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Enterotoxins of EGC Cluster Prevalence in *Staphylococcus aureus* Collected from Clinical Samples of Paraguayan Children

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Abstract: The ability of *S. aureus* to infect humans and cause a wide range of infections so efficiently is a consequence of the many variant virulence factors that it can produce. Superantigens (SAgs) make up a group of them and include staphylococcal enterotoxins (SE). The main objective of this study was to detect enterotoxin gene cluster (EGC) in *S. aureus* isolates that caused infections in Paraguayan children in the year 2017, through an observational and cross-sectional study. The detection of the EGC including the *seg, sei, sem, sen, seo,* and *seu* genes, was carried out by end-point PCR. 181 *S. aureus* isolates were collected, most of them caused skin and soft tissue infections (SSTI, n = 140, 77%) and came from children under 6 years 50% (n = 90). At least one SE gene was detected in 96% (174/181) of isolates and 24% (44/181) carried the entire EGC. An association between methicillin resistance and *seg, sei, seo,* and *seu* gene carriage ($p \leq 0.05$) was observed. A high prevalence of EGC was detected in *S. aureus* isolates collected from infected children attending the main health care centers in Paraguay, its importance is due to the potential of these toxins to aggravate staphylococcal infections.

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Key Contribution: A high prevalence of EGC was detected in *S. aureus* isolates collected from infected children attending the main health care centers in Paraguay, its importance is due to the potential of these toxins to aggravate staphylococcal infections.

1. Introduction

S. aureus is a human pathogenic bacteria that causes a wide range of infections in both, hospital and community settings. Approximately one-third of the population worldwide carries *S. aureus* in nasal cavities, and this asymptomatic colonization is known to be a risk factor for further infections [1]. This bacteria is considered a Serious Threat Level microorganism by the US Centers for Disease Control and Prevention (CDC) and a High Priority by the World Health Organization (WHO), so understanding the infectious process it causes, the discovery of new therapeutic options and its epidemiological surveillance are of extreme urgency, as they would allow concentrated efforts to prevent its spread, as well as the risk of further disease progression [2,3].

The ability of *S. aureus* to infect humans and other animals so efficiently is a consequence of the large number of virulence factors it can produce and release into the tissue in which it is developing infection [4]. Superantigens (SAgs) make up a class of virulence factors and include the toxin that causes toxic shock syndrome (*tsst-1*) and staphylococcal enterotoxins (SEs), associated with foodborne illnesses (FBDs). These SAgs are linked to several conditions including sepsis, endocarditis, and pneumonia, as well as promoting bacterial survival during infection [5,6].

FBDs cover a wide spectrum of ailments and constitute a growing public health problem worldwide, caused by the ingestion of food contaminated by microorganisms or chemicals. *S. aureus* is the most frequent etiological agent among severe food poisoning caused by SE-producing strains,

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which can cause gastroenteritis and have significant importance due to their detection in the food supply chain, by contamination introduced by handlers or by the use of contaminated raw material [7–9]. The impact of SE expression by *S. aureus* on human health lies in that even whether or not they are associated with food poisoning, they can be "markers" of certain specific virulent clones of *S. aureus* and are considered superantigens that activate T cells [10].

Previously only five SEs, SEA to SEE, were known, however, in recent years new SEs and toxins similar to them have been detected [11]. There is a group of six SEs called the Enterotoxin Gene Cluster (EGC), which encodes SEs G, I, M, N, O, and U and are generally associated with long-term mucosal colonization. While these toxins have been associated with asymptomatic colonization, experimental studies in rabbits show that EGC cluster SAgs may be crucial for the development of certain types of infections such as endocarditis or respiratory infections [6].

The role of these virulence factors was exposed in Paraguay in 2009 when there was an outbreak of food poisoning associated with the consumption of ultra-pasteurized milk contaminated with *S. aureus* producing enterotoxins C and D, with the serious outcome of the death of a minor infant [12]. In this context, and given the possibility of carrying other enterotoxin-coding genes in *S. aureus* isolates and their potential risk of causing FDBs and "markers" of virulent clones, our research group aimed to expand the ability to detect a greater number of enterotoxin-coding genes. The importance of this innovation would be the potential use of molecular methods for the characterization of *S. aureus* in future outbreak situations, food poisoning, and carriage control in food handlers, as well as in several epidemiological studies.

The present study aimed to detect genes coding for EGC SEs G, I, M, N, O, and U in *S. aureus* isolates that caused infections in Paraguayan children in 2017.

