



ORIGINAL ARTICLE

Molecular characterization of invasive *Streptococcus dysgalactiae* subsp. *equisimilis*. Multicenter study: Argentina 2011–2012



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Streptococcus dysgalactiae subsp. *equisimilis*;
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Abstract *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) has virulence factors similar to those of *Streptococcus pyogenes*. Therefore, it causes pharyngitis and severe infections indistinguishable from those caused by the classic pathogen. The objectives of this study were: to know the prevalence of SDSE invasive infections in Argentina, to study the genetic diversity, to determine the presence of virulence genes, to study antibiotic susceptibility and to detect antibiotic resistance genes. Conventional methods of identification were used. Antibiotic susceptibility was determined by the disk diffusion and the agar dilution methods and the E-test. Twenty eight centers from 16 Argentinean cities participated in the study. Twenty three isolates (16 group G and 7 group C) were obtained between July 1 2011 and June 30 2012. Two adult patients died (8.7%). Most of the isolates were recovered from blood (60.9%). All isolates carried *speJ* and *ssa* genes. *stG62647*, *stG653* and *stG840* were the most frequent *emm* types. Nineteen different PFGE patterns were detected. All isolates were susceptible to penicillin and levofloxacin, 6 (26.1%) showed resistance or reduced susceptibility to erythromycin [1 *mef*(A), 3 *erm*(TR), 1 *mef*(A) + *erm*(TR) and 1 *erm*(TR) + *erm*(B)] and 7 (30.4%) were resistant or exhibited reduced susceptibility to tetracycline [2 *tet*(M), 5 *tet*(M) + *tet*(O)]. The prevalence in Argentina was of at least 23 invasive infections by SDSE. A wide genetic diversity was observed. All isolates

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◇ The complete list of centers of the National Collaborative Group for the Study of Streptococci and Related Bacteria is included in [Appendix 1](#).

PALABRAS CLAVE

Streptococcus dysgalactiae subsp. *equisimilis*;
Infección invasiva;
Estreptococos del grupo C;
Estreptococos del grupo G

carried *speJ* and *ssa* genes. Similarly to other studies, macrolide resistance (26.1%) was mainly associated to the MLS_B phenotype.

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Caracterización molecular de cepas invasivas de *Streptococcus dysgalactiae* subsp. *equisimilis*. Estudio multicéntrico Argentina 2011–2012

Resumen *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) posee factores de virulencia similares a *Streptococcus pyogenes* y, en consecuencia, produce faringitis e infecciones graves indistinguibles de las generadas por este patógeno clásico. Los objetivos del estudio fueron conocer la prevalencia de SDSE en infecciones invasivas en Argentina, estudiar su diversidad genética, determinar la presencia de genes de virulencia, ensayar su sensibilidad a los antibióticos y conocer los genes de resistencia. Se emplearon métodos convencionales de identificación. La sensibilidad se determinó por difusión, Etest y dilución en agar. Participaron 28 centros de 16 ciudades argentinas. Se obtuvieron 23 aislamientos (16 del grupo G y 7 del grupo C) desde el 1-7-2011 hasta el 30-6-2012. Se registraron 2 muertes en adultos (8,7%). La mayoría de los aislamientos fueron obtenidos de sangre (60,9%). Todos eran portadores de los genes *speJ* y *ssa*. Los genotipos más frecuentes fueron *stG62647*, *stG653* y *stG840*. Se detectaron 19 pulsotipos distintos. Todos los aislamientos fueron sensibles a penicilina y levofloxacina, 6 (26,1%) presentaron resistencia o sensibilidad disminuida a eritromicina (1 *mef*[A], 3 *erm*[TR], 1 *mef*[A] + *erm*[TR] y 1 *erm*[TR] + *erm*[B]) y 7 (30,4%) fueron resistentes o tuvieron sensibilidad disminuida a tetraciclina (2 *tet*[M], 5 *tet*[M] + *tet*[O]). La prevalencia anual en la Argentina fue de al menos 23 infecciones invasivas por SDSE y se observó una amplia diversidad genética. Todos los aislamientos presentaron los genes *ssa* y *speJ*. Como en otros estudios, la resistencia a macrólidos (26,1%) estuvo asociada, principalmente, al fenotipo MLS_B.

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Introduction

β-Hemolytic streptococci are common pathogens that usually cause community-acquired infections.

In general, β-hemolytic streptococci isolated from human beings belong to Lancefield groups A, B, C, G, F, or more rarely, to L¹⁸. Until recently, group A streptococci (GAS, *Streptococcus pyogenes*)⁶³ and group B streptococci (GBS, *Streptococcus agalactiae*)^{45,60} were considered the most important pathogens of this group of microorganisms in clinical settings.

GAS is responsible for a broad spectrum of human infections that result in significant morbidity and mortality, including pharyngitis, scarlet fever, skin and soft tissue infection (SSTI), streptococcal toxic shock syndrome (STSS), septicemia, pneumonia and rarely meningitis¹³. It is estimated that severe GAS diseases lead to more than 500,000 deaths each year *via* infections such as acute rheumatic fever, rheumatic heart disease, post streptococcal glomerulonephritis and invasive diseases⁸.

In contrast, group C and G *Streptococcus dysgalactiae* subsp. *equisimilis* (GCS and GGS) were long considered to be commensal organisms that only rarely cause invasive infections as opportunistic pathogens.

