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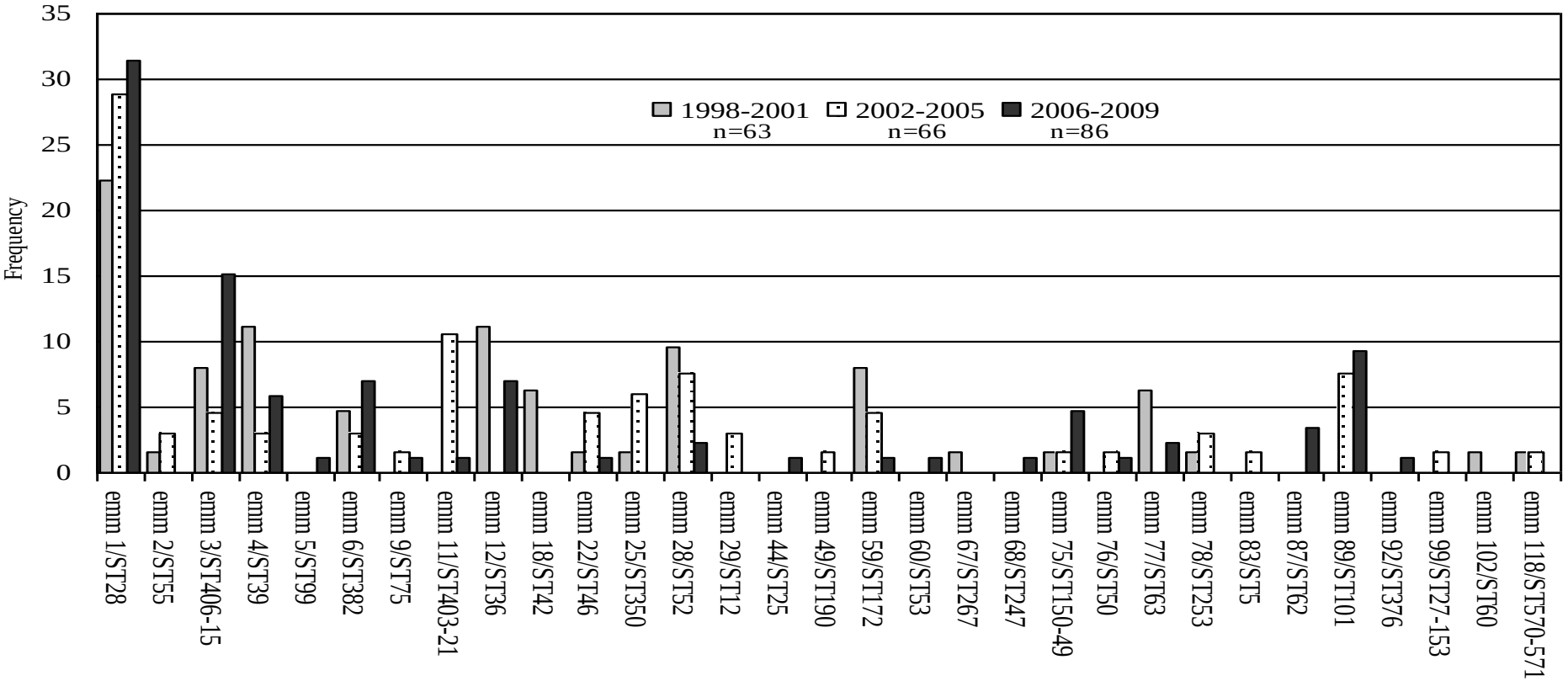

Original article

Title: **Epidemiological and Molecular Analysis of *Streptococcus pyogenes* Isolates Causing Invasive Disease in Spain (1998-2009). Comparison with Non-Invasive Isolates.**

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Running title: Invasive Streptococcus pyogenes in Spain