2. Materials and Methods

To achieve the postulated objectives, a descriptive, cross-sectional, observational, descriptive study was carried out with a non-probabilistic sampling of consecutive cases.

The *S. aureus* isolates analyzed in the present study came from the Biobank of the Instituto de Investigaciones en Ciencias de la Salud (IICS-UNA), stored in 15% BHI-glycerol medium at −80 °C and. Were isolated from different types of biological samples collected from Paraguayan children aged 0 (zero) to 17 (seventeen) years who had infections, were collected and processed in three reference hospitals in Asunción and the Central Department of Paraguay: Hospital de Clínicas (HCL, FCM-UNA), Instituto de Previsión Social (IPS) and Hospital General Pediátrico Niños de Acosta Ñú (HGP) - Ministerio de Salud Pública y Bienestar Social del Paraguay (MSPyBS), in prior research projects.

All isolates had clinical and epidemiological data such as biochemical identification, antibiotic susceptibility, type of material, date of collection, clinical picture, age, and sex of the patient from whom they were isolated, provided by the microbiology laboratory of the health care centers where the samples were collected. The children from whom the isolates were taken were classified according to age: infants (0-23 months), preschool (24–71 months), school children (6–12 years), and adolescents (>12 years).

2.1. Sample analysis and processing

2.1.1. Phenotypic characterization of isolates

Biochemical identification and antibiotic susceptibility analysis were performed in each microbiology laboratory in which the isolates were collected. They were performed according to standard criteria established in the CLSI (Clinical and Laboratory Standards Institute) guidelines 2017 version [33].

Biochemical identification included culture on ram's blood agar for beta-hemolysis detection and colony morphology analysis, as well as Gram staining and biochemical tube assays with catalase and coagulase.

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Antimicrobial susceptibility profiles of all strains were determined by Kirby-Bauer diffusion disk assays using Oxoid/BBI disks and the automated Vitek® system (BioMérieux, La Balme, French). The following antibiotics were tested in the antibiotic susceptibility assay: cefoxitin (FOX), penicillin (PEN), gentamicin (GEN), ciprofloxacin (CIP), rifampicin (RIF), trimethoprim/sulfamethoxazole (SXT), erythromycin (ERI), clindamycin (CLI), tetracycline (TET) and vancomycin (VAN). In addition, the D-test was performed on all MRSA isolates that were sensitive to clindamycin and resistant to erythromycin.

2.1.2. Extraction of genetic material

Isolates identified as *S. aureus* by phenotypic methods were plated on TSA agar (Tryptone Soy Agar, Difco, Le Pont de Claix, France) and incubated at 35 °C for 24–48 h in the presence of 5% CO2 for subsequent extraction of genomic DNA, using a commercial kit (Wizard Genomic, Promega, Madison, USA), following the manufacturer's instructions.

2.1.3. Detection of Virulence Factor Coding Genes

To amplify the genes coding for enterotoxins G, I, M, N, O, and U, the reaction conditions for individual PCR were standardized, using oligonucleotides described by Jarraud et al. 2002 for the genes *seg*, *sei*, *sem*, *sen*, and *seo* [34] and the one described by Holtfreter et al. 2004 [35]. The above assays were performed at a final concentration 1× Buffer (Invitrogen, Thermo Fisher Scientific, California, USA), 0.03 U/µL Taq polymerase (Invitrogen, Thermo Fisher Scientific, California, USA), 1 µM oligonucleotides (Macrogen, South Korea), 3 mM $MgCl_2$ (Invitrogen, Thermo Fisher Scientific, California, USA) and 0.4 mM dNTP (Promega Corporation, Madison, Wisconsin, USA).

For each PCR reaction, 1.5 µL of template DNA was used in a total reaction volume of 15 µL. The oligonucleotides used were synthesized by Macrogen Inc. (Seoul, Korea) and amplification was performed in Veriti thermal cyclers (AppliedBiosystems, Thermo Fisher Scientific, USA). The optimal conditions for the PCR reaction were: initial denaturation 95 °C - 5 min, 35 cycles of denaturation (95 °C for 30 s), banding (54–60 °C for 30 s), and extension (72 °C for 30 s) and finally a final extension cycle of 72 °C for 5 min. The banding temperature was variable according to the amplification gene: *seM* (54 °C), *seG*, *seI*, *seN* (58 °C), and *seO-seU* (60 °C).

Positive and negative controls were included for each of the PCR amplification reactions.

