Presence of genes encoding enterotoxins in *Staphylococcus aureus* isolates recovered from food, food establishment surfaces and cases of foodborne diseases

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ABSTRACT

The aim of this study was to describe the microbiological characteristics and profile of genes encoding enterotoxins in 95 *Staphylococcus aureus* isolates obtained between April 2011 and December 2014 from foodstuffs, persons and surfaces of retail food stores. After microbiological identification and antimicrobial susceptibility testing, polymerase chain reactions (PCR) were performed, targeting *sea, seb, sec, sed* and *see* genes that code for classical enterotoxins (ET) A-E, and three additional genes: *seg*, *seh* and *sei*, coding for so-called "new enterotoxins" G, H and I. The isolates were characterized by Pulsed Field Gel Electrophoresis (PFGE), and five selected isolates were further analyzed through Multi Locus Sequence Typing (MLST). It is noteworthy that 54.7% of the examined isolates harbored one or more of the investigated ET gene types. Most positive isolates carried more than one ET gene up to five types; *seg* was the most frequent ET gene, followed by *sei*. Five enterotoxin-coding isolates also coded for some antimicrobial resistance genes. Two of them, and four additional non-enterotoxic isolates carried *erm* genes expressing inducible clindamycin resistance. PFGE-types were numerous and diverse, even among enterotoxin-coding strains, because most isolates did not belong to known foodborne outbreaks and the sampling period was long. MLST profiles were also varied, and a new ST 3840 was described within this species. ST 88 and ST 72 enterotoxin-coding isolates have been identified in other regions in association with foodborne outbreaks. This manuscript reports the first systematic investigation of enterotoxin genes in *S. aureus* isolates obtained from foodstuffs and infected people in Uruguay.

KEYWORDS: *Staphylococcus aureus*. Enterotoxin genes. Foods. Antimicrobial resistance. Multi Locus Sequence Typing. Pulsed Field Gel Electrophoresis.

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INTRODUCTION

Foodborne diseases include a broad group of illnesses caused by contaminated food or water intake. Although digestive manifestations are usually mild and self-limited, severe cases can occur, and sequelae may result. Infectious etiology is the most frequent. Common bacterial agents are *Salmonella enterica*, *Shigella spp, Campylobacter jejuni, Clostridium botulinum,* diarrheagenic *Escherichia coli, Yersinia enterocolitica* and *Staphylococcus aureus,* among others[1].

S. aureus is a Gram-positive coccus that belongs to the human microbiota, but can also be responsible for multiple disease conditions, including cases of "food poisoning" due to its capacity of producing heat stable enterotoxins. These are short chain proteins produced in the food when S. aureus proliferates, and can cause disease in spite of correct meal cooking. Once ingested, enterotoxins soon produce

the sudden onset of vomiting, abdominal pain and diarrhoea. In vulnerable groups such as the elderly and children, complications may occur. More than 20 enterotoxins and related superantigens have been described, but not all of them have an emetic effect. Those capable of producing intense vomiting in primates are ETA, ETB, ETC, ETD, ETE, ETG, ETI, ETR and ETT[2]. The other enterotoxins are known as ET-like toxins (ETL or ET/L) and this group includes the toxic shock syndrome toxin (TSST)[3]. The enterotoxin-coding genes are usually carried in mobile and transmissible genetic elements as phages, transposons or plasmids; a defined group of genes (*seg, sei, seo, sem, sen*) can be located in vSaβ genomic island of *S. aureus*[3]. This EGC operon (Enterotoxin Gene Cluster) may also include other enzyme- and toxin-coding sequences[4-8].

As there were no studies in Uruguay on circulating staphylococcal enterotoxins, we decided to describe the microbiological characteristics of *S. aureus* isolates from foodstuffs, persons and surfaces of food establishments; to define if they carried enterotoxin-coding genes, and their types; to examine the antimicrobial susceptibility profile of all the isolates, and their comparative genome characteristics through *Sma*I-PFGE and MLST.