Since the mid-1980s there has been a marked increase in reported invasive group A infections, including cases of STSS in Europe and North America^{15,33,57}.

These fulminant infections were characterized by acute hypotension, shock, multiorgan impairment and death. However, the factors underlying the worldwide resurgence of this pathogen remain unknown¹⁶.

In 1996, Vandamme et al⁶² proposed that a novel subspecies, *S. dysgalactiae* subsp. *equisimilis* (SDSE), was a clinically significant pathogen. This microorganism possesses group C or G antigens (rarely A or L), and exhibits strong β-hemolysis.

Beginning around the year 2000, invasive infections such as bacteremia caused by SDSE as well as those caused by GAS and GBS^{6,12,38,59} have been increasingly reported worldwide.

M proteins of GAS, which are encoded by *emm* genes and form elongated structures on the bacterial surface, play an important role in the pathogenesis of this microorganism. M proteins are known to be critical antiphagocytic constituents of GAS due to their role in resistance to opsonization^{4,14}. GCS and GGS have shown a similar pathogenic pattern to *S. pyogenes*²¹, coincidentally expressing homologs of the M virulence proteins of *S. pyogenes*⁷. Moreover, some strains contain superantigen genes firstly characterized in *S. pyogenes*^{25,29}. As with the *emm* genes of *S. pyogenes*, GCS and GGS homologs have been used for sequence-based typing^{3,17,25}.

We conducted a prospective study to assess the relative prevalence of SDSE *versus* GAS in invasive infections in Argentina during a twelve-month period. The objective of

this study was to determine their epidemiological features, to explore the molecular characteristics of the infecting strains, to study their genetic diversity, virulence genes, antibiotic susceptibility and antibiotic resistance genes.

Materials and methods

All *S. pyogenes* and SDSE isolated from invasive infections in 28 centers of 16 Argentinean cities from July 1, 2011 to June 30, 2012 were studied.

A microbiologist from each hospital was required to complete a data sheet of the isolates. During the study period, each laboratory collected one isolate per patient in accordance with the case definition. Isolates were appropriately sent to the reference center where they were preserved in 300 μ l of sheep blood at -80°C .

Definitions

Infections in deep tissues, blood, cerebrospinal fluid, or other liquids obtained by puncture, in which causative organisms were isolated from otherwise sterile samples, were defined as invasive infections.

STSS was defined as an invasive infection due to the presence of a β -hemolytic streptococcus and causing hypotension and two or more of the following conditions: renal impairment, coagulopathy, liver abnormalities, acute respiratory distress syndrome, extensive tissue necrosis and erythematous rash⁵⁵.

Identification

S. pyogenes and SDSE were identified according to the differentiating characteristics described by Ruoff et al⁴⁹. Hemolysis was detected on 5% sheep blood Columbia agar and the observation of chains was performed in Gram smears prepared with drops of overnight cultures in thioglycolate broth. Conventional identification included the following tests: pyrrolidonyl- β -naphthylamide hydrolysis (PYR), bacitracin susceptibility, cell morphology and Gram staining characteristics, Voges-Proskauer, arginine dihydrolase, esculin, starch, sorbitol and trehalose fermentation, α -galactosidase and β -glucuronidase. PYR and bacitracin tests were performed by using Britania[®] disks (Buenos Aires, Argentina). Sorbitol, trehalose, α -galactosidase and β -glucuronidase were tested by using DIATABS[®] commercial tablets (Rosco, Taastrup, Denmark) according to the manufacturer's instructions. Voges-Proskauer, arginine dihydrolase, esculin, and starch tests were performed by standard methods. Moreover, identification was performed using API 20 Strep[®] (Biomérieux Argentina). Identification and grouping were completed by using the latex agglutination method (Slidex Strepto kit[®], Biomérieux Argentina).

emm typing

GAS *emm* DNA fragment preparation was conducted according to the protocol of the Centers for Disease Control and Prevention (CDC)⁹.

As specified by the CDC protocol, primers 1 and 2 were used for amplifying the N-terminal region of the *emm*

gene [primer 1 was 5'-TATT(C/G) GCTTAGAAAATTA-3', and primer 2 was 5'-GCAAGTCTTCAGCTTGTTT-3']. The DNA amplicons were sent for DNA sequencing. The first 160 bases of the 5' end of the *emm* gene were compared with those in the CDC *emm* sequence database (<http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm>). An *emm* type showing more than 98% identity with a CDC reference strain was identified as that particular *emm* type.

PFGE analysis

All isolates were digested by *Sma*I (Promega, Madison, WI, USA). Pulsed field gel electrophoresis (PFGE) was carried out according to the previously described protocol¹⁰.

The digested fragments of DNA were separated by agarose gel 1.2% (TBE 0.5X) in the CHEF-DR III System (Bio-Rad, Hercules, CA, EE.UU.) for 22 h (0.5–40 s pulses at 6 V/cm, 120 $^{\circ}\text{C}$).

The genetic relationship between the bacterial strains was evaluated based on the levels of similarity among the *Sma*I PFGE patterns. A dendrogram was constructed using the unweighted-pair group method with arithmetic mean (UPGMA) algorithm using BioNumerics software version 6.0 (Applied Maths, Kortrijk, Belgium).

