from both factories was contaminated with *S. aureus*, which seems to validate the effectiveness of the efforts of the industry to produce safe products that comply to the current regulations (Brazil, 2001; European Commission, 2005; FDA, 2011) on the efforts of the industry to ensure food safety. However, high concentrations of *se*-carrying *S. aureus* were found in several processing control points of both factories, which generate a high risk of cross-contamination of the tilapia fillets with SEs. The heat stability and resistance to proteolytic degradation can allow enterotoxins retain their emetic activity after food processing and cooking (Landgraf and Destro, 2013; Omoe et al., 2005), increasing the possibility of a food-borne intoxication. It was estimated that concentrations of *S. aureus* of 5 log CFU/g of food are able to produce 1 μg of enterotoxin (Bhatia and Zahoor, 2007), the minimum dose necessary to produce symptoms of staphylococcal food poisoning in a healthy adult after 1–6 h of the ingestion (Pinchuk et al., 2010).

In Factory A, cleaning procedures applied in the initial areas of processing (from slaughter room to filleting room) seemed to be not in concordance with the daily workload (40 t of tilapia processed per day). As a result, the remaining organic matter present on surfaces could have considerably reduced the efficacy of disinfectants applied (Marriott and Gravani, 2006; Wirtanen and Salo, 2003), allowing the surveillance of *se*-carrying *S. aureus* in these initial areas. In contrast, hygienic procedures applied in the final areas of Factory A managed to control properly this bacterial pathogen, but tilapia fillets could be already affected by SEs. Mass processing of tilapias and a high degree of handling also generated the accumulation of high amounts of *se*-carrying *S. aureus* in the gloves of handlers, which can enhance the spread of this pathogen to food and