MATERIALS AND METHODS

Gathering of isolates

Ninety-five bacterial cultures previously identified as *S. aureus* were received from April 2011 to December 2014. They derived from food samples taken by public food control institutions, and from food processing and selling stores examined by private laboratories. No food or clinical sample was directly analysed. Forty-nine isolates were collected from surfaces and environment of food establishments; 40 from foodstuffs; four isolates were also obtained from food handlers and two from clients of a grocery store. Two food isolates were known to come from cheese and chicken salad, respectively. One isolate was obtained from cheese involved in a small family outbreak, and a related isolate was cultured from the store that sold the cheese. All received cultures were confirmed to be *S. aureus* by classical phenotypic assays. After spread-plating on blood agar, obtained colonies were examined through catalase, tube coagulase and DNase tests[9]; commercial DNase agar C/COL with methyl green was employed (BIOKEY lab. S.A. Uruguay).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined according to conventional Kirby & Bauer technique, modified as defined by Clinical & Laboratory Standards Institute (CLSI) guidelines[10]. Cefoxitin, clindamycin, erythromycin, ciprofloxacin, sulfamethoxazole-trimethoprim and gentamicin were tested. The erythromycin and clindamycin discs were closely placed 15 to 25 mm apart, for detecting clindamycin-inducible resistance when a D zone could be observed[11]. After 18-24 h incubation at 35 °C, the inhibition zones diameters were measured and results were interpreted as susceptible (S), Intermediate (I) or Resistant (R). The clindamycin resistance was reported as inducible when a D zone was visible, or constitutive when R size zones surrounded both antibiotic discs.

DNA extraction

Three or four colonies of *S. aureus* were suspended in 100 μL of a 1U/μL solution of Achromopeptidase enzyme (Sigma-Aldrich, St. Louis, MO.). They were exposed to 94 °C for 10 min and for further 10 min at 55 °C. The mixture was then centrifuged at 10,000 g; 80 μL of the supernatant were recovered and frozen until PCR[12] analysis.

PCR for enterotoxin genes detection

Table 1 shows the primers employed for investigating five genes that code for classical enterotoxins (*sea, seb, sec, sed* and *see*), three coding for new ETs (*seg, seh* and *sei*), and the expected molecular weight of the amplicons. The primers sequences and the amplification protocols have been previously described by Manfredi *et al.*[13] and after minor modifications, they were applied for searching classical enterotoxin genes. The protocols published by Omoe *et al.*[14] were used for investigating the new ET genes. Single reactions were performed for each gene instead of multiplex PCR procedures. The reaction master mix was the same for all genes, combining reagents in a final volume of 25 μL with the following concentrations: 0.5 μM primers; 0.5 mM dNTPs; 1X PCR Buffer; 3 mM MgCl$_2$, 1unit of Taq polymerase and 4 μL of DNA template. The following conditions were used for both groups of reactions: an initial denaturation step at 94 ºC for 4 min, 30 cycles of 94 °C 60 s, 57 °C 60 s and 72 °C 60 s, and a final extension at 72 ºC for 4 min. Reactions were carried out in a Gene Amp PCR System 2700 thermocycler (Applied Biosystems®, California, USA). Amplification products were mixed with 5 X loading buffer, loaded in 1.5% agarose gels prepared with 0.5 X Tris-Borate-EDTA (TBE) buffer, and separated by electrophoresis run at 70 V. ATCC 6538 strain carrying

Table 1 - Primer sequences and amplicon molecular weight in PCRs to detect ET genes.

Primer	(5'-3') sequence	Amplicon molecular weight	Primer	(5'-3') sequence	Amplicon molecular weight
sea F	GCAGGGAACAGCTTTAGGC	521	*see* R	CTCTTTGCACCTTACCGC	121
sea R	GTTCTGTAGAAGTATGAAACACG		*16 s rARN* F	GTAGGTGGCAAGCGTTATCC	228
seb F	ACATGTAATTTTGATATTCGCACTG	667	*16 s rARN* R	CGCACATCAGCGTCAG	
seb R	TGCAGGCATCATGTCATACCA		*seg* F	AAGTAGACATTTTTGGCGTTCC	287
sec F	CTTGTATGTATGGAGGAATAACAA	284	*seg* R	AGAACCATCAAACTCGTATAGC	
sec R	TGCAGGCATCATATCATACCA		*seh* F	GTCTATATGGAGGTACAACACT	213
sed F	GTGGTGAAATAGATAGGACTGC	385	*seh* R	GACCTTTACTTATTTCGCTGTC	
sed R	ATATGAAGGTGCTCTGTGG		*sei* F	GGTGATATTGGTGTAGGTAAC	454
see F	TACCAATTAACTTGTGGATAGAC	121	*sei* R	ATCCATATTCTTTGCCTTTACCAG	

