J Microbiol Immunol Infect
2005;38:105-111

Clinical and genetic analysis of invasive and non-invasive group A streptococcal infections in central Taiwan

Chia-Hui Kao[1,2], Po-Yen Chen[2], Fang-Liang Huang[2], Chih-Wei Chen[2], Ching-Shiang Chi[2], Yu-Hui Lin[3], Chi-Yen Shih[3], Bor-Shen Hu[3], Chia-Ro Li[3], Jui-Shan Ma[4], Yeu-Jun Lau[4], Kun-Chia Lu[5], Hsiu-Wen Yu[5]

[1]Department of Pediatrics, Tungs' Taichung MetroHarbor Hospital, Taichung; [2]Department of Pediatrics and [3]Section of Infectious Diseases, Department of Medicine, Taichung Veterans General Hospital, Taichung; [4]Section of Infectious Diseases, Show-Chwan Memorial Hospital, Changhua; and [5]Department of Pediatrics, Chen Ching Hospital, Taiwan

Received: July 10, 2004 Revised: September 8, 2004 Accepted: October 18, 2004

To evaluate the clinical, bacteriologic, and genetic relatedness between invasive and non-invasive infections caused by group A *Streptococcus* (GAS), we retrospectively analyzed the GAS isolates in our hospital from the past decade. A total of 70 GAS-infected cases were enrolled in our study from the period 1993 to 2002. Twenty one cases had invasive disease, and 49 were non-invasive. Their medical records were reviewed, and demographic data were collected for analysis. Antimicrobial susceptibility testing was conducted according to the National Committee for Clinical Laboratory Standards for *Streptococcus* spp. Isolates were subjected to chromosomal *Sma*I (Invitrogen) digestion of pulsed-field gel electrophoresis (PFGE), and *emm* typing was also performed. The mean age of the invasive group was 41.1 ± 22.4 years compared with 13.0 ± 16.6 years for the non-invasive group ($p<0.05$). Eighty one percent of the invasive group had underlying diseases. Diabetes and malignancy were the 2 most common medical conditions. All isolates were susceptible to penicillin. The resistance rate was 42.8% and 55.1% for erythromycin in the invasive and non-invasive groups, respectively. A total of 51 different PFGE types were identified among the GAS isolates without particular genotypes. Serotype M12 was the most common one (28.4%), followed by M4 (19.4%). Our study demonstrated that the patients in the invasive group were older, with more underlying diseases, and with a higher mortality rate. Antimicrobial susceptibility of the isolates was the same in both groups. There was no epidemic strain, nor did PFGE reveal a more invasive clone.

Key words: Bacterial drug resistance, group A *Streptococcus*, risk factors, serotyping, streptococcal M protein

Group A *Streptococcus* (*Streptococcus pyogenes)* [GAS], a Gram-positive, facultative anaerobic bacterium, causes a wide variety of infections in both children and adults worldwide [1]. It is generally noninvasive and is usually associated with localized infection of nasopharyngeal mucosal surfaces and the skin. However, an increasing number of reports on invasive infections have been described. These invasive infections of GAS include bacteremia, pneumonia, necrotizing fasciitis, toxic shock-like syndrome (STSS), etc. Several factors have been implicated in the pathogenesis of severe GAS infections, including decreased host immunity and the introduction of highly virulent mutant strains [2]. Accurate identification and type discrimination of GAS isolates are crucial for effective control of outbreaks.

Corresponding author: Dr. Po-Yen Chen, Department of Pediatrics, Taichung Veterans General Hospital, 160, Section 3, Chung-Kang Road, Taichung 407, Taiwan.
E-mail: pychen@vghtc.vghtc.gov.tw

The major virulence factor of *S. pyogenes* is the filamentous M protein, which protects the bacteria from host phagocytosis of polymorphonuclear leukocytes. Although serotyping of T and M proteins has been considered the gold standard for GAS typing, it is not feasible for most clinical microbiology laboratories in many countries, including Taiwan [1].