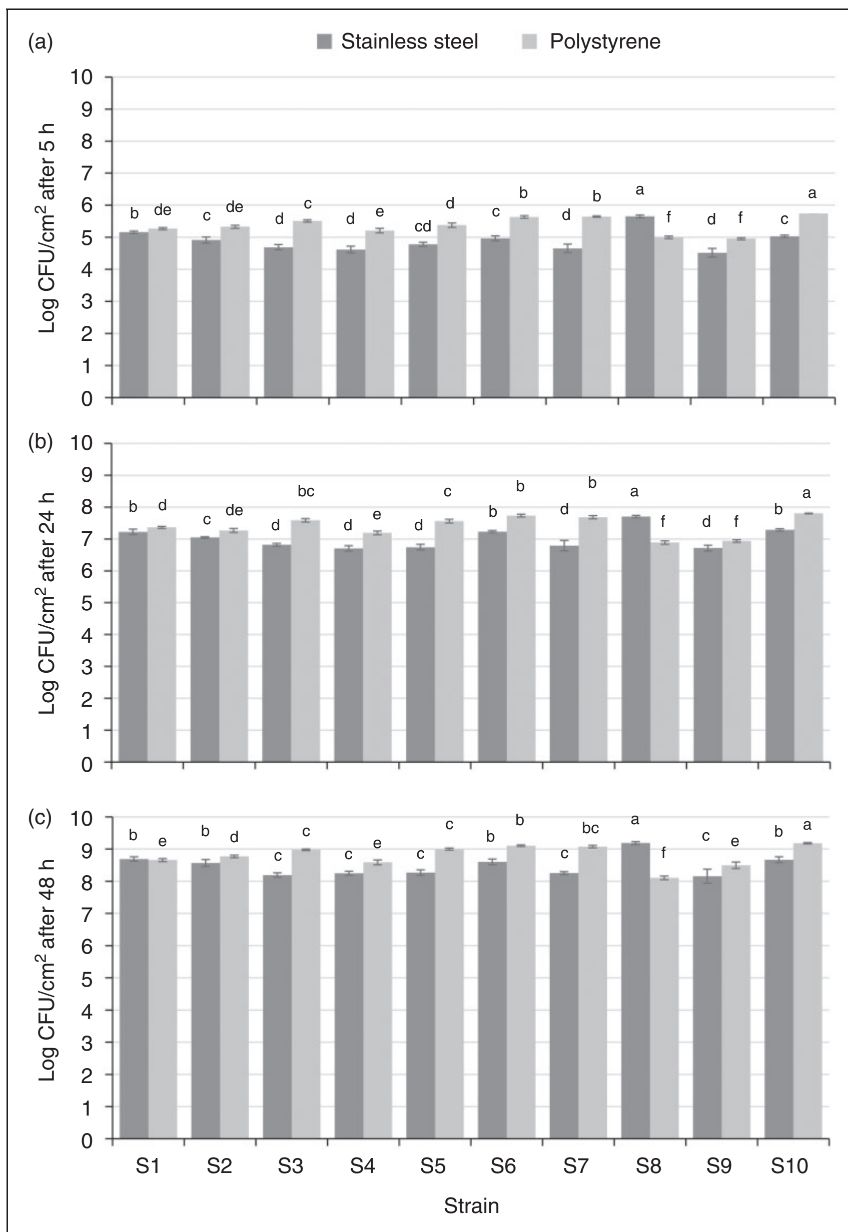

Figure 3. Biofilm-forming ability of *S. aureus* strains on stainless steel and polystyrene after 5 h (a), 24 h (b) and 48 h (c) at 25 °C. Letters indicate significant ($P < 0.05$) differences between strains in each surface.

Table 4. Correlations between biofilm formation of *S. aureus* on stainless steel and polystyrene at 25°C and the type of origin surface (metallic or plastic), expressed as *r* values

	Stainless steel			Polystyrene		
Parameter	5 h	24 h	48 h	5 h	24 h	48 h
Metallic origin surface	0.959[a]	0.914[a]	0.945[a]	−0.545[a]	−0.487[a]	−0.529[a]
Plastic origin surface	−0.381[b]	−0.424[b]	−0.488[a]	0.640[a]	0.641[a]	0.708[a]

An *r* value of zero indicates no correlation, whereas a value of 1 or −1 indicates a perfect positive or negative correlation.
[a]$P < 0.01$.
[b]$P < 0.05$.

