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Original Article

Virulence genes and resistance to antibiotics of beta-hemolytic streptococci isolated from children in Chiapas, Mexico

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Abstract

Introduction: Among beta-hemolytic streptococci, *Streptococcus pyogenes* causes a wide variety of human disease including pharyngitis, necrotizing fasciitis and streptococcal toxic syndrome. Group A Streptococcus (GAS) uses a variety of virulence traits to colonize and then cause damage to the host; others species of beta-hemolytic streptococci are considered as emerging pathogens for humans. Despite its recognized virulence, only few studies have investigated virulence factors of GAS strains isolated in Mexico.

Methodology: We conducted an epidemiological study to investigate the prevalence of GAS strains in child illnesses in Chiapas Mexico. Virulence genes encoding proteases, DNases, superantigens, as well as susceptibility to antibiotics were investigated.

Results: During 2010, 2013 and 2014, beta-hemolytic streptococci (N=12) were isolated from cases of bacterial infections including pharyngitis and bacteremia, with a prevalence of 0.42, 0.04 and 0.20%, respectively. *S. pyogenes* was the most frequent species (33%) followed by *S. agalactiae* and *S. dysgalactiae* subsp. *equisimilis* (25%, each). Most GAS strains encoded genes for proteases: *scpA, speB, spyCEP* and *mac* (75%), followed by *sdaD and sdaB* (DNases) (50%), *speA* and *speG* (superantigens; 50 and 25%, respectively). The *scpA* gene was amplified in all *S. agalactiae* strains and in ~35% of SDSE strains. Strains were all susceptible to beta-lactams, cephalosporins and quinolones.

Conclusions: The present study provides evidence on the epidemiology of beta-hemolytic streptococci infecting children at the southeast Mexico, their virulence traits and sensitivity to first-line antibiotics.

Key words: *Streptococcus pyogenes*; virulence factors; antimicrobial susceptibility; Chiapas.

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Introduction

For almost 20 years (1985-2004), streptococcal pharyngitis and tonsillitis (International Code Diseases -ICD- numbers J02.0, J03.0) were located among the 20 major diseases which affected the Mexican population, mainly children of 1 to 9 years of age. From 2012 to 2014 the diseases reappeared with incidence rates exceeding the national rate (113.73, 113.08 and 134.08 new cases for 100,000 inhabitants in 2012, 2013 and 2014, respectively), being Tamaulipas, Coahuila, Sonora and Zacatecas (Mexican northeast) and Guerrero and Oaxaca (Mexican southeast) the states presenting the largest quantity of new cases [1]. This kind of infections are mainly caused by *Streptococcus pyogenes* or GAS (Group A *Streptococcus*) which causes direct infections on the skin, soft tissues, pharynx, bones, bacteraemia, toxin-mediated infections (scarlet fever, Streptococcal toxic shock syndrome, necrotising fasciitis) and some other affections mediated by the immune system [2]. Other species that are potentially pathogenic for humans are the opportunistic *Streptococcus agalactiae* or Group B *Streptococcus* (GBS) which colonize the gastrointestinal and gastro urinary tracts in adults causing pneumonia, septicemia and meningitis in neonates, being a factor of morbimortality in pregnant women, elders and immunocompromised persons [3]. *S. dysgalactiae subsp. equisimilis* (SDSE) causes