Beginning in the 1980s, reports showed an increased incidence of invasive GAS infections [3-9]. Laboratory studies also revealed an increasing tendency towards GAS isolates that express M protein types 1 and 3 [10-12]. Despite the fact that invasive GAS disease may not be restricted to the spread of 1 or 2 virulent clones, recent study has suggested that 1 serotype can cause invasive as well as non-invasive infections [13]. To determine the difference between invasive and non-invasive GAS infections, we retrospectively surveyed the microbiologic and medical records of patients with GAS infections who had been

treated at Taichung Veterans General Hospital from 1993 to 2002. Genotyping, *emm* typing, and antimicrobial susceptibility were used to evaluate possible differences between invasive and non-invasive GAS infection in central Taiwan during the past decade.

Materials and Methods

We conducted a retrospective study on GAS infection from Taichung Veterans General Hospital from the period 1993 to 2002. All subjects had clinical documented infection and GAS isolated from blood, a normal sterile site or the nasopharynx. A total of 70 cases were enrolled. Twenty one of them were invasive, and 49 were non-invasive. Their medical records, demographic data and clinical data including age, gender, site of infection, and risk factors were analyzed. Of the non-invasive group, the clinical presentations were acute pharyngitis (38 of 49 cases), scarlet fever (8 of 49 cases), post-streptococcal glomerulonephritis (2 of 49 cases), and streptococcal scaled skin syndrome (1 of 49 cases). Of the invasive group, the clinical presentations were sepsis with bacteremia (13 of 21 cases), necrotizing fasciitis (6 of 21 cases) and toxic shock syndrome (2 of 21 cases).

Case definition

Invasive GAS infection was defined by the isolation of *S. pyogenes* from blood, normal sterile body fluid (spinal, synovial, peritoneal, or pleural fluid) or from a wound accompanied by the clinical symptoms of STSS or necrotizing fasciitis. Non-invasive infection was defined by the isolation of *S. pyogenes* from a nasopharyngeal swab with clinical presentations of sore throat, fever and symptoms of upper airway infection, and from superficial skin infections such as impetigo. Immunocompromised status was defined as receipt of chemotherapy, steroid therapy or infection of human immunodeficiency virus at least 2 weeks before the development of GAS infection.

Identification of GAS isolates

GAS are β-hemolytic. They were identified by susceptibility to 0.04 U of bacitracin and agglutinated with group-specific antiserum by the latex agglutination test (Streptex, Murex, USA).

Antimicrobial susceptibility testing

The antimicrobial susceptibilities of these isolates were tested by the broth microdilution method and the E-test. Broth microdilution was performed according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [14-17]. The minimal inhibitory concentrations (MICs) were determined for penicillin G (Sigma Chemical Co., St. Louis, MO, USA), erythromycin (Sigma), ciprofloxacin (Sigma), and tetracycline (Sigma) by broth dilution with Mueller-Hinton broth (Oxoid, Wesel, Germany), containing 5% sheep blood under room air incubation for 18 h. The E-test (AB Biodisk, Solna, Sweden) was conducted according to previous uses [1] for penicillin G and erythromycin to determine the antimicrobial susceptibilities.

Pulsed-field gel electrophoresis for genotyping

Genomic DNA was prepared as described previously [18]. The bacterial suspension was prepared by the scratching of bacterial colonies directly from overnight incubated cultures onto nutrient agar and was adjusted to a concentration of 10^9 colony-forming units/mL in SE buffer (75 mM NaCl and 25 mM ethylenediamine tetra-acetic acid [EDTA], pH 7.5) with a VITEK colorimeter (Hach Company, Loveland, CO, USA). Briefly, in situ cell lysis was carried out by incubation for 2 h at 37°C in lysis buffer [19]. Proteolysis was achieved through 2 h of incubation at 56°C with proteinase K (Sigma) in proteinase K buffer [19]. The plugs were then washed thoroughly [19]. Isolates were subjected to chromosomal *Sma*I (Invitrogen) digestion as previously described [1]. Restriction fragments of DNA were separated by pulsed-field gel electrophoresis (PFGE) with a contour-clamped homogeneous electric field CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, CA, USA) through 1.2% Sealem GTG agarose gel (FMC Bioproducts, Rockland, Maine, USA). The fragmented DNA was run at a field strength of 6 V/cm for 22 h at 14°C, and the pulse time was increased from 5 to 40 sec. A lambda ladder (Bio-Rad Laboratories) was used as the molecular size marker. The genetic relatedness between any 2 isolates was estimated by calculation of the Dice coefficient of similarity as follows: 2 × number of matching bands/total number of bands of both strains. Isolates were considered to be within a cluster if the range of relatedness was >0.80. The PFGE spun down patterns were analyzed by the Windows of Gelcompar, version 3.1b (Applied Math, Kortrijk, Belgium) [20]. Different PFGE types were defined when 3 or more band differences between 2 patterns were used as a criterion to define a PFGE type [21-23].