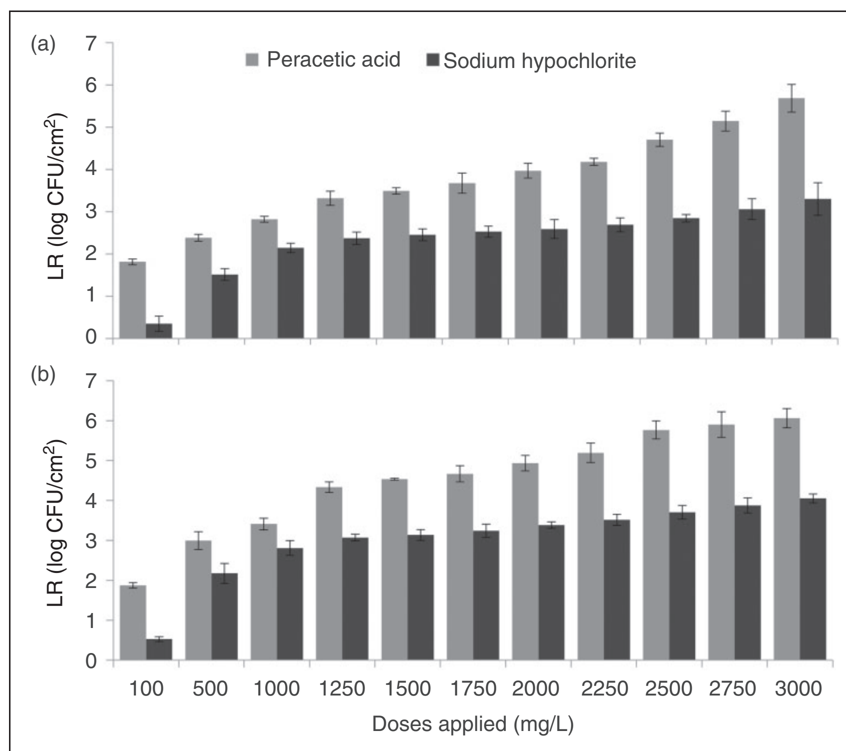

Figure 4. LR of viable cells caused by 30 min of exposure to disinfectants in 24-h-old biofilms formed at 25 °C by *S. aureus* strains S8 and S10 on stainless steel (a) and polystyrene (b), respectively.

food-contact surfaces (DeVita et al., 2007; Sattar et al., 2001; Simon and Sanjeev, 2007; Sospedra et al., 2012). Therefore, gloves should be changed more frequently during the work shifts in Factory A. A limited food safety knowledge of food handlers, inappropriate attitudes (e.g. incorrect use of masks) or a deficient personal hygiene could be also related with *S. aureus* contamination in Factory A (Lee et al., 2017). Meanwhile, the presence of *S. aureus* on cutting boards of the inspection room of Factory B could be due to the use of unsuitable sanitizing procedures (i.e. low efficacy of disinfectants, low frequency of application, short exposure times or, possibly, the application of sub-lethal doses). Moreover, the presence of a reception pool in Factory B allowed removing *S. aureus* present on tilapia tegument. Boari et al. (2008) also reported that viscera of tilapias can contain *S. aureus*. However, none of the factories examined in the present study eviscerate the tilapias to avoid the contamination of fillets with spoilage and pathogenic bacteria. Data obtained in the present study demonstrated the importance of use case-by-case approaches to assess the sources and degree of contamination present in food-processing facilities and, thus, apply accurate control strategies that avoid, or at least reduce, the emergence of antimicrobial resistance.

S. aureus strains isolated from tilapia-processing facilities showed a wide diversity of *se* gene profiles, most being multi-*se*-carriers. Genes *seg*, *seh* and *sei* were more frequently detected than genes coding the classical staphylococcal enterotoxins (SEA–SEE). A high presence of *seg*, *seh* and *sei* genes were also reported in strains isolated from different food-contact surfaces present in Spanish dairy, meat and seafood industries (Gutiérrez et al., 2012), as well as in *S. aureus* involved in food poisoning outbreaks occurred in South Korea (Cha et al., 2006). Nonetheless, these enterotoxins have been considered to play a minor role in staphylococcal food poisonings in comparison with classical enterotoxins (Chen et al., 2004). In fact, SEA-SEE caused 95% of staphylococcal food poisoning outbreaks worldwide, including those reported in Brazil (Carmo et al., 2002; Colombari et al., 2007).

The persistence in food-related environments mainly depends on the ability of *S. aureus* to form biofilms, which considerably increases the stress tolerance in comparison with free-living cells (Bridier et al., 2015; Gutiérrez et al., 2012; Van-Houdt and Michiels, 2010; Vázquez-Sánchez et al., 2014). All *S. aureus* strains isolated in tilapia-processing facilities showed a remarkable biofilm-forming ability on stainless steel and polystyrene under experimental conditions simulating situations normally found in the food industry. Therefore, these materials commonly found as food-contact surfaces (e.g. food-processing equipment made of stainless steel, expanded polystyrene boxes

used for storage of food products) can serve as reservoirs of *S. aureus*. Several studies also reported the presence of *S. aureus* biofilms on different food-processing surfaces (Bagge-Ravn et al., 2003; Sattar et al., 2001; Sospedra et al., 2012). Nevertheless, data obtained in this study showed that biofilm cells seemed to be highly adapted to the type of origin surface. Thus, strains isolated from plastic surfaces showed high biofilm-forming ability on polystyrene, whereas strains from metallic surfaces had high biofilm formation on stainless steel. In addition, the presence of scratches in some cutting boards and carrier ramps investigated in the tilapia-processing facilities probably allowed the long-term presence of *S. aureus*, as they enhance the adherence of biofilms and impede to achieve a proper sanitization of surfaces (Kim et al., 2017).