no enterotoxin genes was added to the study as a negative control. Positive controls were obtained from *S. aureus* strains kindly provided by Dr. E. A. Manfredi (Servicio Fisiopatogenia, Departamento Bacteriologia, Instituto Nacional de Enfermedades Infecciosas – ANLIS "Dr. Carlos G. Malbran" Buenos Aires, Argentina). A PCR mix without DNA for procedure validation, a pair of primers for amplifying the *S. aureus* 16S rDNA as internal control, and a 100 bp ladder for comparing amplicon sizes were also included. After staining with 0.3 ug/ml ethidium bromide, amplicons were visualized with UV light in the FOTO/Analyst investigator Eclipse FOTODYNE* system (Thermo Fisher Scientific, Waltham, MA, USA). The amplified gene type was identified by comparing the obtained molecular weight with known sizes of amplicons shown in tables. ET genes-coding strains were notified to the official agency so that the necessary precautions were taken to protect the population's health.

Amplicon sequencing

For additional control, the amplicons obtained from two investigated genes (*seh* and *sei*) were sequenced. After purifying the DNA with a commercial kit (QIAGEN Inc., Hilden, Germany), the sequencing was performed at the Institut Pasteur of Montevideo. The obtained sequences were compared with those deposited in GenBank using the NCBI BLAST tool (Basic Local Alignment Search Tool).

Pulsed Field Gel Electrophoresis (PFGE)

A regional standard protocol was followed[15]. Plugs were prepared, a pre-restriction step was performed, and they were then exposed to *Sma*I restriction enzyme (New England Biolabs Inc., Ipswich, MA, USA) for 17 h at

25 ºC. The running gel was prepared with ultrapure agarose (Bio-Rad, Hercules, CA, USA, 100 ml) and 0.5 X TBE buffer. Separation was performed in CHEF-DRII equipment (Bio-Rad, Melville, NY) with the following conditions: initial switching time of 5 s, final switching time of 35 s., 6 V/cm or 200 V, running time of 23 h, at 11.3 ºC of temperature and 70 of pump speed. The gel was stained in a 1 μg/mL ethidium bromide solution during 30 min, and visualized as already mentioned. The degree of homology of obtained band patterns was analyzed with the Applied Maths Bionumerics program, using the Dice similarity coefficient with 1.5% of optimization and 1.5% of tolerance settings. Two dendrograms were obtained with UPGMA (Unweighted Pair Group Method with Arithmetic Mean). In one of them, only positive isolates for enterotoxin genes were analyzed; all the studied strains were compared in the other one. Each pattern depicts a hierarchical relationship among different isolates. Strains showing a degree of similarity ≥ 95% were considered identical; if the percentage of similarity of PFGE-types was ≥ 80, they were considered as belonging to the same PFGE-group or cluster that can include two or more subtypes.

Multilocus sequence typing (MLST)

Five strains were selected for further analysis by MLST. Extracted DNA was subjected to PCR for the amplification of defined sequences from each of the seven "housekeeping" genes: *arcC* (carbamate kinase), *aroE* (shikimate dehydrogenase), *glpF* (glycerol facilitator), *gmk* (guanylate kinase), *pta* (phosphotransacetylase), *tpi* (triose phosphate isomerase) and *yqiL* (acetyl coenzyme A acetyltransferase). Both strands of the DNA amplicons from each of the genes were sequenced using forward and

reverse primers. The allelic profile and sequence type (ST) of the examined strains were determined by matching their sequences with known MLST sequences from the website. Comparisons were made through BURST analyses of found STs with the regional *S. aureus* types reported on the corresponding pubmlst website, trying to identify groups, relationships, single variants (SV) or double variants (DV).

