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Bacteremic Pneumonia Caused by a Single Clone of *Streptococcus pneumoniae* with Different Optochin Susceptibilities

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Two isolates of *Streptococcus pneumoniae* having different optochin susceptibilities were recovered from a blood sample of a 2-year-old boy with community-acquired pneumonia. The two isolates were documented to belong to a single clone on the basis of the isolates' identical serotype (23F), antibiograms by the E-test, random amplified polymorphic DNA patterns generated by arbitrarily primed PCR, pulsed-field gel electrophoresis, and restriction fragment length polymorphism of the penicillin-binding protein genes *pbp2b* and *pbp2x*.

CASE REPORT

A 2-year-3-month-old boy was admitted to our ward with the chief problem of cough and rhinorrhea for 2 weeks and high fever with generalized tonic-clonic convulsion on the day of admission. He had a history of febrile convulsion. Physical findings were unremarkable except for an injected throat. His activity was fair without any meningeal sign. A chest radiograph showed infiltration over bilateral lung fields. Laboratory testing revealed a leukocyte count of 37,650/mm^3 with 88% neutrophils and a markedly elevated C-reactive protein level (14.2 mg/dl, normal level being <0.5 mg/dl). Two sets of blood cultures yielded *Streptococcus pneumoniae*, with an inhibition zone around the oxacillin disk (1-μg disk) (BBL Microbiology Systems, Cockeysville, Md.) of 8 mm. Cefazolin (100 mg/kg of body weight/day) was given for 2 days, and the fever subsided before the blood culture result was available. Since the patient's general condition was healthy, cefazolin was given for a further 3 days, treatment was then shifted to oral cephalexin (25 mg/kg/day) for 1 week, and the patient was discharged in stable condition. The child did well in the following 6 months.

Microbiological investigation. Two sets of blood cultures both grew gram-positive cocci in BACTEC 6A aerobic bottles (Becton Dickinson, Sparks, Md.). After subculture, the organisms grew well on Trypticase soy agar supplemented with 5% sheep blood agar plates (BBL Microbiology Systems) at 37°C in ambient air. The colonies were alpha-hemolytic and nonmucoid. The optochin sensitivity test for the organisms was performed on Trypticase soy agar supplemented with 5% sheep blood agar according to a previous description (18). An inhibition zone of 15 mm in diameter around the 6-mm optochin disk was identified, and several colonies (isolate A) also grew within the inhibition zone (7 to 9 mm from the center of the disk). When the colonies grown inside (isolate A) and outside (isolate B) the inhibition zone, respectively, were inoculated onto two plates of Trypticase soy agar supplemented with 5% sheep blood and were incubated for 24 h, no inhibition zone around the optochin disk was found for isolate A and a complete inhibition zone of 15 mm around the disk (no scattered colonies within the zone) was demonstrated for isolate B. Isolates A and B were positive by the bile solubility test, and their biochemical profiles generated by the API 32 Strep System (bioMeriuex Vitek, Marcy l'Etoile, France) were in accordance with the identification of *S. pneumoniae*.

The two isolates of *S. pneumoniae* (isolates A and B) both belonged to serotype 23F as determined by the capsular swelling test (quellung reaction) by specific antisera as previously described (8). For both isolates, penicillin and cefotaxime MICs were 2 and 1 μg/ml, respectively, determined by means of the E-test (PDM Epsilometer; AB Biodisk, Solna, Sweden). MICs of other agents for these two isolates were also identical: erythromycin, ≥256 μg/ml; rifampin, 1 μg/ml; clindamycin, ≥256 μg/ml; and vancomycin, 0.5 μg/ml. Molecular typing of the two isolates by random amplified polymorphic DNA (RAPD) analysis of chromosomal DNA generated by arbitrarily primed PCR and pulsed-field gel electrophoresis and restriction fragment length polymorphism (RFLP) profiles of penicillin-binding protein genes (*pbp2b* and *pbp2x*) after digestion with restriction enzymes *Hin*fI and *Alu*I (Gibco BRL, Gaithersburg, Md.) were performed in accordance with previous descriptions (7, 12, 19). For RAPD analysis, three primers were used: ERICI (5′-GTGAATCCCCAGGAGCTTACAT-3′), M13 (5′-GAGGGTGGCGGTTCT-3′), and OPA-7 (5′-GAAACGGGTG-3′). For molecular typing studies, one *S. pneumoniae* isolate recovered from another patient was included as a control strain. The two isolates had identical RAPD patterns (Fig. 1), pulsotypes, and RFLP profiles of *pbp2b* and *pbp2x* (Fig. 2).

