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In Vivo Emergence of High-Level Macrolide Resistance in *Streptococcus pneumoniae* following a Single Dose of Azithromycin

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Resistance to macrolides in the pneumococcus is generally by virtue of an efflux pump (encoded by the *mefA* or *mefE* gene) or the presence of a ribosomal methylase (encoded by the *ermB* or, rarely, the *ermA* gene). Horizontal spread of these genes can occur through inter- and intraspecies recombination (2). Exposure of the pneumococcus to macrolides can also lead to the spontaneous generation of resistant mutants in vivo and in vitro (9); mutations in the 23S rRNA and ribosomal proteins L4 and L22 have been described (12). A recent randomized controlled study clearly demonstrated the effect of macrolide use on selection of resistant strains of pharyngeal streptococci (7).

The extent of macrolide use in remote indigenous communities in Australia is not well documented. Mass azithromycin treatment campaigns for trachoma eradication have been linked to selection of macrolide-resistant pneumococcal strains in the nasopharynx (5, 6) and conjunctiva (3). However, in populations where macrolide resistance was rare among pneumococci, selection for resistant strains following azithromycin administration for trachoma control was not evident (1).

As a part of a pneumococcal carriage study of young children in remote indigenous communities, we report nasopharyngeal carriage of serial serotype 22F pneumococcal isolates in a 2.5-month-old indigenous infant. The 22F isolate developed resistance to azithromycin (while remaining sensitive to penicillin, tetracycline, chloramphenicol, and cotrimoxazole) after the infant received a single dose of azithromycin (125 mg) as routine prophylaxis following a trachoma contact. As shown in Table 1, the pre- and posttreatment isolates had identical BOX typing (13) patterns and multilocus sequence types (4). The *mefA*/*E* and *ermB* genes and mutations in the ribosomal protein L4 and L22 genes (8, 10–12) were not found in the isolates. However, the previously described 23S rRNA A2059G mutation (*Escherichia coli* numbering) (12) was detected in the posttreatment isolate.

Each subsequent monthly nasal swab from the child over the following 8 months cultured macrolide-susceptible isolates of serotype 16F or presumptive nonencapsulated pneumococci. Five percent of the pneumococcal carriage isolates in the study were macrolide resistant (erythromycin MIC, >2 μg/ml). However, the resistant 22F clone was not detected again despite intensive surveillance in that community.

The A2059G mutation in pneumococcal passaged mutants was previously shown to decrease susceptibility to macrolides; changes at two alleles were associated with an increase in the azithromycin MIC from 0.02 μg/ml to >200 μg/ml (12). This mutation is also believed to slow the replication rate (12) and would potentially provide a fitness cost.

In another case of de novo development of resistance, a serotype 3 pneumococcus developed resistance to erythromycin, azithromycin, and quinupristin-dalfopristin (MIC, 2 to 4 μg/ml) during azithromycin treatment for pneumococcal pneumonia, with a fatal outcome. The mechanism of resistance was reported to be a mutation in ribosomal protein L22 (9).

Our findings and those of others have important implications for practice. Strains highly resistant to azithromycin can arise de novo in a previously sensitive strain, independently and in the absence of azithromycin resistance in the population. Although this may be an unusual occurrence, an important consideration during empirical azithromycin treatment for a pneumococcal infection is that clinical failure may occur even when the risk of selecting a preexisting azithromycin-resistant strain is low.

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TABLE 1. Analysis of serial serotype 22F pneumococcal isolates from nasal swabs collected from an indigenous child given a single dose of azithromycin

Date[a]	Serotype	Azithromycin MIC (μg/ml)	BOX type	MLST[b]	*ermB*	*mefA/E*	23S rRNA	L4	L22
4 Dec 2001	22F	0.5	A	698	Neg[c]	Neg	2059A	Neg	Neg
3 Jan 2002	22F	>256	A	698	Neg	Neg	2059G	Neg	Neg
29 Jan 2002	22F	>256	A	698	Neg	Neg	ND[d]	ND	ND

[a] A single 125-mg dose of azithromycin was given on 6 December 2001.
[b] MLST, multilocus sequence type.
[c] Neg, negative.
[d] ND, not done.

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