Resistance genes investigation

The *mecA* gene was investigated in methicillin resistant isolates as previously described[16]; *ermA*, *ermB*, *ermC*, *msrA* and *msrB* were investigated only in macrolides- and lincosamides-resistant isolates. PCR for *erm* genes were performed in 25 μL of reaction master mix with the following composition: 0.8 μM of the target sequence primers; 0.2 mM of dNTPs; 1 X PCR Buffer; $MgCl_2$ 2 mM; 1 U of Taq polymerase and 4 μL of template DNA. The amplifications conditions were: 94 °C for 5 min, 30 cycles of 30 s at 94 °C, 50 °C (for *ermA* and *ermC*; 54 °C for *ermB*) for 30 s and 72 °C for 60 s, and a final extension at 72 °C during 4 min. The PCR master mix for *mrs A* and *B* had a similar reagents composition, excepting for a double $Mg Cl_2$ concentration. Amplification conditions were: 94 °C for 5 min, 30 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1.5 min, and a final extension of 10 min at 72 °C[17]. Known *Streptococcus agalactiae* strains carrying these genes were used as positive controls, and a negative sample containing the regular master mix of reagents but without template DNA was used for procedure control. Agarose gel electrophoresis conditions were the same that have already been described.

RESULTS

Identification of isolates

The microbiological studies and the positive result of the specific *16S* DNA PCR of all the received strains confirmed that they belonged to *Staphylococcus aureus* species.

Identified enterotoxin genes

Fifty-two strains (54.7 %) carried some of the investigated genes. Of these, in 20 (38.5 %) a single gene could be detected; 20 isolates carried 2 genes, 9 had 3 genes, 3 yielded 4 genes and one isolate from a food selling store contained 5 enterotoxin-coding genes (*seb, sec, sed, seg* and *sei)* (Table 2). The most frequently found gene, irrespective of the isolate origin (foodstuffs or food premises), was *seg,* in 31 strains: in six isolates as a single

Table 2 - Type and number of enterotoxin genes found in 52 *S. aureus* isolates.

Gene or combination of genes	Number of isolates	Genes	Number of isolates
sea	2	*sec seg*	3
seb	1	*see seg*	1
sec	1	*seg sei*	5
sed	3	*sea seb sec*	2
see	2	*sea sed seg*	1
seg	6	*seb sed seg*	2
seh	2	*seb seg sei*	2
sei	2	*sec seg sei*	1
sea seh	1	*sed seg sei*	2
seb sec	3	*sec sed seg sei*	2
seb seh	1	*seb sec seg sei*	1
sed seg	4	*seb sec sed seg sei*	1
seb sei	1	TOTAL	52

sequence, and in 26 in association with some other one. The second most frequently identified gene was *sei*, in 17 strains, usually associated with other gene sequences (with a single exception) (Table 3). The most frequent association was *sei* and *seg,* found in six isolates. Thirty-eight out of 95 isolates coded for genes of classic enterotoxins, being *sed* the most frequent of them (Table 3). The outbreak-associated *S. aureus* isolates cultured from cheese and the store that sold the cheese carried *sea*, *seb* and *sec* DNA sequences.

Table 3 - Number of strains carrying each ET gene, alone or in combinations.

Gene	Number of strains	%
sea	7	6.8
seb	13	12.5
sec	14	13.5
sed	15	14.4
see	3	2.9
seg	**31**	29.8
seh	4	3.8
sei	17	16.3
Total	**104**	100

Sequencing results of *seh* and *sei* amplicons

The sequencing of PCR products from these new enterotoxin genes confirmed that they belonged to *seh* and *sei* types.

Antimicrobial susceptibility

Almost 90% of isolates (85/95) were susceptible to all tested antibiotics. Of the remaining 10%, two were resistant to methicillin, carrying the *mecA* gene; two were only resistant to erythromycin and positive for both *mrsA* and *mrsB* genes; the remaining six were resistant to erythromycin with inducible resistance to clindamycin. Two of these six carried the *ermA* gene, 2 had *ermC* sequences and the other two had both, the *ermB* and the *ermC* genes (Table 4). None of six carried *mrsA* or *mrsB* genes. The resistance coding sequences were present in five strains carrying *seg* and *sei* or *seh* genes, but also in five isolates without any ET coding genes.

