Adult Invasive and Noninvasive Infections Due to *Streptococcus dysgalactiae* subsp. *equisimilis* in France from 2006 to 2010

Julien Loubinoux,^{a,b} Céline Plainvert,^{a,b,c,d} Gislène Collobert,^a Gérald Touak,^a Anne Bouvet,^{a,b} Claire Poyart,^{a,b,c,d} on behalf of the CNR-Strep Network

Centre National de Référence des Streptocoques, Groupe Hospitalier Cochin-Hôtel Dieu-Broca Assistance Publique Hôpitaux de Paris, Paris, France^a; Faculté de Médecine, Université Paris Descartes, Paris, France^b; INSERM 1016, Institut Cochin, Paris, France^c; CNRS, UMR8104, Paris, France^d

We characterized 182 *Streptococcus dysgalactiae* subsp. *equisimilis* isolates and analyzed the epidemiological data on the corresponding infections. *stG6*, *stG485*, and *stG6792* were the 3 most prevalent invasive *emm* types among the 27 different *emm* types recovered. High rates of antimicrobial resistance were observed for macrolides (26.4%) and tetracycline (34.6%).

Taxonomic studies have delineated *Streptococcus dysgalactiae* into two different subspecies, *S. dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis*, isolated from animals and humans (1, 2, 3). Human strains belong to *S. dysgalactiae* subsp. *equisimilis*. *S. dysgalactiae* subsp. *equisimilis* is phylogenetically very close to *Streptococcus pyogenes*, which explains the overlapping spectrum of infections caused by these species (3). The *S. dysgalactiae* subsp. *equisimilis* strains are increasingly recognized as the cause of severe diseases associated with streptococcal toxic shock syndrome (STSS) (4, 5). Horizontal gene transfer between *S. pyogenes* and *S. dysgalactiae* subsp. *equisimilis* are responsible for common virulence determinants (3, 4). The M protein, a major virulence factor, is a fimbrial surface protein encoded by the *emm* gene, and more than 50 different *emm* types have been described for *S. dysgalactiae* subsp. *equisimilis* (4, 6, 7). Determination of the *emm* type is mandatory for epidemiological investigations. Streptococcal pyrogenic exotoxins (Spe), streptococcal superantigen A (SSA), and streptococcal mitogenic exotoxin Z (SmeZ) have been identified as superantigens of *S. pyogenes* (8, 9, 10). Interestingly, *speG* was described in *S. dysgalactiae* subsp. *equisimilis* as a homologue of the *S. pyogenes* gene *speG* and may have a role in the virulence of *S. dysgalactiae* subsp. *equisimilis* strains (11). *S. dysgalactiae* subsp. *equisimilis* isolates remain susceptible to β -lactams, which are the treatment of choice for *S. dysgalactiae* subsp. *equisimilis* infections (4). Macrolides are recommended as an alternative treatment of pharyngitis in case of allergy to β -lactams. Clindamycin may be used in combination with penicillin for treatment of severe disease (12, 13). In case of STSS, treatment with intravenous immunoglobulin preparations may reduce the inflammatory activity (14). The aim of this work was to study human invasive and noninvasive *S. dysgalactiae* subsp. *equisimilis* infections in France and to characterize the isolates responsible for these infections.

S. dysgalactiae subsp. *equisimilis* invasive infection is defined as the isolation of *S. dysgalactiae* subsp. *equisimilis* from a usually sterile site or from samples obtained from a nonsterile site in combination with clinical signs of necrotizing fasciitis or STSS. STSS was defined according to the U.S. Working Group on Severe Streptococcal Infections definitions (15). All nonredundant invasive and noninvasive *S. dysgalactiae* subsp. *equisimilis* isolates from patients ≥ 18 years old received at the National Reference Center for Streptococci (CNR-Strep; <http://www.cnr-strep.fr>) be-

tween January 2006 and December 2010 were studied. The isolates were sent from 58 centers located throughout France in 17 out of 22 regions of metropolitan France. Clinical data were obtained from questionnaires completed on a voluntary basis and submitted with the isolates. Identification of isolates was confirmed by 16S rRNA gene sequencing as previously described (16). Lancefield group carbohydrate antigens were determined by specific latex agglutination for groups A, B, C, F, and G with the Prolex streptococcal grouping latex kit (Pro-Lab Diagnostics) after a chemical extraction (nitrous acid). The *emm* type was determined by sequencing the variable 5'-end of the *emm* gene after amplification by PCR according to the Centers for Disease Control and Prevention recommendations (<http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm>). All the isolates were tested by a multiplex PCR method for the presence of *S. pyogenes* genes encoding the exotoxins or superantigens SpeA, SpeB, SpeC, Ssa, and SmeZ (17, 18). The presence of *speG* was also tested, as previously described (19). Antibiotic susceptibilities to benzylpenicillin, erythromycin, clindamycin, tetracycline, and vancomycin were determined by the disk diffusion method according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org/clinical_breakpoints/). Erythromycin and tetracycline resistance genes were detected by multiplex PCR according to a previously described procedure (20).