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Abstract

The incidence, clinical manifestations and circulating clones involved in *Streptococcus pyogenes* invasive disease was analyzed in two regions of Spain between 1998 and 2009. The annual average incidence of invasive disease was 2 episodes per 100 000 inhabitants (3.1 for children and 1.9 for adults). The most frequent clinical manifestations were cellulitis (41.3%), bacteraemia without focus (19.0%), streptococcal toxic shock syndrome (12.6%), and pneumonia (7.7%). Among 247 invasive isolates analyzed, the most prevalent clones were *emm*1/ST28 (27.9%), *emm*3/ST15-406 (9.8%), and *emm*4/ST39 (6.5%). The *emm*1/ST28 clone was the only clone detected each year throughout the study period and was associated with more than one third of all fatal outcomes. When invasive isolates were compared with 1,189 non-invasive isolates, the *emm*1/ST28 clone was significantly associated with invasive disease. The *speA* and *ssa* genes were more frequent among invasive *emm*1 and *emm*4 isolates, respectively. Forty-two (17%) invasive isolates were resistant to erythromycin (21 harboured the *mef* gene and 21 the *erm*B or *erm*A genes). Twenty-two (8.9%) isolates had reduced susceptibility to ciprofloxacin (MIC 2-8 µg/mL) and thirty-two (13%) were tetracycline-resistant (*tet*M or *tet*O gene). In conclusion, the *emm*1 type was overrepresented among invasive cases and was associated with high mortality rates.

Key words: Group A streptococcus, invasive disease, incidence, *emm*-type, superantigen,

INTRODUCTION

Streptococcus pyogenes, or group A streptococcus (GAS), are ubiquitous human pathogens. The global burden of invasive *S. pyogenes* disease is high, with at least 663 000 new cases and 163 000 deaths each year [1]. In high-income countries the most common manifestation of *S. pyogenes* infection is pharyngitis, but invasive disease, which has shown a re-emergence since the late 1980s in the United States and Europe [2-9], remains a major public health concern.

The M protein (*emm*-type) is a major virulence factor of *S. pyogenes* that confers type-specific immunity [10]. Strains belonging to *emm*1, *emm*3, *emm*28 or *emm*18 types have often been related to invasive disease [2-9], although many *S. pyogenes emm*-types are known to be capable of causing severe disease.

Although a variety of streptococcal vaccines using type-specific and conserved candidates are under study [11], only the 26-valent recombinant M protein vaccine has successfully completed a phase I/II clinical trial involving adults [12]. Data estimate that this multivalent vaccine would provide a good coverage in North America and most other developed countries [6,8,13]. Nevertheless, due to the variability in the diversity and predominance of *emm*-types and emergence of new *emm*-types in different parts of the world [2-9,13-15] the appropriateness of this vaccine to address the global needs is uncertain. Hence, molecular epidemiological data of circulating isolates have important implications in guiding vaccine design and in providing support for new prevention approaches.

The main aim of this study was to describe the incidence, clinical manifestations, and circulating clones involved in invasive *S. pyogenes* disease in a population-based surveillance study conducted in two regions of Spain from 1998 to 2009. A secondary aim was to compare the molecular characteristics of invasive and non-invasive isolates obtained in the same period in order to establish differences among them.

METHODS

Subjects and study area. We performed a laboratory-based study of invasive disease caused by *S. pyogenes* in two Spanish hospitals during a 12-year period (1998 to 2009). Hospital Donostia, located in Gipuzkoa (northern Spain), serves a population of 405 745 inhabitants including children and adults. Hospital Belltvitge, in Barcelona (eastern Spain), serves a population of 626 015 adults. Episodes were defined as growth of *S. pyogenes* in blood or another normally sterile body site. Only one isolate per episode and patient was included. Medical records and outcomes of all patients with invasive *S. pyogenes* disease were analyzed, including 30- and 90-day mortality.

Non-invasive isolate sample. One of every five isolates causing non-invasive disease (pharyngitis, otitis, vaginitis and skin infection) were collected at Hospital Donostia from January 2005 to December 2008 (2005 n=306; 2006 n=299; 2007 n=264; 2008 n=320) for comparison with the sample of invasive isolates.

Isolate characterization. All clinical isolates were characterized as GAS according to their colony morphology, β-haemolysis on blood agar plates, bacitracin susceptibility and/or latex agglutination with specific antisera (Slidex Strepto-kit; bioMérieux, Marcy l'Etoile, France). *emm* typing was performed according to guidelines provided by the Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm) and multilocus sequence typing (MLST) according to recommendations provided at http://spyogenes.mlst.net. *emm*-type was done by a restriction fragment length polymorphism (PCR-RFLP) assay as previously described [16] and at least 10% of isolates of each different *emm*-type was sequenced .Finally, *emm* gene of 55% of invasive isolates and 18% of non-invasive isolates was sequenced. Clones were defined by the combination of *emm* and sequence types.