2.2. Data analysis

The isolate data obtained from the epidemiological, microbiological, and molecular cards were entered and stored in an electronic spreadsheet and statistical calculations were performed using Microsoft Excel version 2013 and Epiinfo Version 7.2.2.6 software. Descriptive statistics were used to refer to the characteristics of the population, gene frequency, and detected profiles.

3. Results

3.1. Epidemiological aspects

We included 181 *S. aureus* isolates causing invasive infections (n = 41, 23%) and skin and soft tissue infections (IPPB, n = 140, 77%) in Paraguayan children aged 15 days to 17 years, collected from January 2017 to February 2018. Fifty percent (n = 91) of the isolates analyzed were from the IPS and 50% (n = 90) of them were under 6 years of age (Table 1).

Table 1. Clinical and demographic characteristics of patients with *S. aureus* isolates (n=181).

Feature		*S. aureus*, n (%)
Biological sex	Male	110 (61)
	Female	71 (39)
Age range	Infant (0–23 meses)	43 (24)
	Pre-school (24–71 meses)	47 (26)

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	School (6–12 años)	60 (33)
	Adolescent (>12 años)	31 (17)
Period of isolation	Spring-Summer	133 (73)
	Autumn-Winter	48 (27)
Hospital	IPS	91 (50)
	HGP	79 (44)
	HCL	11 (6)
Biological material	Purulent secretion	150 (83)
	Blood	26 (14)
	Fluids (CBF, Pleural, Peritoneal)	5 (3)

Abbreviations: IPS: Instituto de Previsión Social, HGP: Hospital General Pediátrico Niños de Acosta Ñú, MSPyBS, HCL: Hospital de Clínicas, Facultad de Ciencias Médicas, UNA.

The relationship between the occurrence of infections according to the seasonal period was calculated and a statistically significant difference was found between the occurrence of SSTI in Spring-Summer ($p = 0.0004$, $p \leq 0.05$ Chi-square or Fisher's exact test as appropriate).

The complete antibiotic susceptibility profile and according to susceptibility to methicillin are shown in Table 2. 100% of the isolates tested were found to be sensitive to vancomycin. Inducible resistance to clindamycin (Dtest positive) was observed in 21% of the isolates analyzed, 84% of which were methicillin-resistant *S. aureus* (MRSA). A statistically significant difference was observed between methicillin susceptibility and tetracycline resistance ($p \leq 0.05$ Chi-square or Fisher's exact test as appropriate). Intermediate susceptibilities to erythromycin (n=3, 2%), gentamicin (n = 3, 2%), rifampicin (n = 5, 3%), and ciprofloxacin (n = 5, 3%) were also observed.

Table 2. Antibiotic susceptibility of *S. aureus* isolates (n = 181).

	***S. aureus* n = 181 (100%)**		**MRSA n=144 (80%)**		**MSSA n = 37 (21%)**		***p****
ATB	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	
PEN	1 (1)	180 (99)	0 (0)	144(100)	1 (3)	36 (97)	0.2044
CLI	137 (76)	44 (24)	108 (75)	36 (25)	29 (78)	8 (22)	0.8304
ERI	136 (75)	42 (23)	107 (74)	34 (24)	29 (78)	8 (22)	0.8307
GEN	161 (89)	17 (9)	127 (88)	15 (10)	34 (92)	2 (5)	0.5302
RIF	172 (95)	4 (2)	135 (94)	4 (3)	37 (100)	0 (0)	0.5805
CIP	174 (96)	2 (1)	137 (95)	2 (1)	37 (100)	0 (0)	1.0000
TET	179 (99)	2 (1)	144(100)	0 (0)	35 (95)	2 (5)	**0.0409**
TMS	179 (99)	2 (1)	142 (99)	2 (1)	37 (100)	0 (0)	1.0000

Abbreviations: MRSA, Methicillin-resistant *S. aureus*; MSSA, Methicillin-susceptible *S. aureus*; S, Susceptible; R, Resistant; ATB, antibiotic; PEN, Penicillin; GEN, Gentamicin; ERI, Erythromycin; CLI, Clindamycin; TET, Tetracycline; CIP, Ciprofloxacin; RIF, Rifampicin; TMS, Trimethroprime-sulfamethoxazole. *Chi-squared or Fisher's exact test.