Superantigen (SAg) gene detection

The isolates were tested for the presence of SAg genes, including *speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speI*, *speJ*, *ssa*, and *smeZ*²⁵.

Antimicrobial susceptibility tests

Disk diffusion tests were performed by the Bauer and Kirby method according to CLSI guidelines with 5% sheep blood Mueller-Hinton agar¹¹.

Testing disks containing penicillin (PEN 10 U), erythromycin (ERY 15 μ g), clindamycin (CLI 2 μ g), tetracycline (TET 30 μ g), ofloxacin (5 μ g), pefloxacin (5 μ g), norfloxacin (10 μ g) and levofloxacin (5 μ g) were from Britania[®] Laboratories (Buenos Aires, Argentina). Incubation was performed at 35 $^{\circ}\text{C}$ for 24 h in ambient air. *Streptococcus pneumoniae* ATCC[®] 4961, *Staphylococcus aureus* ATCC[®] 25923, *Escherichia coli* ATCC[®] 25922 and *Pseudomonas aeruginosa* ATCC[®] 27853 were used as reference strains.

The agar dilution method was used for susceptibility testing of five antibiotics (PEN, ERY, CLI, TET and LEV) with 5% sheep blood Mueller-Hinton agar plates according to CLSI guidelines¹¹. Antibiotics were gently provided by the Servicio de Antimicrobianos, INEI-ANLIS "Dr. Carlos G. Malbrán", Buenos Aires. *Staphylococcus aureus* ATCC[®] 29213 and *Enterococcus faecalis* ATCC[®] 29212 were used as reference strains.

Phenotypic expression of MLS resistance

When performing the disk diffusion tests, blunting of the CLI inhibition zone near an ERY disk placed at 12 mm from the edge of the CLI disk, indicated an inducible type of

resistance to macrolides, lincosamides, and streptogramin B (MLSB), while no blunting indicated the probability of the efflux-mediated M resistance phenotype (resistance only to 14- and 15-membered macrolides). Resistance to both CLI and ERY indicated a constitutive type of MLS_B resistance⁵⁰.

Detection of different macrolide and tetracycline resistance genes

Genotypic characterization of antimicrobial resistance genes was carried out by multiplex PCR [*erm*(B), *erm*(TR), *mef*(A), *tet*(M) and *tet*(O) genes]. Methods used to detect antibiotic resistance genes have been previously described^{27,41}.

Results

Epidemiological, clinical and demographic characteristics of the patients with SDSE invasive disease

One hundred and eleven invasive infections due to groups A, C, or G β -hemolytic streptococci were recorded. Eighty eight of them (79.3%) were identified as *S. pyogenes*, 16(14.4%) as GGS and 7(6.3%) as GCS. The annual prevalence in Argentina (2011–2012) was of at least 23 SDSE invasive infections.

Most cases of SDSE invasive infections (65.2%) were diagnosed in Buenos Aires City and its surroundings (n: 9 cases in Ciudad Autónoma de Buenos Aires, n: 4 in San Martín, and n: 2 in Lanús). The rest of the cases were detected in Mar del Plata (2), Bahía Blanca (1), General Pico (La Pampa) (2), Santa Fe (2) and Concordia (Entre Ríos) (1). STSS was recorded in 11 cases (9 GAS, 1 SDSE group C and 1 SDSE group G) and mortality was 6.3% (4 adults and 1 child died as a consequence of a *S. pyogenes* infection and 2 adults died of GGS SDSE infections).

Most of the SDSE isolates were obtained from blood (60.9%). Other foci of infection were osteoarticular (21.7%) and soft tissue (17.4%). Only GAS was isolated from pleural effusion (n: 5) and ascites (n: 3).

The total number of patients was 111. Fifty-four of them were adults (≥ 16 years old) and fifty-seven were children (<16 years old). With regard to invasive infections by SDSE, nineteen patients were adults and four were children. The median age of the patients was 54 years old (20 months–90 years old) and 69.6% of the patients were male.

Almost 70% (69.6%) of the infected patients presented at least one predisposing condition and 17.4% more than one. The most frequent predisposing conditions for invasive disease were diabetes (n: 4), renal disease (n: 2), arterial hypertension (n: 2) and HIV infection (n: 2).

More than 80% (82.6%) of the patients had been treated with antibiotics. Clindamycin was only administered to 5 patients whereas immunoglobulins were not administered at all. None of the patients who died were treated with clindamycin.

Phenotypic identification

All GCS and GGS isolates obtained from invasive infections (n: 21) were identified by conventional biochemical tests

and API20 Strep. Fourteen of them were isolated from blood, 3 from soft tissue, 2 from bone and 2 from joint-bone fluid.

Table 1 shows the results of the manual biochemical tests and Table 2 summarizes API 20 Strep results with the percentage of positive values obtained from the bibliography. The percentage of positive tests is summarized at the end of both tables.

Six distinct biochemical profiles were obtained using the API 20 Strep identification system: 19% of the strains fermented lactose and the same percentage hydrolyzed esculin, 86% fermented ribose and only 5% fermented glyco-

Genetic diversity

A total of 23 isolates, 16 GGS and 7 GCS were isolated from invasive infections and 11 different *emm* genes were identified. Three types, *stG62647* (n: 4), *stG840* (n: 3) and *stG653* (n: 3) predominated among the 21 *emm* invasive strains typed.

