Applied and Environmental Microbiology, Nov. 2006, p. 7057–7062 Vol. 72, No. 11
0099-2240/06/$08.00+0 doi:10.1128/AEM.00198-06

Staphylococcus aureus and Staphylococcal Enterotoxin A in Breaded Chicken Products: Detection and Behavior during the Cooking Process

Olimpia Pepe,* Giuseppe Blaiotta, Francesca Bucci, Marilena Anastasio, Maria Aponte, and Francesco Villani

Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli Federico II, 80055 Portici, Italy

Received 25 January 2006/Accepted 10 July 2006

In this study we examined the presence of *Staphylococcus aureus* and staphylococcal enterotoxin A (SEA) in 20 industrial breaded chicken products obtained from different retail butchers and supermarket stores in Italy. The levels of contamination in the products analyzed were quite low, although the pH values and water activities (a_w) in the samples considered were in ranges favorable for *S. aureus* growth. As demonstrated by phenotypic and molecular characterization, in spite of the high percentage of coagulase-positive *Staphylococcus* strains, only three strains could be referred to the species *S. aureus*. Moreover, all the strains were negative in PCR assays targeting staphylococcal enterotoxin genes (*se*A to *se*E, *se*G to *se*J, and *se*M to *se*O), as well as the toxic shock syndrome toxin 1 gene, and no SEA was detected in the retail breaded chicken samples analyzed by a reversed passive latex agglutination assay or by Western blotting. Hence, we evaluated the thermal resistance of two strains of SEA-producing *S. aureus* in a laboratory-scale preparation of precooked breaded chicken cutlets. The heat treatment employed in the manufacture determined the inactivation of *S. aureus* cells, but the preformed SEA remained active during product storage at 4°C. The presence of the staphylococci and, in particular, of *S. aureus* in the retail breaded chicken products analyzed is a potential health risk for consumers since the pH and a_w values of these kinds of products are favorable for *S. aureus* growth. The thermal process used during their manufacture can limit staphylococcal contamination but cannot eliminate preformed toxins.

Staphylococcus aureus is a significant cause of avian disease and may thus contaminate foods as a result of processed carcasses (30). Enterotoxin-producing *S. aureus* is the most common cause of food-borne human illness throughout the world (9, 27). The foods that most frequently cause this type of poisoning are red meat and poultry and their products (1, 14, 25, 39). In the United Kingdom, for example, 53% of the staphylococcal food poisoning cases reported between 1969 and 1990 were due to meat products and meat-based dishes, and 22% of the cases were due to poultry and poultry-based meals (27, 39). While staphylococci commonly occur on the skin and nasopharynx of healthy poultry (30), it is primarily *S. aureus* which can survive, colonize, and persist at various processing stages in commercial poultry processing plants due to the expression of various key properties, including adhesion (8, 29) and chlorine resistance (10, 21). In a typical processing operation, after slaughtering and defeathering, fresh chicken carcasses are eviscerated and washed. Then the meat is minced, shaped, breaded, and precooked (only for industrial products). After chilling, the products are packaged and stored at refrigeration temperature (16). These procedures, especially defeathering, increase the contamination by *S. aureus*. Therefore, poultry processing plants are favorable environments for the survival and transmission of various commensal, spoilage, and potentially pathogenic bacteria in the human food chain (21). Staphylococci are one of the most predominant groups during the slaughtering and processing of poultry, and they have been recovered from air samples (11), neck skin of chicken carcasses (15, 32), and equipment and machinery surfaces (15, 21). Moreover, *S. aureus* strains carried by poultry (diseased or healthy) are toxin C and D producers, although recent studies reported that retail raw chicken meat in Japan was contaminated primarily by *S. aureus* belonging to the poultry biotype in which staphylococcal enterotoxin A (SEA) and SEB were most frequently found in isolates from raw chicken meat (25).

Precooked breaded chicken products are being consumed increasingly since they offer added value in terms of both convenience (simple and ready for use) and storage. More innovation in the development of poultry convenience food is likely to bring about further increases in the per capita consumption of chicken meat in Italy. Surprisingly, despite the risk of microbial contamination associated with the transformation of freshly slaughtered chickens into raw or precooked meat fillets, few studies have been performed with this kind of product, and there have been no studies of the incidence of *S. aureus*. Thus, the purposes of this study were to investigate the occurrence of *S. aureus* in Italian industrial breaded chicken products, to characterize staphylococcal isolates at the phenotypic and genotypic levels, and to ascertain the behavior of *S. aureus* and enterotoxin A during the manufacture of breaded chicken cutlets.

* Corresponding author. Mailing address: Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli Federico II, I-80055 Portici, Italy. Phone: 39 081 2539410. Fax: 39 081 2539407. E-mail: olipepe@unina.it.

MATERIALS AND METHODS

Breaded chicken samples. Twenty samples of industrial breaded chicken products, including samples of the most popular Italian brands, were obtained from retail batches and supermarket stores in southern Italy over a 1-year period. The

products were cutlets or fillets of breaded minced meat prepared by a totally mechanized industrial process using standardized technology that included one or more cooking steps. The samples were refrigerated at 4°C, transported to the laboratory, and immediately analyzed. Each kind of breaded chicken product was sampled twice and analyzed in duplicate.