From January 2006 to December 2010, 182 nonredundant isolates of *S. dysgalactiae* subsp. *equisimilis* obtained from 181 patients were studied (Table 1). The isolation dates were distributed regularly throughout the period of the study. The median age of patients was 62 years (range, 18 to 102 years), and 58% were male. The number of cases increased with the age of the patients. Among the 109 invasive *S. dysgalactiae* subsp. *equisimilis* infections, 83 cases had a documented bacteremia. STSS was present in 16.2% of patients and was more frequent in cases of necrotizing fasciitis. The overall mortality rate was 8.1%, reaching 20% and 25% for

Received 14 May 2013 Accepted 15 May 2013

Published ahead of print 22 May 2013

Address correspondence to Claire Poyart, claire.poyart@cch.aphp.fr, or Julien Loubinoux, julien.loubinoux@htd.aphp.fr.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.01262-13

TABLE 1 Ages of patients and clinical manifestations of adult *S. dysgalactiae* subsp. *equisimilis* infections in France, 2006 to 2010

Infection site and clinical manifestation (no. of cases)	Total no. of cases by gender		No. of patients with clinical manifestation by age group (yrs) and gender													
			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
Invasive infection (109)	57	52	3	1	1	2	9	1	11	7	10	10	16	11	7	20
Skin/soft tissue infection (48)																
Necrotizing fasciitis	8	12					2		2	1		2	2	5	2	4
Erysipelas	8	9	1						1	2	3		2	2	1	5
Other	9	2			1		2		1	2			5			
Bacteremia without focus (22)	12	10					2		2	2		2	5	2	3	4
Bone and joint infections (22)																
Septic arthritis	5	3							2		1	1	1	1	1	1
Osteomyelitis	6	2	1						1		4	2				
Prosthesis infection	1	3									1			1		2
Bursitis	1						1									
Spondylodiscitis	1		1													
Other invasive infection (17)																
Endocarditis	3	3		1			1		1			1	1			1
Endometritis		3				2		1								
Meningitis	1	1							1							1
Bacteremia with digestive origin	1	1					1									1
Pyelonephritis		1														1
Transfusion-transmitted infection		1										1				
Pharyngitis		1										1				
Pneumonia	1										1					
Noninvasive infection (72)	48	24	5	5	6	2	4	5	17	1	5	5	10	1	1	5
Total (181)	105	76	8	6	7	4	13	6	28	8	15	15	26	12	8	25

necrotizing fasciitis and STSS, respectively. The 72 noninvasive *S. dysgalactiae* subsp. *equisimilis* infections by site were as follows: skin ($n = 56$), pharynx ($n = 7$), uro-genital tract ($n = 5$), eye ($n = 3$), digestive tract ($n = 1$). The 182 clinical isolates of *S. dysgalactiae* subsp. *equisimilis* belonged to Lancefield group G ($n = 143$), group C ($n = 29$), or group A ($n = 9$). One isolate was nongroupable. A total of 27 different *emm* types were identified. The distribution of Lancefield group antigens according to *emm* types was variable (Fig. 1A). The nongroupable isolate belonged to *stL2764*. This isolate could express a Lancefield group antigen not tested, such as group L. *stG6*, *stG485*, and *stG6792* were the 3 most common invasive *emm* types (Fig. 1B). In spite of the relatively high prevalence (18.3%) of *stG6* in invasive infections, no statistically significant association was found between *stG6* and invasive infection. None of the *S. pyogenes* genes encoding the exotoxins or superantigens was detected. However, *spegg* was present in 52.2% of all isolates, but it was not associated with invasive infection. *spegg* was associated with certain *emm* types. *spegg* was always detected in *stG10*, *stG62647*, and *stC74* isolates and was present in 9 out of 10 *stG480* isolates. All *S. dysgalactiae* subsp. *equisimilis* isolates were susceptible to β -lactams and vancomycin. The rate of erythromycin resistance was 26.4% ($n = 48$). The resistance was stable between 2006 and 2010 and was distributed among 14 different *emm* types. Among the 48 erythromycin-resistant isolates, 25 were constitutively resistant to clindamycin, 13 harbored an inducible clindamycin-resistant phenotype, and 10 were susceptible to clindamycin. Erythromycin resistance was correlated with