Polymerase chain reaction for emm genes

S. pyogenes DNA was prepared according to the outline of the Centers for Disease Control and Prevention (CDC). At least 100 known M serospecificities of *S. pyogenes* have been recognized (http://www.cdc.gov/ncidod/biotech/strep/doc.htm). A fresh growth (half of a standard loop-full) was picked up by a loop, and then re-suspended in 300 μL 0.85% NaCl. After that, samples were heated at 70°C for 15 min, and then spun down. The pellets were resuspended in 50 μL TE (10 mM Tris, 1 mM EDTA, pH 8), 10 μL mutanolysin (3000 units/mL), and 2 μL hyaluronidase (30 mg/mL), and then incubated at 37°C for 30 min. Finally, they were heated at 100°C for 10 min, and then immediately underwent polymerase chain reaction **(**PCR). The PCR primers used for detection of the emm genes were derived from the CDC protocol and previous descriptions [24]. The forward and reverse primers were TATT(C/G)GCTTAGAAAATTAA and GCAAGTTCTTCAGCTTGTTT. The PCR process was performed according to the CDC protocol described in the website (http://www.cdc.gov/ ncidod/ biotech/ strep/ protocols.htm). Sequencing was obtained according to the guidelines detailed by the CDC for *S. pyogenes* sequence at the CDC website (http://www.cdc.gov/ncidod/biotech/strep/protocols.htm) [25,26]. We tried another method with primers MF2 (GGATCCATAA GGAGCATAAA AATGGCTA) and MR1 (TGATA GCTTA GTTTTCTTCT TTGCGTTTT) and changed the annealing temperature to 55-60°C. However, 3 of the 70 GAS isolates still failed to be amplified for *emm* typing.

Table 1. Characteristics and clinical features of patients

Category	Invasive group n = 21 (%)	Non-invasive group n = 49 (%)	*p*
Mean age (years)	41.07	13.03	0.000
Male	11 (52.3)	30 (61.22)	0.492
Female	10 (47.62)	19 (38.78)	
Mortality	5 (23.81)	0	1. 004
Survival	16 (76.19)	49 (100)	
Comorbid diagnoses	17 (80.95)	1 (2.04)	0.000

Statistical evaluation

Paired *t* test and chi-squared tests were used for statistical analysis. The statistical software used was Sigma Plot 4.0 software (SPSS, Chicago, IL, USA). A *p* value less than 0.05 was considered statistically significant.

Results

Characteristics and clinical features of patients

During the 10-year study period between January 1, 1993 and December 31, 2002, 70 patients with GAS infections were identified at Taichung Veterans General Hospital. Of these patients, 21 (30%) had invasive infections, and 49 (70%) had non-invasive infections. The fatality rate of invasive GAS infections was 23.8%, while none died in the non-invasive infection group. The overall mortality rate was 7.1% (Table 1).

The mean age of the invasive group was 41.1 years compared with 13.0 years for the non-invasive group ($p<0.05$). The male-to-female ratio was 1.1 and 1.6 for the invasive and non-invasive groups, respectively

