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Invasive group A streptococcal infections in adults, France (2006–2010)

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Abstract

Severe invasive group A streptococcal diseases have re-emerged during the past 10–20 years. In order to provide a better insight into the current epidemiological situation in France, we analysed the questionnaires regarding all invasive strains received at the National Reference Center for Streptococci (CNR-Strep) between 2006 and 2010 from patients aged ≥18 and characterized them by *emm* typing, *spe* gene detection and antibiotic resistance. Among the 1542 invasive GAS strains studied, 78% (*n* = 1206) were from blood cultures, and a streptococcal toxic shock syndrome (STSS) was described in 22% (*n* = 340) of cases, mainly associated with necrotizing fasciitis (NF) and pleuro-pulmonary infections (p <0.001). The in-hospital fatality rate was 15%. A total of 83 different *emm* types were recovered but the three predominant *emm* types, representing almost 60% of the isolates, were *emm*1 (24%), *emm*28 (17%) and *emm*89 (15%). The preponderance of each *emm* type varied according to the year, with a significant constant increase of *emm*28 strains, whereas *emm*1 strains, representing approximately 32% of GAS invasive isolates in 2007 and 2008, dropped to <15% in 2010 (p <0.001). The distribution of phage-associated superantigen genes (*spe*A, *spe*C and *ssa*) was linked to certain *emm* types. Between 2006 and 2010, the percentage that was macrolide-resistant decreased from 11% to 5%, confirming the trend observed in 2007. Fortunately, *emm*1 strains associated with the most life-threatening clinical manifestations remain susceptible to all anti-streptococcal antibiotics.

Keywords: Antibiotic resistance, *emm* type, epidemiology, group A streptococcus, invasive infections, *Streptococcus pyogenes*
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Introduction

Streptococcus pyogenes or group A streptococcus (GAS) is a human pathogen with a worldwide distribution [1]. Most often it causes mild superficial infections of the skin, such as impetigo, or of the upper respiratory tract, such as pharyngitis. However, bacteraemia and necrotizing fasciitis (NF) associated or not with streptococcal toxic shock syndrome (STSS) are life-threatening GAS infections. An increase of invasive GAS infections during the 1980s has been noticed worldwide [1]. In France, between 2000 and 2009, the incidence of GAS bacteraemia (i.e. positive blood and cerebrospinal fluid cultures) increased from 1.5 to 2.6 cases per 100 000 population according to the report of the Epibac national hospital-based laboratory network (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). In 2007 in France, the overall incidence of invasive GAS infections, including deep infections, such as pneumonia and postpartum endometritis, and STSS without positive blood cultures, was estimated to be 3.1 (95% CI, 2.9–3.2) cases per 100 000 population [2]. This trend seems to be stable as incidence rates of invasive GAS infections in 2008 and 2009 were 2.4 and 2.6 per 100 000 population, respectively.

Several important virulence factors involved in GAS pathogenicity have been described, including the M protein and

the exotoxins [3]. The M protein is a fimbrial surface protein encoded by the *emm* gene and more than 160 different M protein gene sequence types (*emm* types) have been described. Determination of the *emm* type is mandatory for epidemiological investigations of GAS infections [4,5]. Streptococcal pyrogenic exotoxins SpeA, SpeC, SpeG to SpeM, streptococcal superantigen A (SSA), and streptococcal mitogenic exotoxin Z (SmeZ) have been identified as superantigens (SA) [6,7]. The proteins SpeB and SpeF, initially described as toxins, were shown to be a cysteine protease and a DNase, respectively. Usually, isolates of the same *emm* type share a similar SA profile but variants differing by the presence or absence of SA may occur [8].

Group A streptococcus strains remain susceptible to β-lactams, which is the treatment of choice for GAS infections. However, in patients with β-lactam allergy, macrolides and lincosamides are first-line alternative therapeutics. Moreover, clindamycin is used in association with penicillin to treat STSS to reduce, as a protein synthesis inhibitor, the synthesis of virulence factors [9]. In recent years, macrolide- and lincosamide-resistant GAS strains have gradually spread [10–13], in particular among certain *emm* types [14,15].

The aim of this study was to provide a better insight into the current epidemiological situation regarding adult invasive GAS infections in France. We analysed the questionnaires and characterized all invasive strains received at the National Reference Center for Streptococci (CNR-Strep) between 2006 and 2010 from patients aged 18 years and over, by *emm* typing, SA gene detection and antibiotic resistance.

Methods

Case definition

Group A streptococcus invasive infection was defined as the isolation of bacteria from a usually sterile site (e.g. blood, cerebrospinal fluid, bone or joint fluid), or from samples obtained from a non-sterile site in combination with clinical signs of NF or STSS. STSS was defined according to the definitions of the US Working Group on Severe Streptococcal Infections [16]. Bacteraemia was considered to be without focus when no focal symptoms could be identified. In this report, all non-redundant invasive GAS strains isolated from patients ≥18 years, between January 2006 and December 2010 and sent to the CNR-Strep, were studied.

Collection of clinical data and strains

The French national reference centre for streptococci (CNR-Strep) collects, prospectively, GAS strains isolated from invasive infections from a stable network of 232 laboratories located throughout the 22 French administrative regions. Forty-three (18%) laboratories belonged to university hospitals, 157 (68%) were located in general hospitals, and the remaining 32 (14%) were private laboratories. Clinical characteristics were obtained from questionnaires sent on a voluntary basis with invasive isolates. Data collected included sex, date of birth, date and origin of the sample, geographical area and clinical manifestations. Strains were stored in 2% glycerol Todd Hewitt broth at −80°C.

Strains identification

Group A streptococcus isolates were confirmed to be *S. pyogenes* using morphological and growth characteristics, including β haemolysis on sheep blood agar, production of pyrrolydonyl arylamidase, and grouping of carbohydrate with Lancefield group A specific antigen.

emm sequence typing

The *emm* sequence type was determined by sequencing the variable 5′-end of the *emm* gene and comparing sequences with the database of the Center for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/doc.htm).

Superantigen detection

All the strains were tested by a multiplex PCR method for the presence of genes encoding the toxins or superantigens SpeA, SpeB, SpeC and Ssa [17,18].

