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Potential Nonpneumococcal Confounding of PCR-Based Determination of Serotype in Carriage

Monitoring pneumococcal carriage serotype distributions is increasingly used to study pneumococcal biology, disease epidemiology, and vaccine impact. Potentially complicating DNA-based carriage assessment is the well-documented history of genetic recombination between pneumococci and related species that colonize the upper respiratory tract (4).

Recently, when assessing pneumococcal carriage in western Kenya (1) with a multiplex PCR (mPCR) assay for 40 capsular serotypes or serogroups (2), we noted a high frequency of mPCR positivity for cocarried serotypes. This was especially evident in adults who had naso- and oropharyngeal swab specimens combined; from these, 122 of 158 (77%) exhibited 4 or more mPCR serotypes (1). These included 24 of 27 specimens that were negative for the pneumococcus-specific *lytA* PCR assay (3; unpublished data). The mPCR amplicons sg10F/10C/33C and sg18A/18B/18C/18F occurred among 59 to 80% of adults (1). Sequencing of sg10F/10C/33C amplicons from 11 carriage specimens revealed five 192-bp sg10F/10C/33C sequence types, including three from *lytA*-negative specimens, which shared 90.1 to 93.8% identity to corresponding pneumococcal reference 10F, 33C, and 35B sequences (Table 1). Analysis of sg18A/18B/18C/18F amplicons from 17 specimens (including three *lytA*-negative specimens) revealed five sequence types with 89 to 93% identity to the 4 serogroup 18 reference sequences (Table 2). In contrast, sg10F/10C/33C and sg18A/18B/18C/18F mPCR amplicons from carriage specimens that yielded corresponding serotype 18A, 18C, or 10F isolates revealed sequence identity in each instance with the corresponding pneumococcal reference sequence (Tables 1 and 2).

Within a reference collection of 54 strains comprised of 16 nonpneumococcal species, we found 5 mPCR-positive results for sg10F/10C/33C within strains of *Streptococcus oralis* (2 of 3), *Streptococcus infantis* (1 of 1), *Streptococcus gordonii* (1 of 5), and *Streptococcus salivarius* (1 of 3). Strains of *Streptococcus mitis* (4), *Streptococcus pseudopneumoniae* (21), *Streptococcus parasanguinis* (3), *Streptococcus sanguinis* (3), *Streptococcus cristatus* (3), *Streptococcus vestibularis* (2), *Streptococcus peroris*, *Streptococcus australis*, *Streptococcus intestinalis*, *Streptococcus oligofermentans*, *Streptococcus sinensis*, and *Dolosigranulum pigrum* tested negative. Sequence comparisons of the 5 amplicons revealed 87.0 to 95.8% identity to corresponding 10F, 33C, and 35B reference sequences (Table 1). We found that the amplicon from both *S. oralis* reference strains shared sequence identity with an *S. oralis* polysaccharide biosynthetic locus (5) and also with the amplicon

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TABLE 1 Sequence identities (percent) for 192-bp *wzx* gene sequences from nonpneumococcal species and from carriage specimens that coamplify with targeted PCR assay for pneumococcal sg10F/10C[h]

	% identity										
Sequence	Spn 10F[a]	Spn 33C[b]	Spn 35B[c]	*S. infantis* SS1641[d]	*S. gordonii* SS1245[d]	*S. salivarius* SS1061[d]	*S. oralis* SS911, SS1236, and 32[e,f]	Specimen 49[f]	Specimen 248[f]	Specimen 265[f]	Specimen 300[f]
Spn 10F[a]		88.0	87.0	87.0	92.7	92.2	92.7	92.7	93.2	92.7	93.2
Spn 33C[b]			90.1	90.6	94.3	93.2	95.3	93.2	93.8	93.2	93.2
Spn 35B[c]				95.8	89.1	90.6	89.1	90.1	90.6	91.1	91.1
S. infantis SS1641[d]					90.6	89.6	89.6	89.6	90.1	90.6	90.6
S. gordonii SS1245[d]						97.4	99.0	97.9	98.4	97.9	97.9
S. salivarius SS1061[d]							97.4	97.4	97.9	97.4	96.4
32[f]								97.9	98.4	97.9	97.9
49[f]									99.5	99.0	97.9
248[f]										99.5	98.4
265[f]											97.9
83[g]	100										