3.2. Molecular detection of EGC cluster genes

At least one enterotoxin-coding gene was detected in 97% (176/181) of the isolates under study and 23% (42/181) carried the complete EGC cluster (Table 3). A statistically significant difference was observed between methicillin resistance and *seg*, *sei*, *seo*, and *seu* gene carriage ($p \leq 0.05$ Chi-square or Fisher's exact test as appropriate).

Table 3. Association between carriage of virulence factor encoding genes with *S. aureus* susceptibility or resistance to methicillin.

Virulence factor gene	***seg***	***sei***	***sem***	***sen***	***seo***	***Seu***
***S. aureus* n = 181 (100%)**	161 (89)	152 (84)	113 (62)	85 (47)	146 (81)	141 (78)

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MRSA n = 144 (80%)	133 (92)	127 (88)	91 (63)	73 (51)	121 (84)	121 (84)
MSSA n = 37 (20%)	28 (76)	25 (68)	22 (60)	12 (32)	25 (68)	20 (54)
p*	**0.0076**	**0.0047**	0.7060	0.0641	**0.0344**	**0.0002**

*Chi-squared or Fisher's exact test.

The most frequent toxin in isolates causing invasive infections was seg (Table 4), followed by *seo* and *sei*. Seventeen percent (7/41) of the isolates with invasive infections carried the complete group of toxins studied and 98% (40/41) carried at least one of the genes studied. A statistically significant difference was observed between the occurrence of invasive infections and the carriage of *sei* and *seu* genes ($p \leq 0.05$ Chi-square or Fisher's exact test as appropriate).

Table 4. Association of the carriage of virulence factor encoding genes with the type of infection caused by the microorganism.

Virulence factor gene	*seg*	*sei*	*sem*	*sen*	*seo*	*Seu*
***S. aureus* n=181 (100%)**	161 (89)	151 (84)	113 (62)	85 (47)	146 (81)	141 (78)
Invasive n=41 (23%)	35 (85)	30 (73)	24 (59)	17 (41)	32 (78)	24 (59)
SSTI n=140 (77%)	126 (70)	121 (67)	89 (64)	68 (49)	114 (81)	117 (84)
p*	0.4035	**0.0501**	0.5856	0.4789	0.6554	**0.0012**

*Chi-squared or Fisher's exact test.

3 (2%) of the MRSA isolates analyzed produced invasive infectious conditions that led to the death of the patient (between 2 months and 7 years), carrying the *sem* (3/3), *seg* (2/3), *sei* (2/3), *seo* (2/3) and *seu* (1/3) genes.

Using the present study, the molecular detection of genes belonging to the EGC cluster: *seg, sei, sem, sen, seo*, and *seu* in isolates of *S. aureus* causing infections in Paraguayan children was achieved.

4. Discussion

S. aureus is a pathogenic microorganism equipped with a large repertoire of virulence factors, including several toxins that have superantigenic properties (SAgs), which often play a significant role in the development of various diseases such as toxic shock syndrome (TSS), staphylococcal foodborne diseases (SFBD), scalded skin syndrome, endocarditis, and sepsis, all potentially fatal [1,13].

The post-genomic era of *S. aureus,* with the study of publicly available genomic sequences, has further elucidated the existing knowledge on virulence and resistance of clinical isolates, revealing the presence of previously unknown virulence factors. In this context, the genomic analysis of a dozen *S. aureus* isolates belonging to the largest biobank of its kind in Paraguay [14], allowed our research group to identify in the genome of these isolates genes encoding virulence and antibiotic resistance factors never studied before in our country, such as those of the enterotoxins belonging to the EGC cluster (*seg, sei, sem, sen, seo* and *seu*). This important qualitative leap opened up a range of new possibilities for new research projects and assays to be developed to further deepen and deepen the existing knowledge on the epidemiology of circulating *S. aureus* in Paraguay [1,15].

The SAgs encoded in the EGC cluster (G, I, M, N, O, U) are currently considered to be the most prevalent staphylococcal toxins among clinical and colonizing isolates, present in 50-70% of nasal carriers, and their expression may be crucial for the development and aggravation of certain infections. Although initially associated with asymptomatic colonization, several studies show that EGC cluster SAgs may be crucial in the development of respiratory infections and endocarditis [6,16].

In the present study, high carriage of genes encoding enterotoxins belonging to EGC cluster G (89%), I (84%), O (81%), U (78%), M (62%), and N (47%) was detected in *S. aureus* from clinical infectious conditions, mostly (77%) skin and soft tissue infections and 23% invasive infections such as sepsis, necrotizing pneumonia, osteomyelitis, and cystic fibrosis (CF), which provides a further indication that the SAgs encoded in the EGC cluster could be involved in the development of staphylococcal infectious processes and not only accompany the carriage of the microorganism in the human host [6,16].