Antibiotic susceptibility testing

Antibiotic susceptibility to penicillin G, amoxicillin, erythromycin, clindamycin, tetracycline, gentamicin, levofloxacin and vancomycin was determined by the disk diffusion method on Mueller–Hinton agar with 5% sheep blood according to CASFM guidelines (http://www.sfm.asso.fr). Macrolides and tetracycline-resistant genes were detected by multiplex PCR as described [19].

Statistical analysis

The chi-square test for trend was used and $p < 0.05$ was considered significant for all tests.

Results

Number of cases and demographic characteristics

From January 2006 to December 2010, 1542 cases of invasive GAS infections were reported to the CNR-Strep by the microbiologists of the 232 laboratories located throughout the 22 French administrative regions (range by year, 183–

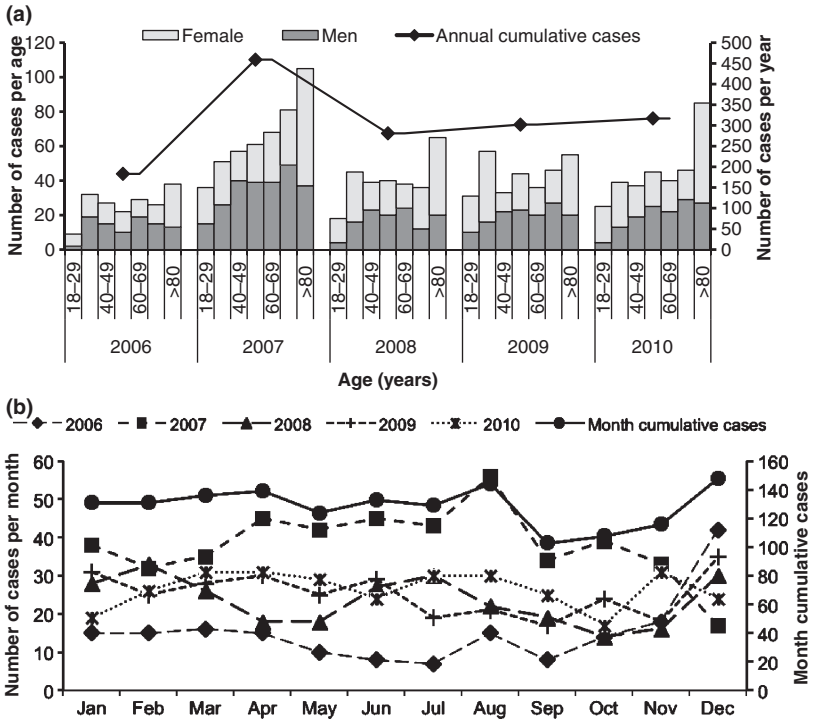

FIG. 1. Distribution of adult invasive GAS strains according to sex, age and year of isolation (a) and to the month of the year (b).

459) (Fig. 1). An over-representation of cases in 2007 was linked to the French national survey performed in collaboration with the Institut National de Veille Sanitaire (InVS) [2]. The number of GAS strains collected by region was correlated with the population density. Therefore two regions, Ile de France and Rhône-Alpes, provided 18% and 12% of GAS isolates, respectively (data not shown). Seasonal patterns of invasive GAS infections were observed but varied depending on the year. However, for all years, cases gradually increased from the end of October to April (Fig. 1b). The median age was 60.3 years (range 18–103) and 52.4% were female (Fig. 1). The number of cases increased with the age of the patients. Among those between 40 and 79 years old, cases were more frequent in men than in women (p <.0001).

Clinical manifestations

Among the 1542 invasive GAS infections included in the study, 1206 (78.2%) cases have a documented bacteraemia. Various clinical manifestations were reported (Table 1). Skin or soft tissue infections were the most frequent, accounting for 43.7% of the cases. NF was reported in 21.8% of cases, accounting for 50% of skin and soft tissue infections, and was 2-fold more frequent in men between 30 and 59 years old than in women (p <0.01). The frequency of other streptococcal diseases was as follows: gynaeco-obstetrical sepsis, 8.9%; pleuro-pulmonary infections, 8.8%; osteoarticular, 6.9%; and other clinical manifestations such as intra-abdominal infections, upper respiratory tract or central nervous system infections, <5% of cases. Bacteraemia without focal symptoms accounted for 391 (25.4%) of the cases. Gynaeco-obstetrical sepsis represented 53.2% of cases in women of childbearing age (18–39 years, n = 218). An STSS was described in 22% (n = 340) of cases. It was, respectively, 3- and 1.9-fold more frequently observed in cases of NF and pleuro-pulmonary infections (p <0.001) than in other clinical manifestations. STSS was more frequently reported in persons aged 50–69 years (29.6% vs. 19.2%, p <0.001). The in-hospital fatality rate, available in 231 cases, was 15%; it reached 18.1% of NF and 44.9% of STSS. The lowest fatality rate (<1%) was observed in young women with postpartum sepsis.

emm sequence types and streptococcal diseases

A total of 83 different *emm* sequence types were identified. All strains belonged to an already described *emm* type. Three predominant *emm* types, namely *emm*1 (24%), *emm*28 (17%) and *emm*89 (15%), accounted for 56% of all isolates over the 5-year period. The distribution of *emm* types varied according to the year considered (Fig. 2a). A significant increase of *emm*28 strains was observed from 2006 (14%) to 2010 (25%, p <.01). A similar trend, although not significant, was observed for *emm*3 strains during the 5-year period (6% in 2006 to 8.5% in 2010). Consequently, *emm*1 strains, which represented approximately 32% of GAS invasive isolates in

