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How Valid Is Single-Colony Isolation for Surveillance of *Streptococcus pneumoniae* Carriage?▽

Surveillance of nasopharyngeal pneumococcal carriage strains is frequently used to approximate the prevalent phenotypes causing invasive disease and has an important role in the design of multivalent vaccines (11). Although multiple strains can colonize simultaneously (4, 5, 7–10), most surveillance studies characterize only single colonies—but how valid is this information (12)? Nasopharyngeal swabs capture more pneumococci than throat swabs (1, 6, 14), but throat swabs can uncover different phenotypes (13, 16). Our carriage studies (submitted for publication) characterizing multiple colonies from nose and throat samples revealed that Tanzanian children were colonized by a mean of three different serogroups or -types (SGT) and two different antibiograms for penicillin and cotrimoxazole. Thus, is picking a single colony a valid method for determining SGT and antibiotic susceptibility in community surveillance?

Nose and throat swab samples were obtained monthly for 6 months from Tanzanian children (mean age, 3.3 years) living on a sugar plantation with no recent history of antibiotic treatment. Up to 10 pneumococcal colonies were characterized from each nose and throat pneumococcal selective plate (2). Penicillin and cotrimoxazole susceptibilities were determined using CLSI guidelines (3). The SGT present in the 23-valent pneumococcal vaccine were identified using standard methodology (15). The nontypeable colonies were excluded from this analysis. The colony from which the SGT and antibiotic susceptibility data were included was selected using a random number generator (http://www.randomizer.org/form.htm) from the entire multiple-colony data at each time point. The average and coefficient of variance (CV) were calculated from five independent selections. Single-colony data were compared with multiple-colony data and between matched nose and throat data.

The community prevalence of SGT calculated from single-colony data for common SGT was similar to that for multiple colonies and to that for paired nose and throat colonies (Table 1) except for SGT 3, 4, 5, and 15, which were isolated only from nose swabs, and SGT 17, which was significantly ($P = 0.0018$) underrepresented in throat samples (Table 1). The average sampling variability was 28.4% CV for the five most prevalent (≥5%) SGT and was more reliable than for the rare (<5%) SGT (112.3% CV). Nose swabs captured 76% and throat swabs captured only 37.2% of all the SGT captured. Importantly, the same SGT were captured from both sites in only 13.2% of occasions. The summary antibiotic susceptibility data from single colonies reflected reliably the multiple-colony data (penicillin, 15.3 ± 8.3% CV; cotrimoxazole, 8.0 ± 1.9% CV), with no differences observed between nose and throat colonies.

Our data indicate that when seeking average prevalences of common SGT or antibiotic susceptibility, single-colony methodologies are valid. To capture all of the circulating SGT or to accurately map multiple colonization events, both nose and throat swabs should be used. We propose, therefore, that to study the diversity of pneumococcal colonization in children, multiple-colony methodology should be applied.

TABLE 1. Community prevalences of SGT as recorded from single, multiple, nose, or throat colonies[a]

SGT	Prevalence (%) in:				
	Single colony[b]		Multiple colonies	Nose colonies	Throat colonies
	Mean	SD			
3	0.0	0.0	0.2	0.0	0.0
4	1.0	1.0	2.0	3.0	0.0
5	0.0	1.0	0.0	1.0	0.0
6	13.0	3.0	11.0	14.0	10.0
7	2.0	1.0	3.0	3.0	4.0
9	5.0	3.0	5.0	4.0	5.0
10	3.0	1.0	3.0	4.0	2.0
11	5.0	2.0	5.0	1.0	4.0
12	0.0	1.0	0.0	0.0	0.0
14	3.0	1.0	1.0	1.0	1.0
15	1.0	1.0	1.0	1.0	0.0
17	10.0	2.0	10.0	15.0	1.0*
18	3.0	2.0	2.0	1.0	5.0
19	4.0	2.0	4.0	6.0	3.0
20	3.0	1.0	2.0	2.0	4.0
22	2.0	2.0	2.0	0.0	4.0
23	1.0	1.0	2.0	2.0	5.0

[a] The nose colony data were compared with matched throat colony data captured from the same colonization event. *, $P = 0.001$ (Fisher's exact test).
[b] Single-colony means and SDs for each SGT are from five independent selections.

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