erm(A) ($n = 20$), *erm(B)* ($n = 14$), *mef(A/E)* ($n = 10$), or both *erm(A)* and *erm(B)* ($n = 2$). For 2 resistant isolates, no resistance gene was found. The rate of tetracycline resistance was 34.6% ($n = 63$). Tetracycline resistance was correlated with *tet(M)* ($n = 51$), *tet(O)* ($n = 7$), both *tet(M)* and *tet(L)* ($n = 2$), *tet(S)* ($n = 1$), *tet(L)* ($n = 1$), or both *tet(M)* and *tet(O)* ($n = 1$).

The demographic characteristics of patients and clinical manifestations of *S. dysgalactiae* subsp. *equisimilis* infections in France are comparable to those previously described in other countries (21, 22, 23, 24, 25, 26, 27). Clinical isolates of *S. dysgalactiae* subsp. *equisimilis* possessing Lancefield group A antigen have already been described (28, 29). The 3 most prevalent invasive *emm* types (*stG6*, *stG485*, and *stG6792*) have been previously reported as the most common invasive *emm* types in other countries (*stG6* in Finland, Israel, Norway, Taiwan, and the United States; *stG485* in Finland, Israel, Japan, Norway, and Taiwan; *stG6792* in Japan) (22, 24, 25, 26, 30, 31). In these studies, the other most prevalent invasive *emm* types were *stC839*, *stG10*, *stG245*, *stG480*, *stG643*, *stG840*, and *stG2078*. Apart from *stC839*, which has been observed in Taiwan, these *emm* types were also identified in France, but less frequently. Most previous studies have characterized strains only from invasive infections. However, three studies compared invasive and noninvasive infections, and these studies found a correlation between *emm* type and invasive disease potential (3, 27, 31). In these studies, *stG6*, *stG10*, *stG480*, *stG485*, *stG2078*, and *stG6792* were described as invasive *emm* types. Interestingly, besides *stG6*, *stG485*, and *stG6792*, *emm* types *stG10*, *stG2078*, and *stG480* were

FIG 1 *emm* types of adult *S. dysgalactiae* subsp. *equisimilis* isolates in France (2006 to 2010). (A) Distribution of Lancefield group antigens of invasive and noninvasive *S. dysgalactiae* subsp. *equisimilis* isolates. (B) Comparison of invasive and noninvasive *emm* types of *S. dysgalactiae* subsp. *equisimilis* isolates. In both panels A and B, the 12 other *emm* types were stG840 (*n* = 2), stG4831 (*n* = 2), stG5420 (*n* = 2), stG7882 (*n* = 2), stC58 (*n* = 1), stC6979 (*n* = 1), stC7934 (*n* = 1), stC9431 (*n* = 1), stCNSRT2 (*n* = 1), stG1750 (*n* = 1), stG5063 (*n* = 1), and stL2764 (*n* = 1).

also prevalent invasive *emm* types in France (the fourth-, fifth-, and sixth-most prevalent, respectively). Thus, even if *emm* types associated with severe streptococcal diseases may vary with time and geographical location, a limited number of *emm* types may be responsible for the majority of *S. dysgalactiae* subsp. *equisimilis* invasive infections. This might be explained either by a higher prevalence in circulating strains or by a higher virulence of these *emm* types. The role of *spe* and other superantigens in the pathogenesis of severe *S. dysgalactiae* subsp. *equisimilis* human diseases is uncertain. *spe* was not associated with invasive infections but was associated with certain *emm* types, as previously reported (26, 32). It should be noted that the rates of erythromycin and tetracycline resistance of *S. dysgalactiae* subsp. *equisimilis* were higher than those described for *S. pyogenes* in France during the same period (6.5% and 13%, respectively) (33). The high rate of macrolide and lincosamide resistance constitutes a major concern, as these antibiotics are recommended for the treatment of *S. dysga-*

lactiae subsp. *equisimilis* infections in penicillin-allergic patients and in cases of STSS.

In conclusion, this is the first study of *S. dysgalactiae* subsp. *equisimilis* infections in France. Further studies with a greater number of isolates are needed to draw a definitive conclusion as to the association between *emm* type and disease, as has been shown for *S. pyogenes*.

ACKNOWLEDGMENTS

We thank the correspondents of the CNR-Strep for sending the strains and completing the questionnaires.