TABLE 1. Clinical manifestations of invasive GAS infections, France, 2006–2010

Clinical manifestations *n* = 1542 (%)	All case-patients (%)		Age group (years)													
			18–29		30–39		40–49		50–59		60–69		70–79		≥80	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Skin/soft tissue infections, *n* = 674 (43.7%)																
Necrotizing fasciitis	189 (12.3)	148 (9.6)	7 (0.5)	9 (0.6)	30 (1.9)	13 (0.8)	38 (2.5)	17 (1.1)	29 (1.9)	25 (1.6)	38 (2.5)	29 (1.9)	27 (1.8)	22 (1.4)	20 (1.3)	33 (2.1)
Erysipelas	61 (4)	89 (5.8)	3 (0.2)	0	3 (0.2)	5 (0.3)	3 (0.2)	6 (0.4)	6 (0.4)	8 (0.5)	10 (0.6)	10 (0.6)	16 (1)	11 (0.7)	20 (1.3)	49 (3.2)
Cellulitis	15 (1)	9 (0.6)	0	2 (0.1)	2 (0.1)	0	0	0	5 (0.3)	3 (0.2)	3 (0.2)	3 (0.2)	3 (0.2)	0 (0)	2 (0.1)	1 (0.1)
Others	83 (5.4)	80 (5.2)	4 (0.3)	0	5 (0.3)	7 (0.5)	13 (0.8)	8 (0.5)	17 (1.1)	7 (0.5)	7 (0.5)	11 (0.7)	20 (1.3)	14 (0.9)	17 (1.1)	33 (2.1)
Bacteraemia without focus, *n* = 391 (25.4%)	187 (12.1)	204 (13.2)	10 (0.6)	12 (0.8)	14 (0.9)	23 (1.5)	28 (1.8)	14 (0.9)	27 (1.8)	23 (1.5)	31 (2)	18 (1.2)	42 (2.7)	34 (2.2)	35 (2.3)	80 (5.2)
Gynaeco-obstetrical sepsis, *n* = 138 (8.9%)																
Endometritis	0 (0)	87 (5.6)	0 (0)	31 (2.0)	0 (0)	51 (3.3)	0 (0)	5 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pelvis peritonitis	0 (0)	29 (1.9)	0 (0)	5 (0.3)	0 (0)	11 (0.7)	0 (0)	6 (0.4)	0 (0)	4 (0.3)	0 (0)	2 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)
Chorioamniotitis	0 (0)	6 (0.4)	0 (0)	2 (0.1)	0 (0)	4 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Others	0 (0)	16 (1)	0 (0)	9 (0.6)	0 (0)	3 (0.2)	0 (0)	2 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)
Pleuro-pulmonary infections, *n* = 136 (8.8%)																
Pneumonia	59 (3.8)	39 (2.5)	1 (0.1)	3 (0.2)	5 (0.3)	3 (0.2)	9 (0.6)	7 (0.5)	7 (0.5)	2 (0.1)	13 (0.8)	2 (0.1)	10 (0.6)	7 (0.5)	14 (0.9)	15 (1.0)
Pleural infection	11 (0.7)	5 (0.3)	0 (0)	0 (0)	2 (0.1)	1 (0.1)	2 (0.1)	0 (0)	3 (0.2)	3 (0.2)	2 (0.1)	0 (0)	1 (0.1)	0 (0)	1 (0.1)	1 (0.1)
Others	9 (0.6)	13 (0.8)	1 (0.1)	3 (0.2)	1 (0.1)	4 (0.3)	2 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	3 (0.2)	2 (0.1)	0 (0)	1 (0.1)	1 (0.1)	1 (0.1)
Osteoarticular infections, *n* = 107 (6.9%)																
Septic arthritis	41 (2.7)	29 (1.9)	2 (0.1)	2 (0.1)	7 (0.5)	1 (0.1)	11 (0.7)	2 (0.1)	9 (0.6)	4 (0.3)	5 (0.3)	6 (0.4)	6 (0.4)	3 (0.2)	1 (0.1)	11 (0.7)
Bursitis	14 (0.9)	2 (0.1)	2 (0.1)	1 (0.1)	6 (0.4)	0 (0)	1 (0.1)	1 (0.1)	2 (0.1)	0 (0)	1 (0.1)	0 (0)	2 (0.1)	0 (0)	0 (0)	0 (0)
Osteomyelitis	11 (0.7)	1 (0.1)	1 (0.1)	0 (0)	3 (0.2)	0 (0)	4 (0.3)	0 (0)	1 (0.1)	0 (0)	1 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	1 (0.1)
Prosthesis infection	4 (0.3)	3 (0.2)	1 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	1 (0.1)	0 (0)	2 (0.1)
Others	1 (0.1)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)	1 (0.1)
Intra-abdominal infections, *n* = 38 (2.5%)	17 (1.1)	21 (1.4)	1 (0.1)	1 (0.1)	6 (0.4)	5 (0.3)	4 (0.3)	3 (0.2)	2 (0.1)	8 (0.5)	3 (0.2)	2 (0.1)	1 (0.1)	2 (0.1)	0 (0)	0 (0)
Central nervous system infections, *n* = 22 (1.4%)																
Meningitis	10 (0.6)	10 (0.6)	1 (0.1)	1 (0.1)	3 (0.2)	0 (0)	1 (0.1)	1 (0.1)	1 (0.1)	3 (0.2)	2 (0.1)	2 (0.1)	2 (0.1)	3 (0.2)	0 (0)	0 (0)
Cerebral abscess	1 (0.1)	1 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Upper respiratory tract infections, *n* = 16 (1%)																
Epiglottitis	3 (0.2)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)
Sinusitis	0 (0)	1 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Others	6 (0.4)	6 (0.4)	0 (0)	1 (0.1)	1 (0.1)	1 (0.1)	2 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	0 (0)	0 (0)	0 (0)	2 (0.1)	2 (0.1)	0 (0)
Others, *n* = 20 (1.3%)																
Endocarditis	7 (0.5)	2 (0.1)	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	3 (0.2)	0 (0)	2 (0.1)	0 (0)	1 (0.1)	1 (0.1)	0 (0)	1 (0.1)
Pyelonephritis	1 (0.1)	5 (0.3)	0 (0)	0 (0)	0 (0)	2 (0.1)	0 (0)	0 (0)	0 (0)	2 (0.1)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)
Central venous catheter infection	4 (0.3)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	2 (0.1)	1 (0.1)
Total	734 (47.6)	808 (52.4)	35 (2.3)	84 (5.4)	90 (5.8)	134 (8.7)	119 (7.7)	74 (4.8)	117 (7.6)	95 (6.2)	124 (8)	87 (5.6)	132 (8.6)	103 (6.7)	117 (7.6)	231 (15.0)