Detection of *S. aureus*. Samples (10 g) were taken aseptically and diluted in 0.25× Ringer's solution (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), and suitable dilutions were spread onto the surfaces of plates of a 3M Petrifilm Rapid Staph Express Count (PSE) system (Laboratoires 3M Santé, Cergy Pontoise Cedex, France) in duplicate. This system consisted of a Petrifilm Staph Express Count plate and a Petrifilm Staph Express disk. After 24 h of incubation at 37°C, the PSE plates were analyzed for presence of *S. aureus*, which appeared as red-violet colonies. The Petrifilm Staph Express disk could be used for visualization of the DNase reaction, as described below.

Detection of SEA. (i) Enterotoxins and antibodies. SEA, rabbit anti-SEA (not affinity purified) polyclonal antibodies, and chemicals were purchased from Sigma-Aldrich S.r.l. (Milan, Italy).

(ii) Preparation and partial purification of the samples. Ten grams of meat along with 10 ml of a 0.85% NaCl solution was subjected to high-speed stomaching. The homogenate was centrifuged at 12,000 × *g* for 20 min, and the supernatant was filtered with a low-protein-binding disk (0.45 μm; Sterile Acrodisc; Gelman Science). Two hundred microliters of an equilibrated cation-exchange carboxymethyl cellulose matrix, prepared as described by Balaban and Rasooly (2), was transferred into a 2-ml minicentrifuge tube. Four hundred microliters of filtered sample was added to the tube, which was rotated for 30 min and centrifuged at 12,000 × *g* for 1 min. The supernatant was removed, and the pellet was washed three times with loading buffer. The material bound to the matrix was eluted by adding sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) loading buffer to the tube. After centrifugation at 14,000 rpm for 5 min, 10 μl of the extract was diluted with 20 μl of sample buffer consisting of 62.5 mM Tris-HCl (pH 6.8), 19% glycerol, 5% β-mercaptoethanol, 2% SDS, and 0.05% bromophenol blue.

(iii) Detection of SEA by RPLA. The presence of SEA in breaded chicken products was detected by the reversed passive latex agglutination (RPLA) method with SET-RPLA (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) by following the manufacturer's instructions.

(iv) Detection of SEA by Western immunoblotting. A Western immunoblotting assay was performed as reported by Rasooly and Rasooly (34), with the following modifications. The SEA band was detected by discontinuous SDS-PAGE (26) performed at a constant voltage (150 V) for 2 h. Samples (15 μl) were heated at 90°C for 4 min and immediately electrophoresed in a Mini-Protean II vertical dual-cell apparatus (Bio-Rad Laboratories, Richmond, CA). The apparent molecular weight was determined using Precision Plus protein standards (10 to 225 kDa; Bio-Rad Laboratories). A known amount of SEA (0.4 μg) was used in the gel as an internal control. The protein profile was visualized by staining with Coomassie brilliant blue R (Sigma). After 2 h of discoloration in an aqueous solution containing methanol (40%) and acetic acid (10%), the gel was washed with running buffer for 2 h at 4°C and with distilled water and phosphate buffer (0.1 M, pH 7) for 15 min. The gel was then electroblotted onto a Sequi-Blot polyvinylidene difluoride membrane (Bio-Rad Laboratories) as described by Rasooly and Rasooly (34). The antigen was detected with an amplified alkaline phosphatase immunblot assay kit (Bio-Rad Laboratories, Hercules, CA) for sensitive qualitative detection.

pH and water activity. pH values were determined at room temperature with diluted samples (5 g in 25 ml of distilled water) using an MP 220 pH meter (Mettler-Toledo, Italy). Water activity (a_w) was determined with 2.5-g samples at 25°C using an Aw-Win system (PBI International). In both cases, values were expressed as means ± standard deviations for triplicate assays.

Isolation and phenotypic characterization of *S. aureus* strains. Isolated red-violet colonies from PSE countable plates were randomly selected and purified by repetitive streaking on tryptic soy agar plates (Oxoid). The cell morphology of isolates that were presumptively *S. aureus* was examined by phase-contrast microscopy, the Gram reaction and catalase activity were determined as described by Gregersen (17), and the coagulase activity was determined by using a latex slide agglutination test (Staphytect Plus; Oxoid). Visualization of the DNase reaction was evaluated directly by placing PSE disks on the red-violet colonies on the PSE plates. After 3 h of incubation at 37°C, DNase activity was shown by the development of a pink area surrounding colonies.

Molecular characterization of staphylococcal isolates. (i) DNA isolation. DNA was extracted by using InstaGene matrix (Bio-Rad Laboratories, Hercules, CA) according to supplier's recommendations. Supernatant containing DNA was stored at −20°C.

(ii) Amplification of the 16S-23S rRNA gene intergenic spacer region. Synthetic oligonucleotide primers G1 (5′-GAAGTCGTAACAAGG-3′) and L1 (5′-CAAGGCATCCACCGT-3′) described by Jensen et al. (23) were used to amplify the 16S-23S rRNA gene intergenic spacer region. The PCR was performed as previously reported (5). About 25 ng of DNA was used for PCR amplification. PCR products were resolved by agarose (2%, wt/vol) gel electrophoresis with Tris-borate-EDTA at 100 V for 2 h.