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Although most of the samples in our study came from SSTI, among the isolates that caused invasive infections there was also a high frequency of carriage of the SE analyzed, and a statistically significant difference was observed between carriage of the *sei* and *seu* genes and the occurrence of invasive conditions such as pneumonia, osteomyelitis, cystic fibrosis, and sepsis.

Several epidemiological studies highlight the high prevalence of specific SAgs in *S. aureus* isolates from patients with infective endocarditis (IE) and chronic respiratory diseases such as CF, specifically carrying *tsst-1, sec* genes and those belonging to the EGC cluster (*seg, sei, sem, sen, seo* and *seu*) [6,17,18].

Experimentally, it has been demonstrated in rabbits infected with *S. aureus,* that deletion of the *tsst-1* and EGC cluster genes increases patient survival, with differential effects, since the *tsst-1* gene occurs at much higher levels than those of the EGC cluster, thus contributing to higher mortality through the development of TSS and/or to a more rapid and complicated progression of the infection generally accompanied by lethal complications such as heart failure and stroke so that its deletion leads to a more marked decrease in lethality. Even so, diseases such as infective endocarditis progress in the presence of EGC cluster enterotoxins, even without the systemic or local effects of TSST-1, evidence that the same is also responsible for the aggravation of the infectious picture, but on a smaller scale due to their level of production [13].

Studies focused on *S. aureus* carriage in patients with allergic rhinitis have shown that these patients are frequently colonized by *S. aureus* and sensitized to its enterotoxins, unlike healthy controls. Likewise, patients with allergic rhinitis and carriers of *S. aureus* have developed more severe allergic symptoms than non-carriers; this is because ES triggers airway inflammation and increases the responsiveness of the airways. Clinically, nasal carriage of *S. aureus* or IgE to ES has been associated with asthma severity and a higher prevalence of sensitization to more than 125 aeroallergens in both adults and children. Thus, there are indications that *S. aureus* and its toxins are associated with the development and/or severity of these allergic conditions [19].

Other studies conducted by our research group, focused on the detection of coding genes of SEs A, B, C, D, and H, frequently associated with foodborne diseases, yielded carriage percentages for these genes lower than 5% [20,21] in samples collected from SSTI in the pediatric population, which reinforces the theory that these SE are strongly related to the production of gastrointestinal infections and those of the EGC cluster are present in all types of clinical samples and even in asymptomatic carriers [22].

The association observed between methicillin resistance (MRSA) and the carriage of *seg, sei, seo,* and *seu* genes could be a result of gene linkage, which occurs by physical proximity concerning the distance in base pairs that exists between them in the genome, but it is still a controversial issue that is still under study. The existing evidence indicates that the *seg, sei, sem, sen, seo,* and *seu* genes belonging to the EGC cluster, as well as the Panton-Valentine leukocidin *lukDE* gene, are located in the *vSaβ* genomic island, unlike the *mecA* gene, coding for methicillin resistance, located in the genetic island known as the *SCCmec* cassette. What is not yet known for certain is the proximity in the genome of these islands [6,23].

Several studies have focused on identifying these genomic islands to understand the mechanisms underlying the emergence of complex patterns of antibiotic resistance and virulence mediated by horizontal gene transfer. It is important to emphasize that these genomic islands and the other mobile genetic elements such as phages, transposons, and chromosomal cassettes together constitute the auxiliary or accessory genome of the microorganism. Previous studies have reported that the genomic core is composed of approximately 95% or more of all *S. aureus* genes, and have revealed recombination hotspots for mobile elements even within the core genome of *S. aureus.* This genetic recombination, in addition to increasing antibiotic resistance, has also collaborated with *S. aureus* for the production of infections and its proliferation in a community setting, as was the acquisition of the Panton-Valentine leukocidin gene by MRSA, which has given rise to community-acquired MRSA (CA-MRSA) [24].