Discussion. *S. pneumoniae* is a major cause of community-acquired pneumonia, otitis media, paranasal sinusitis, bacteremia, and meningitis (16). The emergence of drug-resistant strains of *S. pneumoniae* has complicated treatment of these common infections (3, 4, 6, 8, 10, 11). Pneumococci are identified in the Clinical Microbiology Laboratory of the National

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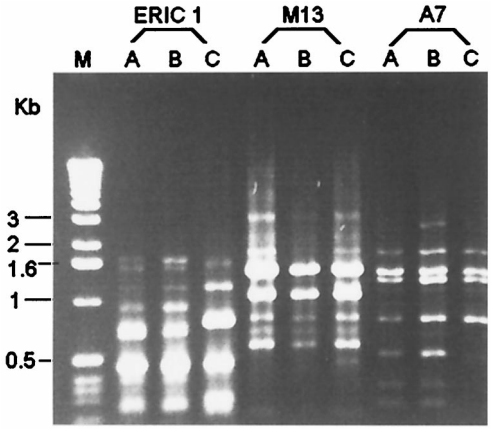

FIG. 1. RAPD patterns generated by arbitrarily primed PCR by three primers, ERICI, M13, and OPA-7. Lane M, molecular markers; lanes C, control strain; lanes A and B, isolates A and B, respectively.

Taiwan University Hospital by the following three reactions: alpha-hemolysis on sheep blood agar, catalase negativity, and solubility in bile salts or susceptibility to ethylhydrocupreine (optochin) (18). In recent years, a number of isolates have been found to be optochin resistant, which has led cautious microbiologists to rely more on the use of bile solubility for definitive identification (9, 15, 17). In fact, it has been reported that up to 5% of *S. pneumoniae* strains may be optochin indeterminate or resistant (1, 15). Furthermore, the bile solubility test is not always specific for *S. pneumoniae* (5). The use of these standard tests can result in ambiguous phenotypes for certain organisms, leading to identification difficulties for routine microbiology laboratories (2, 14).

To our knowledge, bacteremia due to a single clone of *S. pneumoniae* which simultaneously possessed two isolates with different optochin susceptibilities has never been documented previously. By use of the antibiotyping and molecular typing methods, this report described an episode of bacteremia caused by a single clone of serotype 23F and penicillin-resistant *S. pneumoniae* that obviously possessed two subpopulations with different optochin susceptibilities.

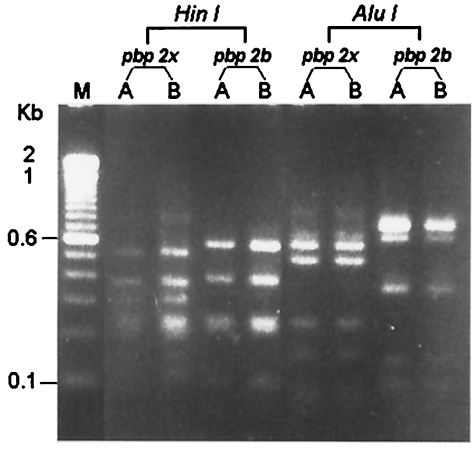

FIG. 2. RFLP patterns of the *pbp2b* and *pbp2x* genes generated with the restriction enzymes *Hin*fI and *Alu*I. See the legend to Fig. 1 for lane definitions.

In summary, we describe a case with invasive infection caused by two isolates of multidrug-resistant *S. pneumoniae* exhibiting different optochin susceptibilities which were later documented to belong to a single clone. Clinical microbiologists need not only to be aware of a continued increase in antimicrobial drug resistance in clinical isolates of *S. pneumoniae* but also to understand the potential difficulties of identification of this organism by conventional methods, particularly the optochin susceptibility test.

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