Serotyping of Pneumococcus from Nasopharyngeal STGG Specimens for the PERCH Study using culture-based and direct microarray techniques

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INTRODUCTION

- Conventional pneumococcal serotyping techniques (Quellung, PCR and Latex agglutination) rely on the culture of a pneumococcal isolate. In the Pneumonia Etiology Research for Child Health study, a proportion of cases and controls had nasopharyngeal (NP) samples that were pneumococcal culture-negative, but PCR-positive. We evaluated culture-based and direct microarray serotyping as an alternative to conventional techniques for serotyping pneumococcus, and of particular use for culture-negative, PCR-positive infections

METHODS

- **PERCH**: a case-control study in seven countries: Bangladesh, The Gambia, Kenya, Mali, South Africa, Thailand and Zambia.
- **Cases** were children aged 1-59 months hospitalized with WHO defined-severe or very severe pneumonia; **controls** were frequency matched by age and season and recruited from the community.
- **NP Specimens:** were collected from cases and controls using a flocked swab (flexible minitip, Copan®), stored in STGG and cultured for pneumococcus using a broth-enrichment step (1,2). NP flocked and rayon OP swabs (Fisher Scientific®) were combined and stored in UTM (Copan®) for quantitative pneumococcal PCR (Fast-track Diagnostics, Sliema, Malta).
- **Conventional Serotyping:** was done on all isolates using either Quellung alone or PCR deduction (3) followed by Quellung resolution.
- **Culture-based Microarray Serotyping** 25uL STGG was plated onto selective media (COBA). If culture had alpha-haemolytic growth it was swept from the dilution plate with heaviest non-confluent distinct colonies and DNA was extracted for microarray analysis (4)
- **Direct Microarray Serotyping**: DNA was extracted from 200uL STGG using enrichment for bacterial DNA plus whole genome amplification (5).
- **Testing Approach:** We first validated the culture-based and direct microarray methods by testing a subset of 45 culture-positive/PCR-positive STGG specimens by both methods and comparing to conventional serotyping results. We then tested 158 STGG culture-negative/ PCR-positive specimens, using culture microarray if colonies grew on COBA and otherwise using direct microarray.

RESULTS

- Pneumococcus was detected in the NP by PCR, but not culture, in approximately 23% of all PERCH cases and 12% of controls (**Table1**). Negative culture results were associated with antibiotic pretreatment prior to specimen collection (44% antibiotic pretreatment in culture(-)/PCR(+) specimens vs. 15% in culture(+)/PCR(+) specimens, p<0.001).
- Of the 45 culture(+)/PCR(+) STGG samples tested, direct microarray detected pneumococcus in 37 (82%), whereas culture-based microarray detected pneumococcus in all 45 (100%). In samples positive by both culture and direct microarray, serotypes were concordant, though for samples with multiple serotypes detected on microarray, the relative abundance (proportion) of each serotype was not always equivalent. Direct microarray serotypes matched the reference Quellung serotype in 34 instances (76% of 45, 91% of 37). Culture-based microarray serotypes matched the Quellung serotype in 43/45 (95%) instances **(Table 2)**.
- 114/158 (72%) culture(-)/PCR(+) specimens were positive on COBA culture, 96 of which were pneumococcus and serotyped by microarray, and the remainder were related species. Of the 44 negative by COBA, direct microarray identified a serotype in 5 (11%). **(Table 2)**
- In total, a pneumococcal serotype was identified for 101/158 (64%) of culture(-)/ PCR(+) specimens. Multiple serotypes were detected in 34/146 (21.2%) of all STGG specimens with pneumococcus detected by microarray (**Table 2**).
- 3/45 (7%) of culture(+) and 29/158 (18%) culture(-) specimens contained a closely-related non-pneumococcal *Streptococcus* species that may contain both *lytA* and *cps* gene homologues.

CONCLUSIONS

- Comparing Quellung serotype results to microarray results for culture(+) STGG samples validated both culture-based and direct microarray approaches for serotyping, although culture-based microarray was more sensitive for the detection of pneumococcus and multiple pneumococcal serotypes.
- Using culture-based and direct microarray yielded a serotype in 101/158 (64%) of PCR positive specimens which would have otherwise not had a serotype identified. This may be in part due to the increased pneumococcal yield for STGG plated on to COBA rather than broth enrichment.
- The relatively higher proportion of non-pneumococcal closely related species in culture(-)/PCR(+) vs. culture(+)/PCR(+) specimens may indicate non-specificity of our PCR assay for identifying *Streptococcus pneumoniae.*

TABLE 1

Detection of Pneumococcus by Culture and PCR

	Culture(-)/ PCR(-)	Culture(+)/ PCR(+)	Culture(-)/ PCR(+)	Culture(+)/ PCR(-)	Culture(-)/ PCR(+) >6.9 log(10) copies/ml
Cases (n=4,049)					
n(%)	977 (23.9)	2,003 (48.9)	946 (23.1)	168 (0.4)	59 (0.1)
Mean PCR density (log(10) copies/ml) (SD)	-	6.1 (1.1)	4.9 (1.3)	-	7.3 (0.3)
Cases, excluding antibiotic pretreated (n=1,998)					
n(%)	433 (21.7)	1,207 (60.4)	279 (14.0)	79 (4.0)	20 (1.0)
Mean PCR density (log(10) copies/ml) (SD)	-	6.2 (1.1)	4.8 (1.4)	-	7.3 (0.3)
Controls (n=5,144)					
n(%)	955 (18.6)	3,309 (64.3)	630 (12.2)	250 (4.9)	28 (0.1)
Mean PCR density (log(10) copies/ml) (SD)	-	5.8 (1.0)	4.8 (1.3)	-	7.2 (0.2)

TABLE 2

Culture-based and Direct Microarray Evaluation

	Culture(+)/PCR(+) n=45	Culture(-)/PCR(+) n=158
Pneumococcus positive by culture-based microarray	45/45 (100%)	96/158 (61%)
Pneumococcus positive by direct microarray	37/45 (82%)	5/44[1] (11%)
Samples with multiple serotypes identified	14/45 (31%)	20/101 (20%)
Proportion of culture-based microarray results with matching[2] Quellung result	43/45 (95%)	n/a
Proportion of direct microarray results with matching[2] Quellung result	34/45 (76%)	n/a

[1]Of those COBA negative
[2]For those with >1 serotype detected by microarray, at least one of the serotypes matched the Quellung result

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