Genetic Relatedness of *Streptococcus pneumoniae* Isolates from Paired Blood and Respiratory Specimens

L. J. Harrell, S. K. Sharps, R. A. Bean, and L. B. Reller

Departments of Medicine, Molecular Genetics and Microbiology, and Pathology, Duke University School of Medicine, and Clinical Microbiology Laboratory, Duke University Health System, Durham, North Carolina

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To assess the relatedness of *Streptococcus pneumoniae* isolates recovered concurrently from blood and respiratory tract specimens from patients with pneumonia, we analyzed 24 paired isolates by pulsed-field gel electrophoresis (PFGE), serotyping, and antimicrobial susceptibility testing. PFGE, serotype, and/or susceptibility patterns were identical for 22 of 24 pairs. Susceptibility results for blood isolates should guide therapy.

*S. pneumoniae* often colonizes the nasopharynx, and one or more serotypes can be found as normal throat flora in 5 to 10% of adults and 20 to 40% of children (6). However, this microorganism commonly causes community-acquired pneumonia and also is an important cause of more invasive infections, including bacteremia. As many as 25% of untreated patients with pneumococcal pneumonia may also have bacteremia (7). For patients who have both pneumonia and bacteremia, the respiratory isolates may be more resistant due to prior therapy for respiratory tract infections (2, 4).

To assess genetic and phenotypic relatedness, we analyzed paired blood and respiratory isolates of *S. pneumoniae* to determine if they had the same pulsed-field gel electrophoresis (PFGE) profile, serotype, and antimicrobial susceptibility pattern. In total, 24 pairs of *S. pneumoniae* isolates recovered concurrently from both blood and respiratory tract cultures between April 1998 and November 2003 were tested. Most of the isolates were from patients at Duke University-affiliated hospitals (23 pairs); 1 pair was from a patient at a nearby hospital. The patients comprised 18 males and 6 females with an age range of 1 month to 73 years. There were 2 pediatric patients (aged 1 month and 6 years), but most (20/24) of the patients were children (1 month to 14 years old).

The respiratory isolates were obtained by sputum collection (n = 11), bronchial washing (n = 3), and sinus aspiration (n = 1). Isolates were identified by standard microbiological methods (10), including alpha-hemolysis on sheep blood agar, Gram staining, and the bile solubility test. Isolates were frozen at −70°C until further testing.

DNA preparation and restriction were done with the Bio-Rad universal module and GenePath group I reagent kit as specified in the manufacturer’s package insert (Bio-Rad Laboratories, Hercules, CA). Briefly, isolates were grown overnight (16 to 18 h) at 37°C on sheep blood agar plates, and colonies were suspended in 1.0 ml of sterile water. This suspension was centrifuged, and the pellet was resuspended in lysis buffer and placed in agarose to form plug molds. The agarose-embedded cells were lysed, after which the DNA was extracted, deproteinized, and restricted with SmaI enzyme.

PFGE was performed with the Bio-Rad GenePath system using program 12, which separates DNA in the size range of 25 to 300 kb. Gels were stained with ethidium bromide and photographed under UV transillumination. Gels were visually inspected and interpreted by the criteria of Tenover et al. (11).

Serotyping was performed by the quellung test (10) using antcapsular antiserum from Statens Seruminstitut (Copenhagen, Denmark). Briefly, colonies were mixed with a drop of antiserum plus methylene blue and were examined microscopically. Encapsulated *S. pneumoniae* isolates reacted with the serotype-specific antiserum and caused a “halo” effect around the cells. Serotyping was performed in the Maxwell Finland Laboratory for Infectious Diseases at Boston University.

Susceptibility testing was performed by disk diffusion (Becton Dickinson and Company, Sparks, MD) according to the CLSI (formerly NCCLS) guidelines (8) and by Etest (AB Biodisk, Solna, Sweden). The following antimicrobials were tested: clindamycin, erythromycin, levofloxacin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin (by disk diffusion) as well as ceftriaxone and penicillin (by Etest).