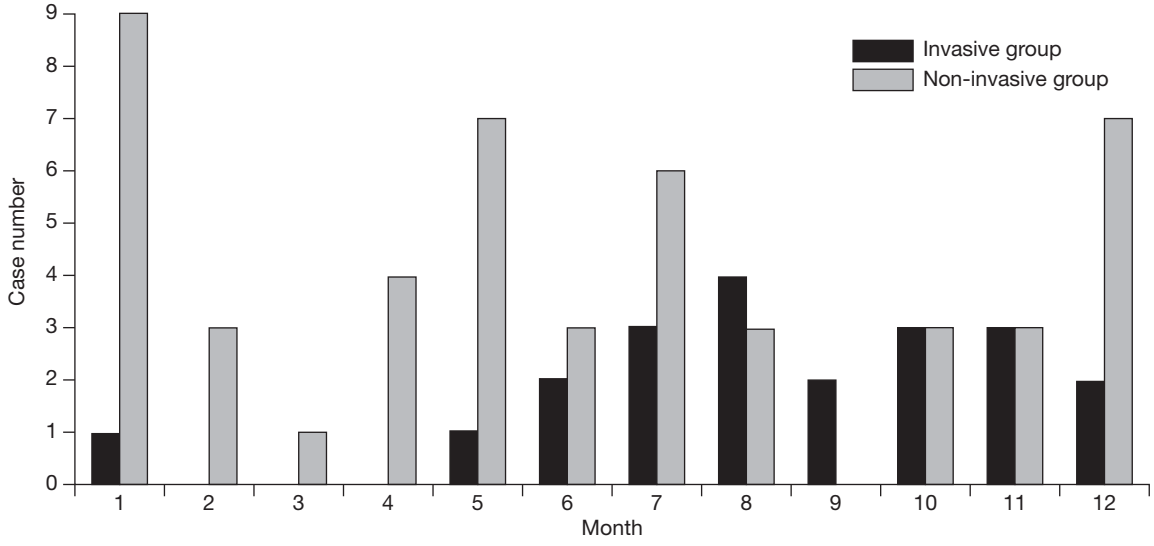

Fig. 1. Distribution of group A *Streptococcus* infection cases by month.

($p>0.05$). Eighty one percent (17/21) of patients had comorbid disease, including diabetes mellitus (DM) and hypertension (3 cases), DM and recent trauma (2 cases), hypertension (1 case), immunocompromised status (1 case), intravenous drug abuse (1 case), renal failure (2 cases), malignancy under chemotherapy (2 cases), malignancy with liver cirrhosis (1 case), alcohol consumption (1 case), chronic lung disease (1 case) and recent trauma (2 cases), among the invasive group compared with 2% (1/49) of those in the non-invasive group (only 1 case with alcohol consumption) [$p<0.05$]. Diabetes mellitus and malignancy were 2 of the most common underlying diseases associated with invasive disease. The distribution of cases in different months is shown in Fig. 1.

Antimicrobial susceptibility testing

MICs were recorded for penicillin G, erythromycin, ciprofloxacin, and tetracycline by the broth microdilution method. All 70 isolates were sensitive to penicillin (Table 2). For erythromycin and tetracycline, there was no difference between the invasive group and the non-invasive group (Table 2).

Table 2. In vitro activity of antimicrobial agents by E-test and microdilution

Antimicrobial agents and susceptibility	Invasive group n = 21 (%)	Non-invasive group n = 49 (%)	*p*
E-test			
Penicllin			
Susceptible	21 (100)	49 (100)	NS
Intermediate	0	0	
Resistant	0	0	NS
Erythromycin			
Susceptible	11 (52.4)	22 (44.9)	0.33
Intermediate	1 (4.8)	0	
Resistant	9 (42.8)	27 (55.1)	0.88
Microdilution			
Penicllin			
Susceptible	21 (100)	49 (100)	NS
Intermediate	0	0	
Resistant	0	0	
Erythromycin			
Susceptible	11 (52.4)	23 (46.9)	0.17
Intermediate	0	1 (2.0)	
Resistant	10 (47.6)	25 (51.0)	0.07
Tetracycline			
Susceptible	9 (42.9)	21 (42.9)	NS
Intermediate	0	0	
Resistant	12 (57.1)	28 (57.1)	NS

Abbreviation: NS = not significant

Table 3. In vitro activity (broth microdilution method) of antimicrobial agents tested against isolates

Antimicrobial agents	MIC (µg/mL)			
	Invasive group n = 21		Non-invasive group n = 49	
	50%	90%	50%	90%
Penicillin	<0.01	<0.01	<0.01	<0.01
Erythromycin	0.03	8	2	>512
Tetracycline	16	32	16	32
Cefazolin	0.06	0.25	0.125	0.125
Ciprofloxcin	0.25	0.125	0.125	0.5

Abbreviation: MIC = minimum inhibitory concentration

The E-test and broth microdilution methods for antimicrobial susceptibility showed no difference in this study. For penicillin, both methods revealed the same susceptibility rate of 100%. The erythromycin susceptibility rate was also the same in the same group tested by the E-test and broth microdilution method ($p>0.05$) [Table 2]. In vitro activity of antimicrobial agents against isolates is shown in Table 3.

