In Vivo Penicillin MIC Drift to Extremely High Resistance in Serotype 14 *Streptococcus pneumoniae* Persistently Colonizing the Nasopharynx of an Infant with Chronic Suppurative Lung Disease: a Case Study

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Received 5 December 2001/Returned for modification 29 January 2002/Accepted 5 August 2002

This is the first report of in vivo pneumococcal penicillin MIC drift from 4.0 to 16.0 mg/liter, possibly associated with alterations in the *pbp1a* gene. The case presented here is of an infant with early onset recurrent pneumonia and chronic bronchitis requiring repeated antibiotics.

The relationship between antibiotic use, the proliferation and spread of antibiotic-resistant pneumococci, and the rate of de novo emergence of resistance is poorly understood. Australia's level of pneumococcal penicillin resistance increased from 1% in 1989 to 25% in 1997 (3). More judicious antibiotic use is currently advocated in most developed countries, following similar trends worldwide. Young Australian Aboriginal children in disadvantaged communities demonstrate high cumulative incidence rates of severe bacterial respiratory disease, including chronic suppurative otitis media (10 to 40%) (A. J. Leach, P. S. Morris, G. McCallum, C. Wigger, and the PROMPT and PRIORiTI Investigators, Int. Symp. Pneumococci Pneumococcal Dis., abstr. PO8 47A, p. 94, 2002), chronic suppurative lung disease (1 to 5%), and invasive pneumococcal disease (1 to 4%) (7, 18). Repeated courses of antibiotics are often required in our region (10). In such children, the relationship between antibiotic use and absolute rates of disease and disease outcomes is not known.

In April 1996 a randomized placebo-controlled triple-blind trial of long-term (up to 6 months) amoxicillin treatment (50 mg/kg/day) for prevention of acute otitis media with perforation and chronic suppurative otitis media commenced in a remote Aboriginal community. The study was unblinded in August 2001. Enrolled infants were examined (by P. S. Morris), and nasopharyngeal swabs were collected monthly (8). Routine culture methods were used. This case study is of a child enrolled at 82 days of age and randomized at 193 days of age.

Pneumococcal isolates were serotyped with antisera from the Statens Serum Institut, Copenhagen, Denmark. The MICs were determined by the E-test (AB Biodisk). For PCR studies, DNA was prepared using a slight modification of a method described elsewhere (12). Enterobacterial repetitive intergenic consensus (ERIC) PCR fingerprinting (19) and *pbp1a* and *pbp2b* gene fingerprinting were performed as described previously (4, 5).

Recurrent bronchiolitis and pneumonia delayed randomization (to the placebo group) of this child until 193 days of age. Assessments occurred at ages 95, 129, 163, 193, and 219 days, with collection of nasopharyngeal swabs. Between 17 and 193 days of age the child also made 27 clinic presentations, was admitted to the hospital four times with lower respiratory tract infections, and was prescribed 88 days of β-lactam antibiotics. Compliance with oral antibiotics was estimated as being a minimum of 23%. At age 219 days, the child was diagnosed with chronic suppurative lung disease and commenced long-term antibiotic therapy. Bronchitis and bilateral otitis media with effusion have persisted.

At age 163 days the nasopharyngeal swab culture showed heavy growth of serotype 14 pneumococci with penicillin MICs of 16 mg/liter and ceftriaxone MIC of 0.2 mg/liter. Dual pneumococcal serotypes 6B (E-test penicillin MIC of 0.5 mg/liter and ceftriaxone MIC of 0.2 mg/liter) and 14 (E-test penicillin MIC of 4 mg/liter and ceftriaxone MIC of 3.0 mg/liter). At ages 193 days and 219 days, swab cultures showed a heavy growth of serotype 14 pneumococci with penicillin MICs of 16 mg/liter and ceftriaxone MICs of 4.0 and 6.0 mg/liter, respectively. The high MIC and the uniqueness of this isolate in Australia was confirmed by Jan Bell of the Australian Group on Antimicrobial Resistance. ERIC typing patterns for the three serotype 14 isolates with penicillin MICs of 4, 16, and 16 mg/liter were identical but unique in this population (five ERIC types were allocated to the 25 serotype 14 isolates recovered from children in the same community between April 1996 and October 1997). Restriction fragment length polymorphism analysis revealed identical *pbp2b* patterns for the three isolates; however, the two high-MIC isolates had identical *pbp1a* patterns that were different from the *pbp1a* profile of the lower-MIC isolate. The *HaeIII* cut of the 2.45-kb PCR product for the isolate with a MIC of 16 mg/liter produced bands at 1.3, 0.6, and 0.4 kb, whereas *HaeIII* repeatedly failed to cut *pbp1a* of the isolate for which the MIC was 4 mg/liter.

Subsequent nasopharyngeal swabs at ages 394 and 452 days both contained multidrug-resistant serotype 6B with a penicillin MIC of 2.0 mg/liter. To date, high-level penicillin-resistant serotype 14 pneumococci have not been detected in other infants or children living in this community. The child's mother was carrying pneumococci on three occasions, at infant ages 129 days, 163 days, and 193 days; all isolates tested were fully susceptible to penicillin.

Reports of pneumococci with penicillin MICs of 16 mg/liter or more are extremely rare. To our knowledge this is the first...
such isolate to be reported in Australia and the first report of in vivo drift from a 4 mg/liter MIC to 16 mg/liter to be described in the medical literature (2, 13). The ordered multistep process of penicillin resistance development seems to begin with an alteration of pbp2x, followed by pbp2b and then pbp1a (14). Mechanisms other than penicillin-binding protein alterations may be involved (15, 16). We now show that in vivo the final step towards the highest-level MIC may be associated with further alterations to pbp1a.

The relative contributions by clonal (organisms) or horizontal (genes) mechanisms to the spread of resistance are unknown. For pneumococci, clonal spread has been described globally (11, 17) and locally within this child’s community and beyond (1, 9). In the case presented, a highly resistant clone colonized in almost pure culture. However, subsequent spread in the community was not detected either in the short or long term, despite extensive antibiotic use within the community. The failure of this highly resistant clone to spread may indicate that organisms with extremely high MICs, probably mediated by low-affinity PBPIA, are not stable in natural populations. Whether the antibiotics used directly influenced these in vivo genetic events is not known. It may be that in children with a high bacterial load, such as those living in disadvantaged populations where infections commence early in life, the rate of transformation is greater, regardless of antibiotic selective pressure. The dilemma facing health practitioners in these areas is what constitutes appropriate antibiotic use in this era of increasing antibiotic resistance (6). More longitudinal data are needed to improve our understanding of how antibiotic-resistant strains arise and spread and of the clinical implications of policies that aim to reduce antibiotic use, particularly in populations with high rates of bacterial infection.

We thank the family involved, the Tiwi Health Board, and the NHMRC.

REFERENCES