S. aureus strains with the highest biofilm-forming ability were also highly resistant to peracetic acid and sodium hypochlorite, two disinfectants widely used in the food industry. In fact, biofilms resisted doses considerably higher than that recommended by manufacturers for peracetic acid (50–350 mg/l) and sodium hypochlorite (50–800 mg/l) (Gaulin et al., 2011). The emergence of this antimicrobial resistance in *S. aureus* strains was probably due to the application of short exposure times and sub-lethal doses of these disinfectants in both tilapia-processing facilities (Langsrud et al., 2003; Sheridan et al., 2012; Vázquez-Sánchez et al., 2014). Peracetic acid showed a higher effectiveness than sodium hypochlorite against *S. aureus* planktonic cells and biofilms formed on stainless steel and polystyrene. Several studies also reported a higher effectiveness of peracetic acid than sodium hypochlorite against *S. aureus* biofilms formed on different food-contact surfaces (Meira et al., 2012; Vázquez-Sánchez et al., 2014). Although both disinfectants are non-specific powerful oxidizing agents (Kitis, 2004; Russell, 2003), sodium hypochlorite may have a limited diffusion in the biofilm, which reduces its effectiveness drastically (De-Beer et al., 1994). The inadequate use of disinfectants with similar targets or mechanisms of action increases the risk of cross-resistance, particularly in biofilms (Braoudaki and Hilton, 2004; Chapman, 2003; Langsrud et al., 2004). Therefore, the use of novel working-safe, environmentally-friendly and cost-effective biocides and the development of innovative sanitizing procedures are recommended to avoid, or at least reduce, the risk of biofilm formation and antimicrobial resistance.

CONCLUSIONS

Case-by-case approaches are recommended to determine the sources and degree of contamination present in each factory, which would allow applying precise responses that avoid, or at least reduce, the presence of bacterial pathogens and the emergence of antimicrobial resistance. In this study, although none of final products were affected by *S. aureus*, high concentrations of *se*-carriers were found in several processing control points of both factories, which generate a high risk of cross-contamination of the tilapia fillets with SEs. Hygienic and handling procedures should be particularly revised to be in concordance with the daily workload of each factory, to preserve the effectiveness of sanitizers and to avoid the accumulation of high amounts of bacteria. Stainless steel and polystyrene surfaces can act as reservoirs of *S. aureus* in the food industry, involving a serious food safety risk unless proper control procedures are applied. Moreover, the high biofilm-forming ability and resistance to peracetic acid and sodium hypochlorite, two disinfectants widely used in the food industry, could explain the surveillance of *S. aureus* in some points of the tilapia-processing facilities.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the São Paulo Research Foundation (FAPESP 2014/20590-0).

REFERENCES

Abee T and Wouters JA. (1999). Microbial stress response in minimal processing. *International Journal of Food Microbiology* 50(1–2): 65–91.

APHA (2013). Chapter 2. Sampling plans, sample collection, shipment, and preparation for analysis *In:* APHA (ed.) *Compendium of Methods for the Microbiological Examination of Foods*. Washington, DC: American Public Health Association.

Bagge-Ravn D, Ng Y, Hjelm M, Christiansen J, Johansen C and Gram L. (2003). The microbial ecology of processing equipment in different fish industries – Analysis of the microflora during processing and following cleaning and disinfection. *International Journal of Food Microbiology* 87(3): 239–250.

Bartolomeu DAFS, Dallabona BR, Macedo REF and Kirschnik PG. (2011). Contaminação microbiológica durante as etapas de processamento de filé de tilápia (*Oreochromis niloticus*). *Archives of Veterinary Science* 16(1): 21–30.

Becker K, Roth R and Peters G. (1998). Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes,

exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *Journal of Clinical Microbiology* 36(9): 2548–2553.