infections in the pharynx and skin, as well as acute rheumatic fever and bacteremia favored by its ability of adding platelets, internalizing in the human endothelium [4,5]. *S. constellatus subsp. pharyngis* colonizes the human pharynx causing pharyngitis [6]. Pathogenicity among these species is measured by different factors as hemolysins, adhesins, proteases, DNAses and superantigens [7,8]. Proteases may degrade antibodies (SpeB), chemotactic cytokines as IL-8 (SpyCEP), proteins from complement system (SCPA and SIC) or prevent phagocytosis (Mac) [8]; DNases (as Spd3, Sdc, SdaB and SdaD) degrade the neutrophil extracellular traps (NETs) allowing the bacteria to escape from phagocytosis, meanwhile superantigens (SpeG, SpeJ, SMEZ, SpeA, SpeC, SpeH, SpeI, SpeJ, SpeK, SpeL, SpeM, and SSA) induce the massive secretion of cytokines which increase the severity of infections [9]. In a greater proportion, infections caused by GAS affect children and young people from developing countries, besides the fact that it is located amongst the group of neglected diseases [2]. In Mexico, since 2010, the state of Chiapas (in the Mexican southeast) holds the first place of poverty [10] and for this reason, the child population may be more susceptible to this kind of infectious diseases. The purpose of this work was to investigate the prevalence, pathogenicity profile and antimicrobial susceptibility among strains of beta-hemolytic streptococci isolated from children living in a major city of South Mexico, Tuxtla Gutiérrez, Chiapas.

Methodology

Children cohort and isolation of bacterial strains.

In this study, children under the age of 18 who attended to the "Hospital de Especialidades Pediátricas" –HEP- (Tuxtla Gutiérrez, Chiapas) [11] during 2010, and 2013-2014, with clinical diagnosis of streptococcal diseases that included pharyngitis/tonsillitis [12], skin and soft tissue infections, invasive infections (.i.e., bacteremia), necrotizing fasciitis [13], and streptococcal toxic shock syndrome [14] were included.

Bacteriological cultures were performed at the Microbiology and Pathology Laboratory of HEP, from tonsillar exudates, bronchial aspirates, blood, urine, abscess and ulcers, according to procedures from Spanish Society for Infectious Diseases and Clinical Microbiology [15-18]. Identification at the species level was conducted with a Vitek 2 Compact (bioMérieux, Marcy l'Etoile, France) system, by using the Vitek 2 GP (Gram-positive) and Vitek 2 GN (Gram-negative) ID cards; the Vitek 2 AST-ST01 cards were used for the susceptibility test. Bacterial strains were cryopreserved in skim milk (10% v/v) at -80º C and placed at the UNICACH bacterial culture collection [19]. *S. pyogenes* strain ATCC (American Type Culture Collection, Manassas, USA) 19615 was used as a control. This project was approved by the HEP Ethics Committee (approval ID DPEI/00394/13) offering the possibility to obtain different information as: age, sex, type of infection and treatment of the affected child.

Lancefield group's determination

The serological group was investigated with a commercial kit (Pastorex Strep, Biorad, Marnes La Coquette, France) according to the manufacturer´s specifications.

PCR amplification of virulence genes encoded by beta-hemolytic streptococci

Multiplex PCR reactions were used to amplify genes encoding proteases (*speB*, *spyCEP*, *scpA*, *mac*), superantigens (*speL*, *speK*, *speM*, *speC*, *smeZ*, *speI*, *ssa*, *speA*, *speH*, *speG*, *speJ*) and DNases (*spd3*, *sdc*, *sdaB*, *sdaD*). PCRs were conducted as described by Borek *et al.* (2012) with minor modifications detailed below. Bacterial DNA was extracted following an improved phenol/chloroform technique [20,21]; the cell pellet was washed twice with 400 µL of 0.5 M STE buffer (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH = 8.0), resuspended with 200 µL of TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH = 8.0) and 100 µL of Tris-saturated phenol (pH = 8.0). Afterwards, 40 µL of TE buffer plus 100 µL of chloroform was added to the aqueous phase. Three washings were performed with chloroform until this was transparent. The preparation was then treated with 40 µL of TE buffer and 5 µL of RNase (10 mg/mL), incubated at 37°C for 10 minutes after which 100 µL of chloroform were added to obtain the aqueous phase containing purified DNA. The concentration of DNA preps was determined with a BioSpectromether (Eppendorf, Hamburg, Germany.) and then stored at -20°C until used. Primers sequences used were reported elsewhere [21]. Primer mixtures were prepared to contain a final concentration of 0.7 µM: mixture 1 (*speL*, *speK* and *speM*); mixture 2 (*speC*, *smeZ* and *speI*); mixture 3 (*ssa*, *speA*, *speH*, *speG* and *speJ*); mixture 4 (*spd3*, *sdc*, *sdaB* and *sdaD*) and mixture 5 (*speB*, *spyCEP*, *scpA*, *mac* and *sic*). PCR reactions contained 0.5 U Taq polymerase DNA (Invitrogen, Carlsbad, USA), 1X PCR buffer (Invitrogen, Carlsbad, USA), 5 mM $MgCl_2$ (Invitrogen, Carlsbad, USA), dNTP´s mix (0.2 mM each; Invitrogen, Carlsbad, USA) and ~100 ng of genomic DNA. Reactions were run in a thermal cycler