Polymerase chain reaction for emm genes

Of the 67 GAS isolates that underwent *emm* typing successfully, type 12 was the most common (19 of 67, 28.4%), followed by type 4 (13 of 67, 19.4%) [Fig. 2]. Seven different emm types were noted among the invasive group, including M1, M4, M12, M13, M27, M92, and M102, and 8 of 21 were untypable. In the non-invasive group, there were also 7 emm types, including M1, M4, M6, M11, M12, M13, and M63, and 3 of 46 were untypable. Among the typable GAS strains, the M1 type was the most common, followed by the M4 type in the invasive group, while in the non-invasive group the M12 type was the most prevalent, followed by the M4 type. In total, there were 14 untypable GAS strains when using the PCR typing method.

Pulsed-field gel electrophoresis for genotyping

All 70 GAS isolates were subjected to PFGE analysis by using *Sma*I restriction enzyme. Visual and computerized analysis of the *Sma*I patterns revealed 48 unrelated GAS PFGE types. This demonstrates very high genetic variability among GAS in this study. The most common strains, designated as PFGE type 01, constituted 10% of the clinical isolates examined. The second most common strain, designated as PFGE type 02, constituted 7.1% of the clinical isolates examined (Table 4). There was no predominant genotype in the invasive group when compared with the non-invasive group.

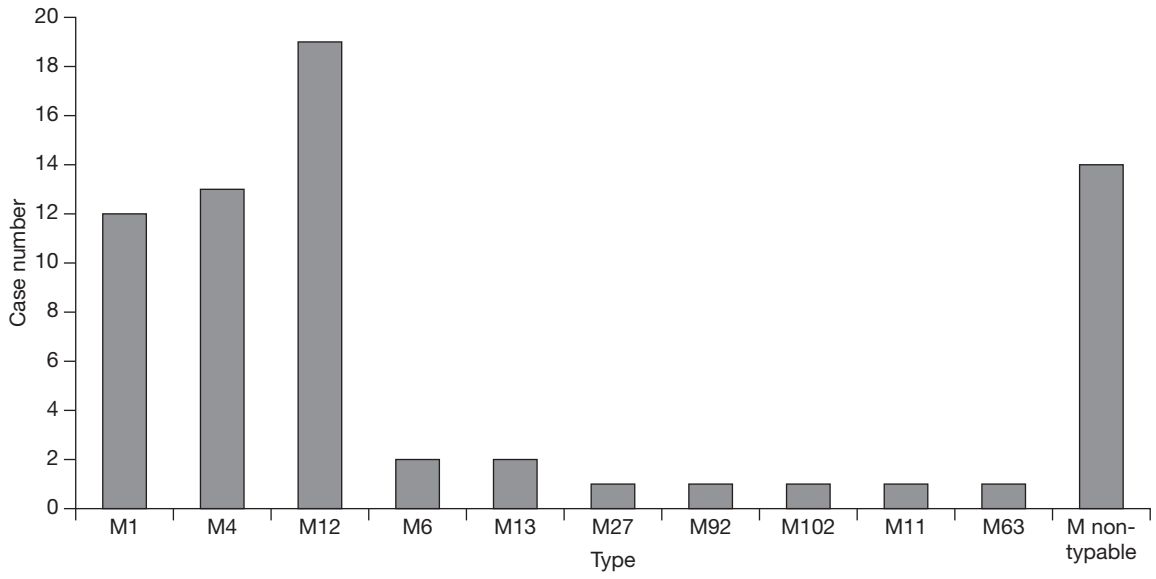

Fig. 2. Distribution of M types of 67 group A streptococci isolates that underwent *emm* typing. Type 12 was the most common (19 of 67, 28.4%), followed by type 4 (13 of 67, 19.4%).