Bhatia A and Zahoor S. (2007). *Staphylococcus aureus* enterotoxins: A review. *Journal of Clinical Diagnoses Research* 1(3): 188–197.

Boari CA, Pereira GI, Valeriano C, Silva BC, Morais VM and Figueiredo HCP. (2008). Bacterial ecology of tilapia fresh fillets and some factors that can influence their microbial quality. *Ciência e Tecnologia de Alimentos* 28(4): 863–867.

Braoudaki M and Hilton AC. (2004). Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. *Journal of Clinical Microbiology* 42(1): 73–78.

Brazil. (2001). *Regulamento Técnico Sobre os Padrões Microbiológicos Para Alimentos*. Resolução No. 12, de 2 de janeiro de 2001. Brasília: Diário Oficial da República Federativa do Brasil.

Bridier A, Sanchez-Vizuete P, Guilbaud M, Piard J-C, Naïtali M and Briandet R. (2015). Biofilm-associated persistence of food-borne pathogens. *Food Microbiology* 45: 167–178.

Carbonera N, Cappelletti BM and Espírito Santo MLP. (2011). ISO 22000/HACCP associated with the presence of microorganisms in the processing of tilapia (*Oreochromis niloticus*) fillets. *The Quality Assurance Journal* 14(3–4): 50–60.

Carmo SL, Dias RS, Linardi VR, Sena JM, Santos AD, Faria EM, et al. (2002). Food poisoning due to enterotoxigenic strains of *Staphylococcus* present in minas cheese and raw milk in Brazil. *Food Microbiology* 19(1): 9–14.

Cha JO, Lee JK, Jung YH, Yoo JI, Park YK, Kim BS, et al. (2006). Molecular analysis of *Staphylococcus aureus* isolates associated with staphylococcal food poisoning in South Korea. *Journal of Applied Microbiology* 101(4): 864–871.

Chapman JS. (2003). Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *International Biodeterioration and Biodegradation* 51(4): 271–276.

Chen T-R, Chiou C-S and Tsen H-Y. (2004). Use of novel PCR primers specific to the genes of staphylococcal enterotoxin G, H, I for the survey of *Staphylococcus aureus* strains isolated from food-poisoning cases and food samples in Taiwan. *International Journal of Food Microbiology* 92(2): 189–197.

Colombari V, Mayer MDB, Laicini ZM, Mamizuka E, Franco BDGM, Destro MT, et al. (2007). Foodborne outbreak caused by *Staphylococcus aureus*: Phenotypic and genotypic characterization of strains of food and human sources. *Journal of Food Protection* 70(2): 489–493.

De-Beer D, Srinivasan R and Stewart PS. (1994). Direct measurement of chlorine penetration into biofilms during disinfection. *Applied and Environmental Microbiology* 60(12): 4339–4344.

DeVita MD, Wadhera RK, Theis ML and Ingham SC. (2007). Assessing the potential of *Streptococcus pyogenes* and *Staphylococcus aureus* transfer to foods and customers via a survey of hands, hand-contact surfaces and food-contact surfaces at foodservice facilities. *Journal of Foodservice* 18: 76–79.

Dice LR. (1945). Measures of the amount of ecologic association between species. *Ecology* 26(3): 297–302.

EFSA. (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal* 14(12): 4634.

European Commission. (2005). Commission regulation (EC) no. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union* L338: 1–26.

FAO. (2016). *FAO Yearbook*. *Fishery and Aquaculture Statistics 2014*. Rome: Food and Agriculture Organization of the United Nations.

FDA. (2011). *Guidance for the Industry: Fish and Fishery Products Hazards and Controls Guidance*. 4th ed. Silver Spring, MD: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition.

Gaulin C, Lê M, Shum M and Fong D. (2011). Disinfectants and sanitizers for use on food contact surfaces. *National Collaborative Centre for Environment Health* August: 1–15.