(Eppendorf, Hamburg, Germany) with following conditions: for superantigens/DNases, an initial denaturation for 3 minutes at 95º C; 40 cycles of 15 seconds at 95º C, 20 seconds at 60º C, and 2 minutes at 72º C; a final extension step of 7 minutes at 72º C. For proteases/sic gene, both initial and later denaturation as well as final extension and cycles number were as stated previously; 45 seconds at 52.5º C, and 3 minutes at 72º C were used for annealing and extension steps, respectively [20]. PCR products were separated by electrophoresis in agarose gel (1.5%), stained with ethidium bromide (0.5 µg/mL) and documented in a transiluminator (Enduro GDS, Labnet International, Inc., Edison, USA). A 100 bp Plus DNA ladder was used as a molecular weight marker (Fermentas, Waltham, USA).

Data analysis

Age, as well as categorical variables such as sex, biologic material, *Streptococus* species, virulence genes and the antimicrobial susceptibility profile was analyzed through descriptive statistics. The Fisher´s exact test was used to evaluate the association between the non-parametric variables, establishing a statistical significance value if $p \leq 0.05$. Analyses were conducted using the SPSS software (version 20; Armonk, New York: IBM Corp.).

Results

The average mean age of the affected patients was 5.6 years; most of these were boys (59.7%; N = 387), followed by girls (40.3%; N = 261).

During three years, a total of 8,285 biological materials stated above were cultured as follows: 699 in 2010; 4,087 in 2013, and 3,499 in 2014; Gram-positive and Gram-negative bacteria were isolated in 7.8% (N = 648) and 12.1% (N = 1,003) from those materials, respectively. *Staphylococcaceae and Streptococcaceae* were mainly recovered from blood (55.3 and 2%, respectively), while *Enterobacteriaceae* and *Pseudomonadaceae* from urine and bronchial aspirates (39.4 and 10.3%, respectively (Table 1).

Regarding beta-hemolytic streptococci, a total of 12 strains were isolated during 2010, 2013 and 2014 with a prevalence of 0.42, 0.04 and 0.20%, respectively. *S. pyogenes* strains were the most frequent pathogen isolated (N = 4; 33%) from all biological material tested (except for blood and urine), followed by *S. agalactiae* and *S. dysgalactiae subsp. equisimilis* (SDSE) strains (N = 3; 25% each) from urine and tonsillar exudates, respectively (Table 2).Serological analysis showed that strains of *S. pyogenes* belonged to the Lancefield group A, *S. agalactiae* to group B, SDSE to Lancefield group G, and *S. constellatus subsp. pharyngis* (N = 2) to Lancefield groups C and non-typeable, respectively.

Table 1. Bacterial strains isolated from children of Chiapas, Mexico.