(iii) Identification of *S. aureus* strains by PCR. The thermostable nuclease (*nuc*) and 60-kDa heat shock protein (*hsp60*) genes were amplified using sets of primers described by Brakstad et al. (7) and Blaiotta et al. (6), respectively. *S. aureus* DSM 20231^T was used as a positive control. PCR analyses were performed as previously reported (6). The PCR products were resolved by agarose (2%, wt/vol) gel electrophoresis with Tris-borate-EDTA at 100 V for 1 h.

(iv) Detection of staphylococcal enterotoxin and TSST-1 genes by PCR. For detection of staphylococcal enterotoxin genes (*se*A to *se*E, *se*G to *se*J, and *se*M to *se*O) and the toxic shock syndrome toxin 1 (TSST-1) gene, 13 different primer sets were used for PCR, as previously described by Blaiotta et al. (6). The following *S. aureus* strains were used as controls: FRI326 (*se*E$^+$), D4508 (*se*A$^+$ *se*H$^+$), ATCC 14458 (*se*B$^+$), ATCC 25923 (*egc*$^+$ [= *se*G *se*I *se*M *se*N *se*O]), FRI137 (*se*C$^+$*se*H$^+$ *egc*$^+$), A900322 (*egc*$^+$), NCTC9393 (*se*D$^+$ *se*J$^+$ *egc*$^+$), RIMD31092 (*se*B$^+$ *se*C$^+$ *egc*$^+$ *tsst*1), and DSM 20231^T (nontoxigenic).

Impact of cooking on *S. aureus* and SEA activity during laboratory-scale manufacture of breaded chicken cutlets. Breaded chicken cutlets were prepared by kneading at medium speed 1,000 g of minced chicken breast in a mixer (model KPM50 Professional; KitchenAid, St. Joseph, MI) for 5 min at room temperature. Overnight cultures in brain heart infusion broth of two enterotoxigenic *S. aureus* clinical isolates producing SEA, strains K6278/94 and A4178/98, were used to inoculate meat dough at a final concentration of about 1.5×10^5 viable cells g^{-1}. Cutlets, each weighing 100 g, were buttered (with a mixture of wheat flour and water), breaded, prefried at 180°C for 55 s, and baked at 180°C for 13 min. The temperature used for frying and the temperature of the oven were continuously monitored with a thermocouple probe linked to a digital thermometer (model DO9416; Delta OHM, Italy). The cutlets were then cooled and stored at 4°C.

The presence of *S. aureus* and the thermal resistance of SEA were evaluated by counting on PSE plates and by the RPLA method, respectively, as previously described. Samples were analyzed immediately after preparation, after frying, after baking, and after 1 week of storage at the refrigeration temperature (4°C). After inoculation all the experimental materials were handled and treated like biohazardous materials. The experiment was performed in duplicate.

Statistics. For statistical analysis standard deviations were determined.

RESULTS

Detection of *S. aureus*, a_w, and pH in breaded chicken products. The typical red-violet colonies grown on PSE plates were counted as presumptive *S. aureus* colonies. As shown in Table 1, 75% of the breaded meat cutlets tested contained from 10 to 10^3 CFU g^{-1} of presumptive *S. aureus*, whereas five samples (samples G, L, H, F, and AO) had undetectable levels of the pathogen.

The pH values of the samples ranged from 5.9 to 6.8, and for 40% of the samples the pH was ≥6.5. The a_w values ranged from 0.95 to 0.98, and 85% of the samples had an a_w value less than 0.98.

Detection of staphylococcal enterotoxin A in breaded chicken products. As SEA was not found in breaded chicken products by using the RPLA assay, Western immunoblotting was carried out to improve the sensitivity of detection. As shown in Fig. 1B, no SEA was detected in the breaded chicken extracts (lanes c to h), while a colorimetric signal for purified SEA (Fig. 1A and B, lane b), used as positive control, was clearly observed at 27 kDa.

Phenotypic and genotypic characterization of presumptive *S. aureus* isolates. Red-violet colonies were randomly isolated from PSE plates, purified, and subcultured. On the basis of phenotypic tests (Gram reaction and catalase, coagulase, and

TABLE 1. Concentration of *S. aureus*, pH, and a_w in breaded chicken products

Sample[a]	*S. aureus* concn (log CFU g^{-1})[b]	pH[c]	a_w[d]
B	1.2×10^2	6.2	0.96
E	1.0×10	6.3	0.98
G	ND	6.7	0.98
AD	1.2×10^2	6.5	0.96
L	ND	6.5	0.97
AG	1.3×10^3	6.5	0.95
D	1.2×10^3	6.2	0.96
AF	5.0×10	6.5	0.97
AE	3.9×10^2	6.8	0.96
C	2.0×10	6.1	0.96
H	ND	6.2	0.97
F	ND	6.3	0.98
AM	1.5×10^2	6.3	0.97
AN	9.8×10^2	6.3	0.96
AH	1.8×10^2	5.9	0.96
AI	9.0×10	6.2	0.96
A	1.0×10	6.2	0.97
AL	7.0×10^2	6.5	0.96
AO	ND	6.5	0.95
AP	1.5×10^3	6.2	0.95

[a] The products were industrial breaded chicken products obtained from retail butchers and supermarket stores in southern Italy. Cutlets or fillets of breaded minced meat were prepared using standardized technology. The methods used to analyze the products are described in the text.