Antibiotic susceptibility testing. Antibiotic susceptibility testing was determined in all invasive isolates by a broth microdilution method using Sensititre microtiter trays (Trek

Diagnostic Systems, East Sussex, UK) and Mueller-Hinton II broth (BioMerieux, Mercy l'Etoile, France) supplemented with lysed horse blood (3-5% v/v). In addition, in a sample of 41 invasive isolates daptomycin, linezolid and tigecycline antimicrobials were tested using the E-test (AB BIODISK, Sweden). Minimum inhibitory concentrations (MIC) were interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) [17].

***Antibiotic resistance gene detection*.** Macrolide resistance (*erm*B, *erm*A (TR) and *mef*) and tetracycline resistance (*tet*M and *tet*O) gene detection was performed by PCR, as previously described [18].

Superantigen profiling. Two multiplex PCRs were performed for detection of exotoxin genes: one for *ssa*, *spe*A, *spe*C, and *sme*Z genes, and another for *Spe*B and *slo genes* [16].

Ethical statement. The study and publication of their results was approved by the 'Comité Ético de Investigación Clínica del Área Sanitaria de Gipuzkoa'.

***Statistical analysis*.** Data were analyzed with the Instat3 program. Chi square and Fisher's exact probability tests were used to perform comparisons between groups.

RESULTS

***Incidence, clinical findings and outcome*.** A total of 247 cases of invasive *S. pyogenes* disease were identified (103 in Gipuzkoa and 144 in Barcelona) during the study period (January 1998 to December 2009). Out of them, 223 were isolated from blood, 10 from joint fluid, 8 from pleural fluid, 3 from sinovial tissue, 2 from cerebrospinal fluid, and 1 from ascitic fluid.

Children younger than 15 years old represented 19.4% of cases detected in Gipuzkoa, with an annual average incidence of 3.1 episodes/100 000 children. Among 227 adult cases, 51.5% were aged between 15 and 64 years old and 48.5% were older than 64 years. The adult annual average incidence was 1.9 episodes/100 000 inhabitants with no significant differences between Gipuzkoa and Barcelona.

By gender, 42.9 % of affected patients were women and 57.1 % were men (rate1:1.3). By seasons, 32.4 % cases were detected in winter, 26.7 % in spring, 21.9 % in summer, and 19 % in fall. No significant differences in the case fatality rate by season were found (16.3% among cases in winter, 21.2% in spring, 18.5% in summer and 17% in fall).

The most common clinical manifestation was cellulitis as sole manifestation (41.3 %), followed by bacteraemia without focus (19%), streptococcal toxic shock syndrome (STSS) (12.6%), arthritis (8.9%) and pneumonia (7.7%), having also other 6 cases with lower respiratory tract infections (five empyema and one lung abscess). The overall case fatality rate was 18.2% (45/247) (Table 1). Seventy-six percent of patients with a fatal outcome died within 1 week and all but one, who succumbed at 33 days, died within 30 days. Of these 45 patients with a fatal outcome, all (80%) except 9 were aged more than 64 years old or had comorbidities. STSS was associated with the highest mortality rate. Necrotizing fasciitis was an infrequent clinical manifestation (3.6% 9/247) and three of them also developed STSS. Skin (cellulitis, skin lesions, chickenpox or injecting drug use) was identified as the most probable portal of entry in 51% of patients with invasive infection.

Thirteen invasive infections occurred in injecting drug users (IDU) with the following clinical manifestations: cellulitis in 9, arthritis in 3, and bacteraemia in 1 and all recovered successfully. Ten puerperal sepsis cases caused by *S. pyogenes* were detected, being two of them complicated with STSS.

The most common risk or associate factors for invasive *S. pyogenes* infection were age older than 64 years (44.6%), immunosuppression (16.2%), respiratory diseases (11.3%), diabetes type II (6.1%), cirrhosis (2.4%) and chickenpox (2.4%).

