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Meningitis Due to Mixed Infection with Penicillin-Resistant and Penicillin-Susceptible Strains of *Streptococcus pneumoniae*

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Streptococcus pneumoniae **is the major cause of bacterial meningitis. We report a case of meningitis due to a mixed infection with two distinct strains of *S. pneumoniae*: one penicillin-resistant strain of serotype 9V and one penicillin-susceptible strain of serotype 7. The two strains exhibited different pulsed-field gel electrophoresis profiles.**

CASE REPORT

A 60-year-old male patient with a medical history of arterial hypertension, diabetes mellitus, alcohol abuse, and heavy smoking presented to the emergency service with dizziness, fever, and signs of meningism. One week prior to this, he had been diagnosed with acute otitis media and received therapy with oral amoxicillin-clavulanate. At the time of admission to the intensive care unit the patient was acutely ill. A lumbar puncture yielded a cloudy cerebrospinal fluid (CSF) sample, and laboratory analysis revealed a white blood cell count of 3,600 cells/ml (97% polymorphonuclear cells), a protein level of 224 mg/dl, and a glucose level of 144 mg/dl (blood glucose level, 276 mg/dl). Gram staining of the CSF showed gram-positive cocci in pairs and chains. The patient was treated empirically with ceftriaxone, vancomycin, and ampicillin. Two days after admission, a central nervous system computed tomography scan showed evidence of mastoiditis, and the area was drained surgically. Cultures of CSF and blood yielded *Streptococcus pneumoniae*. Susceptibility testing of the CSF isolate was performed by the E-test strip method (AB Biodisk, Solna, Sweden). The ellipse of organism growth inhibition intercepted the penicillin strip at 0.012 μg/ml, but due to the growth of discrete colonies with identical morphology within the zone of inhibition up to a MIC of 2 μg/ml, the isolate was reported as resistant to penicillin (4). The penicillin-resistant isolate was also found to be susceptible to cefotaxime, vancomycin, erythromycin, and clindamycin. On days 3 and 4 of hospitalization, treatment with ampicillin and vancomycin, respectively, was discontinued. The patient completed a course of antimicrobial treatment with ceftriaxone over a period of 14 days and was discharged in good health.

We decided to investigate the intriguing presence of colonies within the zone of inhibition of penicillin. Two possible explanations were considered: selection of chromosomally mediated mutations of the penicillin-binding proteins within a single strain of *S. pneumoniae* or, alternatively, a mixed infection with two or more different strains of the organism, at least one of which was resistant to penicillin. Colonies from the zone close to the 2-μg/ml mark on the penicillin strip and from the confluent zone of growth were subcultured, retested by the broth microdilution method, and serotyped. Colonies exhibiting resistance to penicillin (isolate 1; MIC, 2 μg/ml) were identified as serogroup 9V and showed intermediate resistance to cefotaxime (MIC, 1 μg/ml) and erythromycin (MIC, 0.5 μg/ml) and susceptibility to vancomycin (MIC, 0.5 μg/ml), tetracycline (MIC, 0.5 μg/ml), and chloramphenicol (MIC, 2 μg/ml). Colonies that exhibited full susceptibility to penicillin (isolate 2; MIC, 0.012 μg/ml) were identified as serogroup 7 and were susceptible to cefotaxime (MIC, 0.015 μg/ml), erythromycin (MIC, 0.12 μg/ml), vancomycin (MIC, 0.5 μg/ml), tetracycline (MIC, 0.25 μg/ml), and chloramphenicol (MIC, 2 μg/ml).

Although clinical isolates of *S. pneumoniae* within a clonal group typically share the same serotype, capsular switching within a well-defined *S. pneumoniae* clone has been described elsewhere (5, 8). For this reason and to determine whether the two isolates belonged to the same clonal group, both were characterized by standard pulsed-field gel electrophoresis (PFGE) (7) with the restriction endonuclease *Sma*I. PFGE analysis of the DNA revealed that the *S. pneumoniae* isolates exhibited more than eight band differences, which clearly qualifies them for their designation at two separate strains (Fig. 1, lanes 3 and 4). We interpreted these results to indicate that the patient's meningitis was due to a mixed infection with two entirely distinct strains: one penicillin resistant, of serotype 9V, and the other penicillin susceptible, of serotype 7.