[b] The values are means ± standard deviations ($0.1 \leq$ standard deviation ≥ 1.0) ($n = 4$). ND, not detectable.

[c] The values are means ± standard deviations ($0.1 \leq$ standard deviation ≥ 0.5) ($n = 4$).

[d] The values are means ± standard deviations ($0.001 \leq$ standard deviation ≥ 0.005) ($n = 4$).

DNase activities) and genotypic tests (16S-23S rRNA gene spacer analysis, PCR-amplified *nuc*, and PCR-amplified *hsp60*), the 34 gram- and catalase-positive cocci were divided into 10 different groups. As shown in Table 2, all strains were negative for the DNase reaction, and 11 (32%) were coagulase positive (CPS).

Groups 1 and 7 included three strains positive for both the coagulase reaction and amplification of the *nuc* and *hsp60* genes that could be considered members of the species *S. aureus*. These strains were isolated from two different breaded chicken samples (samples B and AH) and were characterized by two different 16S-23S rRNA gene spacer profiles (profiles C and J).

Groups 2, 3, and 5 contained eight coagulase-positive but *nuc*- and *hsp60*-negative strains. They could be discriminated on the basis of their 16S-23S rRNA spacer profiles (profiles F, G, and H). The remaining 23 strains (68%) were characterized by a lack of coagulase production and a lack of *nuc* and *hsp60* gene amplification (groups 4, 6, 8, 9, and 10). The latter strains could also be differentiated by their 16S-23S rRNA gene spacer profiles (profiles G, I, L, M, and N). Moreover, all 34 strains were negative in the PCR assays targeting staphylococcal enterotoxin genes (*se*A to *se*E, *se*G to *se*J, and *se*M to *se*O) and the TSST-1 gene.

Impact of processing on *S. aureus* and SEA activity during manufacture of breaded chicken cutlets. Laboratory-scale manufacture of breaded chicken cutlets was performed with the aim of clarifying how the heat treatments used in industrial production of poultry products can affect the viability of *S. aureus* and its toxin resistance. A mixture containing SEA-producing *S. aureus* strains K6278/94 and A4178/98 was used to test breaded chicken cutlets in order to minimize the potential diversity in sensitivity to the processing conditions. The effect of cooking on the survival of *S. aureus* and on SEA activity was monitored during manufacture at each processing step.

Figure 2 shows the manufacturing procedure and the following sampling points: immediately after preparation of breaded cutlets (S0), after incubation at 37°C (S1), after frying (S2), after baking (S3), and after storage (S4). At the end of cutlet preparation, the size of the *S. aureus* population was about 1.6×10^5 CFU g^{-1}, and it increased to 3.6×10^7 CFU g^{-1} after 7 h of incubation at 37°C (Table 3). After prefrying and baking, as well as after 1 week of storage at 4°C, the pathogen was not detectable in 0.1-g samples (Table 3). The thermal treatments resulted in an approximately 7-log CFU g^{-1} reduction. By contrast, SEA was detected in the cutlets after the incubation period and after both cooking steps (frying and baking), thus indicating that SEA resisted double heat treatment at 180°C. SEA was still present after 1 week of storage at 4°C (Table 3).

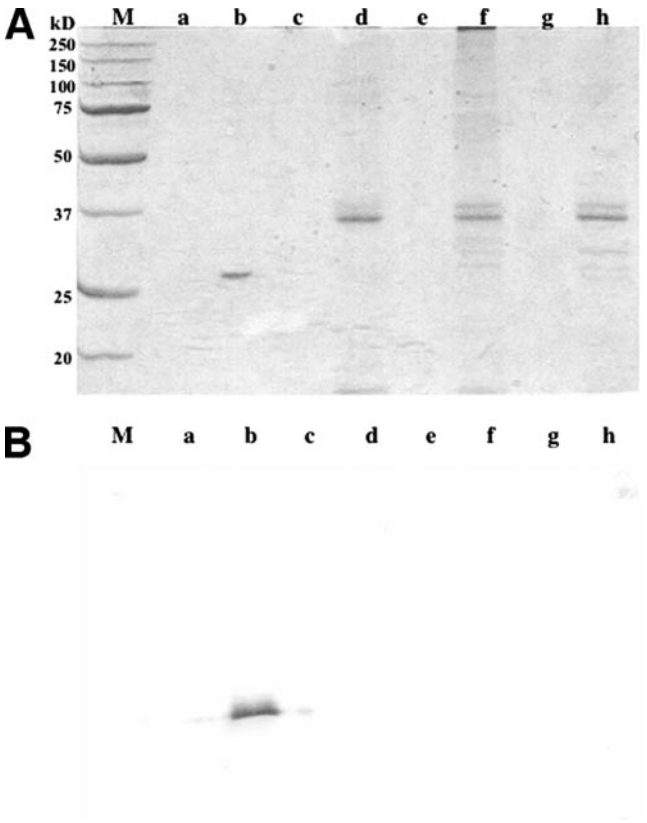

FIG. 1. Identification of staphylococcal enterotoxin A in breaded chicken products by Western immunoblotting. (A) Coomassie blue-stained gel from SDS-PAGE analysis. (B) Western immunoblot of the gel shown in panel A. Lane M, molecular weight markers; lanes a, c, e, and g, sample buffer; lane b, 400 ng of SEA; lanes d, f, and h, samples D, AO, and AG, respectively.