***Clone distribution among invasive isolates*.** A total of 31 different *emm*-types and 36 different sequence types (STs) were detected (Figure 1). The most prevalent clones were *emm*1/ST28 (27.9%), *emm*3/ST15-406 (9.8%), *emm*4/ST39 (6.5%), *emm*28/ST52 (6%), *emm*12/ST36 (6%), and *emm*89/ST101 (6%). The *emm*1/ST28 clone was the only one detected

each year throughout the study period (annual mean 5, SD 3.79) and was the most prevalent clone associated to fatal outcome, being responsible for 16 deaths (35.6% of total 45 deceased patients or 41% of those with a known *emm*-type). The prevalence of the majority of the clones did not show statistically significant changes throughout the study period. A peak of incidence of *emm11*/ST403-21 clone was observed in 2002-2005. This clone increased from 0% in 1998-2001 to 10.6% in 2002-2005 (p=0.013) and decreased up to 1.2% in 2006-2009 period (p=0.021). The *emm12*/ST36 clone showed an off-peak: from 11.1% (1998-2001) to 0% (2002-2005, p=0.006) and to 7.0% (2006-2009, p=0.036). The *emm18*-ST42 clone significantly decreased and was not detected after 2002. The *emm89*/ST101 clone emerged in 2002-2005 period (0% 1998-2001 vs 7.6% in 2002-2005 p=0.058) and increased up to 9.3% in 2006-2009 period (Figure 1).

Disease manifestations and emm-type. Among patients with STSS, the predominant *emm*-type was *emm*1 (35.5%) (Table 1), although a further 12 different *emm*-types were also detected. Necrotizing fasciitis and pneumonia were caused mainly by *emm*1 and *emm*3 types. Only two cases (0.8%) of meningitis were detected, both were caused by an *emm*1 type. In other clinical manifestations, such as arthritis, puerperal sepsis, cellulitis and bacteraemia, a more extensive variety of *emm*-types was identified (Table 1). Thirteen infections occurred in IDU patients, all in Barcelona, of them four were caused by *emm*25 isolates and four by *emm*59 isolates.

Comparison of emm-types causing invasive and non-invasive disease. To assess the potential of *emm*-types to cause invasive disease, we compared invasive isolates with a sample of non-invasive isolates collected between 2005 and 2008 in Gipuzkoa. Among 1189 *S. pyogenes* isolates causing non-invasive disease, 89 different *emm*-types were found, the most common being the *emm*4 (12.3%), *emm*87 (11.5%), and *emm*6 (10.4%) types. The difference in the prevalence of *emm*1 type between invasive and non-invasive isolates obtained in Gipuzkoa was significant (p<0.0001), whereas in the remaining *emm* types, no significant variations in the circulation of isolates causing non-invasive and invasive disease were found (Table 2).

Superantigen gene pattern and emm-type association. Among invasive isolates, the *spe*B and *slo* genes were detected in high percentages in most of these isolates (98.1% and 88.4%, respectively), *spe*C and *sme*Z in 43.7%, *spe*A in 39.1% and *ssa* in 20.5%. Strong associations ($p<0.0001$) (Table 3) among certain invasive *emm*-types and some superantigen genes were found: *emm*1 with *spe*A and *sme*Z, *emm*3 with *spe*A and *ssa*, *emm*4 with *spe*C, *ssa* and *sme*Z, and *emm*6 and *emm*28 with the *spe*C gene.

A sample of 161 non-invasive isolates with the same *emm*-types found in the invasive sample was checked for a superantigen gene pattern. The *spe*A gene was significantly more closely associated with invasive *emm*1 isolates (50/60) than with non-invasive *emm*1 isolates (25/42) ($p=0.01$), and the *ssa* gene was significantly more closely associated with *emm*4 invasive isolates (11/14) than with *emm*4 non-invasive isolates (2/18) ($p=0.002$). For the remaining *emm*-types no significant differences in association were found.