Two of the 24 isolate pairs had different PFGE patterns by the criteria of Tenover et al. (11), as seen in Fig. 1. The two pairs that had discordant PFGE patterns were blood-ETS and blood-bronchial wash specimens, respectively; one of these pairs had the same susceptibility pattern. A total of 23 isolate pairs were available for serotyping, and each pair had the same serotype. One pair was nontypable. Most (78%) of the isolate pairs (18/23) had serotypes that are included in the 23-valent pneumococcal vaccine (1). One of the pediatric isolate pairs belonged to serotype 18C, which is included in the new conjugate 7-valent pediatric vaccine (4); the other pediatric patient had serotype 15B, which is included in the 23-valent vaccine (Table 1).

Susceptibility patterns were in agreement for 22 of 24 isolate pairs. One isolate pair with discordant PFGE patterns (blood-ETS) had discordant susceptibility results for three of eight antimicrobials tested (penicillin, tetracycline, and trimethoprim-sulfamethoxazole). Four of the 24 patients had respiratory isolates...
that were penicillin resistant (MIC, >0.06 µg/ml; range, 1.5 to 3 µg/ml [by Etest]), but none of the isolates was resistant to levofloxacin or ceftriaxone.

To our knowledge, this is the first published study to determine the genetic relatedness of S. pneumoniae isolates from the blood and respiratory tract. The preponderance of older adults rather than children in our series is consonant with their relative risk for pneumococcal pneumonia versus occult bacteremia. Previous attempts to correlate strain relatedness for 420 children who had simultaneous blood cultures and tracheal aspirates disclosed only 1 pair of pneumococcal isolates (13). It is assumed that patients with pneumococcal pneumonia and bacteremia are infected with the same strain. However, there are at least 90 different serotypes of S. pneumoniae (3), and the respiratory tracts of healthy people can be colonized with multiple serotypes (9). Thus, it is possible that isolates with different susceptibility patterns as well as different molecular patterns may be found at the two sites. It is important to know if the isolates from the blood and respiratory tract are indeed the same, because of emerging antibiotic resistance to penicillin and other antimicrobials used for treating pneumococcal infections (2, 9). Additionally, surveillance for changes in the epidemiology of invasive serotypes of pneumococci is crucial to preserving the reductions in the frequency of bacteremia, pneumonia, and otitis media that have followed the introduction of pneumococcal vaccines. Such surveillance is especially important given the reality of capsular switching and the emergence of antimicrobial resistance even in genetically related strains of pneumococci (5, 12). We conclude that strains of pneumococci recovered from the blood and respiratory tracts of patients with pneumonia usually (about 90% of the time) have the same PFGE and susceptibility patterns as well as the same serotypes. When available, susceptibility results for isolates from blood should guide therapy for pneumococcal pneumonia, because bacteremia with S. pneumoniae provides the most robust proof of etiology.

(These data were presented in part at the 104th General Meeting of the American Society for Microbiology in 2004 [abstr. C234].)

![PFGE patterns of SmaI-restricted chromosomal DNA from paired blood and respiratory isolates of S. pneumoniae](image)

**FIG. 1.** Representative PFGE patterns of SmaI-restricted chromosomal DNA from paired blood and respiratory isolates of S. pneumoniae. Lanes: 1 and 20, molecular size marker (phage lambda DNA ladder); 2 and 19, staphylococcal controls; A, C, D, E, G, and H, pairs of blood and respiratory isolates with identical (indistinguishable) or closely related patterns; B and F, pairs of blood and respiratory isolates with different PFGE patterns.

| Serotype(s) | No. of pairs |
|------------|-------------|
| 3          | 3           |
| 4          | 1           |
| 6A         | 1           |
| 6B         | 1           |
| 9A         | 1           |
| 15B        | 1 (child)   |
| 16F        | 1           |
| 18C        | 2 (1 child) |
| 19A        | 3           |
| 19F        | 3           |
| 22F        | 3           |
| 23A        | 1           |
| 23F        | 1           |
| 29, 35, 42 | 1           |

*Most of the patients (18/23) had serotypes included in a vaccine. Boldfaced serotypes are included in the 23-valent pneumococcal vaccine (1). Isolates from one of the pediatric patients had a serotype (18C) that is included in the new conjugate 7-valent pediatric vaccine (4).
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