Gutiérrez D, Delgado S, Vázquez-Sánchez D, Martínez B, Cabo ML, Rodríguez A, et al. (2012). Incidence of *Staphylococcus aureus* and analysis of associated bacterial communities on food industry surfaces. *Applied and Environmental Microbiology* 78(24): 8547–8554.

Havelaar AH, Brul S, Jong A, Jonge R, Zwietering MH and Kuile BHT. (2010). Future challenges to microbial food safety. *International Journal of Food Microbiology* 139: 79–94.

Hennekinne J-A, De-Buyser M-L and Dragacci S. (2012). *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiology Reviews* 36(4): 815–836.

Hunter PR and Gaston MA. (1988). Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. *Journal of Clinical Microbiology* 26(11): 2465–2466.

IBGE. (2015). *Produção da Pecuária Municipal*. Rio de Janeiro: Ministério do Planejamento, Desenvolvimento e Gestão.

Junior PG, Assunção AWA, Baldin JC and Amaral LA. (2014). Microbiological quality of whole and filleted shelf-tilapia. *Aquaculture* 433: 196–200.

Kadariya J, Smith TC and Thapaliya D. (2014). *Staphylococcus aureus* and staphylococcal food-borne disease: An ongoing challenge in public health. *BioMed Research International* 2014: 827965.

Kim CY, Ryu GJ, Park HY and Ryu K. (2017). Resistance of *Staphylococcus aureus* on food contact surfaces with different surface characteristics to chemical sanitizers. *Journal of Food Safety* 00: e12354.

Kitis M. (2004). Disinfection of wastewater with peracetic acid: A review. *Environment International* 30(1): 47–55.

Landgraf M and Destro MT. (2013). Staphylococcal food poisoning. In: Morris GJ and Potter M (eds) *Foodborne Infections and Intoxications*. 4th ed. London: Food Science and Technology, pp. 389–400.

Langsrud S, Sidhu MS, Holck AL and Heir E. (2003). Bacterial disinfectant resistance – A challenge for the