Antibiotic susceptibility and resistance gene detection. All invasive and non-invasive isolates were susceptible to penicillin (MIC < 0.06 µg/mL). Overall, 17% (42/247) of invasive isolates were resistant to erythromycin ranging from 44.4% (4/9) in 2002 to 7.7% (1/13) in 2009. Among erythromycin-resistant isolates, 50% (21/42) displayed the M phenotype, harbouring the *mef* gene, and the remaining half had the MLS_B phenotype associated with the *erm*B and *erm*A (TR) genes. The most frequent clones among MLS_B and M phenotypes were *emm*11/ST403 and *emm*4/ST39, respectively. Reduced susceptibility to ciprofloxacin (MIC 2-8 µg/mL) was found in 8.9% (22/247) of isolates. Tetracycline resistance was detected in 13% (32/247) of isolates, 93.8% (30/32) harbouring the *tet*M gene and 6.2% (2/32) the *tet*O gene. All 41 isolates studied against daptomycin, linezolid and tigecycline showed full susceptibility (MIC <0.06, <2, and <0.06 µg/mL, respectively).

DISCUSSION

The epidemiological study of severe *S. pyogenes* infections in Europe was analyzed by Strep-EURO program in 2003-2004 period [6]. However, no data from Spain were included in this study. The present study, which analyzes the molecular epidemiology of 247 cases of invasive *S. pyogenes* disease in Spain, extends the knowledge of *S. pyogenes* in Europe.

The annual incidence of invasive disease ranged from 0.93 to 3.2 cases per 100 000 inhabitants, showing an increasing trend since 2005. The incidence was similar to that published in other European countries [2,4,5,7,19], Canada [20] and the United States [8], but was much lower than the figure published for Oceania (38 x 100,000 inhabitants) [14]. The most prevalent clinical manifestation by far was cellulitis, comprising 41.3% of all invasive cases, matching descriptions of United Kingdom [21], although the mortality in the present study was lower. In accordance with reports in Europe and the United States [6,8], the overall case fatality rate was 18.2%. As expected, STSS was associated with the highest case fatality rate, 11 out of 15 patients that succumbed, died within 1 week. In our series, only 2 (one with STSS) patients with necrotizing fasciitis died in contrast to other published series [8,21]. As previously reported [2,4,5,7,8,19,21], greater age (>64 years old) and underlying diseases such as immunosuppression, diabetes or chickenpox were important associate factors that influenced infection outcome. Unlike to that found by to Lamagni *et al* [21], we did not detect seasonal case fatality rate differences.

The overall erythromycin resistance percentage found among invasive *S. pyogenes* isolates was unexpectedly high (17%) compared with other studies that reported values of 3-7% [4,5,7,22-24]. Resistance in Barcelona and Gipuzkoa (20.1% vs 13.6%) roughly approximated to the resistance found in non-invasive isolates in each geographic area [16,22,25]. The role of superantigens in invasive *S. pyogenes* disease remains a matter for discussion. It has been described that isolates sharing the same *emm*-type, causing invasive or non-invasive disease, often harbour the same exotoxin gene profile [3,19,26]. We also observed that, independently of

the source of the sample, most isolates sharing the same *emm*-type, also had the same exotoxin gene profile. Nevertheless, the *spe*A and *ssa* genes were significantly associated with *emm*1 and *emm*4 invasive isolates, respectively.

As reported elsewhere [2-8,13,14,19], a wide diversity of *emm*-types was found among invasive isolates, although in this study, six clones (*emm*1/ST28, *emm*3/ST15-406, *emm*4/ST39, *emm*28/ST52, *emm*12/ST36, and *emm*89/ST101) accounted for 62.3% of invasive isolates. The question of whether isolates causing invasive disease have features conferring a special ability to invade sterile body sites or whether they are simply a reflection of the main circulating clones in the community remains unclear [6,27,28]. We observed that most *emm* types had a similar prevalence among isolates causing invasive and non-invasive disease, suggesting that the general population represents an important reservoir of isolates capable of causing severe disease, as previously reported [13,28,29]. Nevertheless, when invasive isolates were compared with a sample of non-invasive isolates, we found that *emm*1 type was the only type significantly associated with invasive disease. In this study, *emm*1 type was the most prevalent invasive type (27.9%), it was responsible for more than one third of all fatal outcomes. The only type present throughout the entire study period was *emm*1 type, in agreement with previous observations that reported maintenance of this type in the last three decades [3,13,27]. The highly virulent *emm3* type ranked second among invasive isolates, in agreement with previous reports from Europe [6] and the US [8]. The *emm*1 and *emm*3 types were the most important cause of necrotizing fasciitis and pneumonia.

