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CASE REPORTS

Genetic Relationship between *Streptococcus pneumoniae* Isolates from Nasopharyngeal and Cerebrospinal Fluid of Two Infants with Pneumococcal Meningitis

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The molecular epidemiology of *Streptococcus pneumoniae* isolates from carriage and cerebrospinal fluid (CSF) concurrently recovered from the same individual has not yet been reported. By using pulsed-field gel electrophoresis, we demonstrated the genetic linkage among strains from CSF and nasopharynges of two children with pneumococcal meningitis.

CASE REPORTS

Case 1. A boy 4 years and 10 months old was admitted to the Tropical Diseases Hospital in Goiânia, Brazil, in July 2000 with a 2-day history of prostration, vomiting, and fever. The child had been well until 7 days earlier, when he developed flu-like symptoms and acute otitis media. No antimicrobial therapy had been administered before admission. The patient had never received any kind of bacterial meningitis vaccine and was not attending any day-care center. On examination, he was lethargic and presented signs of meningeal irritation. The axillary temperature was 38.5°C, and the pulse was 78 beats/min. A lumbar puncture disclosed cloudy cerebrospinal fluid (CSF), and laboratorial analysis revealed a white cell count of 390 cells/ml (85% polymorphonuclear cells), a protein level of 116 mg/dl, and a glucose level of 2.5 mg/dl. Gram staining of the CSF showed gram-positive cocci in pairs and chains. The child was treated with ceftriaxone (1.5 g/day intravenously). Culturing of CSF and blood was carried out according to standard procedures (11) and World Health Organization guidelines (18) and yielded *Streptococcus pneumoniae* susceptible to penicillin and also to other antimicrobial agents (Table 1). The child completed a 12-day course of treatment with ceftriaxone and was discharged from the hospital in good health.

Case 2. A 4-month-old boy was admitted to the same hospital in August 2000. He had been well until 1 week before admission, when he developed flu-like symptoms and subsequent yellow nasal discharge. He received erythromycin for 3 days. One day before admission, he presented with fever, vomiting, and irritability. On examination, the axillary temperature was 37.6°C, the pulse was 120 beats/min, and he had a stiff neck and a bulging fontanelle. The child did not attend any day-care center. He had received one dose of *Haemophilus influenzae* type b (Hib) vaccine, but no pneumococcal vaccine had been administered. A lumbar puncture yielded cloudy CSF, and laboratorial analysis revealed a white cell count of 2,340 cells/ml (98% polymorphonuclear cells), a protein level of 110 mg/dl, and a glucose level of 2.5 mg/dl. Gram staining of the CSF showed gram-positive cocci in pairs and chains. In accordance with a local protocol, treatment with ampicillin and chloramphenicol was then initiated. In our institution, this drug combination is an alternative to ceftriaxone and has also been employed in empirical therapy of bacterial meningitis in children, pending CSF and blood culture results. Since the child did not show clinical improvement, the drugs used for antimicrobial therapy had to be switched to ceftriaxone. Culture of CSF yielded *S. pneumoniae*, and no pathogen was recovered from blood culture. The isolate was found to be nonsusceptible to penicillin (MIC = 0.125 μg/ml), and results of assays of susceptibility to other antimicrobials are shown in Table 1. The child was discharged from the hospital in good health after 14 days of antimicrobial therapy.

As part of an ongoing molecular epidemiological surveillance of *S. pneumoniae* in children that is being carried out in the municipality of Goiânia (A. L. S. S. Andrade, Program Abstr. Book 3rd Int. Symp. Pneumococci Pneumococcal Dis., p. 19, 2002 [online]), nasopharyngeal (NP) transwabs have been collected from all patients with suspected cases of bacterial meningitis (18) at the time of admission, concurrently with lumbar puncture and blood specimens. For this reason, NP

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TABLE 1. Phenotyping and PFGE patterns of isolates from CSF, blood, and nasopharynges of two children with *S. pneumoniae* meningitis

Case no. (patient age, gender)	Origin of *S. pneumoniae* isolate	Serotype of isolate	MIC (μg/ml) of[a]:						PFGE pattern
			Pen	Cm	Ctx	TSX	Van	Ery	
1 (58 months, male)	CSF	9V	0.06	2.0	0.03	0.5	0.25	0.03	A
	Blood	9V	0.03	2.0	0.03	0.25	0.5	0.06	A
	Nasopharynx	9V	0.06	1.0	0.03	0.25	0.5	0.03	A
2 (4 months, male)	CSF	14	0.125	1.0	0.06	2.0	0.5	0.03	B
	Nasopharynx	14	0.125	1.0	0.06	2.0	0.5	0.03	B

[a] Pen, penicillin; Cm, chloramphenicol; Ctx, ceftriaxone; TSX, trimethoprim-sulfamethoxazole; Van, vancomycin; Ery, erythromycin.

