Detection and prediction of *Streptococcus pneumoniae* serotypes directly from nasopharyngeal swabs using PCR

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Monitoring *Streptococcus pneumoniae* serotype distribution is important to assess the impact and effectiveness of pneumococcal vaccine programs. With the challenges of Quellung serotyping, PCR-based serotype prediction is increasingly being used for large-scale epidemiological studies. This study used real-time (RT)-PCR targeting the genes encoding autolysin (*lytA*) and capsular biosynthesis gene A (*cpsA*) of *S. pneumoniae* in nucleic acids extracted directly from nasopharyngeal (NP) swabs submitted for viral studies. If the specimen was *lytA* or *cpsA* PCR-positive, then serotype prediction was performed on the same nucleic acid using eight conventional multiplex PCRs (cmPCRs) and seven real-time multiplex PCRs (rmPCRs). Of 1770 NP swabs, 132 (7.5%) were *lytA*-positive and 122 (6.9%) were positive for both targets (*lytA* and *cpsA*). Of the 122 *lytA* and *cpsA* positive specimens, a serotype could be assigned in 52 (41.8%) using cmPCR alone and the yield was increased to 70 (57.4%) with the addition of rmPCR. Based on sensitivity, incremental yield and more efficient workflow, an algorithm was proposed where *lytA* and *cpsA* RT-PCR screening was followed by serotype deduction using rmPCR and a modified set of four instead of eight cmPCRs. This algorithm was validated for use on NP swabs, and the distribution of *S. pneumoniae* serotypes deduced from this approach showed good concordance with those obtained with cultured isolates serotyped by Quellung and PCR. Overall, molecular detection and serotyping of *S. pneumoniae* from NP swabs was found to be a valuable tool to assess *S. pneumoniae* colonization and monitor trends in serotype distribution.

INTRODUCTION

*Streptococcus pneumoniae* (or pneumococcus) is a bacterium that colonizes the nasopharynx of humans (Gray et al., 1980; Bogaert et al., 2004; Pasinato et al., 2014). Whilst colonization is often asymptomatic, infections do occur, manifesting as community-acquired pneumonia (CAP) and infections of normally sterile sites (e.g. pleural fluid, cerebrospinal fluid and blood), which are collectively called invasive pneumococcal disease (IPD). CAP and IPD are major causes of morbidity and mortality, and pose a significant burden on our healthcare system (O’Brien et al., 2009; File & Marrie, 2010; Bonten et al., 2015).

To date, 94 different *S. pneumoniae* serotypes have been identified and pneumococcal vaccines have been developed against the most predominant serotypes causing IPD (Huss et al., 2009). Whilst vaccination has had success in reducing the burden of disease caused by vaccine-type *S. pneumoniae* (Huss et al., 2009; Bonten et al., 2015), ongoing surveillance is important to monitor trends in serotype distribution as the benefits of vaccination could be offset by increased rates of pneumococcal disease caused by non-vaccine serotypes (Hicks et al., 2007; Weinberger et al., 2011; Demczuk et al., 2013; Zuccotti et al., 2014). Recently, the 13-valent pneumococcal conjugate vaccine was shown to reduce CAP and IPD caused by vaccine-type pneumococci in healthy adults, prompting a renewed interest in
monitoring *S. pneumoniae* serotype distribution in Canadian adult populations (Bonten et al., 2015).

Establishment of the true burden of pneumococcal disease can be challenging as there is no gold standard for the diagnosis of *S. pneumoniae* (Blaschke, 2011). CAP and IPD surveillance often rely on a number of laboratory tests, including bacterial culture and antigen detection (Facklam & Washington, 1991; Spellerberg & Brandt, 2007; Harris et al., 2014). Increasingly, molecular methods such as PCR are being used for the detection of *S. pneumoniae* as they are highly sensitive compared with culture, can be applied to various specimen types and are not predicated on the growth of viable bacteria (Carvalho et al., 2010; Resti et al., 2010; Kim et al., 2012; Cvitkovic Spik et al., 2013; Grijalva et al., 2014; Pernica et al., 2014; Strálin et al., 2014). To assess *S. pneumoniae* serotype distribution, serotyping methods using Quellung reactions or latex agglutination are often used, but these require viable pneumococci and large panels of antisera (Austrian, 1976; Lovgren et al., 1998; Siira et al., 2012; Ortika et al., 2013). Molecular methods to deduce *S. pneumoniae* serotypes, such as conventional multiplex PCRs (cmPCR) and real-time multiplex PCRs (rmPCR), have been shown to be a more practical alternative for large epidemiological studies, and have been applied to bacterial isolates and clinical specimens (Pai et al., 2006; Dias et al., 2007, Morais et al., 2007; Azzari et al., 2008; Lafourcade et al., 2010; Jourdain et al., 2011; Pimenta et al., 2013; Richter et al., 2013; Shakrin et al., 2013). This study evaluated the feasibility of PCR-based detection and serotyping of pneumococci directly in nasopharyngeal (NP) swabs routinely collected for viral studies to add to the repertoire of non-culture methods used for pneumococcal surveillance.

