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# A Pneumococcal Clinical Isolate with High-Level Resistance to Cefotaxime and Ceftriaxone

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**A beta-lactam-resistant serotype 23F *Streptococcus pneumoniae* clinical isolate from the cerebrospinal fluid of a pediatric patient from California is unusual in that the MICs of cefotaxime and ceftriaxone (2.5 μg/ml each) are higher than that of benzylpenicillin (0.3 μg/ml); the isolate also has patterns of penicillin-binding proteins and of cell wall peptides which are atypical compared with those of previously examined penicillin-resistant pneumococci.**

A common property of all penicillin-resistant *Streptococcus pneumoniae* isolates examined so far was that the MICs of penicillin were always 2 to 4 times higher than or at least equal to the MICs of expanded-spectrum cephalosporins against the same isolates (5, 6, 11). For this reason and also because of their superior pharmacokinetic properties, cefotaxime and ceftriaxone have been considered the chemotherapeutic agents of choice for the treatment of pneumococcal disease (particularly meningitis) in areas of the world with a high incidence of penicillin-resistant pneumococci (10).

However, a recent report described a pneumococcal clinical isolate of serotype 23F from California for which the MICs of cefotaxime and ceftriaxone were unusually high (1). Three additional isolates of the same serotype for which the MICs of the expanded-spectrum cephalosporins were high have since been reported from Tennessee (9a). The pediatric patient from whom the California pneumococcus was isolated has had frequent episodes of otitis media, starting at the age of 5 months, for which he received 140 days of oral antibiotic therapy prior to his meningitic illness. Prior therapy included both penicillins (amoxicillin and amoxicillin-clavulanic acid) and a cephalosporin (cefixime). In this communication, we describe some unusual properties of this isolate.

**Relative susceptibility to beta-lactam antibiotics.** Figure 1 shows the relative MICs of benzylpenicillin, cefotaxime, and ceftriaxone for a number of penicillin-resistant pneumococcal strains. Bacterial cultures were grown in a synthetic medium to the middle of the exponential phase of growth ($2 \times 10^8$ to $5 \times 10^8$ CFU/ml) and were tested for MICs at two cell concentrations ($10^6$ and $10^4$ CFU per plate) in an agar dilution assay with Mueller-Hinton agar supplemented with 3% sterile sheep blood. The strains were selected to include representatives of five distinct penicillin-binding protein (PBP) families classified on the basis of criteria described elsewhere (4). The isolates from Hungary and Czechoslovakia were obtained through the courtesy of Anna Marton (Budapest, Hungary) and Pavla Urbaskova (Prague, Czechoslovakia). It may be seen that for all isolates, the MICs (in micrograms per milliliter) of the two cephalosporins were either 2 to 4 times less than or, for some strains, equal to the respective MIC of penicillin. The MICs of benzylpenicillin, cefotaxime, and ceftriaxone for the standard penicillin-susceptible laboratory strain R36A in this assay system were 0.005, 0.05, and 0.05 μg/ml, respectively.

In sharp contrast to results for all the penicillin-resistant clinical isolates examined, the penicillin MIC for California resistant strain (referred to as CFTR, for ceftriaxone resistance) was 0.3 to 0.6 μg/ml, while the MICs of cefotaxime and ceftriaxone were 2.5 μg/ml.

This inversion of the MICs of penicillin compared with those of expanded-spectrum cephalosporins makes isolate CFTR unique among all the beta-lactam-resistant pneumococcal clinical isolates examined so far. The purpose of the studies described here was to examine isolate CFTR for some additional characteristics that have been correlated with the mechanism of beta-lactam resistance in clinical pneumococcal isolates.