This study was partly funded by the Institut National de Veille Sanitaire, the Assistance Publique-Hôpitaux de Paris, INSERM, and by a grant from ANR of ERA-Net PathoGenoMics.

C. Poyart has received reimbursement for attending meetings from bioMérieux, Bio-Rad, Cepheid, and Novartis and has received research funding from Institut Mérieux, Wyeth, and Siemens.

REFERENCES

- Vandamme P, Pot B, Falsen E, Kersters K, Devriese LA. 1996. Taxonomic study of Lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. *Int. J. Syst. Bacteriol.* 46:774–781.
- Vieira VV, Teixeira LM, Zahner V, Momen H, Facklam RR, Steigerwalt AG, Brenner DJ, Castro ACD. 1998. Genetic relationships among the different phenotypes of *Streptococcus dysgalactiae* strains. *Int. J. Syst. Bacteriol.* 48:1231–1243.
- Jensen A, Kilian M. 2012. Delineation of *Streptococcus dysgalactiae*, its subspecies, and its clinical and phylogenetic relationship to *Streptococcus pyogenes*. *J. Clin. Microbiol.* 50:113–126.
- Brandt CM, Spellerberg B. 2009. Human infections due to *Streptococcus dysgalactiae* subsp. *equisimilis*. *Clin. Infect. Dis.* 49:766–772.
- Takahashi T, Ubukata K, Watanabe H. 2011. Invasive infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis*: characteristics of strains and clinical features. *J. Infect. Chemother.* 17:1–10.
- Fischetti VA. 1989. Streptococcal M protein: molecular design and biological behavior. *Clin. Microbiol. Rev.* 2:285–314.
- McMillan DJ, Bessen DE, Pinho M, Ford C, Hall GS, Melo-Cristino J, Ramirez M. 2010. Population genetics of *Streptococcus dysgalactiae* subsp. *equisimilis* reveals widely dispersed clones and extensive recombination. *PLoS One* 5:e11741. doi:10.1371/journal.pone.0011741.
- Stevens DL. 2001. Invasive streptococcal infections. *J. Infect. Chemother.* 7:69–80.
- Fraser JD, Proft T. 2008. The bacterial superantigen and superantigen-like proteins. *Immunol. Rev.* 225:226–243.
- Norrby-Teglund A, Thulin P, Gan BS, Kotb M, McGeer A, Andersson J, Low DE. 2001. Evidence for superantigen involvement in severe group A streptococcal tissue infections. *J. Infect. Dis.* 184:853–860.
- Sachse S, Seidel P, Gerlach D, Günther E, Rödel J, Straube E, Schmidt KH. 2002. Superantigen-like gene(s) in human pathogenic *Streptococcus dysgalactiae* subsp. *equisimilis*: genomic localisation of the gene encoding streptococcal pyrogenic exotoxin G (*speG^{lys}*). *FEMS Immunol. Med. Microbiol.* 34:159–167.
- Sawai J, Hasegawa T, Kamimura T, Okamoto A, Ohmori D, Nosaka N, Yamada K, Torii K, Ohta M. 2007. Growth phase-dependent effect of clindamycin on production of exoproteins by *Streptococcus pyogenes*. *Antimicrob. Agents Chemother.* 51:461–467.
- Stevens DL, Gibbons AE, Bergstrom R, Winn V. 1988. The Eagle effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. *J. Infect. Dis.* 158:23–28.
- Anthony RM, Wermeling F, Karlsson MC, Ravetch JV. 2008. Identification of a receptor required for the anti-inflammatory activity of IVIG. *Proc. Natl. Acad. Sci. U. S. A.* 105:19571–19578.
- Working Group on Severe Streptococcal Infections. 1993. Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. *JAMA* 269:390–391.
- Loubinoux J, Rio B, Mihaila L, Foïs E, Le Fleche A, Grimont PAD, Marie JP, Bouvet A. 2005. Bacteremia caused by an undescribed species of *Janibacter*. *J. Clin. Microbiol.* 43:3564–3566.
- Chatellier S, Ihendyane N, Kansal RG, Khambaty F, Basma H, Norrby-Teglund A, Low DE, McGeer A, Kotb M. 2000. Genetic relatedness and superantigen expression in group A streptococcus serotype M1 isolates from patients with severe and nonsevere invasive diseases. *Infect. Immun.* 68:3523–3534.
- Tyler SD, Johnson WM, Huang JC, Ashton FE, Wang G, Low DE, Rozee KR. 1992. 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.* 30:3127–3131.