TABLE 2. Phenotypic and genotypic characterization of presumptive *S. aureus* strains isolated from breaded chicken cutlets[a]

Group or strain	No. of strains tested	Source (sample[s])	Phenotypic and genotypic characteristics						
			Gram reaction	Catalase	Coagulase	DNase reaction	16S-23S rRNA gene spacer profile	PCR-amplified *nuc*	PCR-amplified *hsp60*
1	2	B	+	+	+	−	C	+	+
2	2	AD, AG	+	+	+	−	F	−	−
3	4	AD, AH, AI, AP	+	+	+	−	G	−	−
4	3	B	+	+	−	−	G	−	−
5	2	AC, AP	+	+	+	−	H	−	−
6	10	AF, AD, AL AG, AH, AN	+	+	−	−	I	−	−
7	1	AH	+	+	+	−	J	+	+
8	1	AL	+	+	−	−	L	−	−
9	5	D, AM	+	+	−	−	M	−	−
10	4	D, AC	+	+	−	−	N	−	−
S. aureus DSM 2023I^T			+	+	+	+	J	+	+

[a] All phenotypic and genotypic methods used are described in the text.

DISCUSSION

Despite the increasing consumption of processed breaded chicken, to our knowledge there have been no previous studies of the occurrence of *S. aureus* in this kind of food. Based on the assumption that the pathogen can be introduced into the process through raw materials, handling, and unsanitary procedures and equipment, research was undertaken to assess the presence of *S. aureus* in such poultry products.

Use of the PSE system resulted in detection of *S. aureus* at a relatively high frequency in the samples analyzed (75%), although further steps for identification at the genotypic level of the isolates reduced the frequency to 5%. These results demonstrated that the specificity of the PSE system was quite low. Therefore, the level of contamination in the breaded chicken products analyzed was quite low, even if the pH and a_w values for the samples considered were in ranges favorable for *S. aureus* growth. Indeed, this gram-positive bacterium has no particular nutritional or environmental requirements. Its growth is limited at a_w values less than 0.86 to 0.87, although the precise boundary of the growth–no-growth interface for *S. aureus* can be affected by the types of humectants used, as well as by the physical properties of the solutes and their effects on biological systems (37, 38). In particular, the decrease in water bioavailability affects the lag phase more than the generation time. In optimal temperature and acidity conditions, at an a_w of 0.99 the lag phase is 1 h; at an a_w of 0.96 the lag phase is 6 h, and it increases to 3 days, after which the a_w is 0.90. The osmoadaptive strategies of *S. aureus* are related to accumulation of certain low-molecular-weight compounds termed compatible solutes, such as glycine, betaine, proline, and carnitine (33). In the same way, a pH less than 4.8 and a temperature below 8 to 9°C inhibited *S. aureus* growth (31). In particular, as reported by Ingham et al. (22), the *S. aureus* concentration decreased for fermented ready-to-eat meat products vacuum packaged at a pH of ≤5.1 with a wide range of salt concentra-

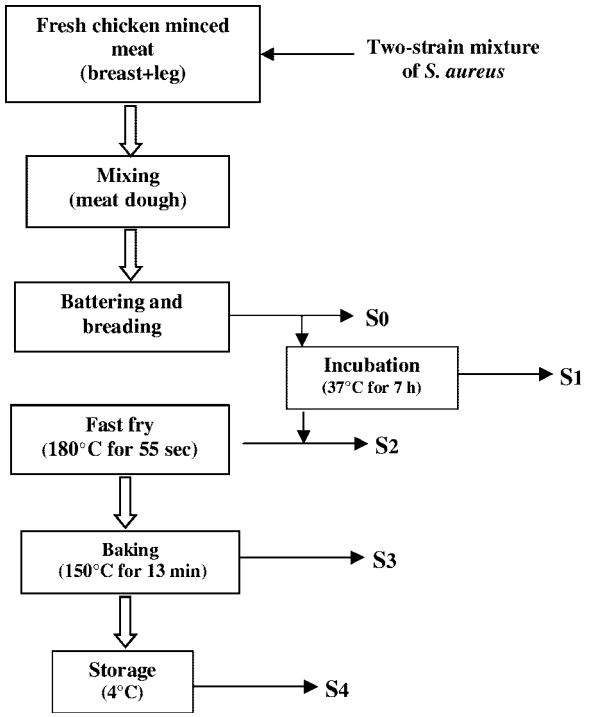

FIG. 2. Laboratory-scale procedure for manufacturing breaded chicken cutlets and sampling points, including immediately after preparation of breaded cutlets (S0), after incubation (S1), after prefrying (S2), after baking (S3), and after storage (S4).