Bacterial meningitis is the most severe and frequent infection of the central nervous system, with a mortality rate of up to 20% and an adverse neurological outcome in up to 50% of survivors. *S. pneumoniae* has come to be recognized as the major cause of bacterial meningitis in developed countries (3). In Spain, 43% of pneumococci recovered from CSF express intermediate or full resistance to penicillin, presenting serious problems for treatment (2). Twenty-five percent of these cases are due to the more prevalent serotypes with resistance to penicillin (23F, 9V, and 6B) (1). Notably, the prevalence of penicillin-susceptible strains of serogroup 7 has recently shown evidence of a decline, from 6% of all isolates in 1990 to 1% in 1996 (2).

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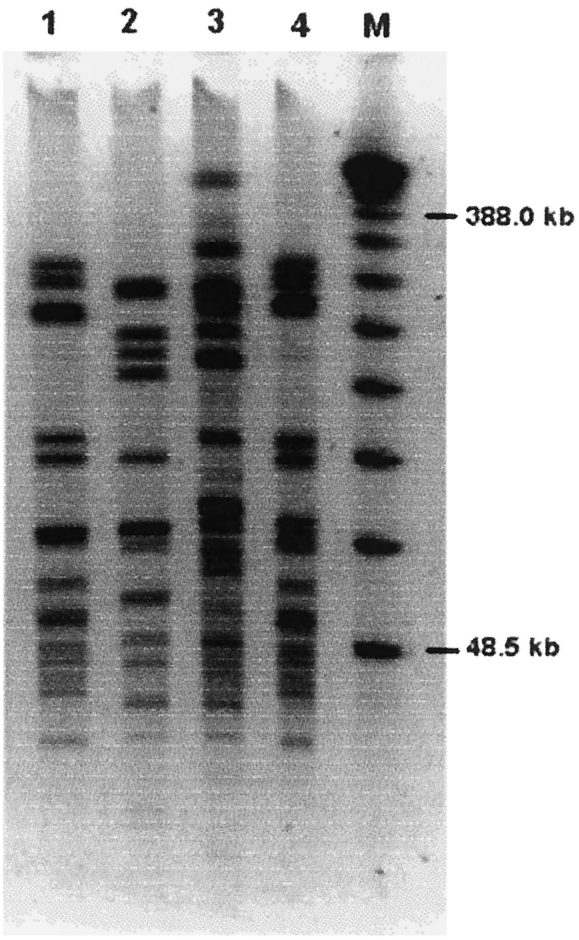

FIG. 1. PFGE of clinical isolates of *S. pneumoniae*. Lane 1, isolate from blood (serotype 14); lane 2, isolate from catheter (serotype 3). Both were unrelated strains from other patients. Lanes 3 and 4, isolates from CSF (see text); lane M, molecular size marker.

To the best of our knowledge, this is the first time that a case of meningitis due to a mixed infection with different strains of *S. pneumoniae* has been described. Recently, Tsai et al. reported a case of bacteremic pneumonia caused by a single clone of *S. pneumoniae*, individual colonies from which exhibited different optochin susceptibilities (9). Pikis et al. also described three isolates of *S. pneumoniae* obtained from the CSF, blood, and nasopharynx of three different patients. These were all shown to contain mixed populations of optochin-susceptible and -resistant organisms, although the two variants exhibited the same antibiogram, serotype, and PFGE profiles (6). The frequency with which mixed pneumococcal infections occur is unknown. This phenomenon might easily be underreported because discrete colonies within the zone of inhibition of an antimicrobial agent are typically considered to represent a minor subpopulation of resistant mutants of the same strain. Full identification and characterization of these organisms are therefore not routinely performed. Furthermore, in cases of meningitis involving fully susceptible *S. pneumoniae*, the possibility of mixed infection with different serotypes or genotypes is not usually considered. The clinical implications of infection with a mixture of susceptible and resistant strains of *S. pneumoniae* are serious if the condition is not recognized in a timely manner and the appropriate therapy is not provided. The administration of inappropriate treatment might lead to selective growth of the resistant population and severe complications for the patient. Our observations highlight the importance of the recommendation of the National Committee for Clinical Laboratory Standards for testing a mixture of several pneumococcal colonies instead of analysis of individual colonies of *S. pneumoniae*, in order to obtain an accurate susceptibility report.

Whenever there is evidence of a mixture of susceptible and resistant organisms in a population, it is important to consider the possible clinical implications. Enhanced surveillance is necessary in order to determine the frequency with which infections due to mixed populations occur and to track the emergence of serotypes with enhanced virulence.

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