Figure 1 shows the distribution of invasive types. The comparison with the types found in the 1998–1999 Argentinian multicenter study is also shown. All the sequences were compared with the CDC *emm*-sequence-database.

Nineteen distinct PFGE patterns were detected, with no significant relationship among them. The PFGE profiles of invasive strains digested with the restriction enzyme *Sma*I are shown in Figure 2. Strains 11, 13 and 18 exhibited the same pattern and all were *stG62647* type.

Virulence factors

Toxigenic genes *speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speJ*, *smeZ* and *ssa* were analyzed. All the strains only carried *speJ* and *ssa* genes simultaneously.

Antibiotic susceptibility and macrolide and tetracycline resistance gene study

All SDSE isolates showed *in vitro* susceptibility to PEN (MIC₉₀ 0.016 μ g/ml) and to LEV (MIC₉₀ 0.5 μ g/ml).

The percentage of isolates not susceptible to CLI was 19.0% (MIC₉₀ 0.25 μ g/ml), to ERY 26.1% (MIC₉₀ 4 μ g/ml) and to TET 30.4% (MIC₉₀ 32 μ g/ml).

Only one isolate was found to be less susceptible to NOR, PEF and OFL by the disk diffusion method (13 mm, 6 mm and 19 mm, respectively). Breakpoints were only available for ofloxacin¹¹ and 19 mm was inside the category of susceptibility.

Table 3 summarizes the minimum inhibitory concentration (MIC) ranges, MIC₅₀ and MIC₉₀ values of antimicrobial agents for SDSE. Table 4 summarizes the macrolide and TET resistance gene study.

The six isolates with resistance or diminished susceptibility to ERY presented the following phenotypes and genotypes: 1M [*mef*(A)], 2 iMLS_B[*erm*(TR)], and 1 cMLS_B[*erm*(TR)]. Isolate 14 presented two subpopulations: one resistant to ERY and CLI (CIM > 256 μ g/ml; cMLS_B) and another resistant to ERY (CIM > 256 μ g/ml) but susceptible to CLI (iMLS_B phenotype). Isolate 24 was susceptible to ERY

Table 1 Characteristics of *Streptococcus dysgalactiae* subsp. *equisimilis* isolated from invasive infections in Argentina (2011–2012). The percentage of positive tests is summarize at the end of the table

Case	ID	Sample	Group	BA	PYR	VP	ADH	ESC	STR	SBL	TRE	α -GAL	β -GUR
1	1	Blood	G	R	—	—	+	—	—	—	+	—	+
2	19	Blood	G	R	—	—	+	—	—	—	+	—	+
3	37	Blood	C	R	—	—	+	—	—	—	+	—	+
4	39	Blood	G	R	—	—	+	+	—	—	+	—	+
5	42	Blood	G	R	—	—	+	—	—	—	+	—	+
6	59	Bone, SST	C	R	—	—	+	—	—	—	+	—	+
7	67	Joint	G	R	—	—	+	—	—	—	+	—	+
8	69	Blood	G	R	—	—	+	—	—	—	+	—	+
9	73	Blood	G	R	—	—	+	—	—	—	+	—	+
10	89	Blood	G	R	—	—	+	—	—	—	+	—	+
11	93	Blood	C	R	—	—	+	+	—	—	+	—	+
12	143	Blood	G	R	—	—	+	—	—	—	+	—	+
13	179	Blood	C	R	—	—	+	—	—	—	+	—	+
14	187	Blood	C	R	—	—	+	—	—	—	+	—	+
15	195	Blood	G	R	—	—	+	—	—	—	+	—	+
18	227	Blood	C	R	—	—	+	—	—	—	+	—	+
19	235	Joint	G	R	—	—	+	+	—	—	+	—	+
20	237	SST	C	R	—	—	+	+	—	—	+	—	+
21	257	SST	G	R	—	—	+	—	—	—	+	—	+
23	264	SST, DBT foot	G	R	—	—	+	—	—	—	+	—	+
24	266	Bone, SST	G	R	—	—	+	—	—	—	+	—	+
% of positive reactions (n = 21)					0	0	100	19	0	0	100	0	100
					—	—	+	V	—	—	+	—	+

BA: bacitracin, PYR: pirrolidoni-arilamidase, VP: Voges–Proskauer, ADH: arginine dihydrolase, ESC: esculin, STR: starch, SBL: sorbitol, TRE: trehalose, α -GAL: α -galactosidase, β -GUR: β -D-glucuronidase, +: positive, —: negative, V: variable, SST: skin and soft tissue, and DBT: diabetic.

by the disk diffusion method but showed low level resistance to ERY (MIC = 3 μ g/ml) and carried resistance genes *mef*(A) and *erm*(TR).

Two TET resistant strains harbored only the *tet*(M) gene. The other five strains carried both the *tet*(M) and *tet*(O) genes.

Discussion

Invasive SDSE infections included sepsis with an unknown focus, cellulitis, septic arthritis, pneumonia, necrotizing fasciitis, meningitis, infectious endocarditis, STSS, abscesses at sites other than skin, osteomyelitis, and others.

In our study most of the SDSE isolates were obtained from blood (n: 14). The rest of the isolates were obtained from bone, joint fluid or soft tissue (n: 7).