**Penicillin-induced lysis.** The great majority (>80%) of penicillin-resistant clinical pneumococcal isolates were shown to be defective in antibiotic-induced autolysis (8). Isolate CFTR differed from this majority of resistant isolates in its autolytic properties. Strain CFTR, two Spanish penicillin-resistant clinical isolates (53/55 and 2/55, both of serotype 23F; the penicillin MIC for both strains was 2 μg/ml), and the penicillin-susceptible control strain R36A (for which the penicillin MIC was 0.005 μg/ml) were grown in a synthetic medium at 37°C to the middle of the exponential phase, when they received penicillin at concentrations 10 times the respective MICs, and the rates of change of the optical densities of the suspensions were monitored. The two penicillin-resistant strains were chosen because they have reduced autolysis rates characteristic of the majority of these isolates (8).

In contrast to the two resistant strains, which showed reduced autolysis, CFTR lysed as fast as the susceptible control strain when treated with penicillin (Fig. 2).

**PBP pattern and penicillin affinity.** The PBPs of penicillin-resistant clinical isolates of pneumococci differ from the PBPs of penicillin-susceptible strains in two major ways. (i) Resistant isolates for which penicillin MICs were 0.5 μg/ml or higher have altered PBP electrophoretic profiles (i.e., abnormal molecular sizes and numbers of PBPs), and (ii) the high-molecular-weight PBPs in such isolates have reduced

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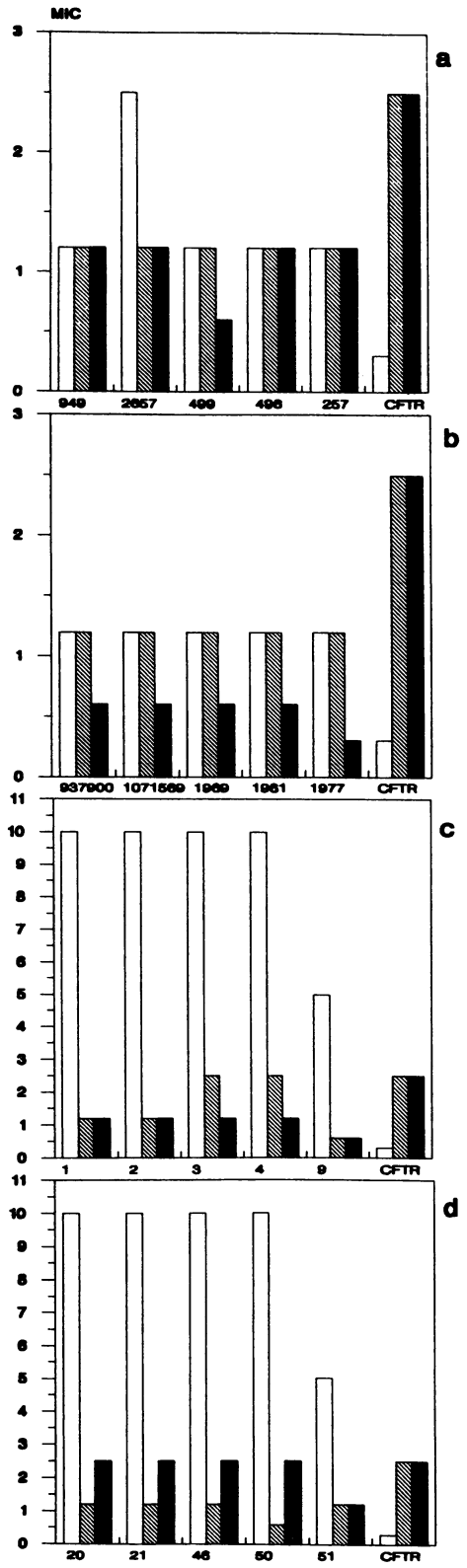


FIG. 1. Relative susceptibilities of pneumococcal clinical isolates to penicillin, cefotaxime, and ceftriaxone. (a) Spanish isolates of serotype 23F; (b) isolates from Cleveland, Ohio, of serotype 23F; (c) isolates from Hungary of serotype 19A; (d) isolates from Czechoslovakia. MICs (micrograms per milliliter) of penicillin (□), cefotaxime (▨), and ceftriaxone (■) are shown. Identification numbers of the isolates are at the bottom of each panel.