Discussion

In our study, the mean age of the invasive group was much older than that of the non-invasive group. The overall mortality rate among the patients with invasive GAS infection was 23.81%, while all the patients of non-invasive GAS infection had a good prognosis. Most (81%) of the patients in the invasive group had underlying conditions, with DM and malignancy being the 2 most common. In contrast, most of the non-invasive group were previously healthy (2% with underlying conditions).

Previous reports have indicated that several M serotypes make up the majority of invasive GAS infection, including M1, M3, M6, M12, and M18 [23]. Moses et al reported that the M3 serotype was the most common strain, followed by the M28 serotype and M2 serotype among 401 isolates of invasive GAS infection [27]. In our study, among the typable GAS strains, the M1 type was the most common one, followed by the M4 type in invasive group, while the M12 type was the most prevalent in the non-invasive group, followed by the M4 type. A study conducted by Ho et al revealed that types M1, M4, and M12 were the most prevalent in both invasive and non-invasive GAS infections in Hong Kong [28]. We are unable make any definitive conclusions on the relationship between clinical disease and the M serotype from our study.

Table 4. Distribution of *Sma*I types and *emm* type

PFGE genotype	M type (*emm* type)	Number of GAS strains
01	*emm* 1	7
02	*emm* 12	5
03	*emm* 12	4
04	*emm* 1	3
05	*emm* 1	3
06	*emm* 4	3
07	*emm* 4	3
08	*emm* 12	3
Others (09-51)	Non-typable	37
Total		68

Abbreviations: PFGE = pulsed-field gel electrophoresis; GAS = group A *Streptococcus*

All of the 70 GAS isolates in our study, regardless of whether invasive or non-invasive, were susceptible to penicillin. The erythromycin susceptibility rate demonstrated no difference between invasive and non-invasive groups. Similar results were produced with other antimicrobial agents tested in this study (tetracycline and ciprofloxacin).

According to several studies, the prevalence of erythromycin-resistant GAS appears to be on the rise globally [28-35]. We found an erythromycin resistance rate of 42.8% among the invasive group and 55.1% among the non-invasive group (average 51.4%), compared with 44% in a report in Finland and 4.6% in a report in Canada [31]. A high rate of erythromycin-resistant GAS in Taiwan has been recognized since the mid-1990s when 37-60% of GAS strains were found to be resistant to erythromycin. Yan et al conducted a

study in southern Taiwan in 1997 and 1998, demonstrating an erythromycin-resistant rate of 63.2% [32].

This study revealed that the M4 serotype and M12 serotype make up the majority of erythromycin-resistant strains. Janne et al found that the erythromycin-resistant strain is associated with the M4 serotype [33], and Colman et al reported that the M4 serotype and M12 serotype are the most prevalent among erythromycin-resistant strains [36]. As for the high erythromycin resistance rate in Taiwan, compared with the previously reported studies in other countries, the probable cause may be related to the increasing consumption of macrolides in Taiwan [14,32]. However, the fact that GAS isolates are still universally susceptible to penicillin, despite 50 years of widespread consumption worldwide, makes the pathology a little more complex [28].

Several limitations in this study must be taken into consideration. The major limitation of this study is that limited clinical information was available regarding the course of hospitalization because of the nature of the retrospective study. We were unable to evaluate the role of previous antimicrobial agents used in each case prior to admission. Furthermore, the case number was limited. Additional studies involving a greater number of patients should be carried out to further study invasive and non-invasive GAS infections.

In summary, data from the present study demonstrate that the differences between an invasive GAS group and non-invasive group are age, underlying conditions, and the mortality rate. Antimicrobial susceptibility makes no difference between the 2 groups by both broth microdilution and the E-test method. No definitive conclusions on the relationship between clinical disease and the M serotype can be made from this study. The erythromycin-resistant rate in our study is comparable with results previously reported in Taiwan [29]. M serotyping results in our study are also comparable with a previous study in Hong Kong, where the M1, M4, and M12 serotypes were the most common [28].

Acknowledgments

The authors are grateful to the Laboratories of Molecular Diagnosis at the Department of Pediatrics and the Section of Infectious Diseases at Taichung Veterans General Hospital.