On the other hand, the diversity of MRSA is well known, even among strains belonging to the same clone and circulating in the same geographical region. Despite this, Challagundla et al. in a

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study focused on the phylogenomic classification of MRSA of clonal complex 5, demonstrated relatively low rates of recombination between strains of the same clonal complex: CC5, the second most frequent in Paraguay [23,25]. In that study, they traced the evolution of selected CC5 traits and found that some of the genomic changes occurred before the expansion of the largest currently existing clades and represented convergent instances of evolution. This convergent acquisition of broadly prescribed antibiotic resistance is a recurring theme in the history of successful MRSA clones, the tendency for the number of toxins to decrease with distance from the root of the tree and loss of other virulence factors, findings that suggest that MRSA clones as they acquire greater antibiotic resistance become less virulent, with a consequent increase in the ability to spread geographically [23].

Given the above, the association observed between methicillin resistance (MRSA) and the carriage of *sei*, *seg*, *seo*, and *seu* genes in this study is an issue whose causes should be further explored in future studies that include genomic analysis of the isolates under study, to determine the physical proximity between the genetic islands carrying the genes involved.

The levels of penicillin resistance observed at the general level, whether the isolates were MRSA or MSSA, coincide with other regional and national studies that report levels of penicillin resistance in community *S. aureus* isolates greater than 90% in Latin America [21,25–27]. Regarding the levels of resistance to clindamycin and erythromycin reported in this study (24% and 23%, respectively), a significant increase is seen, compared to reports made by our research group in previous studies in bacterial populations collected under the same conditions and geographical regions, but in different temporalities: in 2016, Guillén et al. reported clindamycin and erythromycin resistance levels of 6.9% for both antibiotics and in 2020, Rodriguez et al. reported clindamycin and erythromycin resistance levels of 15% and 17% respectively [25,27]. Similar results were found by other authors who reported resistance percentages between 12 to 32% to erythromycin and between 9 to 20% in the case of clindamycin, in MRSA isolates associated with the community. The preferential use of clindamycin for the treatment of SSTI produced by MRSA, most in our study is probably the cause that favors the emergence of resistance to it during treatment, as well as the use of empirical treatment schemes not always adjusted to specific microbiological results of the patient [1].

Analyzing the type of clinical picture produced, higher frequencies of isolates causing SSTI were observed (77%) concerning invasive infections (23%); this could be attributed to the characteristics of the stages of life covered by the sampling: breastfeeding, childhood, and adolescence, in which situations such as blows, falls, skin scratches, which damage the epidermis, which is exposed to microorganisms that enter and cause infections, occur more frequently, which is why children are more susceptible to developing this type of infections caused by *S. aureus* [29], to which is added maternal colonization by *S. aureus* during pregnancy, which would increase the likelihood of the child being colonized by this microorganism at birth [30,31]. Half of the samples came from children under six years of age, so the insufficient development of the immune system at this age, added to the dermatitis caused by moisture in the diaper area and the proximity of children in schools and daycare centers could further promote the occurrence of infectious conditions that could be aggravated in certain situations, and could even lead to systemic infections [29,32].

Almost 3/4 of the cases were recorded during the spring-summer months, from September to March in the southern hemisphere, 85% of which corresponded to infectious conditions originating from SSTI. The climatic conditions at this time of the year, characterized by average temperatures above 30°C and relative humidity between 60%-80%, is a favorable combination for the occurrence of skin infections associated with *S. aureus* [21,27]. Although in the autumn-winter months, the number of isolates collected decreased, the trend of higher frequency of isolates causing skin and soft tissue infections was maintained, probably because in Paraguay the winter is very mild, with average temperatures between 16-19°C due to its subtropical climate.

Using the present study, the molecular detection of genes encoding enterotoxins belonging to the EGC cluster, considered a genetic marker of virulent clones, was achieved and it was established that these genes are highly prevalent in *S. aureus* isolates from infectious conditions in children, collected in the main health care centers of Paraguay, the importance of the finding lies in the

potential that these toxins present for the development of serious infectious conditions produced by the bacterium.

It is also important to analyze the relationship between the detected genes and their expression, for which the experimental measurement of the secreted toxin, the protein encoded by the *seg*, *sei*, *sem*, *sen*, *seo*, and *seu* genes, by protein analysis or by measurement of mRNA expression by qPCR, should be carried out.

Therefore, we postulate as perspectives of the present study to analyze the expression of the detected genes that allow correlating the presence or absence of the encoded toxins with their expression, to establish if they are active or not in the infectious process.

5. Conclusions

The molecular detection of genes encoding enterotoxins belonging to the EGC cluster, considered a genetic marker of virulent clones, was achieved and it was established that these genes are highly prevalent in *S. aureus* isolates from infectious conditions in Paraguayan children, their importance lies in the potential that these toxins present for the development of severe infectious conditions produced by the bacterium.

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