All our strains were correctly identified by both systems (manual and API 20 Strep) as SDSE. The correlation was excellent between the tests with ROSCO tablets (esculin, sorbitol, trehalose, α -galactosidase and β -glucuronidase) and the same biochemical tests in the API 20 Strep panel.

The frequency of invasive SDSE infection has increased in Asia^{5,38}, in Europe^{19,34,36,37,48,53,54}, and in America^{1,6,40} over the years. In particular, since 2003, the number of invasive SDSE infections, including STSS and severe soft tissue infection^{22,26,44}, has gradually increased in Japan each year.

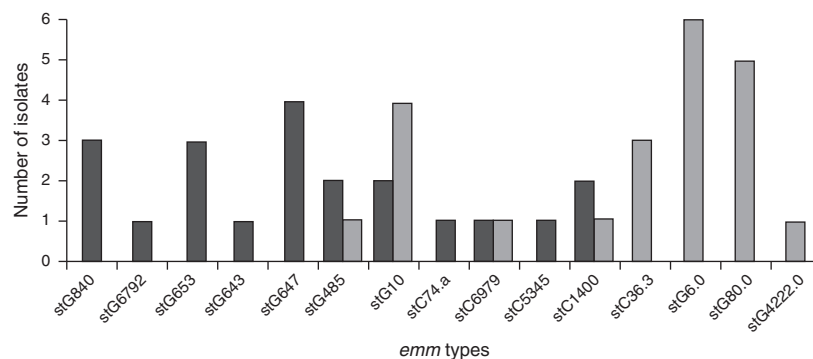


Figure 1 Distribution of invasive *emm* types (n = 21, black bars) and comparison with types found in the Argentinean multicenter study 1998–1999 (n = 22, gray bars).

Table 2 Phenotypic characteristics determined by the API 20 Strep system for *Streptococcus dysgalactiae* subsp. *equisimilis* isolated from invasive infections in Argentina (2011–2012). The percentage of positive tests is summarize at the end of the table and results with the percentage of positive values obtained from bibliography (identification table API 20 strep V 7.0)

Case	ID	Sample	Group	VP	HIP	ESC	PYR	α-GAL	β-GUR	β-GAL	PAL	LAP	ADH	RIB	ARA	MAN	SBL	LAC	TRE	INU	RAF	AMD	GLYG	HEM	Profile
1	1	Blood	G	–	–	–	–	–	+	–	+	+	+	–	–	–	–	–	+	–	–	+	–	+	0 4 6 1 0 1 5
2	19	Blood	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	0 4 6 3 0 1 5
3	37	Blood	C	–	–	–	–	–	+	–	+	+	+	–	–	–	–	–	+	–	–	+	–	+	0 4 6 1 0 1 5
4	39	Blood	G	–	–	+	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	4 4 6 3 0 1 5
5	42	Blood	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	+	+	0 4 6 3 0 1 7
6	59	Bone, SST	C	–	–	–	–	–	+	–	+	+	+	+	–	–	–	+	+	–	–	+	–	+	0 4 6 3 4 1 5
7	67	Joint	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	0 4 6 3 0 1 5
8	69	Blood	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	+	+	–	–	+	–	+	0 4 6 3 4 1 5
9	73	Blood	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	0 4 6 3 0 1 5
10	89	Blood	G	–	–	–	–	–	+	–	+	+	+	–	–	–	–	–	+	–	–	+	–	+	0 4 6 1 0 1 5
11	93	Blood	C	–	–	+	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	4 4 6 3 0 1 5
12	143	Blood	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	0 4 6 3 0 1 5
13	179	Blood	C	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	0 4 6 3 4 1 5
14	187	Blood	C	–	–	–	–	–	+	–	+	+	+	+	–	–	–	+	+	–	–	+	–	+	0 4 6 3 4 1 5
15	195	Blood	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	4 4 6 3 0 1 5
18	227	Blood	C	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	4 4 6 3 0 1 5
19	235	Joint	G	–	–	+	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	4 4 6 3 0 1 5
20	237	SST	C	–	–	+	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	4 4 6 3 0 1 5
21	257	SST	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	0 4 6 3 0 1 5
23	264	SST, DBT foot	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	–	+	–	–	+	–	+	0 4 6 3 0 1 5
24	266	Bone, SST	G	–	–	–	–	–	+	–	+	+	+	+	–	–	–	+	+	–	–	+	–	+	0 4 6 3 4 1 5
% of positive reactions (n = 21)				0	0	19	0	0	100	0	100	100	100	86	0	0	0	19	100	0	0	100	5	100	
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>				0	1	25	1	1	99	1	99	100	97	97	1	1	1	45	99	0	1	98	40	94	

VP: Voges–Proskauer, HIP: hippurate, ESC: esculin, PYR: pyrrolidonyl-β-naphthylamide hydrolysis, α-GAL: α-galactosidase, β-GUR: β-glucuronidase, β-GAL: β-galactosidase, PAL: alkaline phosphatase, LAP: leucinaminopeptidase, ADH: arginine dihydrolase, RIB: ribose, ARA: arabinose, MAN: manitol, SBL: sorbitol, LAC: lactose, TRE: trehalose, INU: inulin, RAF: raffinose, AMD: amigdaline, GLYG: glycogen, HEM: hemolysis, +: positive, –: negative, SST: skin and soft tissue, and DBT: diabetic.