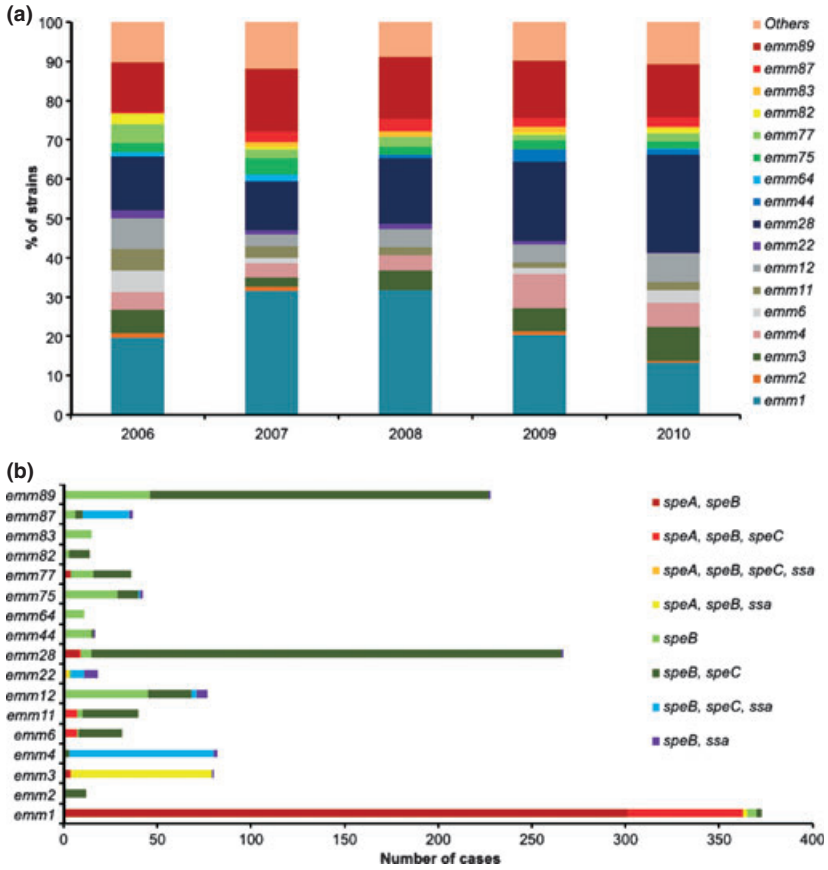

FIG. 2. Molecular characterization of GAS isolated from invasive infections in adults, France 2006–2010. (a) Distribution of the top 17 *emm* types, for which more than ≥10 strains were isolated; 'others' designates all strains belonging to rare *emm* types (≤9 strains): *emm*5, *emm*8, *emm*9, *emm*18, *emm*25, *emm*27G, *emm*29, *emm*32, *emm*33, *emm*42, *emm*43, *emm*46, *emm*49, *emm*50, *emm*53, *emm*56, *emm*58, *emm*59, *emm*60, *emm*61, *emm*63, *emm*65, *emm*68, *emm*69, *emm*71, *emm*73, *emm*74, *emm*76, *emm*78, *emm*79, *emm*81, *emm*85, *emm*88, *emm*90, *emm*92, *emm*93, *emm*94, *emm*101, *emm*102, *emm*104, *emm*106, *emm*108, *emm*109, *emm*110, *emm*112, *emm*113, *emm*116, *emm*118, *emm*122, *emm*124, st211, st221, st587, st809, st854, st1389, st2037, st2147, st2460, st2861UK, st2904, st3757, stD432, stG1750, stNS1033, stXH1. (b) Superantigen profiles according to *emm* types.

2007 and 2008, dropped to <15% in 2010 (p <0.001). *emm*1 strains were recovered from 32% (*n* = 108/337) of NF and 37.9% of STSS (*n* = 129/340) (Table 2) cases. Three other major genotypes within STSS cases were *emm*3 (*n* = 35/340), *emm*89 (*n* = 35/340) and *emm*28 (*n* = 34/340). Each of them was, respectively, responsible for <11% of STSS cases. Among skin and soft tissue infections, *emm*1 strains were responsible for most of the NF cases (p <0.001) whereas erysipelas were mainly caused by *emm*28 strains (p <0.001). Moreover, *emm*28 strains seemed to be associated with septic arthritis and gynaeco-obstetrical infections (p <0.01 and p <0.001, respectively). GAS strains isolated from pulmonary infections were predominantly *emm*1 (p <0.001). *emm*87 strains were identified mostly in patients aged 50–59 years old, whereas *emm*22 were predominantly found in those under 30 years (p <0.001) unless there was a particular link with any clinical manifestation.

Superantigen gene patterns and *emm* types

speB, encoding the chromosomal cysteine protease, was detected in all strains. *speA* carried by prophages was detected among 492 strains, primarily in *emm*1 (*n* = 365; 74.2%) and *emm*3 (*n* = 79; 16%) strains (p <0.001) (Fig. 2b). The remainder *speA*-positive isolates (48; 9.8%) included *emm*6, *emm*11, *emm*22, *emm*28, *emm*77, *emm*82, *emm*83, *emm*87 and *emm*89 strains. However, *speA* was not detected in *emm*2, *emm*4, *emm*12, *emm*44, *emm*64, *emm*75 or *emm*83 strains. *speC* was detected in 15 out of the 17 most prevalent *emm* types and in more than 90% of *emm*2, *emm*4, *emm*6, *emm*11 and *emm*28 strains. Ten different *emm* types were positive for *ssa*, including more than 70% of *emm*3, *emm*4, *emm*22 and *emm*87 strains (p <0.001). Conversely, *ssa* was never detected in *emm*2, *emm*6, *emm*11, *emm*64, *emm*77, *emm*82 or *emm*83 types. STSS occurred more frequently in GAS strains harbouring *speA* or *speC* SA genes (p <0.001) than *ssa*.