transwabs were collected from the two infants, placed in Stuart transport medium (Medical Wire & Equipment, Corsham, United Kingdom), and sent to the bacteriology laboratory at the Instituto de Patologia Tropical e Saúde Pública-Universidade Federal de Goiás, Goiânia, Brazil. Cultures of swabs of the two children yielded *S. pneumoniae*. Serotyping of pneumococcal isolates from CSF, blood, and NP swabs was done by using the Quellung reaction with sera obtained from the Statens Seruminstitut, Copenhagen, Denmark (16). Serotypes 9V and 14 were identified, respectively, in the isolates from case 1 (CSF, blood, and NP swab) and case 2 (CSF and NP swab) (Table 1). To investigate the genetic relatedness of the isolates recovered from the distinct specimen types from the same child, whole-cell DNA analysis of the *S. pneumoniae* isolates was performed at the Adolfo Lutz Institute—São Paulo, the National Reference Center for *S. pneumoniae* in Brazil, by using pulsed-field gel electrophoresis (PFGE) as previously described (3). Strains were ascertained to be of the same lineage when no more than a three-band difference was observed between individual PFGE profiles by visual inspection (17). Table 1 summarizes the phenotypes and genotypes of the pneumococcal isolates from the different clinical specimens in the two cases. Isolates recovered from NP swabs and CSF and, in case 1, blood of the same child displayed the same chromosomal DNA restriction patterns, meaning that the colonizing pneumococcal strain was the one related to the present invasive disease in each child. Furthermore, the antimicrobial susceptibility profiles of the NP isolates matched those of the isolates recovered from the CSF and blood (case 1) and from the CSF (case 2).

S. pneumoniae is a relevant cause of invasive diseases in children under 5 years old, accounting for significant morbidity and mortality rates in developing regions (7). With the notable decline of Hib meningitis in several countries, accomplished by immunization with the Hib conjugate vaccine, it has been speculated that *S. pneumoniae* will become the leading bacterial pathogen in childhood meningitis (9), as has already been noticed by surveillance programs in developed settings (14). In Brazil, since the introduction of the Hib vaccine in 1999, *S. pneumoniae* ranks as the second most important cause of bacterial meningitis in children, preceded only by *Neisseria meningitidis.* The annual incidence of pneumococcal meningitis is estimated as 3.5 cases per 100,000 children less than 5 years old, with a case fatality rate of 34.8% (National Centre of Epidemiology, Ministry of Health). Despite the availability of the heptavalent conjugate pneumococcal vaccine, the implementation of the vaccine in developing countries has been hampered by the elevated cost of adhering to the preconized schedule. Up to now, the vaccine has been available only for patients able to afford it at private health services in Brazil.

Our local ongoing surveillance program was developed to improve the accuracy of the microbiological diagnosis of meningitis in children less than 5 years of age in Goiânia, Central Brazil (Andrade, Program Abstr. Book 3rd Int. Symp. Pneumococci Pneumococcal Dis.). This initiative allowed us to document the epidemiological relationships of isolates recovered from distinct specimen types in the two cases of pneumococcal meningitis, based upon phenotyping and molecular typing by PFGE. We showed that the same *S. pneumoniae* isolate could be found simultaneously in the nasopharynx and the blood or CSF. Since the clinical specimens were collected at the same time, the temporal sequence between NP swab and CSF pneumococcal acquisition could be questioned. However, it has been well established that NP colonization by *S. pneumoniae* is the first step in bloodstream dissemination and ultimate progress to pneumococcal meningitis, and it has also been shown that invasive disease is most likely to occur soon after NP colonization (4, 6). Thus, the most reasonable explanation for our findings is that the children were first colonized with the isolate, and colonization subsequently led to the invasive disease. Usually, pneumococcal isolates with the same PFGE patterns share the same capsule serotypes; however, some reports have shown that isolates belonging to different capsule serotypes may show the same PFGE patterns. This phenomenon is attributed to the transformation genetic process (12). Nevertheless, there is a lack of information concerning the relatedness of NP swab and CSF pneumococcal isolates recovered from the same individual, as shown by the two cases reported herein.

NP isolates comprise a diverse range of serotypes and genotypes (8, 13) compared to invasive pneumococci, which usually belong to a limited number of serotypes, mainly those related to the conjugate vaccine (1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F) (1, 5). A recent study using data from the Brazilian surveillance system found types 9V and 14 (those colonizing patients in cases 1 and 2) to rank among the most frequent serotypes in pediatric meningitis cases, with an estimated prevalence of 3.1 and 19.1%, respectively (2). It has been hypothesized that invasive isolates have a transient residence in the nasopharynx (15). Follow-up studies have shown that different isolates belonging to the same or distinct serotypes may colonize the nasopharynx at the same time or at different periods of time, followed by local replacement with distinct serotypes (6). Although NP colonization precedes invasion, progress to clinical disease (meningitis, bacteremia, and otitis media) is expected to occur in up to 15% of children who are colonized (6).

A previous study has already demonstrated that, frequently, carriage clones are associated with cases of invasive diseases (10). Nonetheless, the translation of this assumption to an individual basis requires evidence supported by studies using molecular typing of a larger number of isolates. Because isolates that are epidemiologically related may not be genetically related, molecular tools such as PFGE, when incorporated into surveillance systems, provide valuable information enabling determination of the frequency with which this phenomenon occurs.

The identical DNA profiles of isolates from NP swabs and CSF of the two patients illustrate the link between carriage and CSF pneumococcal strains in the pathogenesis of meningitis in children. To our knowledge, this is the first study to address the molecular epidemiology of carriage and invasive *S. pneumoniae* isolates concurrently recovered from the same individual. To what extent carriage isolates represent strains causing meningitis at the individual level deserves investigation by further studies with extended numbers of isolates.

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