- Hashikawa S, Iinuma Y, Furushita M, Ohkura T, Nada T, Torii K, Hasegawa T, Ohta M. 2004. Characterization of group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J. Clin. Microbiol.* 42:186–192.
- Malhotra-Kumar S, Lammens C, Piessens J, Goossens H. 2005. Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. *Antimicrob. Agents Chemother.* 49:4798–4800.
- Ekelund K, Skinhoj P, Madsen J, Konradsen HB. 2005. Invasive group A, B, C and G streptococcal infections in Denmark 1999–2002: epidemiological and clinical aspects. *Clin. Microbiol. Infect.* 11:569–576.
- Cohen-Poradosu R, Jaffe J, Lavi D, Grisariu-Greenzaid S, Nir-Paz R, Valinsky L, Dan-Goor M, Block C, Beall B, Moses AE. 2004. Group G streptococcal bacteremia in Jerusalem. *Emerg. Infect. Dis.* 10:1455–1460.
- Takahashi T, Sunaoshi K, Sunakawa K, Fujishima S, Watanabe H, Ubukata K, Invasive Streptococcal Disease Working Group. 2010. Clinical aspects of invasive infections with *Streptococcus dysgalactiae* subsp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections. *Clin. Microbiol. Infect.* 16:1097–1103.
- Broyles LN, Van Beneden C, Beall B, Facklam R, Shewmaker PL, Malpiedi P, Daily P, Reingold A, Farley MM. 2009. Population-based study of invasive disease due to β -hemolytic streptococci of groups other than A and B. *Clin. Infect. Dis.* 48:706–712.
- Rantala S, Vähäkuopus S, Vuopio-Varkila J, Vuento R, Syrjänen J. 2010. *Streptococcus dysgalactiae* subsp. *equisimilis* bacteremia, Finland, 1995–2004. *Emerg. Infect. Dis.* 16:843–846.
- Kittang BR, Skrede S, Langeland N, Haanshuus CG, Mylvaganam H. 2011. *emm* gene diversity, superantigen gene profiles and presence of *SlaA* among clinical isolates of group A, C and G streptococci from western Norway. *Eur. J. Clin. Microbiol. Infect. Dis.* 30:423–433.
- Pinho MD, Melo-Cristino J, Ramirez M, Portuguese Group for the Study of Streptococcal Infections. 2006. Clonal relationships between invasive and noninvasive Lancefield group C and G streptococci and *emm*-specific differences in invasiveness. *J. Clin. Microbiol.* 44:841–846.
- Brandt CM, Haase G, Schnitzler N, Zbinden R, Lütticken R. 1999. Characterization of blood culture isolates of *Streptococcus dysgalactiae* subsp. *equisimilis* possessing Lancefield's group A antigen. *J. Clin. Microbiol.* 37:4194–4197.
- Tanaka D, Isobe J, Watahiki M, Nagai Y, Katsukawa C, Kawahara R, Endoh M, Okuno R, Kumagai N, Matsumoto M, Morikawa Y, Ikebe T, Watanabe H, Working Group for Group A Streptococci in Japan. 2008. Genetic features of clinical isolates of *Streptococcus dysgalactiae* subsp. *equisimilis* possessing Lancefield's group A antigen. *J. Clin. Microbiol.* 46:1526–1529.
- Tseng S-P, Lin Y-Y, Tsai J-C, Hsueh P-R, Chen H-J, Hung W-C, Teng L-J. 2010. Distribution of *emm* types and genetic characterization of the *mge* locus in group G *Streptococcus dysgalactiae* subsp. *equisimilis* from a hospital in northern Taiwan. *J. Clin. Microbiol.* 48:2975–2977.
- Sunaoshi K, Murayama SY, Adachi K, Yagoshi M, Okuzumi K, Chiba N, Morozumi M, Ubukata K. 2010. Molecular *emm* genotyping and antibiotic susceptibility of *Streptococcus dysgalactiae* subsp. *equisimilis* isolated from invasive and non-invasive infections. *J. Med. Microbiol.* 59:82–88.
- Kittang BR, Bruun T, Langeland N, Mylvaganam H, Glambeek M, Skrede S. 2011. Invasive group A, C and G streptococcal disease in western Norway: virulence gene profiles, clinical features and outcomes. *Clin. Microbiol. Infect.* 17:358–364.
- Plainvert C, Doloy A, Loubinoux J, Lepoutre A, Collobert G, Touak G, Trieu-Cuot P, Bouvet A, Poyart C on behalf of CNR-Strep Network. 2012. Invasive group A streptococcal infections in adults, France (2006–2010). *Clin. Microbiol. Infect.* 18:702–710.