TABLE 2. Correlation between the top 17 *emm* types encountered in France (2006–2010) and clinical manifestations

	Number of isolates (%)															
	emm-types															
Clinical manifestations	***emm1***	***emm2***	***emm3***	***emm4***	***emm6***	***emm11***	***emm12***	***emm22***	***emm28***	***emm44***	***emm64***	***emm75***	***emm77***	***emm82***	***emm83***	***emm87***	***emm89***
STSS (n = 340) 22%	129 (37.9)	2 (0.6)	35 (10.3)	20 (5.9)	10 (2.9)	5 (1.5)	11 (3.2)	0 (0)	34 (10)	2 (0.6)	2 (0.6)	7 (2.1)	6 (1.8)	1 (0.3)	1 (0.3)	6 (1.8)	35 (10)
Skin/soft tissue infections (n = 674) 43.7%																	
Necrotizing fasciitis (n = 337)	108 (32)	2 (0.6)	21 (6.2)	17 (5)	6 (1.8)	4 (1.2)	15 (4.5)	2 (0.6)	42 (12.5)	6 (1.8)	4 (1.2)	12 (3.6)	6 (1.8)	4 (1.2)	4 (1.2)	7 (2.1)	40 (11.9)
Erysipelas (n = 150)	26 (17.3)	0 (0)	4 (2.7)	8 (5.3)	4 (2.7)	3 (2)	11 (7.3)	0 (0)	38 (25.3)	3 (2)	1 (0.7)	7 (4.7)	1 (0.7)	1 (0.7)	1 (0.7)	2 (1.3)	30 (20)
Cellulitis (n = 24)	1 (4.2)	0 (0)	3 (12.5)	1 (4.2)	0 (0)	0 (0)	2 (8.3)	1 (4.2)	1 (4.2)	0 (0)	0 (0)	1 (4.2)	0 (0)	0 (0)	0 (0)	1 (4.2)	6 (25)
Others (n = 163)	24 (14.7)	3 (1.8)	10 (6.1)	8 (4.9)	3 (1.8)	4 (2.5)	8 (4.9)	1 (0.6)	27 (16.6)	1 (0.6)	2 (1.2)	5 (3.1)	8 (4.9)	3 (1.8)	3 (1.8)	7 (4.3)	24 (14.7)
Bacteraemia without focus n = 391 (25.4%)	89 (22.8)	1 (0.3)	21 (5.4)	23 (5.9)	7 (1.8)	14 (3.6)	21 (5.4)	8 (2)	58 (14.8)	4 (1)	3 (0.8)	7 (1.8)	8 (2)	4 (1)	5 (1.3)	11 (2.8)	63 (16.1)
Gynaeco-obstetrical sepsis (n = 138) 8.9%																	
Endometritis (n = 87)	9 (10.3)	2 (2.3)	2 (2.3)	7 (8)	3 (3.4)	2 (2.3)	2 (2.3)	0 (0)	30 (34.5)	0 (0)	0 (0)	4 (4.6)	4 (4.6)	1 (1.1)	0 (0)	1 (1.1)	17 (19.5)
Pelvis peritonitis (n = 29)	10 (34.5)	0 (0)	0 (0)	2 (6.9)	1 (3.4)	1 (3.4)	0 (0)	0 (0)	11 (37.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.4)	1 (3.4)
Chorioamniotitis (n = 6)	5 (83.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Others (n = 16)	3 (18.8)	0 (0)	0 (0)	0 (0)	1 (6.3)	1 (6.3)	0 (0)	1 (6.3)	5 (31.3)	0 (0)	0 (0)	0 (0)	1 (6.3)	0 (0)	0 (0)	0 (0)	2 (12.5)
Pleuro-pulmonary infections (n = 136) 8.8%																	
Pneumonia (n = 98)	38 (38.8)	1 (1)	7 (7.1)	7 (7.1)	3 (3.1)	2 (2)	8 (8.2)	1 (1)	10 (10.2)	0 (0)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)	2 (2)	8 (8.2)
Pleural infection (n = 16)	5 (31.3)	0 (0)	3 (18.8)	0 (0)	0 (0)	1 (6.3)	0 (0)	0 (0)	2 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (12.5)	2 (12.5)
Others (n = 22)	10 (45.4)	0 (0)	3 (13.6)	1 (4.5)	0 (0)	0 (0)	2 (9.1)	1 (4.5)	2 (9.1)	0 (0)	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	2 (9.1)
Osteoarticular infections (n = 107) 6.9%																	
Septic arthritis (n = 70)	9 (12.9)	1 (1.4)	1 (1.4)	3 (4.3)	0 (0)	3 (4.3)	3 (4.3)	2 (2.9)	17 (24.3)	1 (1.4)	1 (1.4)	2 (2.9)	3 (4.3)	0 (0)	1 (1.4)	1 (1.4)	13 (18.6)
Bursitis (n = 16)	2 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.3)	0 (0)	0 (0)	3 (18.8)	1 (6.3)	0 (0)	1 (6.3)	0 (0)	0 (0)	1 (6.3)	0 (0)	3 (18.8)
Osteomyelitis (n = 12)	0 (0)	1 (8.3)	0 (0)	0 (0)	1 (8.3)	0 (0)	1 (8.3)	0 (0)	1 (8.3)	0 (0)	0 (0)	1 (8.3)	1 (8.3)	0 (0)	0 (0)	0 (0)	2 (16.7)
Prothesis infection (n = 7)	1 (14.3)	0 (0)	0 (0)	2 (28.6)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Others (n = 2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Intra-abdominal infections (n = 38) 2.5%	7 (18.4)	0 (0)	0 (0)	1 (2.6)	1 (2.6)	1 (2.6)	2 (5.3)	1 (2.6)	10 (26.3)	0 (0)	0 (0)	1 (2.6)	1 (2.6)	1 (2.6)	0 (0)	0 (0)	5 (13.2)
Central nervous system infections (n = 22) 1.4%																	
Meningitis (n = 20)	9 (45)	1 (5)	2 (10)	0 (0)	1 (5)	1 (5)	0 (0)	0 (0)	3 (15)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)
Cerebral abscess (n = 2)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Upper respiratory tract infections (n = 16) 1%																	
Epiglottitis (n = 3)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.7)
Sinusitis (n = 1)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Others (n = 12)	4 (33.3)	0 (0)	1 (8.3)	2 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	2 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (25)
Others (n = 20) 1.3%																	
Endocarditis (n = 9)	2 (22.2)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (22.2)	2 (22.2)
Pyelonephritis (n = 6)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	3 (50)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)
Central venous catheter infection (n = 5)	1 (20)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (40)