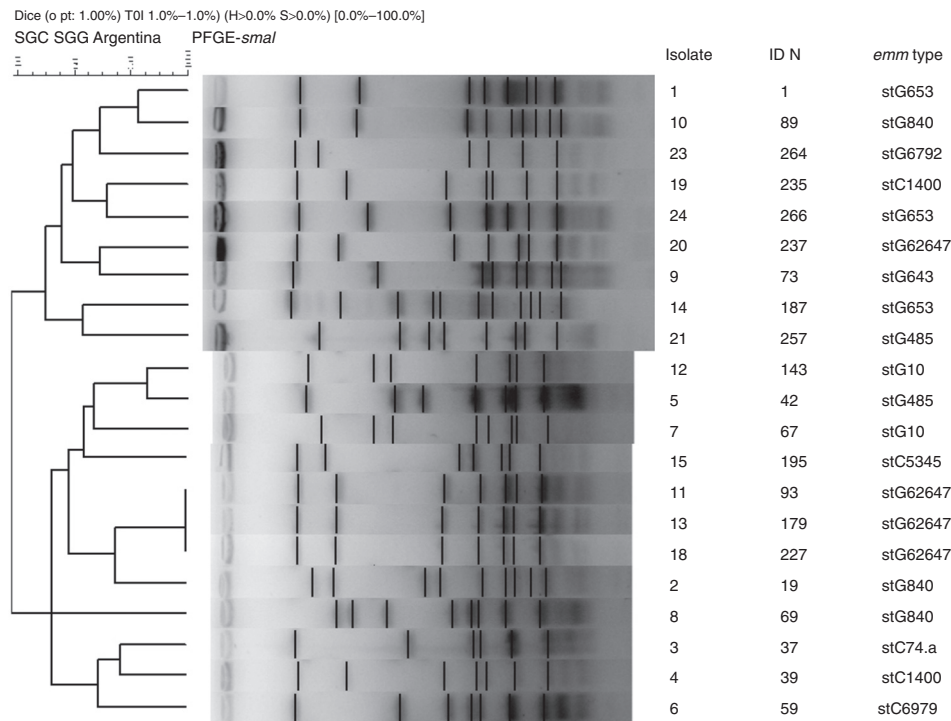


Figure 2 PFGE profiles of invasive *Streptococcus dysgalactiae* subsp. *equisimilis* (n=21). The strains were digested with the restriction enzyme *Sma*I.

Table 3 Minimum inhibitory concentration (MIC) ranges, MIC₅₀ and MIC₉₀ values of antimicrobial agents for *Streptococcus dysgalactiae* subsp. *equisimilis*

Antibiotic	Cut-off	Number of strains tested	MIC ₅₀ (μg/ml)	MIC ₉₀ (μg/ml)	MIC range (μg/ml)
Penicillin	S ≤ 0.125	21	0.016	0.016	0.007–0.015
Levofloxacin	S ≤ 2 R ≥ 8	21	0.5	0.5	0.5–1
Clindamycin	S ≤ 0.25 R ≥ 1	21	0.064	0.25	0.03–2
Erythromycin	S ≤ 0.25 R ≥ 1	21	0.064	4	0.03–2
Tetracycline	S ≤ 2 R ≥ 8	21	2	32	0.125–16

MIC: minimum inhibitory concentration.

Table 4 Characteristics of macrolide and tetracycline resistance among *Streptococcus dysgalactiae* subsp. *equisimilis* from Argentina

Strain no	Tetracycline MIC		<i>tetM</i>	<i>tetO</i>	Erythromycin MIC		Clindamycin MIC		Phenotype	<i>mef(A)</i>	<i>erm(B)</i>	<i>erm(TR)</i>
	AD	Etest			AD	Etest	AD	Etest				
2	8	8	+	–	2	3	0.03	0.125	iMLS _B	–	–	+
5	>16	64	+	+								
7	>16	32	+	+								
8	>16	96	+	+	>2	24	0.25	0.125	M	+	–	–
10	4	4	+	–	2	4	0.06	0.094	iMLS _B	–	–	+
12	>16	48	+	+								
14					>2	>256	>2	>256	iMLS _B /cMLS _B	–	+	+
20					>2	32	>2	>256	cMLS _B	–	–	+
21	>16	48	+	+								
24					0.5	3	0.03	0.125	Susceptible	+	–	+

MIC: minimum inhibitory concentration; AD: agar dilution.

By the year 2011 the Argentine population was 40.73 million inhabitants, therefore, the annual prevalence of SDSE invasive infections (n: 23) was at least 0.06/100,000 in Argentina during the 2011–2012 period. This prevalence seems to be lower than that recorded in a previous multicenter study conducted in 1998–1999, in which 27 invasive SDSE infections were reported during six-months; however, more health institutions participated in that study⁴⁰.

As for age distribution, a population-based surveillance carried out by Broyles et al. (n: 212)⁶ found that most patients suffering from invasive diseases due to SDSE (59.0%) were adults under 65 years old. Moreover, Takahashi et al. analyzed 286 SDSE infections from August 2006 to December 2009⁶⁰ and all patients with invasive infections were adults, often elderly (74.0%), and most of them with underlying diseases. Another investigation⁵⁸ also indicated that invasive SDSE infection (n = 42) mostly occurred in elderly adults (60–80 years old). Severe underlying conditions (*i.e.*, diabetes mellitus, liver or renal dysfunction, and others) were associated with 85.7% of these invasive SDSE infections.

