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Emergence of Invasive Serotype VIII Group B Streptococcal Infections in Denmark

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Serotype VIII group B streptococcus has only rarely been described outside Japan. The Streptococcus Unit, Statens Serum Institut, performed national surveillance of invasive group B streptococcal (GBS) diseases in Denmark in 1999 to 2002 and identified seven clinical GBS isolates of serotype VIII in blood from seven patients admitted to different hospitals.

Group B streptococci (GBS) are a cause of mortality and morbidity in neonates, besides being an emerging cause of invasive infection in elderly patients with underlying diseases (9, 16). GBS are classified into nine serotypes, Ia, Ib, and II to VIII, based on type-specific capsular polysaccharides, and surveillance studies have shown that serotype VIII predominates among GBS isolated from pregnant women in Japan. Outside Japan, serotype VIII is rare and only a few isolates have been described (4, 6, 10, 13, 17). The Streptococcus Unit, Statens Serum Institut, Copenhagen, Denmark, has surveyed the serotype distribution in invasive GBS infections in Denmark since 1999. We present seven clinical GBS isolates with serotype VIII from seven patients admitted to hospitals in Denmark during 2001 and 2002.

The Streptococcus Unit of the Statens Serum Institut serves as the National Streptococcus Reference Center and receives the majority of invasive GBS isolates as pure cultures from local clinical microbiological departments for national surveillance. Included in this study were GBS blood isolates collected from patients admitted to Danish hospitals from 1 January 1999 to 31 December 2002 and two serotype VIII GBS reference strains, JM9 Prague no. 130013 (CNCTC 1/92) and JM9 Prague no. 130669, which were kindly provided by J. Motlova, National Streptococcus and Enterococcus Reference Laboratory, National Institute of Public Health, Prague, Czech Republic.

All isolates were serotyped by precipitin test as described by Lancefield with specific rabbit anti-capsular polysaccharide antibodies (Ia, Ib, and II to VIII; Statens Serum Institut). Nonserotypeable isolates were designated NT (8). Both 0.1 and 0.2 N hydrochloric acid (HCl) solutions were used to extract the capsular polysaccharide antigens (H.-C. Slotved, S. Sauer, and H. B. Konradsen, Letter, J. Clin. Microbiol. **40:**1882-1883, 2002). MICs of benzylpenicillin, gentamicin, moxifloxacin, trimethoprim-sulfamethoxazole, tetracycline, clindamycin, and azithromycin were determined by E test (AB-Biodisk, Solna, Sweden) on Mueller-Hinton agar with 5% lysed horse blood (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Breakpoints were defined in accordance with NCCLS guidelines, except for trimethoprim-sulfamethoxazole and moxifloxacin, for which the breakpoints of the Swedish Reference Group for Antibiotics were used (12). The Swedish Reference Group for Antibiotics breakpoints were obtained at http://www.ltkronoberg.se/ext/raf/raf.htm. The clinical and reference strains were analyzed by pulsed-field gel electrophoresis (PFGE) as described elsewhere (15) with supplementation of the lysis buffer with 22 μl of mutanolysin (Sigma, St. Louis, Mo.) per ml (14). Calculation of similarity between PFGE patterns with the Dice coefficient and clustering by the unweighted pair group method using mathematical averages (UPGMA) was done in BioNummerics (Applied Maths, Kortrijk, Belgium) after the image was captured by Chemi Doc (Bio-Rad Laboratories). Strains were considered to be clonal if the Dice coefficients were greater than 85%.

In the study period, we received 386 invasive GBS isolates from patients admitted to hospitals in Denmark, and seven GBS isolates of serotype VIII were identified from 31 July 2001 to 20 December 2002 in blood from seven patients admitted to different hospitals, which constituted 1 and 6% of the total number of invasive GBS isolates in 2001 and 2002, respectively. The median age of the patients (three female [one pregnant] and four male) was 49.3 (range, 0.0 to 88.7) years. None of the patients had been outside Denmark in the last 3 weeks before hospital admission. Besides bacteremia, three of the patients presented erysipelas and one had clinical cystitis. In two of the patients, skin lesions were reported as a predisposing factor, one patient received immunosuppressive treatment, and one patient had no predisposing factors reported.