Pulsed Field Gel Electrophoresis results

The *Sma*I enzyme macro restriction generated 9 to 12 bands from each genome. When analyzing the banding patterns of the 52 ET genes- positive isolates, a wide variety was found and more than 20 PFGE-types were defined (Figure 1). Nine clusters with two or more PFGE-types each were identified, including 22 isolates in total. Different gene types were similarly distributed among the analyzed PFGE-types. No specific ET genotype predominated in any defined cluster. It is noteworthy that there is a cluster including the strains carrying many ET genes (one isolate with five genes, one with four genes and one with three gene types).

Two strains, cultured from one cheese and its corresponding grocery, showed the same banding pattern with 100% matching, and were considered identical, as was another pair of isolates originated in a single food premise and carrying the same ET gene types. When grouping ET coding strains by gene types, banding pattern similarities were lower than 80%.

When examining all of the isolates (with and without ET genes), more than 50 PFGE-types could be defined. There was an outstanding cluster grouping 10 isolates of similar origin, all of them coming from food premises. Most of them (eight out of 10) did not carry any ET coding gene. Somehow, this group showed different susceptibility phenotypes, and the ones sharing resistance characteristics carried different resistance genes. There was a smaller cluster including only five isolates that had also in common the absence of any of the analyzed ET genes; they were all susceptible to all tested antimicrobials, but they did not share a common origin.

MLST results

Four STs were identified from the five studied isolates: two ST 72, one ST 8, one ST 88 and one newly defined type with a particular allele combination that had not been previously described; it was thus included in the pubmlst database, and ST 3840 was assigned for its identification[18].

Both ST 72 strains were recovered from food premises: one of them was resistant to methicillin and carried the *seg* and the *sei* genes, whereas the other strain was susceptible to methicillin and beared *seb*, *sec sed*, *seg* and *sei* DNA sequences. The ST 8 strain, derived from foodstuff presumptively involved in a foodborne outbreak, carried

Table 4 - Characteristics of antibiotic-resistant strains and of selected isolates analysed by MLST.

Isolate	Origin	ST	Toxin genes	Susceptibility profile	Resistance genes
A 02	Human handler	--	*seg*	MSSA E-Cli	*ermA*
A 08	Human client	--	--	MSSA E-Cli	*ermC*
A 10	Food	--	*seg, sei*	MSSA E	*msrA, msrB*
A 19	Establishment	--	--	MSSA E-Cli	*ermB, ermC*
A 33	Establishment	--	--	MSSA E-Cli	*ermA*
A 39	Establishment	88	*seg*	MSSA E-Cli	*ermB, ermC*
A51	Establishment	--	--	MRSA	*mecA*
A54	Food	--	--	MSSA E-Cli	*ermC*
A 64	Food	3840	*seg*	MSSA	--
A 67	Establishment	72	*seb, sec, sed, seg, sei*	MSSA	--
A 103	Food	--	*seh*	MSSA E	*msrA, msrB*
A 133	Establishment	72	*seg, sei*	MRSA	*mecA*
A 318	Cheese	8	*sea, seb, sec*	MSSA	--

MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*; E-Cli: erythromycin-resistance plus inducible resistance to clindamycin.

sea, *seb* and *sec* genes and it was susceptible to all tested antimicrobials. The ST 88 strain came otherwise from a