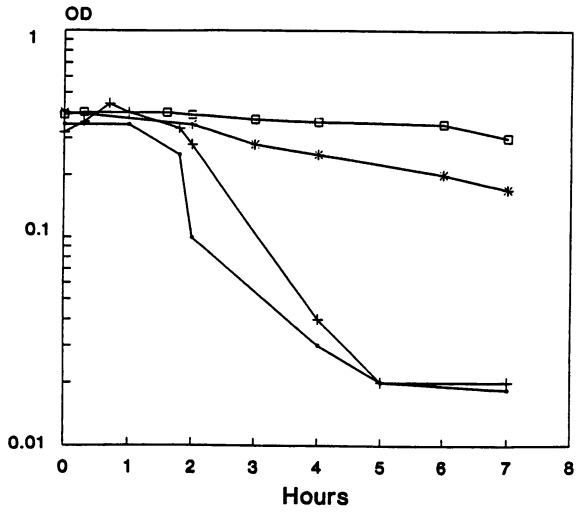


FIG. 2. Lysis of pneumococci during exposure to penicillin. Optical densities (OD) of the cultures of strains CFTR (+), of penicillin-resistant isolates 53/55 penR (□) and 2/55 penR (*), and of the penicillin-susceptible control strain R36A (•) were monitored by a previously described method (3) after the addition of penicillin (at concentrations 10 times the respective MICs) at zero time.

binding capacities for radioactive penicillin. In particular, PBP 2B was no longer detectable by the standard fluorographic assay in penicillin-resistant isolates for which MICs were 0.5 μg/ml or higher. These generalizations were based on the examination of the PBPs in several hundred clinical isolates (4, 9).

Isolate CFTR differed from most penicillin-resistant isolates in these properties also. Isolate CFTR had the same PBP electrophoretic profile as the susceptible strain, and there appeared to be relatively little, if any, affinity change in PBP 2B (Fig. 3). This binding protein was visualized on the fluorograms even at low concentrations (10 ng/ml) of [$^3$H]penicillin. This is in contrast to the situation with isolates for which penicillin MICs were comparable, in which PBP 2B could not be detected even by 500 ng of radioactive penicillin per ml. The most striking PBP alteration in CFTR was the reduction in the penicillin-binding capacity of PBP 1B, the detection of which required 500 ng of [$^3$H]penicillin per ml in strain CFTR, while this PBP was already saturated at 10 ng of penicillin per ml in the susceptible strain R36A (Fig. 3).

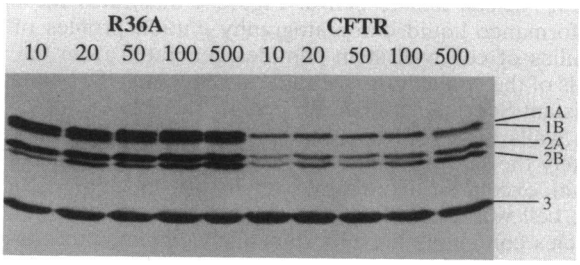


FIG. 3. PBP electrophoretic profiles and penicillin-binding capacities of the PBPs from strain CFTR and the penicillin-susceptible pneumococcal strain R36A. Numbers above the lanes in the fluorograms indicate the concentrations (nanograms per milliliter) of [$^3$H]penicillin used in the assay. PBPs were assayed by the in vivo labeling technique as described in reference 4.