The frequency of the *emm*3/ST406-15 clone increased from 4.5% in 2002-2005 to 15.1% in 2006-2009 period which coincided with an outbreak of mucoid *emm*3 isolates observed in the community of Gipuzkoa in 2009 [30]. The *emm11*/ST403-21, frequently associated with macrolide resistance, had the highest frequency in 2002-2005 period in which the erythromycin-resistance rates were higher in Spain [16,22]. The frequency of the *emm28* clone among invasive isolates was lower than in other European countries and its frequency decreased over the study

period [6,7]. This clone ranked first is most northern European countries as Denmark, Finland or Norway [6,7] and third in the US [8]. We observed an increase in the *emm*89 clone which reached a frequency similar to that found in other European countries in the 2003-2004 period [6]. Nearly 5% of invasive isolates and up to 10.4% of non-invasive belonged to the *emm6*/ST382 clone associated with diminished susceptibility to fluorquinolones [22,31]. Although quinolones are not used in children the dissemination of this clone was recently described in the paediatric population in Portugal, our neighbour country [32].

The *emm*59 and *emm*25 types ranking 8th and 12th, respectively were almost exclusively linked to the IDU patient group, reflecting a clonal spread, as referred in Switzerland and United Kingdom [33,34]. However, a dramatic emergence of invasive disease caused by *emm*59 type was recently documented in Canada from 2006 to 2009 [35].

The 26-valent M protein-based vaccine would cover 82.8% of invasive *S. pyogenes* cases of our study. However, the *emm*4 type, our third most prevalent *emm*-type among invasive infections and the first causing non-invasive disease is not included in the vaccine approach. This *emm*4 type has been ranked among the eight most common types in many countries worldwide [6,13] and has been also related to an invasive outbreak of STSS in a child day care centre in northern Spain [36]. As expected, vaccine coverage promises to be successful for Spain although the limitations of the formulation of a type-specific vaccine come to evidence.

In conclusion, the incidence and epidemiology of invasive *S. pyogenes* in Spain was similar to that found in other European countries. We found an overlap between most *S. pyogenes emm*-types causing both invasive and non-invasive infections, with exception of *emm*1 type. This type was overrepresented among invasive forms and was also associated with high mortality rates, indicating that this type may have some features that enhance its virulence. Furthermore, host factors, such as increased age and underlying diseases, were critical in the outcome of patients with severe *S. pyogenes* infections.

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Conflict of interest: The authors declare that they have no conflict of interest

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Table 1. Disease manifestation, overall and disease associated case fatality rate (CFR) and *emm* type of isolates of 247 invasive *Streptococcus pyogenes* diseases in two regions of Spain, 1998-2009.

Disease manifestation	No. of cases[a]	No of deaths (CFR%)	Overall CFR%	Number of isolates (percentage of cases by disease manifestation)								
				*emm*1	*emm*3	*emm*4	*emm*6	*emm*12	*emm*28	*emm*89	other *emm*	NA[c]
STSS[b]	31	15 (48.4)	6.1	11 (35.5)	7 (22.6)	1 (3.2)	2 (6.5)	1 (3.2)	1 (3.2)	1 (3.2)	6 (19.4)	1 (3.2)
Meningitis	2	1 (50)	0.4	2 (100)	-	-	-	-	-	-	-	-
Pneumonia, empyema and lung abscess	25	6 (24)	2.4	13 (52)	3 (12)	-	1 (4)	2 (8)	-	-	1 (4)	5 (20)
Necrotizing fasciitis	6	1 (16.7)	0.4	3 (50)	1 (16.7)	-	-	-	-	-	-	2 (33.3)
Bacteraemia without focus	47	8 (17)	3.2	10 (21.3)	3 (6.4)	3 (6.4)	4 (8.5)	3 (6.4)	3 (6.4)	4 (8.5)	11 (23.4)	6 (12.8)
Cellulitis	102	12 (11.8)	4.9	18 (17.6)	5 (4.9)	8 (7.8)	4 (3.9)	6 (5.9)	7 (6.9)	6 (5.9)	36 (35.3)	12 (11.8)
Arthritis	22	1 (4.5)	0.4	2 (9.1)	1 (4.5)	1 (4.5)	-	-	1 (4.5)	2 (9.1)	10 (45.5)	5 (22.7)
Puerperal sepsis	8	0 (0)	0	-	-	1 (12.5)	-	1 (12.5)	1 (12.5)	-	4 (50)	1 (12.5)
Endocarditis	2	0 (0)	0	-	-	-	-	-	-	-	2 (100)	-
Unknown	2	1 (50)	0.4	1 (50)	1 (50)	-	-	-	-	-	-	-
TOTAL (no.)	247	45 (18.2)	18.2	60 (24.3)	21 (8.5)	14 (5.7)	11 (4.4)	13 (5.3)	13 (5.3)	13 (5.3)	70 (28.3)	32 (12.9)