In our study, the age range was 21–86 years; 57.1% of the patients were adults older than 50 years old (12 out of 21 patients).

With regard to underlying diseases, in U.S. surveillance reports, 96.2% of patients with invasive infections possessed underlying medical conditions¹³.

Similarly to the study by Sunaoshi et al.⁵⁸, 69.9% of SDSE-infected patients in Argentina presented at least one underlying disease and 17.4% had more than one, being diabetes the most frequent one.

Concerning SDSE disease outcomes, the mortality rate in a Japanese study (12.7%) was similar to that previously described in Hong Kong and the United States (12 and 15%)^{6,60,64}. In our series, the mortality rate due to SDSE invasive infections was 8.7% (2 cases) and both were adults. The same percentage corresponded to STSS cases. Three STSS cases and 3 deaths were recorded in a previous six-month Argentinean study⁴⁰.

Neither fatalities nor SDSE-mediated STSS were detected in children. SDSE-mediated STSS was a consequence of infections due to types *stG840* and *stG62647* and fatal infections were associated with *stC74.a* and *stG62647*.

The dominant *emm* types in Japan (*stG6792* and *stG485*) were different from those in the United States (*stG6*, *stG245*, *stG2078*, and *stG643*). The geographical factor may not be the only cause accounting for such difference since both study periods differed in these studies (2002–2004 vs. 2006–2009)^{6,60}. The *emm* type *stG6792* was the most frequently found in SDSE Japanese isolates (n: 65; 22.7%) and was more strongly related to poor outcome of SDSE diseases than other SDSE *emm* types⁶⁰. Furthermore, those isolates displayed similar DNA profiles with PFGE suggesting the clonal expansion of a specific subpopulation of strains rather than the spread of distinct strains.

In another study⁵⁸, 3 *emm* types, *stG6792*, *stG485*, and *stG2078*, predominated among the 42 invasive strains; strains having the same *emm* type showed uniform DNA profiles by PFGE. Moreover, previous reports described *emm* types and the DNA profiles by PFGE as variable among SDSE strains^{22,26}. In two other SDSE bacteremia studies carried out in the U.S. and Israel, *stG485*, *stG6*, *stG245* and *stG2078* types were the most frequently found^{6,12}.

In our study, 19 distinct DNA profiles by PFGE were detected, not having significant relationship among them. These results were expected because strains were not isolated in the context of an epidemic outbreak and were obtained from different geographic places. Predominant *emm*-types were *stG62647* (n: 4), *stG840* (n: 3) and *stG653* (n: 3). *stG6792* (n: 1), *stG485* (n: 2) and *stG2078* (n: 0) types were less represented in the present study, in contrast to the study conducted by Sunaoshi et al.⁵⁸. The types in this study were also different from those reported in the previous Argentinean multicenter study (1998), in which *stG6.0* (n: 6), *stG480* (n: 5) and *stG10.0* (n: 4) types⁴⁰ predominated.

Therefore, *emm* typing is an excellent comparative epidemiological tool for the analysis of SDSE strains belonging to different geographical regions or from the same region but isolated at different times.

The complete genomic sequence of SDSE GGS_124 (*stG480.0*) isolated from patients with STSS (GenBank accession No. AP010935) have been recently determined. The genome size was 2.1 Mbp, and sequence coverage with GAS genomes was 61–63% identity²⁰. Interestingly, many genes encoding virulence factors in GAS were identified in SDSE.

SDSE possesses many virulence factors shared with GAS²⁸, such as M protein, streptolysin O, streptolysin S, streptokinase, fibronectin binding protein, collagen binding protein and DNase. They also exhibit homology in streptococcal inhibitor of complement (SIC). However, they do not possess certain virulence factors, such as some superantigens, cysteine protease (SPE-B) and the ABC operon⁵². However, it was reported that some strains of SDSE may contain superantigen genes, which were firstly characterized in *S. pyogenes*^{25,29}.

It has been suggested that these factors were transmitted from GAS to SDSE species³⁰.

We analyzed *speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speJ*, *smeZ* and *ssa* genes. All the strains harbored only *speJ* and *ssa* genes simultaneously.

In the study by Ikebe et al. from Japan, all isolates were negative to *speA*, *speB*, *speC*, *speH*, *speI*, *speJ*, *speL* and *speM*, however, 12 out 16 isolates had the *speG* gene²⁶.

As shown in the present study and in previous reports, SDSE secretes only a few previously known superantigen exotoxins^{23,35,46}.

The clinical manifestations of STSS caused by SDSE are similar to EGA. *speA*, *speB*, and *speC* genes were not observed in our study although they are the most frequent detected exotoxins in *S. pyogenes*. Therefore, we suspect that not yet identified superantigen exotoxins or exoenzymes may exist, which could play an important role in the development of STSS caused by SDSE.

Although 60 years have passed since the introduction of PEN, β -hemolytic streptococci still continue being susceptible to this antibiotic, even though some *S. agalactiae* strains showed reduced susceptibility³².

Macrolide, CLI and TET resistance has been a matter of real concern because of the development of ERY-resistant *S. pyogenes* outbreaks in Japan, Australia and several European countries since the 70s^{42,51,56}. Macrolide and TET resistance has been observed both in GAS and SDSE^{31,43}.