Bacterial family	No. (%) of isolates						
	Blood	Urine	Tonsillar exudate	Bronchial aspirate	Abscess	Ulcer	Total
Gram-positive							
Staphylococcaceae	358 (55.3)	65 (10.0)	6 (0.9)	32 (4.9)	28 (4.3)	2 (0.3)	491 (75.8)
Enterococcaceae	24 (3.7)	82 (12.7)	0 (0.0)	4 (0.6)	5 (0.8)	0 (0.0)	115 (17.7)
Streptococcaceae	13 (2.0)	4 (0.6)	10 (1.5)	9 (1.4)	2 (0.3)	2 (0.3)	40 (6.2)
Micrococcaceae	0 (0.0)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.3)
Total	396 (61.1)	153 (23.6)	16 (2.5)	45 (6.9)	35 (5.4)	3 (0.5)	648 (100.0)
Gram-negative							
Enterobacteriaceae	101 (10.1)	395 (39.4)	0 (0.0)	46 (4.6)	16 (1.6)	2 (0.2)	560 (55.8)
Pseudomonadaceae	57 (5.7)	74 (7.4)	1 (0.1)	103 (10.3)	4 (0.4)	0 (0.0)	239 (23.8)
Moraxellaceae	27 (2.7)	8 (0.8)	0 (0.0)	22 (2.2)	0 (0.0)	0 (0.0)	57 (5.7)
Morganellaceae	0 (0.0)	55 (5.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	55 (5.5)
Xanthomonadaceae	9 (0.9)	1 (0.1)	0 (0.0)	32 (3.2)	0 (0.0)	0 (0.0)	42 (4.2)
Yersiniaceae	10 (1.0)	1 (0.1)	0 (0.0)	16 (1.6)	0 (0.0)	0 (0.0)	27 (2.7)
Flavobacteriaceae	7 (0.7)	1 (0.1)	0 (0.0)	2 (0.2)	0 (0.0)	0 (0.0)	10 (1.0)
Alcaligenaceae	5 (0.5)	1 (0.1)	0 (0.0)	2 (0.2)	0 (0.0)	0 (0.0)	8 (0.8)
Aeromonadaceae	3 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.3)
Comamonadaceae	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Sphingomonadaceae	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Total	221 (22.1)	536 (53.4)	1 (0.1)	223 (22.2)	20 (2.0)	2 (0.2)	1003 (100.0)

Table 2. Isolation source of beta-hemolytic streptococci strains from children in Tuxtla Gutierrez, Chiapas, Mexico.

	S. pyogenes	***S. agalactiae***	**SDSE**	**SCSP**		
Biologic material	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**Total**	**P**
Abscess	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0.289
Ulcer	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	
Blood	0 (0.0)	1 (8.3)	1 (8.3)	0 (0.0)	2 (16.7)	
Urine	0 (0.0)	2 (16.7)	0 (0.0)	0 (0.0)	2 (16.7)	
Tonsillar exudate	1 (8.3)	0 (0.0)	2 (16.7)	2 (16.7)	5 (41.7)	
Bronchial aspirate	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	
Total	4 (33.3)	3 (25.0)	3 (25.0)	2 (16.7)	12 (100.0)	

SDSE: *Streptococcus dysgalactiae subspecies equisimilis; SCSP:S. constellatus subsp. pharyngis;* P: *p* value by Fisher´s exact test.

Table 3. Beta-hemolytic streptococci strains isolated from patients of Tuxtla Gutierrez, Chiapas, México.