[a] Only available pneumococcal serotype 10F reference *wzx* sequence (GenBank accession no. CR931652), from which mPCR assay primers derived (192 bp after subtracting mPCR primer sequences) (2), which is also identical to the serotype 10C reference mPCR amplicon sequence (GenBank accession no. CR931651) .
[b] The corresponding pneumococcal serotype 33C *wzx* sequence (192 bp, from GenBank accession no. cr931700).
[c] The corresponding pneumococcal serotype 35B *wzx* sequence (192 bp, from GenBank accession no. cr931705).
[d] These sequences are available at the CDC *Streptococcus* Lab website at http://www.cdc.gov/ncidod/biotech/strep/pcr.htm (see PCR deduction protocols, point 3).
[e] These 3 amplicons shared sequence identity and were also identical within their overlap with GenBank submission AB289547 describing the *S. oralis* surface polysaccharide synthetic gene cluster. All included amplicons were of the exact same length of 248 bp.
[f] Sequences corresponding to amplicons representative of multiple carriage specimens PCR positive for sg10F/10C. Specimens either were *lytA* negative or were positive for confirmed pneumococcal isolates of nonrelated serotypes. Sequences corresponding to specimens 49, 248, and 300 are available at the CDC *Streptococcus* Lab website. Specimens 32 and 265 are identical within their overlaps with recent *S. oralis* GenBank submissions AB289547 and FR720602, respectively.
[g] Sequence corresponds to amplicon from the single carriage specimen that yielded a serotype 10F pneumococcal isolate. Specimen was mPCR positive for 10F/10C and was *lytA* positive.
[h] Shading indicates corresponding pneumococcal reference sequences for serotypes 10F, 33C, and 35B. Spn, *S. pneumoniae*.

TABLE 2 Sequence identities (percent) for suspected nonpneumococcal 507-bp *wzy* gene sequences from carriage specimens that coamplify with targeted PCR assay for pneumococcal sg18A/18B/18C/18F with pneumococcal references 18A, 18C, and 18F[e]

Sequence(s)	% identity						
	18A[a]	18C[a]	18F[a]	257[b]	262[b]	269[b]	368[b]
18A		97.6	97.8	89.5	90.5	92.1	90.7
18C			99.0	89.3	90.7	91.9	90.9
18F				90.3	90.9	92.5	91.1
257					92.1	92.7	91.9
262						96.3	99.4
269							96.4
8, 33, 103, 323[c]	100						
117, 369, 395[d]		100					

[a] The indicated 507-bp reference amplicon sequences (after subtracting primer sequences [2]) are within GenBank accessions CR931671 (18A), CR931673 (18C), and CR931674 (18F). The 18C and 18B reference amplicons share the identical 507-bp sequence. All amplicons in the table are of the same length (573 bp).

[b] Sequence corresponds to mPCR amplicon for sg18A/18B/18C/18F from indicated carriage specimen. Specimens either were *lytA* negative or were positive for confirmed pneumococcal isolates of non-sg18 serotypes. These sequences are available in GenBank as JX104734 (specimen 257), JX105735 (specimen 262), JX105736 (specimen 269), and JX105737 (specimen 368).

[c] Sequence corresponding to amplicons representative of carriage specimens PCR positive for sg18A/18B/18C/18F. Specimens were *lytA* positive and yielded serotype 18A pneumococcal isolates.

[d] Sequences corresponding to amplicons representative of carriage specimens PCR positive for sg18A/18B/18C/18F. Specimens were *lytA* positive and yielded serotype 18C pneumococcal isolates.

[e] Shading indicates pneumococcal references 18A, 18C, and 18F.

obtained from *lytA*-negative specimen 32. We did not find positive mPCR results for sg18A/18B/18C/18F within these reference strains; however, it is unlikely that this collection provided adequate representation of commensal streptococcal species diversity.

In summary, mPCR amplicon sequences for sg10F/10C/33C and sg18A/18B/18C/18F within *lytA*-negative specimens were divergent compared to the reference pneumococcal serotype sequences (Tables 1 and 2). From within the same population, multiple specimens that yielded isolates that expressed the serotypes in question invariably revealed amplicons (from the specimens and isolates) with sequence identity to the corresponding 10F, 18A, and 18C references. We found divergent homologs of a pneumococcal capsular biosynthetic locus within a small nonpneumococcal species collection. We also encountered similar issues in this population with additional vaccine serotypes besides 18C (2, 5, 7F, and 33F) that are vaccine components (unpublished data). Until further clarification, PCR-based serotyping for carriage studies should be employed upon identified pneumococcal isolates only.

This preliminary letter describes our concern. There has been an unforeseen delay in the availability of these carriage specimens to us for isolation and characterization of potentially nonpneumococcal confounding species. While aware that this letter represents an incomplete study, we feel that further delay in conveying this information is unacceptable.

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