TABLE 3. Impact of cooking on *S. aureus* and SEA activity during laboratory-scale manufacture of breaded chicken cutlets

Time during manufacture of breaded chicken cutlets[a]	*S. aureus* mixed-culture concn (log CFU g^{-1})[b]	Presence of SEA (n = 4)
S0	$1.6 \times 10^5 \pm 0.2 \times 10^5$	−
S1	$3.5 \times 10^7 \pm 0.6 \times 10^7$	+
S2	Absent in 0.1 g	+
S3	Absent in 0.1 g	+
S4	Absent in 0.1 g	+

[a] A breaded chicken cutlet was analyzed immediately after preparation (S0), after 7 h of incubation at 37°C (S1), after prefrying for 55 s at 180°C (S2), after baking for 13 min at 180°C (S3), and after 1 week of storage at 4°C (S4). For details concerning the methods used see the text.

[b] The values are means ± standard deviations (n = 4).

tions and moisture contents, while products that were not fermented or dried supported *S. aureus* growth.

Breaded chicken products contained CPS strains and coagulase-negative staphylococcal strains, both of which could contain enterotoxin genes, as previously determined by Rodriguez et al. (35) using molecular assays. In particular, among the coagulase-negative strains, *Staphylococcus cohnii*, *Staphylococcus epidermidis*, *Staphylococcus xylosus*, and *Staphylococcus haemolyticus* strains were found to produce one or several staphylococcal endotoxins (3). In spite of the elevated percentage of CPS strains that we found in precooked chicken products compared to the data reported by Normanno et al. (31), only three strains could be actually referred to the species *S. aureus*. In particular, a study conducted by Normanno et al. (31) showed that 23.1% of fresh meat products and preparations marketed in Italy were contaminated by CPS strains and that a large majority of the enterotoxigenic *S. aureus* isolates produced SEA and SEC. Nevertheless, in the present study, no SEA was found in the retail breaded chicken samples analyzed either by the RPLA method, which allowed detection of up to 1 ng/g of toxin, or by Western blotting. The latter method allows removal of extraneous substances from samples as the toxin is captured by the matrix used in the chromatography, and denatured proteins can be solubilized, thus determining the characteristics of the antigen that reacts with the antibody. Rasooly and Rasooly (34) demonstrated that the Western blot assay is a very sensitive method capable of detecting very small amounts of SEA (0.1 ng/ml). In our experiments, the method was further improved by using the polyvinylidene difluoride membrane and an amplified AP immunoblot kit that increased the sensitivity of colorimetric Western blotting to more than 10 pg of protein bound to the blotting membrane. SEA is the most common toxin involved in staphylococcal food poisoning outbreaks (14, 19, 20). The amount of SEA necessary to cause intoxication is variable since the emetic dose necessary ranges from 20 to 0.2 μg (4, 12). Although staphylococcal endotoxins are generally considered to be heat stable, evidence that heat resistance depends on several factors has been obtained. Partial or complete inactivation of SEA was obtained by incubation at 80°C or 100°C for 10 min in some media at different pH values, and the loss of immunological activity was accompanied by a concomitant loss of biological activity (36). Moreover, as reported by Balaban and Rasooly (1), the analytical method is a useful tool for detecting SEA in heat-treated foods since staphylococcal endotoxins are also resistant to inactivation by heat treatment for 8 to 28 min at 121°C. Hence, we evaluated the thermal resistance of two strains of *S. aureus* and of their SEA in laboratory-scale preparations of precooked breaded chicken cutlets. In accordance with the results obtained by other authors (34), the *S. aureus* strains used in the experiment were grown in the cutlets under favorable conditions to allow SEA production. Indeed, in highly contaminated mushrooms under good growth conditions, the toxin was produced in less than 3 h (34). Our challenge test demonstrated that the cooking process, beginning with prefrying at 180°C for 55 s, resulted in *S. aureus* inactivation, even with a high initial level of contamination of the raw chicken meat. It was previously reported (28) that an *S. aureus* strain is more heat resistant than *Salmonella enterica* serotypes and *Listeria monocytogenes* in high-solids egg mixes. In particular, *S. aureus* had D values of about 1.3 to 1.7 min at 64°C and of 0.30 to 0.40 min at 70°C. In addition, Kennedy et al. (24) demonstrated that chilling of *S. aureus* did not increase resistance to thermal destruction during a process such as cooking. Hence, the presence of the staphylococci, particularly *S. aureus*, in the retail breaded chicken products analyzed indicated that the sanitary conditions during processing were poor, which may have created a potential health risk for consumers. In fact, as demonstrated by the challenge test, the heat treatment employed in the manufacture of breaded chicken meat could determine the inactivation of *S. aureus* cells, but the preformed SEA remained active until storage of the product. Moreover, postprocessing contamination by *S. aureus* represents a significant health hazard due to the elimination of microbes that would normally outcompete *S. aureus* (18).