Case no.	**Gender/age of patient**	**Streptococci species/ Strain designation**	**Type of infection/Biologic material**	**Disease/Treatment**
1	Male, 4 y.o.	SDSE/SP005	Direct/Blood	Myeloproliferative syndrome/patient deceased.
2	Male, 11 y.o.	*S. pyogenes/* SP006	Direct/Tonsillar exudate	Chronic tonsillitis/ Treated and cured with benzyl penicillin IM 50,000 IU/kg, single dose.
3	Female, 11 y.o.	*S. pyogenes/*SP007	Direct/Bronchial aspirate	Fever of unknown origin/ Without treatment because parents requested leave the hospital as a voluntary patient.
4	Female, 2.4 y.o.	*S. pyogenes*/SP001	Direct/Abscess	Erysipelas/Abscess treated with both iodine and 0.02% chlorhexidine solutions. Patient cured with dicloxacillin IV (100 mg/kg/day for 10 days).
5	Female, 7.9 y.o.	*S. pyogenes/*SP002	Toxin-mediated/Ulcer	Necrotizing fasciitis/ Cured with cefotaxime IV (200 mg/kg/day) plus clindamycin IV (40 mg/kg/day) for 14 days.
6	Female, 14 y.o.	*S. agalactiae*/SP008	Direct/Urine	UTI/Patient cured with ampicillin 500 mg orally, every 8 hours/10 days.
7	Female, 17.9 y.o.	SCSP/ SP013	Direct/Tonsillar exudate	*Systemic lupus erythematosus* (SLE)/ Without treatment due to absent of clinical signs.
8	Female, 14.4 y.o.	SDSE/SP011	Direct/Tonsillar exudate	Pharyngitis/Treated and cured with benzyl penicillin IM 50,000 IU/kg, single dose.
9	Female, 6.0y.o.	SDSE/SP009	Direct/Tonsillar exudate	*Chronic sinusitis/*Treated and cured with c*larithromycin 15 mg/kg/day, orally.*
10	Female, 11.8 y.o.	SCSP/SP010	Direct/Tonsillar exudate	Dermographism/ Without treatment due to absent of clinical signs.
11	Female, 5.0 y.o.	*S. agalactiae*/SP012	Direct/Blood	Childhood acute lymphoblastic leukemia/*Patient treated with vancomycin IV (40 mg/kg/day for 3 days); patient died.*
12	Male, 0.5 y.o.	*S. agalactiae*/SP014	Direct/Urine	UTI, Congenital Bilateral hydronephrosis/ Treated and cured with ampicillin 50 mg/kg/day orally every 6 hours, 10-14 days.

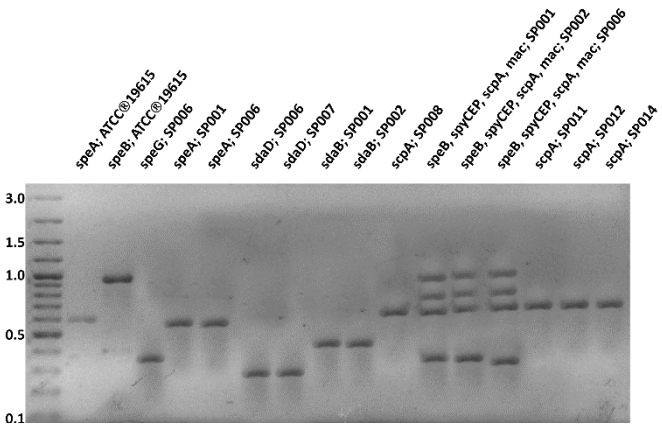
y.o.: years old; SDSE: *Streptococcus dysgalactiae subspecies equisimilis*; SCSP: *S. constellatus subsp. pharyngis*; IM: intramuscular; IV: intravenous; IU: International Units; UTI: Urinary Tract Infection.

More than a half of the beta-hemolytic streptococci strains were isolated from patients suffering of primary infections such as tonsillitis and bacteraemia (N = 9; 75.0%), followed by one case of toxin-mediated infection (N = 1, 8.3%) (Table 3); due to the low isolation rate, an association between the *Streptococcus* species and these diseases could not be established (data not shown).

Regarding virulence genes carried by strains, most of *S. pyogenes* isolates (75%) carried genes encoding for *scpA*, *speB*, *spyCEP* and *mac* proteases, whereas 50% of strains encoded *sdaD* and *sdaB* DNases and *speA* superantigen while only 25% of strains carried the *speG* superantigen gene. All *S. agalactiae* strains carried the *scpA* gene but only 33% of the SDSE strains exhibited this gene (Figure 1). There was no significant association between any *Streptococcus* species with genes codifying for these virulence factors (Table 4).