food store, showed inducible resistance to clindamycin, and coded for ET G. Finally, the isolate with the newly assigned
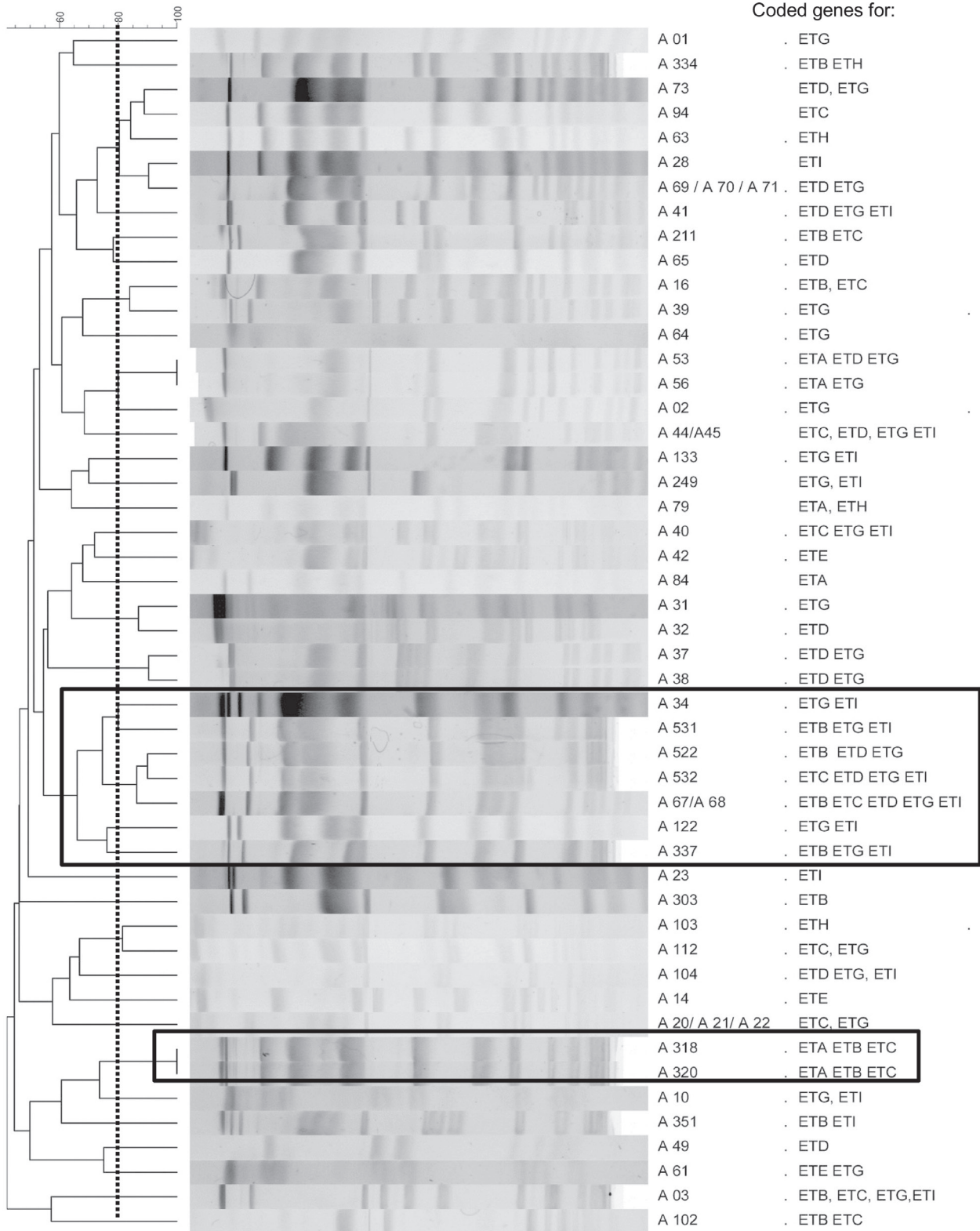

Figure 1 - PFGE profiles of 52 *S. aureus* isolates that carry ET genes. Dotted line = 80% homology; Main box = PFGE-group of isolates with multiple enterotoxin genes; Small box = PFGE-group of isolates from a human disease outbreak.

ST 3840 carried enterotoxin G sequence type and it was susceptible to all tested antibiotics.

DISCUSSION

Foodborne diseases are important causes of human morbidity and mortality, worldwide. In Uruguay, they are subject to compulsory notification. *Staphylococcus aureus* has been identified as the second most common cause of foodborne outbreaks, after *Salmonella*[19,20]. However, established investigation protocols are not usually fulfilled, and medical, epidemiological or laboratory data are frequently missing. Clinical illnesses are usually short, so that patients ignore medical consultations, or medical doctors do not request laboratory exams, hindering the recovery of etiologic agents from outbreaks. This manuscript reports the first systematic investigation of enterotoxins in *S. aureus* isolates obtained from foodstuffs in Uruguay.

Half of the studied bacterial strains were isolated from samples taken from food establishments, from raw material and from food contact surfaces. A similar number was directly cultured from foods. Two closely related isolates were included: one obtained from contaminated cheese, presumptively involved in a small foodborne outbreak, and the second isolate from the corresponding food selling store.