SDSE isolates (n = 212) collected in a multicenter surveillance study by Broyles et al.⁶ in the United States showed

resistance rates of 28.8% to ERY, 4.2% to CLI, and 0.9% to fluoroquinolones.

In Korea³⁹, high frequency of the TET resistance-mediating *tet(S)* gene was demonstrated (68.8%), while ERY, CLI, and chloramphenicol resistance rates were low (9.4, 3.1 and 9.4%, respectively)⁶¹.

Of 231 SDSE isolates in the study by Takahashi et al.⁶⁰, four harbored the *mef(A)* gene, and 13 and six isolates carried the *erm(A)* and *erm(B)* genes, respectively.

In our study, 19.0% were non-susceptible to CLI and 26.1% to ERY. Only one strain was resistant to ERY (iMLS_B phenotype, *ermTR* positive) and no CLI constitutive resistance was detected in the previous multicenter study⁴⁰.

In our study, TET resistance was 30.4%. Two tetracycline-resistant strains only carried the *tet(M)* gen while five others carried both the *tet(M)* and *tet(O)* genes.

TET resistance was also common in SDSE during the 1998–1999 period in Argentina (33.3% in group C SDSE and 40% in group G SDSE)⁴⁰. In such study, TET resistance was 40.7% and all TET resistant strains carried only the *tet(M)* gen.

In Portugal, a high rate of fluoroquinolone resistance (12%) was detected between 1998 and 2005⁴⁷, although a previous study from Europe and the United States detected less than 1% resistance toward this group of antibiotics².

All our isolates were *in vitro* susceptible to LEV. Only one isolate (strain 9) was found to have reduced susceptibility to NOR, PEF and OFL.

Fluoroquinolones act by inhibition of bacterial DNA gyrase and DNA polymerase. The alterations of these enzymes were the prevalent mechanism of resistance in the *Streptococcus* genus²⁴. The mutation in the *gyrA* subunits (gyrase DNA) and *parC* (DNA topoisomerase) are found in the so-called quinolone resistance determining regions (QRDR). The mechanisms involved in the small diameters observed by the disk diffusion test with strain 9 deserve to be further studied.

In the future, we should continue to survey invasive SDSE infections to further clarify the prevalence of infection, the study of new virulence factors, the distribution of *emm* types and to monitor antibiotic susceptibility rates in the Argentinean population.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix 1. National Collaborative Group for the Study of Streptococci and Related Bacteria

The “National Collaborative Group for the Study of Streptococci and Related Bacteria” consists of twenty eight centers belonging to a collaborative group for the study of streptococci and related bacteria: 5 centers from Buenos Aires City (C. Hernández, L. Casimir, M. Litterio and M. del C. Ceinos, Hospital de Pediatría Prof. Dr. Juan P Garrahan; A. Famiglietti, C. Rodríguez and S. García, Hospital de Clínicas G. J. San Martín; C. Vay, Sanatorio Mater Dei; D. Ballester and F. Amalfa, Hospital Parmenio Piñero; R. Pereda, Hospital Pedro Elizalde), 4 from Rosario (N. Borda and R. Notario, Hospital Español; E. Sutich, J. Pérez, G. Cera, M. J. Spoletti, I. Demaría and D. Aguila, Hospital Provincial del Centenario; A. Ernst, A. Badano and A. Aletti, Hospital de Niños Víctor J. Vilela; A. Ponessa, R. Notario, T. Gambandé and L. All, Cátedra de Microbiología, Facultad de Ciencias Médicas Universidad Nacional de Rosario), 2 from Santa Fe (S. Virgolini, M. R. Barone and G. Ezcurra, Hospital de Niños Dr. O. Alassia; E. Méndez, Hospital Dr. José María Cullen) 2 from Córdoba (M. Bottiglieri, Clínica Privada Reina Fabiola; P. Montanaro, A. Oreccini and A. Garnero, Hospital de Niños de la Santísima Trinidad), 2 from San Juan (H. Castro, Hospital Marcial Quiroga; O. Navarro, M. López and M. Mengual, Hospital Guillermo Rawson) and 1 each from Mar del Plata (M. Vallejo, N. Rosales, V. Fanjul and M. Gordovil, Hospital Privado de la Comunidad), San Rafael (A. Acosta and C. Baldoni, Hospital Teodoro Schestakow), Mendoza (M. A. Di Stefano and L. Contreras, Hospital Central de Mendoza), Lanús (A. Togneri, L. Podestá and M. Pérez, Hospital Evita), La Plata (A. Pacha, Hospital San Juan de Dios, Pilar (V. Vilches, Hospital Austral), Bahía Blanca (M. Rizzo, Ma. Luz Benvenuti and L. Giordano, Hospital General de Agudos Dr. José Penna), Neuquén (M. R. Núñez, Hospital Provincial Dr. Castro Rendón), Gral. Pico (S. Cirimele, A. Baroni and D. Ruderman, Establecimiento Asistencial Gobernador Centeno), Santa Rosa (G. Almada, Hospital Lucio Molas), Esquel (O. Daher, Hospital Zonal de Esquel), Posadas (M.E. von Specht, L. Leguizamón and Oscar López, Hospital Provincial de Pediatría Dr. F. Barreyro), and Concordia (Ma. Ofelia Moulins, L. Otaegui and L. Bernhardt, Hospital Masvernat).

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