Resistance to tetracycline was observed among all *S. agalactiae* strains (N = 3, 100%), followed by SDSE and *S. constellatus subsp. pharyngis* strains (N = 1; 33.3% each); 33.3% of *S. agalactiae* strains exhibited also resistance to erythromycin; all beta-hemolytic streptococci were susceptible to beta-lactams, cephalosporins and quinolones (Figure 2).

Figure 1. Detection of virulence genes among beta-hemolytic *Streptococcus* isolates.



Lanes: 1, MWM (kb are noted at left); 2-3, primers amplifying the speA and speB genes (576 and 952 bp, respectively), DNA template from *S. pyogenes* ATCC® 19615™; 4, speG gene (384 bp) from *S. pyogenes* SP006 strain; 5-6, speA gene (576 bp) from *S. pyogenes* SP001 and SP006 strains, respectively; 7-8, sdaD gene (295 bp) from *S. pyogenes* SP006 and SP007 strains, respectively; 9-10, sdaB gene (440 bp) from *S. pyogenes* SP001 and SP002 strains, respectively; 11, 15-17, scpA gene (622 bp) from *S. agalactiae* SP008 strain, SDSE SP011 strain, *S. agalactiae* SP012 and SP014 strains, respectively; 12-14, speB, spyCEP, scpA and mac genes(952, 786, 622, and 389 bp, respectively) from *S. pyogenes* SP001, SP002, and SP006 strains, respectively.

Discussion

Beta-hemolytic streptococci cause a variety of pyogenic infections in humans and animals. GAS is the most important for human beings due to its non supurative effect [22]. In 2002, infections caused by GAS (e.g., invasive infections, post- streptococcal glomerulonephritis, acute rheumatic heart disease, and acute rheumatic fever) were the ninth cause of mortality in the world, mainly affecting persons living in developing countries [8,23]. In these countries, the prevalence of strep throat in schoolchildren and adults is 5-10 fold increase compared to the prevalence observed in developed countries (15 and 4-10%, respectively) [24]. In Mexico, according to official figures of the Ministry of Health, for nearly two decades (1985-2002), strep throat and tonsillitis has been positioned among the first 20 leading causes of disease, with a maximum peak during 1999, with a national rate of 240.4 new cases for 100,000 inhabitants, affecting mainly children from 1-14 years old [1].

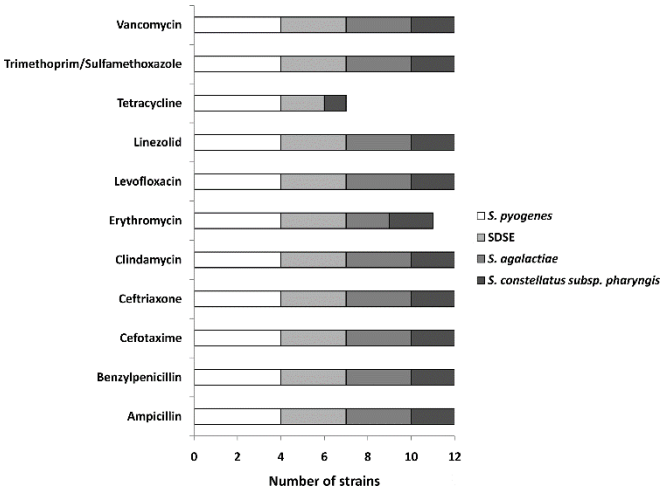
Nonetheless, in Chiapas, the incidence rates caused by these diseases during the period of this study (2010,

Table 4. Virulence genes of beta-hemolytic streptococci strains from Tuxtla Gutierrez, Chiapas, Mexico.