[a] When two or more clinical manifestation per patient was present, only the major of they was included. Patients included in a previous clinical manifestation were excluded from the subsequent ones

[b] STSS: streptococcal toxic shock syndrome

[c] NA: not available for characterization

Table 2. Distribution of *Streptococcus pyogenes emm*-types causing non-invasive and invasive disease in a same region (Gipuzkoa) and period of time (2005-2008)

emm	**Source of isolates**		**OR (95% CI)**	**Fisher's exact test p value**
	No. invasive isolates (%) (n=47)	**No. non-invasive isolates (%) (n=1189)**		
emm 1	15 (31.9)	113 (9.5)	4.5 (2.4 to 8.5)	<0.0001
emm 3	6 (12.8)	94 (7.9)	1.7 (0.7 to 4.1)	ns[a]
emm 12	4 (8.5)	94 (7.9)	1.1 (0.4 to 3.1)	ns
emm 89	4 (8.5)	78 (6.5)	1.3 (0.5 to 3.8)	ns
emm 4	4 (8.5)	146 (12.3)	0.7 (0.2 to 1.9)	ns
emm 6	3 (6.4)	124 (10.4)	0.6 (0.2 to 1.9)	ns
emm 87	2 (4.2)	137 (11.5)	0.4 (0.08 to 1.4)	ns
emm 77	2 (4.2)	62 (5.2)	0.8 (0.2 to 3.4)	ns
emm 28	1 (2.1)	50 (4.2)	0.5 (0.07 to 3.7)	ns
emm 5	1 (2.1)	7 (0.6)	3.7 (0.4 to 30.5)	ns

[a] ns, not significant p value (>0.05).

Table 3. Association between superantigens and mainly *emm*-types among 215 available invasive *Streptococcus pyogenes* isolates for molecular characterization

emm-type (no. isolates)	*spe*A (%)	*spe*C (%)	*Ssa* (%)	*sme*Z (%)	*Slo* (%)	*spe*B (%)
emm 1 (60)	50 (83.3)	27 (45)	-	57 (95)	58 (96.7)	60 (100)
emm 3 (21)	20 (95.2)	-	18 (85.7)	1 (4.8)	17 (80.9)	21 (100)
emm 4 (14)	3 (21.4)	10 (71.4)	11 (78.59)	14 (100)	12 (85.7)	13 (92.8)
emm 6 (11)	-	11 (100)	1 (9.1)	1 (9.1)	6 (54.5)	11 (100)
emm 11 (8)	-	8 (100)	-	-	6 (75)	8 (100)
emm 12 (13)	1 (7.7)	2 (15.4)	-	-	13 (100)	13 (100)
emm 18 (4)	4 (100)	4 (100)	-	2 (50)	4 (100)	4 (100)
emm 22 (5)	3 (60)	-	4 (80)	-	5 (100)	5 (100)
emm 25 (5)	-	1 (20)	4 (80)	-	3 (60)	5 (100)
emm 28 (13)	1 (7.7)	10 (76.9)	-	2 (15.4)	12 (92.3)	12 (92.3)
emm 59 (9)	-	-	-	9 (100)	9 (100)	9 (100)
emm 89 (13)	-	4 (30.8)	-	-	13 (100)	13 (100)

Figure 1.Trends on *emm* type and multilocus sequence type (ST) of 215 invasive *Streptococcus pyogenes* isolates in two regions of Spain, 1998-2009