Table 1 shows the similarity of the *Sma*I PFGE patterns of the two reference strains and the seven epidemiologically unrelated isolates based on the Dice coefficient and clustering by UPGMA, in addition to the results of the antibiotic susceptibility tests. Four of the clinical isolates belonged to the same clone as the reference strains. The remaining three clinical isolates had distinct PGFE patterns.

In Japan, GBS serotype VIII constitutes 36% of the sero-

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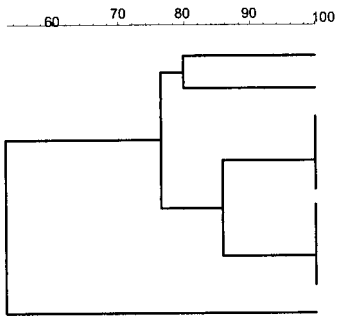
TABLE 1. Cluster analysis of *Sma*I PFGE of seven clinical serotype VIII GBS strains identified in Denmark is 2001 and 2002 in comparison with two reference strains[a]

Dice coefficient clustering by UPGMA	Patient age, sex or strain	Sample date (mo, yr)	Specimen	Nonsusceptibility[b]
	37, female	June 2002	Blood	
	0, male	Dec. 2002	Blood[d]	STX
	40, male	July 2001	Blood	
	49, male	Sept. 2002	Blood	
	70, female	Dec. 2002	Blood	STX, AZM
	87, female	Apr. 2002	Blood	STX
	130013[c]			
	130669[c]			
	89, male	Nov. 2002	Blood	

[a] Similarities are based on the Dice coefficient, and cluster analysis was performed in according to UPGMA in BioNumerics.
[b] STX, trimethoprim-sulfamethoxazole; AZM, azithromycin.
[c] JM9 reference strains CNCTC Prague no. 130013 and Prague no. 130669 were used.
[d] Taken from umbilical veins.

types identified in pregnant women (7), but outside Japan, isolates of serotype VIII have only been sporadically reported. In 2001 and 2002, serotype VIII constituted 1 and 6% of all of the invasive GBS infections in Denmark, respectively. The pathogenicity of serotype VIII has been described to be weaker than that of serotypes III and Ia because serotype VIII is rarely identified in neonatal GBS infections (5, 10). In Maryland, Harrison found one GBS isolate with serotype VIII among 816 invasive isolates from children and adults (4), while Paoletti identified one serotype VIII isolate among 114 GBS isolates colonizing women and children in Boston, Mass. (13). Setting up a group- and serotype-specific PCR, Kong et al. used one serotype VIII strain isolated from Australia (6), and two invasive isolates of serotype VIII were recently identified in Perugia, Italy, in a GBS surveillance of neonates (3). In a surveillance study in Korea, four noninvasive isolates of serotype VIII were detected in urine, female genital, and pus specimens (10). Other GBS surveillance studies have looked for but not identified serotype VIII (18, 19). GBS surveillance limited to serotypes Ia to V has also been performed (1, 11). Our study emphasized the importance of using antisera specific for serotypes Ia to VIII when describing the complete serotype distribution in GBS surveillance. In addition, we have been able to reduce the number of strains originally determined as NT by supplementing 0.2 N HCl with 0.1 N HCl in the extraction of capsular antigen (Slotved et al., letter, 2002).

The susceptibility testing revealed only minor differences between the two reference strains and the seven clinical isolates. The PFGE results showed that several lineages of GBS serotype VIII are causing invasive disease in Denmark. Four of the clinical isolates showed relatedness to the reference strains at a similarity level of greater than 85%, while three isolates belonged to separate lineages. In comparison Benson et al. found great homology among eight strains of serotype VIII (2). We conclude that serotype VIII is causing invasive infections outside Japan; therefore, monitoring of the serotype distribution including all nine serotypes (Ia to VIII) is necessary to sustain complete surveillance of invasive GBS infections.

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