Virulence gene	GAS N (%)	GBS N (%)	SDSE N (%)	SCSP N (%)	P
Proteases					
scpA	3 (75.0)	3 (100.0)	1 (33.3)	0 (0.0)	0.189
speB	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.073
spyCEP	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.073
mac	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.073
DNases					
sdaB	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.379
sdaD	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.379
Superantigens					
speA	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.379
speG	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000

GAS: Group A *Streptococcus* or *S. pyogenes*; GBS: Group B *Streptococcus* or *S. agalactiae*; SDSE: *Streptococcusdysgalactiae* subsp. *equisimilis*; SCSP: *S. constellatus subsp. pharyngis*; N: number of strains; %, percent of strains; P: *p* value by Fisher´s exact test.

Figure 2. Antibiotic susceptibility of beta-hemolytic *Streptococcus* isolates from Chiapas, Mexico. SDSE: *Streptococcus dysgalactiae subsp. equisimilis*.



2013 and 2014) were of 1.21, 12.48 and 46.72 new cases for 100,000 inhabitants, respectively, not exceeding national incidence rates (104.92, 113.08 and 134.08, respectively); these trends may explain the low prevalence of beta-hemolytic streptococci reported here. In contrast, during the same years the state of Tamaulipas (Mexican northeast) had incidence rates for strep throat, 8 times greater (1151.93, 1018.91 and 1061.95, respectively) in relation to national rates [1]. Perhaps this disparity is related to the weather because *S. pyogenes* infections are more prevalent in regions with dry weather, such as Tamaulipas, in comparison to regions with rainy weather as the case for Chiapas [25].

In this study, *S. pyogenes* was the species with more prevalence among the studied population (Table 2). In 2005, at the global level, it was estimated that at least 18.1 million persons were affected by invasive diseases caused by this species; besides it showed an incidence of 1.78 million of new cases per year, plus more than 111 and 616 million cases of streptococcal pyoderma and pharyngitis, respectively [23]. After *S. pyogenes*, *S. agalactiae* and SDSE strains were the ones that mostly caused UTI and pharynx colonization, respectively (Table 3).

SDSE strains isolated during the current work belong to Group G Lancefield; in fact, 90% of SDSE strains appertain to this group, even though a minority may belong to Group C [7]. SDSE has a virulence profile that causes a range of infections similar to GAS [26]. One of the analyzed SDSE strains in the current work was isolated from blood of a patient with leukemia; it has been reported that malignancy, as well others underlying diseases constitute a predisposing factors in bacteremia caused by SDSE [27]. A multicenter study done in Argentina reported 23 invasive infections (60.9% isolated from blood) caused by SDSE [26].

S. constellatus subsp. *pharyngis* belongs to the Lancefield group C streptococci, strains are mainly found in the human throat where can cause pharyngitis [6]. Whereas it is not a primary pathogen, patients with underlying immune system diseases can carry this bacterium in the upper airways, such as it was demonstrated in the nasopharynx of patients included in this study (Table 3).

The genetic analysis of the *Streptococcus* species analyzed in the current work showed that *S. pyogenes* was the only one exhibiting greater repertoire of virulence genes (proteases, DNases and certain superantigens); this could explain the broad spectrum of diseases caused by this Gram-positive bacteria [2].

Interestingly, in all clinical isolates of GBS and a third of SDSE, we amplified the gene for the protease SCPA (Table 4). This may be due to the mechanism of horizontal gene transfer described between both species, as protease SCPB of GBS exhibits a high genomic and proteomic homology with the SCPA of GAS [28]; besides, this event seems to occur in Group G *Streptococcus*, as a copy of this gene has been described in this species [29]. Along with the gene encoding SCPA protease, genes *speB*, *spyCEP* and *mac* were detected in most clinical isolates of *S. pyogenes* isolated in this work. The gene *speB*, encode a cysteine protease, SpeB, which promotes the dissemination of GAS in the affected tissues, but also plays a role modulating the host immune system and certain virulence factors of such Gram-positive [30]. SpyCEP, which operon *spyCEP* was identified in this study, degrades IL-8 allowing *S. pyogenes* to be refractory to the destruction by neutrophils [31], while Mac/IdeS, encoded by *mac*, is a protease that degrades IgG [32].