Not only can *S. aureus* enter the process on raw materials, but it can also be introduced into foods during processing from unclean hands and unsanitary utensils and equipment. The hazard develops into toxin formation when raw materials and products are exposed to temperatures between 10°C and 21.1°C for more than 12 h or to temperatures greater than 21.1°C for more than 3 h (13).

The level of contamination during industrial processing is due to several factors, such as the bacteriological quality of the raw meat, some factors (pH and a_w) influencing the biological response of microorganisms, food hygiene during processing, the storage temperature, and the product discard policy, which should be carefully monitored to ensure that products are microbiologically stable with enhanced safety. Moreover, consumers have to be informed and follow the basic instructions regarding storage temperature, cooking, and prevention of contamination and cross-contamination.

In conclusion, our results demonstrated that the pH and a_w of breaded chicken products are favorable for *S. aureus* survival and growth and that the thermal process used during manufacture can limit staphylococcal contamination but cannot eliminate preformed toxins. This study suggests that ready-to-eat breaded chicken products are relatively safe. However, when the levels of contamination of the raw minced meat are high and/or when an incorrect temperature is used for storage of the final product before consumption, such precooked products may represent a potential risk for consumers.

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministry of Agricultural and Forestry Policy SIQUALTECA project.

We thank Karsten Becker of the Institut für Medizinische Mikrobiologie, Universitätsklinikum Münster (Münster, Germany), for kindly providing strains K6278/94 and A4178/98.

REFERENCES

1. **Balaban, N., and A. Rasooly.** 2000. Staphylococcal enterotoxins. Int. J. Food Microbiol. **61:**1–10.
2. **Balaban, N., and A. Rasooly.** 2001. Analytical chromatography for recovery of small amounts of staphylococcal enterotoxins from food. Int. J. Food Microbiol. **64:**33–40.
3. **Bautista, L., P. Gaya, M. Medina, and M. Nunez.** 1988. A quantitative study of enterotoxin production by sheep milk staphylococci. Appl. Environ. Microbiol. **54:**566–569.
4. **Bergdoll, M. S.** 1967. The staphylococcal enterotoxins, p. 1–25. *In* R. I. Mateles and G. N. Wogan (ed.), Biochemistry of some foodborne microbial toxins. MIT Press, Cambridge, MA.
5. **Blaiotta, G., C. Pennacchia, D. Ercolini, G. Moschetti, and F. Villani.** 2003. Combining denaturing gradient gel electrophoresis of 16S rDNA V3 region

and 16S-23S rDNA r region polymorphism analyses for the identification of staphylococci from Italian fermented sausages. Syst. Appl. Microbiol. **26:** 423–433.