References

1. Hsueh PR, Lee JT, Lee PI, Yang PC, Huang LM, Chang SC, et al. Outbreak of scarlet fever at a hospital day care center: analysis of strain relatedness with phenotypic and genotypic characteristics. J Hosp Infect 1997;36:191-200.
2. Masccini EM, Jansze M, Schouls LM, Fluit AC, Verhoef J, Van Dijk J. Invasive and noninvasive group A streptococcal isolates with different *spea* alleles in the Netherlands: genetic relatedness and production of pyrogenic exotoxin A and B. J Clin Microbiol 1999;37:3469-74.
3. O'Brien KL, Levine OS, Schwartz B. The changing epidemiology of group A streptococcus infections. Semin Pediatr Infect Dis 1997;8:10-6.
4. Cone LA, Woodard DR, Schlievert PM, Tomory GS. Clinical and bacteriology observations of a toxic shock syndrome. N Engl J Med 1987;317:146-9.
5. Hirbalova V. *Streptooccus pyogenes* and the toxic shock syndrome. Ann Intern Med 1988;108:772.
6. Chiobotaru P, Fraser D, Dagan R. Changing epidemiology of invasive *Streptooccus pyogenes* infections in southern Israel: differences between two ethnic population groups. Pediatr Infect Dis J 1997;16:195-9.
7. Martin PR, Hoiby EA. Streptococcal serogroup A epidemic in Norway 1987-1988. Scand J Infect Dis 1990;22:421-9.
8. Stormberg A, Romanus V, Burman LG. Outbreak of group A streptococcal bacteremia in Sweden: An epidemiologic and clinical study. J Infect Dis 1991;164:595-8.
9. Fata J Jr, Drabkova J, Rehak F, Smat V, Votocek K, Frankova V. Primary peritonitis imitating the toxic shock syndrome (TSS). Prakt Lek (Prague) 1984;64:674-6.
10. O'Brien KL, Beall B, Barrett NL, Cieslak PR, Reingold, Farley MM, et al. Epidemiology of invasive group A *Streptococcus* disease in the United States, 1995-1999. Clin Infect Dis 2002; 35:268-76.
11. Johnson DR, Stevens DL, Kaplan EL. Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. J Infect Dis 1992;166:374-82.
12. Schwartz B, Facklam AR, Breiman RF. Changing epidemiology of group A streptococcal infection in the USA. Lancet 1990;336:1167-71.
13. Chatellier S, Ihendyana N, Kansal RG, Khambaty F, Basma H, Norrby-Teglund A, et al. Genetic relatedness and superantigen expression in group A *Streptococcus* serotype M1 isolates from patients with severe and non-severe invasive diseases. Infect Immun 2000;68:3523-4.
14. York MK, Gibbs L, Perdreau-Remington F, Brooks GF. Characterization of antimicrobial resistance in *Streptococcus pyogenes* isolates from the San Francisco bay area of northern California. J Clin Microbiol 1999;37:1727-31.
15. Ko WC, Lee HC, Wang LR, Lee C, Liu AJ, Wu JJ. Serotyping and antimicrobial susceptibility of group B *Streptococcus* over an eight-year period in southern Taiwan. Eur J Clin Microbiol