Antimicrobial susceptibility

All GAS strains were susceptible to β-lactams and vancomycin, and had a low-level resistance to gentamicin. The overall rate of erythromycin resistance was 6.5% (Table 3). However, a 50% decrease was observed during this 5-year period. In 2006, 10.9% of GAS strains were erythromycin resistant whereas the rate was 5% in 2010 ($p < 0.02$). Two *emm* types, *emm*11 and *emm*28, were over-represented among erythromycin-resistant strains ($p < 0.001$). Among the 100 erythromycin-resistant strains collected during this 5-year period, 71 were constitutively resistant to clindamycin (cMLS), 13 harboured an inducible clindamycin-resistant phenotype (iMLS) and 16 displayed an M phenotype (data not shown). Genetic characterization of erythromycin and clindamycin resistance is shown in Table 3. All 16 isolates harbouring an M phenotype contained *mef*(A); among 71 cMLS strains, *erm*(B) and *erm*(A) accounted for 97% (69/71) and 3% (2/71), respectively. Among the 13 iMLS isolates, *erm*(B) and *erm*(A) accounted for 53.8% (7/13) and 46.2% (6/13), respectively. A significant correlation was observed between *erm*(B) and *emm*11 and *emm*28 strains ($p < 0.001$ and $p < 0.02$, respectively) and between *mef*(A) and *emm*4 strains ($p < 0.001$). The overall rate of tetracycline resistance was 13% (Table 3). In contrast to the observed decrease in macrolide resistance, tetracycline resistance slightly increased and *emm*11, *emm*44, *emm*77 and *emm*83 types were strongly associated with this resistance ($p < 0.001$). Seventy-eight per cent of tetracycline-resistant strains harboured *tet*(M). The remaining tetracycline-resistant strains had *tet*(O) (11.4%), *tet*(M) + *tet*(L)(9%), *tet*(L) (1%) and *tet*(M) + *tet*(K) (0.5%) (Table 3).

Group A streptococcus strains have been tested for fluoroquinolone (FQ) resistance since 2008. Among the 614 GAS strains tested, 82 (13.3%) demonstrated a decrease in FQ susceptibility (data not shown) and were distributed among 20 different *emm* types: 66% (10/15) and 45% (5/13) of *emm*6 and *emm*75 strains, respectively.

Discussion

This study describes the clinical and microbiological data obtained from 1542 cases of invasive GAS infections among adults in France between 2006 and 2010. The incidence rate of GAS invasive infection was estimated in 2007 at 3.1 cases per 100 000 population by the prospective survey conducted in collaboration with InVS [2]. This rate is comparable to those recently reported from surveys in Europe (2.1) and the USA (3.8) [20,21]. Seasonal patterns were observed, but varied depending upon the year. However, as observed in other European countries, GAS invasive infections seem to be more frequent in winter and early spring, which might be due to the vulnerability of patients with viral respiratory infections due to syncytial and influenza viruses [20,22].

The median age of patients was 60.3 years and infection was more frequent in women than in men, as described previously in Denmark, Norway and Sweden, but this is in contrast to what has been observed in other European countries and the USA [20,21,23,24]. The reasons for the increasing incidence of GAS invasive infection in the elderly are not fully understood but it may be explained by the presence of other comorbidities such as diabetes, a clearly identified risk factor, especially in GAS skin and soft tissue infections [20–22]. However, in the age group 40–79 years, most of the infections occurred in men ($p < 0.001$), especially with osteoarticular localizations ($p < 0.02$). Women under 60 were more likely to develop intra-abdominal and gynaeco-obstetrical infections ($p < 0.001$). Among the 340 STSS cases recorded, the majority was associated with NF (36.5%). The

TABLE 3. Characterization of macrolide and tetracycline-resistant GAS strains according to *emm* types, France 2006–2010

		Macrolides resistance					Tetracycline resistance					
		Phenotypic pattern		Genetic characterization			Phenotypic pattern	Genetic characterization				
emm-types	All, *n* (%)	Erythromycin (%)	Clindamycin (%)	*erm*(A) (%)	*erm*(B) (%)	*mef*(A) (%)	Tetracycline (%)	tet(M) (%)	tet(O) (%)	tet(L) (%)	tet(M) + tet(L) (%)	tet(M) + tet(K) (%)
*emm*1	373 (24.2)	1 (0.3)	1 (0.3)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*4	82 (5.3)	6 (7.3)	0 (0)	0 (0)	0 (0)	6 (100)	1 (1.2)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*11	40 (2.6)	22 (55)	21 (52.5)	0 (0)	21 (95)	1 (5)	24 (60)	21 (10.5)	0 (0)	1 (0.5)	1 (0.5)	1 (0.5)
*emm*12	77 (5)	5 (6.5)	1 (1.3)	1 (20)	0 (0)	4 (80)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*22	18 (1.2)	3 (16.7)	3 (16.7)	0 (0)	3 (100)	0 (0)	9 (50)	9 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*28	267 (17.3)	42 (15.7)	42 (15.7)	0 (0)	42 (100)	0 (0)	1 (0.4)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*44	17 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	15 (88.2)	13 (6.5)	0 (0)	0 (0)	2 (1)	0 (0)
*emm*64	11 (0.7)	1 (9.1)	1 (9.1)	1 (100)	0 (0)	0 (0)	9 (81.8)	9 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*75	42 (2.7)	3 (7.1)	1 (2.4)	1 (33)	0 (0)	2 (66)	3 (7.1)	1 (0.5)	1 (0.5)	0 (0)	1 (0.5)	0 (0)
*emm*77	36 (2.3)	2 (5.6)	2 (5.6)	2 (100)	0 (0)	0 (0)	2 (75)	6 (3)	21 (10.5)	0 (0)	0 (0)	0 (0)
*emm*82	14 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (35.7)	5 (2.5)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*83	15 (1.0)	2 (13.3)	2 (13.3)	0 (0)	2 (2)	0 (0)	12 (80)	12 (6)	0 (0)	0 (0)	0 (0)	0 (0)
*emm*89	228 (14.8)	2 (0.9)	2 (0.9)	1 (1)	1 (1)	0 (0)	1 (0.4)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)
Others	162	11 (6.8)	8 (4.9)	2 (2)	6 (6)	3 (3)	94 (58)	79 (39.5)	0 (0)	1 (0.5)	14 (7)	0 (0)
Total	1542	100 (6.5)	84 (5.4)	8 (8)	76 (76)	16 (16)	201 (13)	157 (78.1)	23 (11.4)	2 (1)	18 (9)	1 (0.5)

fatality rate among those STSS cases was 44.9%, a value consistent with the 43% reported in 2007 [2].

