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Pneumolysin Detection Identifies Atypical Isolates of *Streptococcus pneumoniae*

We read with interest the report of Cima-Cabal and colleagues, suggesting that pneumolysin detection be used as a diagnostic test for the identification of *Streptococcus pneumoniae* (2).

We recently screened 278 expectorated sputum samples, using a selective medium (7), for the presence of alpha-hemolytic streptococci showing any degree of sensitivity to optochin. Eighty-five alpha-hemolytic streptococcal isolates were recovered, of which 69 were totally resistant to optochin. The remaining 16 isolates had inhibition zones ranging from 10 to 20 mm in diameter. Eleven of these isolates had typical pneumococcal colonial morphology (optochin inhibition zones: range, 14 to 20 mm; mean, 16.6 mm), whereas five isolates had small dry colonies more in keeping with nonpneumococcal strains (optochin inhibition zones: range, 10 to 15 mm; mean, 12.6 mm). All 16 isolates agglutinated with a latex test for pneumococcal polysaccharide antigen (SLIDEX; BioMerieux, Marcy L'Etoile, France) and those with typical colonial appearances were tube bile soluble, suggesting that they were isolates of *S. pneumoniae*, but the five colonially atypical isolates were bile insoluble.

We therefore examined further these five isolates (i) by a real-time PCR method for the pneumolysin gene which included a hybridization probe for amplicon recognition (4), (ii) by demonstrating the expression of pneumolysin in the lysed organisms using a monoclonal antibody to a recombinant pneumolysin in a Western blot technique (8), and (iii) by a commercial 16S RNA gene probe (AccuProbe; Gene-Probe Inc., San Diego, Calif.). The results are seen in Table 1.

The same tests were applied to 8 of the 11 typical, bile-soluble, *S. pneumoniae* isolates, with uniformly positive results for all isolates in all tests.

The most common strategy for the identification of putative strains of *S. pneumoniae* is screening for optochin sensitivity (zone size > 14 mm) (6). Isolates with intermediate zone sizes (7 to 13 mm) require a confirmatory test, most commonly latex agglutination (LA) for polysaccharide antigen. However, LA tests can cross-react with nonpneumococcal streptococci (3), and the unusual colonial appearances of our isolates led us to use bile solubility, but with unexpectedly negative results. Given the 16S RNA probe identification of these isolates as *S. pneumoniae*, our isolates accord most closely to a group of 10 strains described in the paper by Mundy et al. (6). However, our demonstration of the presence of the pneumolysin gene in these isolates and our detection of pneumolysin expression also confirmed the identity of these atypical isolates. Although we did not test for pneumolysin in the same way as Cima-Cabal et al., our results strongly support their proposition that pneumolysin-mediated agglutination will be a rapid and reliable new method for the identification of *S. pneumoniae*, including atypical strains.

TABLE 1. Results of confirmatory tests on five isolates of *S. pneumoniae* with atypical colonial appearances

Isolate no.	Optochin zone diam (mm)	Bile solubility	Result using:		
			PCR	Pneumolysin Western blot	16S RNA probe
1	15	−[a]	+[b]	+	+
2	12	−	+	+	+
3	14	−	+	+	+
4	10	−	+	+	+
5	12	−	+	+	+

[a] −, negative.
[b] +, positive.

We have no explanation for the emergence of these atypical isolates, although atypical strains of *S. pneumoniae* are well recognized, including ones with diminished optochin sensitivity (5), and may emerge within a given area (1). Recent work by Whatmore and Dowson (submitted), however, clearly identifies a group of organisms that are genetically distinct though closely related to typical capsulate pneumococci, among which are isolates which fall into this atypical category.

In addition to the obvious implications for individual patients, the introduction of new conjugate vaccines against *S. pneumoniae* underscores the need for all isolates of this important pathogen to be swiftly and securely identified. Hence, new rapid techniques are welcome.

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