Half of *S. pyogenes* isolates exhibited genes that encode for DNases, immunogenic proteins conferring bacterial protection against polymorphonuclear leukocytes through its action over the neutrophil extracellular traps (NETs) [33]. Finally, some *S. pyogenes* isolates carried genes encoding SpeA and SpeG superantigens. This information is similar to the one obtained through a study made with 54 *S. pyogenes* strains isolated from children affected by pharingytis (Mexico City), where 61% (N = 33) showed the presence of *speA* gene. Besides, a variability in *sic* gene was found; such gene codifies a protease which inhibits the complement [34]. Another study carried out in Mexico City analyzed 47 *S. pyogenes* strains isolated

from a scarlet fever outbreak and more than a half (67.5%) exhibited the *speA* gene [35].

The strains analyzed in this study were susceptible to cephalosporins and beta-lactams. These findings are similar to the ones of a study made with 100 *S. pyogenes* strains from the "Hospital Infantil de la Ciudad de México" indicating that all the strains were sensitive to beta-lactams and clindamycin [36]. In the same way, another work performed in Mexico City showed that 100% from 197 *S. pyogenes* isolates from adults presenting pharyngotonsillitis were sensitive to moxifloxacin, a third generation quinolone that can be employed as an alternative for the treatment of patients allergic to penicillin or for *S. pyogenes* strains resistant to macrolides [37]. Another study reported that, between 1999 and 2000, all GAS strains isolated in Mexico (N = 467) and USA (N = 174) were susceptible to penicillin, ceftriaxone and vancomycin, while the resistance for erythromycin was low for both groups (4.9 and 5.2% for Mexico and USA, respectively). In this study, the resistance observed for such macrolide was 15.4%, and for this reason, it should be necessary to analyze the presence of certain genes as *mefA* and *ermB* in macrolide resistant strains, as well as its association with this phenotype [38].

Conclusions

The current study revealed a low prevalence of beta-hemolytic streptococci species among children who assist to the HEP (Tuxtla Gutiérrez, Chiapas) demonstrating that: 1) *S. pyogenes* is the most frequent species which causes direct infections and, 2) indicating that its virulence profile is mainly constituted by genes that encode for proteases.

It is important to note that GAS strains were susceptible to a wide range of antibiotics. This information is important in view that this was the first study analyzing virulence factors, and resistance to antibiotics, in Gram-positive bacteria isolated from children of Tuxtla Gutiérrez, Chiapas. The information will guide therapeutics. Future studies should also include strains causing disease in children living in marginalized municipalities of the state. We acknowledge the need of incorporating more sophisticated analysis such as the typification of *emm* genes to study the geographical distribution and its association with symptoms. We will likely identify, in Mexico, a high diversity of *emm* types, as reported in other developing countries [39].

The additional study of these molecular markers among beta-hemolytic streptococci strains from Tuxtla Gutiérrez, Chiapas and other geographical regions of Mexico will enable us to determine the origin of epidemic breakouts, characterize the strains and predict its pathogenic potential to implement preventive strategies such as the use of biological products directed to the central and northern population of Mexico, where it is usual to find strep throat incidence rates greater than the national average.

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Authors´contributions

JGJ: conceived the whole study, assessed methodology, analyzed data and wrote the manuscript;MIMO: performed molecular analysis of bacterial strains; AVS: performed molecular analysis of bacterial strains; LMLC: assessed methodology, and wrote the manuscript; JMFG: performed phenotypic analys as well as antimicrobial susceptibility tests of bacterial strains; JAGH: assessed clinical data, and provided intellectual support to the overall manuscript; JEV: Analyzed data and wrote the manuscript.

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