*emm*1 was the most prevalent *emm* type, which is in accordance with results from the USA, Japan and across Europe, followed by *emm*28 and *emm*89 [25–27]. However, we observed that *emm*1 decreased since 2006, to be replaced by *emm*28, *emm*89 and *emm*3 types (Fig. 2a). In 2010, *emm*1 represented only 13.2% of the strains compared with 24.9% and 13.6% for *emm*28 and *emm*89, respectively. Fluctuations of *emm* types have been observed in other European countries and they may reflect ongoing epidemic waves, herd immunity or population immunity [20,27].

As observed in previous surveys, *emm*1 was associated with NF and *emm*28 with puerperal infections [20]. Additionally, this study highlights a correlation between *emm*28 and erysipelas. Therefore, the fluctuation observed for *emm*1 and *emm*28 is likely to be due to a combination of several factors over the 5-year period: the increase of gynaeco-obstetrical sepsis from 4.9 to 9.8%, the increase of erysipelas from 8.2 to 13.6%, and the decrease of NF from 25.7 to 19.2%.

As already reported, *speA* was detected in almost all *emm*1 (98%) and *emm*3 (99%) types and less frequently among other *emm* types. *speC* was detected in more than 90% of *emm*2, *emm*4, *emm*6, *emm*11 and *emm*28. The *ssa* gene was detected in more than 70% of *emm*3, *emm*4, *emm*22 and *emm*87. Nevertheless, most of the strains within a given *emm* type shared the same SA gene profile, which is that found in other countries [27,28]. The relevance of SA genes relating to invasive infections remains controversial as they are present at the same rate in non-invasive isolates [23].

Up to now, GAS remained universally susceptible to β-lactams and glycopeptides. All strains tested were susceptible to penicillin, amoxicillin and vancomycin, whereas resistances were observed for macrolides and tetracycline. The increase of macrolide and lincosamide resistance rates among GAS observed in many countries constitutes a major concern because these antibiotics are recommended for the treatment of GAS infections in penicillin-allergic patients and in case of STSS. In France, a marked increase was observed in 2004 with a rate reaching 35% [10,29]. In this study, we show that since 2006, erythromycin resistance decreased significantly from 10.9 to 5% ($p < 0.02$), an evolution already observed in Spain and Portugal [30–32]. The reason for this decrease is likely to be due to the reduction of macrolides prescription in France since 2002 [33]. Macrolide resistance was mainly due to the presence of *erm*(B) (76%) among *emm*28 and *emm*11 types (Table 3), as reported in other European countries [30,32,34]. Surprisingly, *emm*1 strains accounting for the majority of STSS and NF are rarely resistant to erythromycin (0.3%). Among *mef*(A)-carrying isolates the predominant clone was *emm*4, which is widespread in Europe [10,19,34–36]. The overall rate of tetracycline resistance reached 13% (Table 3). The prevalence of tetracycline-resistant strains fluctuates over the study period, between 11 and 16.6% (Table 3). *tet*(M) accounted for 78.6% of resistant strains and *emm*77 was the most prevalent *emm* type (13.4%) among tetracycline-resistant strains, as described in Germany [37]. However, *emm*11, *emm*44 and *emm*83 strains were also strongly associated with tetracycline resistance. Finally, non-susceptible FQ strains were due to the emergence of an *emm*6 clone, as described in Spain [38,39].

In conclusion, this study provides accurate data about the current epidemiological situation regarding adult invasive GAS infections in France. *emm*1 still remains the most prevalent *emm* type and is associated with the most life-threatening clinical manifestations. Fortunately, *emm*1 strains remain susceptible to all anti-streptococcal antibiotics. Importantly, the trend observed in 2007 concerning the decrease of erythromycin resistance was confirmed in 2008–2010, an evolution that can be correlated with the improvement of good practices in antibiotic use [33].

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References

1. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. *Lancet Infect Dis* 2005; 5: 685–694.