6. **Blaiotta, G., D. Ercolini, C. Pennacchia, V. Fusco, A. Casaburi, O. Pepe, and F. Villani.** 2004. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products. Evidence for new variants of seG and seI in *S. aureus* AB-8802. J. Appl. Microbiol. **97:**719–730.
7. **Brakstad, O. G., K. Aasbakk, and J. A. Maeland.** 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol. **30:**1654–1660.
8. **Chaffey, B. J., and W. M. Waites.** 1987. The adhesion of *Staphylococcus aureus* isolates from poultry-processing plants. J. Appl. Bacteriol. **63:**R17.
9. **Do Carmo, L. S., C. Cummings, V. R. Linardi, R. S. Dias, J. M. De Suoza, M. J. De Sena, D. A. Dos Santos, J. W. Shupp, R. K. Pereira, and M. Jett.** 2004. A cause of massive staphylococcal food poisoning incident. Foodborne Pathog. Dis. **1:**241–246.
10. **Dodd, G. C., Mead, and W. M. Waites.** 1988. Chlorine resistance of strains of *Staphylococcus aureus* isolated from poultry-processing plants. Lett. Appl. Microbiol. **6:**31–34.
11. **Ellerbroek, L.** 1997. Airborne microflora in poultry slaughtering establishments. Food Microbiol. **14:**527–531.
12. **Evenson, M. L., M. W. Hinds, R. S. Bernstein, and M. S. Bergdoll.** 1988. Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. Int. J. Food Microbiol. **7:**311–316.
13. **Food and Drug Administration Center for Food Safety & Applied Nutrition.** 2001. *Staphylococcus aureus* toxin formation in hydrated batter mixes, p. 201–208. *In* Fish and fisheries products hazards and controls guidance, 3rd ed. Food and Drug Administration Center for Food Safety & Applied Nutrition, Washington, D.C. [Online.] http://www.cfsan.fda.gov/~comm/haccp4o.html.
14. **Genigeorgis, C. A.** 1989. Present state of knowledge on staphylococcal intoxication. Int. J. Food Microbiol. **9:**365–369.
15. **Georgnaras, I., A. Dejesus, E. Vanzyl, and A. von Holy.** 1995. Microbiological survey of a South-African poultry-processing plant. J. Basic Microbiol. **35:** 73–82.
16. **Giordano, L.** 2004. Tecnologia per la produzione industriale di cotolette di pollo. [Online.] http://www.pubit.it/sunti/euc0403x.html.
17. **Gregersen, T.** 1978. Rapid method for distinction of Gram negative from Gram positive bacteria. Eur. J. Appl. Microbiol. Biotechnol. **5:**123–127.
18. **Gundogan, N., S. Citak, N. Yucel, and A. Devren.** 2005. A note on the incidence and antibiotic resistance of *Staphylococcus aureus* isolated from meat and chicken samples. Meat Sci. **69:**807–810.
19. **Halpin-Dohnalek, M. I., and E. H. Marth.** 1989. *Staphylococcus aureus*: production of extracellular compounds and behaviour in foods—a review. J. Food Prot. **52:**267–282.
20. **Holmberg, S. D., and P. A. Blake.** 1984. Staphylococcal food poisoning in the United States. New facts and old misconception. JAMA **251:**487–489.
21. **Huys, G., K. D'Haene, J. V. Eldere, A. Holy, and J. Swings.** 2005. Molecular diversity and characterization of tetracycline-resistant *Staphylococcus aureus* isolates from a poultry processing plant. Appl. Environ. Microbiol. **71:**574–579.
22. **Ingham, S. C., R. A. Engel, M. A. Fanslau, E. L. Schoeller, G. Searls, D. R. Buege, and J. Zhu.** 2005. Fate of *Staphylococcus aureus* on vacuum-packaged ready-to-eat meat products stored at 21 degrees C. J. Food Prot. **68:**1911–1915.
23. **Jensen, M. A., J. A. Webster, and N. Straus.** 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA polymorphisms. Appl. Environ. Microbiol. **59:**945–952.
24. **Kennedy, J., I. S. Blair, D. A. McDowell, and D. J. Bolton.** 2005. An investigation of the thermal inactivation of *Staphylococcus aureus* and the potential for increased thermotolerance as a result of chilled storage. J. Appl. Microbiol. **99:**1229–1235.
25. **Kitai, S., A. Shimizu, J. Kawano, E. Sato, C. Nakano, H. Kitagawa, K. Fujio, K. Matsumura, R. Yasuda, and T. Inamoto.** 2005. Prevalence and characterization of *Staphylococcus aureus* and enterotoxigenic *Staphylococcus aureus* in retail raw chicken meat throughout Japan. J. Vet. Med. Sci. **67:**269–274.
26. **Laemmli, C.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
27. **Le Loir, Y., F. Baron, and M. Gautier.** 2003. *Staphylococcus aureus* and food poisoning. Genet. Mol. Res. **2:**63–76.
28. **Li, X., B. W. Sheldon, and H. R. Ball.** 2005. Thermal resistance of *Salmonella enterica* serotypes, *Listeria monocytogenes*, and *Staphylococcus aureus* in high solids liquid egg mixes. J. Food Prot. **68:**703–710.
29. **Mead, G. C., C. Gibson, and D. B. Tinker.** 1995. A model system for the study of microbial colonization in poultry defeathering machines. Lett. Appl. Microbiol. **20:**134–136.
30. **Mead, G. C., and C. E. R. Dodd.** 1990. Incidence, origin and significance of staphylococci on processed poultry. J. Appl. Bacteriol. Symp. Suppl. **19:**81S–91S.
31. **Normanno, G., A. Firinu, S. Virgilio, G. Mulab, A. Dambrosio, A. Poggiu, L. Decastelli, R. Mioni, 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. Int. J. Food Microbiol. **98:**73–79.
32. **Olivier, M., C. M. Veary, T. E. Cloete, and A. von Holy.** 1996. Microbiological status of selected chicken carcasses from a nonautomated poultry processing plant. J. Basic Microbiol. **36:**41–49.
33. **Qi, Y., and K. J. Miller.** 2000. Effect of low water activity on staphylococcal enterotoxin A and B biosynthesis. J. Food Prot. **63:**473–478.
34. **Rasooly, A., and R. S. Rasooly.** 1998. Detection analysis of staphylococcal enterotoxin A in food by Western immunoblotting. Int. J. Food Microbiol. **41:**205–212.
35. **Rodriguez, M., F. Nunez, J. J. Cordoba, E. Bermudez, and M. A. Asensio.** 1996. Gram-positive, catalase-positive cocci from dry cured Iberian ham and their enterotoxigenic potential. Appl. Environ. Microbiol. **62:**1897–1902.
36. **Schwabe, M., S. Notermans, R. Boot, S. R. Tatini, and J. Krämer.** 1990. Inactivation of staphylococcal enterotoxins by heat and reactivation by high pH treatment. Int. J. Food Microbiol. **10:**33–42.
37. **Scott, W. J.** 1953. Water relations of *Staphylococcus aureus* at 30°C. Aust. J. Biol. Sci. **6:**549–564.
38. **Stewart, C. M., M. B. Cole, J. D. Legan, L. Slade, M. H. Vandeven, and D. W. Schaffer.** 2002. *Staphylococcus aureus* growth boundaries: moving towards mechanistic predictive models based on solute-specific effects. Appl. Environ. Microbiol. **68:**1864–1871.
39. **Wieneke, A. A., D. Roberts, and R. J. Gilbert.** 1993. Staphylococcal food poisoning in the United Kingdom, 1969–1990. Epidemiol. Infect. **110:**519–531.