This study found that 54.7% of examined isolates carried genes coding for some of the studied enterotoxins, and that most of them carried more than one ET gene. These results are novel contributions to the knowledge of *Staphylococcus* enterotoxins distribution in Uruguay, South America and the world. The diversity of origins and genetic profiles of positive isolates and the presence of 10% of resistant variants, revealed the potential health risks they represent.

The most frequently found enterotoxin gene was *seg* (alone or in combination) followed by *sei*; *seg* was both prevalent in isolates obtained from foods, and among those recovered from food contact surfaces, tools and raw materials in food establishments. Strains of this last group were more often carriers of ET genes, and coded for more gene types per strain than isolates coming from foodstuffs.

A recent report from South America, performed in Sao Paulo, Brasil, examined more than 2,000 milk samples of bovine mastitis and 285 *S. aureus* strains, searching for the same enterotoxin genes than we did. The researchers found that the most frequently identified gene was *seg*, followed by *seh*[21]. These findings agree with reports on the presence of *S. aureus* enterotoxins in isolates from foods of different regions of the world. In Ethiopia, 51% of 160 *S. aureus* examined isolates from dairy products carried ET coding genes, being *seg* and *sei* the most frequently found, as in our series[22]. An Iranian metanalysis performed in 2017 that included 34 publications on *S. aureus* strains from contaminated foodstuffs showed that 54% of them were enterotoxigenic, ranging from 13 to 83% in different studies. The most frequently found enterotoxins were ETA and ETG among classical and new resistance genes, respectively[23].In our study, two isolates recovered from a single confirmed outbreak carried *sea*, *seb* and *sec* genes. In published studies of isolates from foodborne outbreaks, classical enterotoxins have been frequently reported as the causes of disease. A recent report from China, including 37 *S. aureus* isolates recovered in 2015 and 2016 from four foodborne outbreaks, described that 12 strains (32%) carried enterotoxin genes, being *sea* the most frequently found (81 %), followed by the combinations *sec, se/l* and *seg, sei, se/m, se/n, se/o, se/u* forming an operon encoded by the enterotoxin gene cluster 2 (*egc*2)[24,25]. Another study, published in 2015, reviewed 76 staphylococcal foodborne outbreaks occurred in France during the last 30 years. Eleven classical and new genes were investigated in 103 examined strains, of which 87 carried one or more ET coding genes. The most frequent gene was *sea*, followed by *seg* and *sei*[26]. Also *sea* (together with *sed*) or *seb* were the classical enterotoxin genes found in *S. aureus* isolates involved in food poisoning outbreaks studied in Argentina[27,28]. Classical and new ET genes were found in an outbreak-associated strain of *S. aureus* described in Central Italy during a post-earthquake reconstruction[29]. However, several recent outbreak studies reported that the causing agent was *Staphylococcus aureus* carrying genes of *egc* cluster (*seg, sei, se/m, se/n, se/o* and *se/u*) without classical ET coding genes. All of these outbreaks shared the same clinical case presentation with an early onset of symptoms after consuming a common food, and the identification of a significant number of *S. aureus* colonies, either in the involved food or in the patients' feces. Classical enterotoxins or their coding genes were not found in any examined isolate, in spite of the thorough investigation perfomed by several techniques (PCR, ELISA)[30,31]. These data confirm the importance of new enterotoxins as the cause of foodborne disease, and the need for further research on this subject, especially on the actual presence and concentration of new enterotoxins in foodstuff contaminated with *S. aureus* that carry new ET coding genes. This is not an easy approach, although commercial detection kits are presently available for the detection of classical and even some newly described toxins. Detection rates are influenced by multiple environmental and metabolic parameters, so the identification of toxigenic strains