Infect Dis 2001;20:334-9.
16. Reinert RR, von Eiff C, Kresken M, Brauers J, Hafner D, Al-Lahham A, et al. Nationwide German multicenter study on the prevalence of antibiotic resistance in streptococcal blood isolates from neutropenic patients and comparative in vitro activities of quinupristin-dalfopristin and eight other antimicrobials. J Clin Microbiol 2001;39:1928-31.
17. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A4. 4th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa., 1997.
18. Toshiyuki M, Reiko S, Ro O, Shiro Y. Characteristics of *Streptococcus pyogenes* serotype M1 and M3 isolates from patients in Japan from 1981 to 1997. J Clin Microbiol 1999; 37:4131-4.
19. Shi ZY, Liu PY, Lau YJ, Lin YH, Hu BS. Epidemiological typing of isolates from an outbreak of infection with multidrug-resistant *Enterobacter cloacae* by repetitive extragenic palindromic unit b1-primed PCR and pulsed-field gel electrophoresis. J Clin Microbiol 1996;34:2784-90.
20. Lau YJ, Hu BS, Wu WL, Lin YH, Chang HY, Shi ZY. Identification of a major cluster of *Klebsiella pnuemoniae* isolates from patients with liver abscess in Taiwan. J Clin Microbiol 2000;38:412-4.
21. Descheemaeker P, Van Loock F, Hauchecorne M, Vandamme P, Goossens H. Molecular characterization of group A *Streptococci* from invasive and non-invasive disease episodes in Beligum during 1993-1994. J Med Microbiol 2000;49: 467-71.
22. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
23. Hsueh PR, Wu JJ, Tsai PJ, Liu JW, Chuang YC, Luh KT. Invasive group A streptococcal disease in Taiwan is not associated with the presence of streptococcal pyrogenic exotoxin genes. Clin Infect Dis 1998;26:584-9.
24. Adrian MW, Michael AK. Horizontal gene transfer in the evolution of group A streptococcal *emm*-like genes: gene mosaics and variation in Vir regulons. Mol Microbiol 1994; 11:364-74.
25. Lancefield RC. Current knowledge of the type specific M antigens of group A *Streptococci*. J Immunol 1962;89:307-13.
26. Whatmore AM, Kapur V, Sullivan DJ, Musser JM, Kehoe MA. Non-congruent relationships between variation in *emm* gene sequences and the population genetic structure of group A streptococci. Mol Microbiol 1994;14:619-31.
27. Moses AE, Goldberg S, Korenman Z, Ravins M, Hanski E, Shapiro M. Invasive group A streptococcal infections, Israel. Emerg Infect Dis 2002;8:421-6.
28. Ho PL, Johnson DR, Yue AW, Tsang DN, Que TL, Beall B, et al. Epidemiologic analysis of invasive and noninvasive Group A streptococcal isolates in Hong Kong. J Clin Microbiol 2003; 41:937-42.
29. Perez-Trallero E, Fernandez-Mazarrasa C, Garcia-Rey C, Bouza E, Aguilar L, Garcia-de-Lomas J. Antimicrobial susceptibilities of 1,684 *Streptococcus pneumoniae* and 2,039 *Streptococcus pyogenes* isolates and their ecological relationships: results of a 1-year (1998-1999) multicenter surveillance study in Spain. Antimicrob Agents Chemother 2001;45:3334-40.
30. El-Bouriv KW, Lewis AM, Okeahialam CA, Wright D, Tanna A, Joynson DH. A community outbreak of invasive and non-invasive group A beta-haemolytic streptococcal disease in a town in South Wales. Epidemiol Infect 1998;121: 515-21.
31. Weiss K, **Aza**vedo JD, Restieri C, G**ala**rne**a**u L**A**, Gourde**a**u M, H**a**rvey P, et al. Phenotypic and genotypic characterization of macrolide-resistant group *A Streptococcus* strains in the province of Quebec, Canada. J Antimicrob Chemother 2001; 47:345-8.
32. Yan JJ, Wu HM, Huang AH, Fu HM, Lee CT, Wu JJ. Prevalence of polyclonal *mef A*-containing isolates among erythromycin-resistant group A streptococci in southern Taiwan. J Clin Microbiol 2000;38:2475-9.
33. Janne K, Pentti H, Anna M, Jaana V-V, Androulla E, Gillian H. Clonal spread of group A *Streptococcus* with the new type of erythromycin resistance. J Infect Dis 1998;177:786-9.
34. Seppala H, Nissinen A, Jarvinen H, Huovinen S, Henriksson T, Herva E, et al. Resistance to erythromycin in group A streptococci. N Engl J Med 1992;326:292-7.
35. Hsueh PR, Chen HM, Huang AH, Wu JJ. Decreased activity of erythromycin against *Streptococcus pyogenes* in Taiwan. Antimicrob Agents Chemother 1995;39:2239-42.
36. Colman G, Tanna A, Efstratiou A, Gaworzewska ET. The serotypes of *Streptococcus pyogenes* present in Britain during 1980-1990 and their association with disease. J Med Microbiol 1993;39:165-78.