food industry. *International Biodeterioration and Biodegradation* 51(4): 283–290.
Langsrud S, Sundheim G and Holck AL. (2004). Cross-resistance to antibiotics of *Escherichia coli* adapted to benzalkonium chloride or exposed to stress-inducers. *Journal of Applied Microbiology* 96(1): 201–208.
Lee HK, Abdul Halim H, Thong KL and Chai LC. (2017). Assessment of food safety knowledge, attitude, self-reported practices, and microbiological hand hygiene of food handlers. *International Journal of Environmental Research and Public Health* 14(1): 55.
Mann CM and Markham JL. (1998). A new method for determining the minimum inhibitory concentration of essential oils. *Journal of Applied Microbiology* 84(4): 538–544.
Marriott N and Gravani RB. (2006). *Principles of Food Sanitation*. 5th ed. New York: Springer.
Martín MC, Fueyo JM, González-Hevia MA and Mendoza MC. (2004). Genetic procedures for identification of enterotoxigenic strains of *Staphylococcus aureus* from three food poisoning outbreaks. *International Journal of Food Microbiology* 94(3): 279–286.
Meira QGS, Barbosa IM, Athayde AJAA, Siqueira-Júnior JP and Souza EL. (2012). Influence of temperature and surface kind on biofilm formation by *Staphylococcus aureus* from food-contact surfaces and sensitivity to sanitizers. *Food Control* 25(2): 469–475.
Novotny L, Dvorska L, Lorencova A, Beran V and Pavlik I. (2004). Fish: A potential source of bacterial pathogens for human beings. *Veterinarni Medicina* 2004(9): 343–358.
Omoe K, Hu D-L, Takahashi-Omoe H, Nakane A and Shinagawa K. (2005). Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. *FEMS Microbiology Letters* 246(2): 191–198.
Omoe K, Ishikawa M, Shimoda Y, Hu D, Ueda S and Shinagawa K. (2002). Detection of seg, seh and sei in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S. aureus* isolates harboring *seg*, *seh*, or *sei* genes. *Journal of Clinical Microbiology* 40(3): 857–862.
Papadopoulou C, Economou E, Zakas G, Salamoura C, Dontorou C and Apostolou J. (2007). Microbiological and pathogenic contaminants of seafood in Greece. *Journal of Food Quality* 30: 28–42.
Pinchuk IV, Beswick EJ and Reyes VE. (2010). Staphylococcal enterotoxins. *Toxins* 2(8): 2177–2197.
Rendueles E, Omer MK, Alvseike O, Alonso-Calleja C, Capita R and Prieto M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT – Food Science and Technology* 44(5): 1251–1260.
Russell AD. (2003). Similarities and differences in the responses of microorganisms to biocides. *The Journal of Antimicrobial Chemotherapy* 52(5): 750–763.
Sattar SA, Springthorpe S, Mani S, Gallant M, Nair RC, Scott E, et al. (2001). Transfer of bacteria from fabrics to hands and other fabrics: Development and application of a quantitative method using *Staphylococcus aureus* as a model. *Journal of Applied Microbiology* 90(6): 962–970.
Schelin J, Wallin-Carlquist N, Cohn MT, Lindqvist R, Barker GC and Radstrom P. (2011). The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence* 2(6): 580–592.
Sheridan À, Lenahan M, Duffy G, Fanning S and Burgess C. (2012). The potential for biocide tolerance in *Escherichia coli* and its impact on the response to food processing stresses. *Food Control* 26(1): 98–106.
Simon SS and Sanjeev S. (2007). Prevalence of enterotoxigenic *Staphylococcus aureus* in fishery products and fish processing factory workers. *Food Control* 18(12): 1565–1568.
Sneath PHA and Sokal RR. (1973). *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. San Francisco, CA: Freeman.
Sospedra I, Mañes J and Soriano JM. (2012). Report of toxic shock syndrome toxin 1 (TSST-1) from *Staphylococcus aureus* isolated in food handlers and surfaces from foodservice establishments. *Ecotoxicology and Environmental Safety* 80: 288–290.
Straub JA, Hertel C and Hammes WP. (1999). A 23S rDNA-targeted polymerase chain reaction-based system for detection of *Staphylococcus aureus* in meat starter cultures and dairy products. *Journal of Food Protection* 62: 1150–1156.
Van-Belkum A, Kluytmans J, Van-Leeuwen W, Bax R, Quint W and Peters E. (1995). Multicenter evaluation of arbitrarily primed PCR for typing of *Staphylococcus aureus* strains. *Journal of Clinical Microbiology* 33(6): 1537–1547.
Van-Houdt R and Michiels CW. (2010). Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of Applied Microbiology* 109(4): 1117–1131.
Vautor E, Abadie G, Pont A and Thiery R. (2008). Evaluation of the presence of the *bap* gene in *Staphylococcus aureus* isolates recovered from human and animals species. *Veterinary Microbiology* 127(3–4): 407–411.
Vázquez-Sánchez D, Cabo ML, Ibusquiza PS and Rodríguez-Herrera JJ. (2014). Biofilm-forming ability and resistance to industrial disinfectants of *Staphylococcus aureus* isolated from fishery products. *Food Control* 39: 8–16.
Vázquez-Sánchez D, López-Cabo M, Saá-Ibusquiza P and Rodríguez-Herrera JJ. (2012). Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain). *International Journal of Food Microbiology* 157(2): 286–296.
Wertheim HFL, Melles DC, Vos MC, Van-Leeuwen W, Van-Belkum A, Verbrugh HA, et al. (2005). The role of nasal carriage in *Staphylococcus aureus* infections. *The Lancet Infectious Diseases* 5(12): 751–762.
Wirtanen G and Salo S. (2003). Disinfection in food processing – Efficacy testing of disinfectants. *Reviews in Environmental Science and Bio/Technology* 2: 293–306.