in food environments that have not actually produced traceable enterotoxins in foodstuffs or do not produce them in laboratory conditions may still be relevant on epidemiological grounds[32,33]. Regarding the antimicrobial susceptibility of recovered strains, it should be noted that two isolates were resistant to methicillin, other two showed resistance only to erythromycin, and six had the most commonly found resistance profile of resistance to erythromycin and capacity to inducible resistance to clindamycin. Two of these isolates were obtained from human samples, three from food establishments, and one directly from contaminated food. The high antimicrobial susceptibility of most recovered strains could be explained by their origin: although contamination of foods and food establishments can derive from food handlers, it is most often caused by contaminated or poorly preserved raw food materials, which at the moment, do not seem to carry highly resistant strains. In line with our data, international reports described inducible rates of resistance to clindamycin of 3% or lower in *S. aureus* obtained from raw milk or derived dairy products[34,35]. This type of resistance has not been extensively studied in local samples of raw foodstuffs of animal origin. However, considering isolates taken in Montevideo, from children's skin and soft tissues infections, inducible resistance to clindamycin was initially described in 30% of the methicillin-resistant isolates[36], and a more recent study of 73 *S. aureus* isolates (most of them susceptible to methicillin), showed that the prevalence of this resistance has declined to only 4%, a similar rate to the frequency observed in isolates of the present study[37] Both MRSA isolates harbored *mecA*, which is an important observation because methicillin-resistant strains bearing the *mecC* genotype have been increasingly described in association with bovine mastitis, animal carriage and human diseases related to occupational exposure[38]. *Sma*I-PFGE defined a higher number of PFGE-types in non-enterotoxigenic strains than among ET gene positive ones. Not surprisingly, there was no predominant PFGE-type among the studied isolates. The large number and diversity of the patterns (more than 50 types) could be explained by the varied and independent origin of isolates and by the extended period of sampling. However, some clusters could be defined, and the collection of gathered molecular data on the locally circulating strains in foods and food establishments may constitute the basis for comparison in future surveillance studies, or in new *S. aureus* foodborne outbreaks studies. Special attention should be paid to the mentioned group of potentially pathogenic isolates that harbour numerous ET genes.

MLST is a trustworthy classification tool, because it is based on sequencing of seven highly conserved genes, providing information on the lineage of examined strains, broadening our understanding on the long-term epidemiology of staphylococci or other bacteria. The obtained data can be easily shared by different laboratories as there is a wide online database that contains the allelic profile of thousands of strains, helping the unification, standardization and interpretation of results. The five *S. aureus* isolates subjected to MLST were selected for having some special characteristics. The first one was the strain associated with a small outbreak, that beared *sea*, *seb* and *sec* genes. The other four isolates carried *seg*, the most frequently found ET gene. One of them coded for four additional enterotoxins, another one was methicillin-resistant, and the other two were susceptible to methicillin, but one of them was resistant to erythromycin and showed an inducible clindamycin resistance. Our MRSA was ST 72 and carried *seg* and *sei* genes, as did MRSA isolates from foods studied in Asian countries[23,39], but the second ST 72 strain was susceptible to antimicrobials and coded for three additional enterotoxins. ST 8 and ST 72 strains from neighbouring South American countries (Argentina, Brasil, Chile, Paraguay) and the prevalent ST8 USA300 had already been recorded in pubmlst database. They were both founder types of group 2 (they shared at least five alleles). The new ST 3840 is part of group 5 and is a SV of founder ST 15, differing only in their *pta* allele. ST 88 strains other than ours have not been included in pubmlst database by other South American countries. When selecting outbreak-associated strains, regardless of their geographical origin for BURST analyses, ST 88 from Vietnam and several ST 72 isolates from South Korea and Vietnam were found, revealing the potential pathogenic role of these locally circulating types. Isolate A 133 (ST 72, *seg* and *sei* positive, MRSA) seems very similar to the prevalent Korean clone described in association with food-borne disease[39].

The frequent presence of enterotoxin coding genes in *S. aureus* strains from foostuffs, raw food materials and establishments, and the identification of one of these strains in association with a foodborne outbreak are facts that have not been previously documented in our country. They are a matter of concern regarding the safety of commonly consumed foods. They also reveal the need for a routine surveillance and diagnostic procedures to evaluate and avoid the risks of disease caused by classic enterotoxins as well as by new enterotoxins that are often recognized as potential food contaminants. Further research is needed and would benefit from the improvement of sample gathering of confirmed outbreaks, through a better education of clinicians, health staff, laboratory workers and the general population so as to optimize the diagnosis, recovery and characterization of relevant *S. aureus* isolates.

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