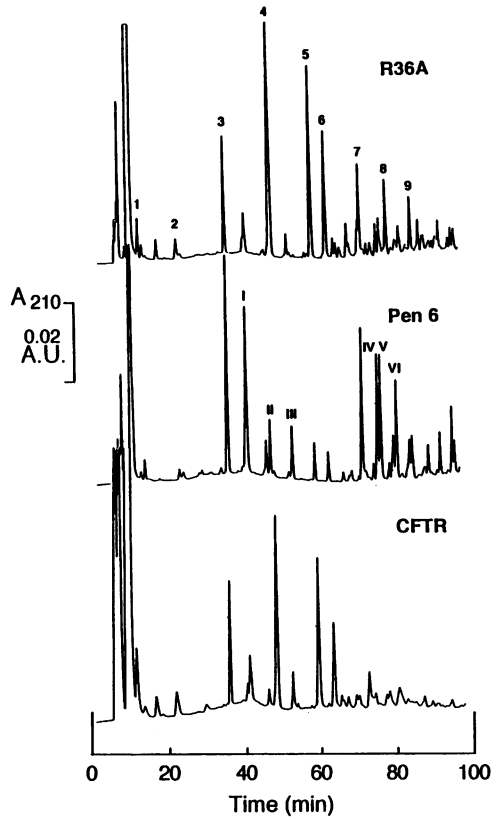


FIG. 4. Muropeptide compositions of the cell walls of the beta-lactam-resistant strains CFTR and pen 6 (South Africa) and the penicillin-susceptible strain R36A. Cell wall muropeptide patterns were identified by reversed-phase high-performance liquid chromatography. Arabic numbers indicate peptides characteristic of penicillin-susceptible pneumococci, while roman numerals refer to the branched peptides found in penicillin-resistant strains. For chemical structures, see reference 3. A.U., absorbance units.

**Cell wall muropeptides.** Penicillin-resistant pneumococcal clinical isolates produce abnormal cell walls with unusual muropeptide compositions (3).

The muropeptide composition of the cell walls of CFTR was unique in that it resembled the pattern typical of penicillin-susceptible pneumococci. This peptide pattern differs sharply from the more dramatically altered peptide patterns seen in other penicillin-resistant isolates, e.g., the South African isolate pen 6. Figure 4 illustrates the high-performance liquid chromatography elution profiles of the families of cell wall stem peptides generated from the cell walls of the penicillin-susceptible strain R36A, the penicillin-resistant South African strain pen 6, and isolate CFTR. The cell walls of CFTR contain the same peptides in approximately the same proportion as those seen in the susceptible strain, except for the relative scarcity of peptides 7, 8, and 9. The cell wall of CFTR did not contain any of the peptide species containing the abnormal alanyl-alanine cross bridge (see peaks labeled with roman numerals in Fig. 4) often seen in other beta-lactam-resistant pneumococcal isolates, such as pen 6 (3).

Biochemical, genetic, and epidemiological data suggest that penicillin-resistant clinical pneumococcal isolates had multiple origins, giving rise to distinct PBP families or clonally related isolates that differ from one another in PBP patterns, serotypes, DNA sequences of the high-molecular-weight PBP genes, and several other properties as well, and these clones may have a uniquely high incidence in some geographic areas (2, 7, 9). Our observations suggest that the California isolate CFTR may represent yet another unique clone of beta-lactam-resistant pneumococci. The strain appears unusual compared with other penicillin-resistant isolates with regard to several properties: it has an inversion of the relative MICs of penicillin compared with those of expanded-spectrum cephalosporins; unlike most penicillin-resistant isolates, CFTR lyses quickly with penicillin; CFTR contains PBP 1B, which has drastically reduced beta-lactam affinity, and PBP 2B, which has a relatively small decrease in penicillin-binding capacity; and CFTR also has a cell wall peptide pattern which is atypical for a beta-lactam-resistant isolate.

Epidemiological data suggest that in many areas of the world with high incidences of beta-lactam-resistant pneumococci, the selective agent was primarily benzylpenicillin (2, 5, 7, 11). The clinical history of isolate CFTR raises the possibility that the unique properties of this strain may be attributable to a direct selection by the cephalosporin used in the prolonged therapy of the infant from whom this isolate originates.

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