2. Lepoutre A, Doloy A, Bidet P *et al.* Epidemiology of invasive *Streptococcus pyogenes* infections in France, 2007. *J Clin Microbiol* 2011; under revision.
3. Bisno AL, Brito MO, Collins CM. Molecular basis of group A streptococcal virulence. *Lancet Infect Dis* 2003; 3: 191–200.
4. Beall B, Facklam R, Thompson T. Sequencing *emm*-specific PCR products for routine and accurate typing of group A streptococci. *J Clin Microbiol* 1996; 34: 953–958.
5. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global *emm* type distribution of group A streptococci: systematic review and implications for vaccine development. *Lancet Infect Dis* 2009; 9: 611–616.
6. Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. *Immunol Rev* 2008; 225: 226–243.
7. Norrby-Teglund A, Thulin P, Gan BS *et al.* Evidence for superantigen involvement in severe group A streptococcal tissue infections. *J Infect Dis* 2001; 184: 853–860.
8. Schmitz FJ, Beyer A, Charpentier E *et al.* Toxin-gene profile heterogeneity among endemic invasive European group A streptococcal isolates. *J Infect Dis* 2003; 188: 1578–1586.
9. Sawai J, Hasegawa T, Kamimura T *et al.* Growth phase-dependent effect of clindamycin on production of exoproteins by *Streptococcus pyogenes*. *Antimicrob Agents Chemother* 2007; 51: 461–467.
10. Bingen E, Bidet P, Mihaila-Amrouche L *et al.* Emergence of macrolide-resistant *Streptococcus pyogenes* strains in French children. *Antimicrob Agents Chemother* 2004; 48: 3559–3562.
11. Martin JM, Green M, Barbadora KA, Wald ER. Erythromycin-resistant group A streptococci in schoolchildren in Pittsburgh. *N Engl J Med* 2002; 346: 1200–1206.
12. Garcia-Rey C, Aguilar L, Baquero F, Casal J, Martin JE. Pharmacoepidemiological analysis of provincial differences between consumption of macrolides and rates of erythromycin resistance among *Streptococcus pyogenes* isolates in Spain. *J Clin Microbiol* 2002; 40: 2959–2963.
13. Jasir A, Schalen C. Survey of macrolide resistance phenotypes in Swedish clinical isolates of *Streptococcus pyogenes*. *J Antimicrob Chemother* 1998; 41: 135–137.
14. Mihaila-Amrouche L, Bouvet A, Loubinoux J. Clonal spread of *emm* type 28 isolates of *Streptococcus pyogenes* that are multiresistant to antibiotics. *J Clin Microbiol* 2004; 42: 3844–3846.
15. Chen I, Kaufisi P, Erdem G. Emergence of erythromycin- and clindamycin-resistant *Streptococcus pyogenes emm* 90 strains in Hawaii. *J Clin Microbiol* 2011; 49: 439–441.
16. Working Group on Severe Streptococcal Infections. Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. *JAMA* 1993; 269: 390–391.
17. Chatellier S, Ihendyane N, Kansal RG *et al.* Genetic relatedness and superantigen expression in group A streptococcus serotype M1 isolates from patients with severe and nonsevere invasive diseases. *Infect Immun* 2000; 68: 3523–3534.
18. Tyler SD, Johnson WM, Huang JC *et al.* Streptococcal erythrogenic toxin genes: detection by polymerase chain reaction and association with disease in strains isolated in Canada from 1940 to 1991. *J Clin Microbiol* 1992; 30: 3127–3131.
19. Malhotra-Kumar S, Lammens C, Piessens J, Goossens H. Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. *Antimicrob Agents Chemother* 2005; 49: 4798–4800.
20. Lamagni TL, Darenberg J, Luca-Harari B *et al.* Epidemiology of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2008; 46: 2359–2367.
21. O'Loughlin RE, Roberson A, Cieslak PR *et al.* The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004. *Clin Infect Dis* 2007; 45: 853–862.
22. Lamagni TL, Neal S, Keshishian C *et al.* Severe *Streptococcus pyogenes* infections, United Kingdom, 2003–2004. *Emerg Infect Dis* 2008; 14: 202–209.
23. Darenberg J, Luca-Harari B, Jasir A *et al.* Molecular and clinical characteristics of invasive group A streptococcal infection in Sweden. *Clin Infect Dis* 2007; 45: 450–458.
24. Meisal R, Andreasson IK, Hoiby EA, Aaberge IS, Michaelsen TE, Caugant DA. *Streptococcus pyogenes* isolates causing severe infections in Norway in 2006 to 2007: emm types, multilocus sequence types, and superantigen profiles. *J Clin Microbiol* 2010; 48: 842–851.
25. O'Brien KL, Beall B, Barrett NL *et al.* Epidemiology of invasive group a *Streptococcus* disease in the United States, 1995–1999. *Clin Infect Dis* 2002; 35: 268–276.
26. Ikebe T, Hirasawa K, Suzuki R *et al.* Distribution of *emm* genotypes among group a *streptococcus* isolates from patients with severe invasive streptococcal infections in Japan, 2001–2005. *Epidemiol Infect* 2007; 135: 1227–1229.
27. Luca-Harari B, Darenberg J, Neal S *et al.* Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2009; 47: 1155–1165.
28. Wahl RU, Lutticken R, Stanzel S, van der Linden M, Reinert RR. Epidemiology of invasive *Streptococcus pyogenes* infections in Germany, 1996–2002: results from a voluntary laboratory surveillance system. *Clin Microbiol Infect* 2007; 13: 1173–1178.
29. Bouvet A, Aubry-Damon H, Péan Y. Emergence de la résistance aux macrolides des *Streptococcus pyogenes* ou streptocoques bêta-hémolytiques du groupe A. *Bull Epidémiol Hebd* 2004; 32–33: 154–155.
30. Ardanuy C, Domenech A, Rolo D *et al.* Molecular characterization of macrolide- and multidrug-resistant *Streptococcus pyogenes* isolated from adult patients in Barcelona, Spain (1993–2008). *J Antimicrob Chemother* 2010; 65: 634–643.
31. Perez-Trallero E, Martin-Herrero JE, Mazon A *et al.* Antimicrobial resistance among respiratory pathogens in Spain: latest data and changes over 11 years (1996–1997 to 2006–2007). *Antimicrob Agents Chemother* 2010; 54: 2953–2959.
32. Silva-Costa C, Pinto FR, Ramirez M, Melo-Cristino J. Decrease in macrolide resistance and clonal instability among *Streptococcus pyogenes* in Portugal. *Clin Microbiol Infect* 2008; 14: 1152–1159.
33. Sabuncu E, David J, Bernede-Bauduin C *et al.* Significant reduction of antibiotic use in the community after a nationwide campaign in France, 2002–2007. *PLoS Med* 2009; 6: e1000084.
34. Malhotra-Kumar S, Lammens C, Chapelle S *et al.* Macrolide- and telithromycin-resistant *Streptococcus pyogenes,* Belgium, 1999–2003. *Emerg Infect Dis* 2005; 11: 939–942.
35. Creti R, Imperi M, Baldassarri L *et al. emm* types, virulence factors, and antibiotic resistance of invasive *Streptococcus pyogenes* isolates from Italy: what has changed in 11 years? *J Clin Microbiol* 2007; 45: 2249–2256.
36. Reinert RR, Franken C, van der Linden M, Lutticken R, Cil M, Al-Lahham A. Molecular characterisation of macrolide resistance mechanisms of *Streptococcus pneumoniae* and *Streptococcus pyogenes* isolated in Germany, 2002–2003. *Int J Antimicrob Agents* 2004; 24: 43–47.
37. Imohl M, Reinert RR, Ocklenburg C, van der Linden M. Epidemiology of invasive *Streptococcus pyogenes* disease in Germany during 2003–2007. *FEMS Immunol Med Microbiol* 2010; 58: 389–396.
38. Montes M, Tamayo E, Orden B, Larruskain J, Perez-Trallero E. Prevalence and clonal characterization of *Streptococcus pyogenes* clinical isolates with reduced fluoroquinolone susceptibility in Spain. *Antimicrob Agents Chemother* 2010; 54: 93–97.
39. Alonso R, Mateo E, Galimand M, Garaizar J, Courvalin P, Cisterna R. Clonal spread of pediatric isolates of ciprofloxacin-resistant, *emm* type 6 *Streptococcus pyogenes*. *J Clin Microbiol* 2005; 43: 2492–2493.