**METHODS**

**Clinical specimens.** The Serious Outcomes Surveillance (SOS) Network of the Canadian Immunization Research Network (CIRN) is a national hospital-based surveillance program established in 2009 to conduct active surveillance for influenza virus, and subsequently for CAP and IPD (McNeil et al., 2014). In the nine participating hospitals, daily admissions were screened by trained study staff for patients admitted with radiographically confirmed CAP or IPD. Sputum and blood were collected from consenting patients, and submitted for bacterial culture and susceptibility testing according to standard laboratory techniques (Facklam & Washington, 1991; Spellerberg & Brandt, 2007), and NP swabs were collected in universal transport media (UTM) (Copan Diagnostics) to allow viral studies and *S. pneumoniae* PCR detection and serotyping. This report details results of specimens collected between 1 December 2010 and 31 December 2012.

**Bacterial culture.** Streptococci isolated from sputum or sterile sites, such as blood or parapneumonic fluids, were cultured at 35 °C under 5% CO₂ on trypticase soy agar plates with 5% sheep blood (Becton Dickinson). Bacterial growth was harvested from overnight cultures and suspended in PBS to a McFarland value of ~2.0. Ten-fold serial dilutions were plated for c.f.u. enumeration and 200 µl of each dilution was subjected to a nucleic acid extraction. Pneumococcal isolates were also characterized by Quellung serotyping at the National Microbiology Laboratory in Winnipeg, Manitoba, Canada. Serotyping was performed by Quellung reaction using commercial pool, group, type and factor antisera (SSI Diagnostica) (Austrian, 1976; Lovgren et al., 1998).

**Nucleic acid extraction.** Nucleic acids were isolated from 200 µl bacterial suspensions or specimens using a Roche MagNA Pure Total Nucleic Acid Isolation kit on a MagnaPure LC instrument, as recommended by the manufacturer. Elution was performed in a volume of 100 µl, and 2.5 µl served as template for *lytA* and *cpsA* real-time (RT)-PCR and 5 µl for cmPCR and rmPCR.

**Detection and serotype deduction using RT-PCR.** RT-PCRs for *lytA* or *cpsA* were carried out using a TaqMan Universal PCR Master Mix kit (Life Technologies) in 25 µl reactions consisting of: 1 × Master Mix, 200 nM primers (*LytA*-F and *LytA*-R or *CpsA*-F and *CpsA*-R), and 200 nM probe (*LytA*-Fb or *CpsA*-Fb) (Table S1, available in the online Supplementary Material). For serotyping, rmPCRs were performed using seven triple reactions as described previously (Pimenta et al., 2013). Each rmPCR was performed in 25 µl reactions consisting of: 1 × Platinum Quantitative PCR SuperMix-UDG (Life Technologies), 50 nM MgCl₂, and primer and probe concentrations and combinations listed in Tables S1 and S2, respectively. Amplifications were performed on an ABI 7500 Fast instrument (Life Technologies): 95 °C for 10 min and 45 cycles of 95 °C for 15 s and 60 °C for 60 s. Threshold cycle (*Cₜ*) values were determined using software provided by the manufacturer. Primers and probes were obtained from Integrated DNA Technologies.

**Serotype deduction using cmPCR.** The cmPCRs were performed in 25 µl volumes that consisted of 1 × enzyme mix from a Multiplex PCR kit (Qiagen), and primers at concentrations and combinations as listed in Tables S1 and S2, respectively. For the modified cmPCR assay (cmPCRmod), different primer combinations were used to account for the serotypes not detectable by rmPCR (Table S1, Fig. 1). When a serotype was detected by cmPCR or cmPCRmod, PCRs were repeated using individual primer pairs to avoid misidentification due to non-specific amplification (Table S3). All reactions contained a primer pair (cmCpsA-F and cmCpsA-R) targeting the *cpsA* gene as an internal control (Table S1). Amplification was performed in 96-well plates using a C1000 thermocycler (Bio-Rad): 95 °C for 90 s, 35 cycles of 95 °C for 30 s, 54 °C for 90 s and 72 °C for 60 s, followed by 72 °C for 10 min. Amplicons were resolved by 1.2% (w/v) agarose gel electrophoresis with 10 µg ethidium bromide ml⁻¹ staining and visualized using a Bio-Rad GelDoc XR+ with ImageLab software (version 5.1). Expected sizes (bp) for PCR products are denoted in Table S2 and molecular markers correspond to the 100 bp ladder (Invitrogen).

**Analytical specificity and sensitivity.** Each PCR method was tested against various *S. pneumoniae* and non-pneumococcal isolates (Table S4). The limit of detection (LoD) for each assay was estimated using Probit analysis (Finney, 1971) at a confidence of 95% using 10-fold serial dilutions in PBS of various *S. pneumoniae* (Table 1). Each dilution was subjected to nucleic acid extraction prior to PCR detection and serotyping, and an aliquot was plated for c.f.u. enumeration. Results are presented as the mean ± SD of triplicate values obtained in five independent experiments. For serotypes identifiable by rmPCR (Table S2), *Cₜ* values were correlated with *Cₜ* values obtained by *lytA* RT-PCR, and comparisons were made with end-point titre for cmPCR and cmPCRmod (Table 1). For serotypes identifiable only by cmPCR or cmPCRmod, *lytA* *Cₜ* values were also used to compare the detection end point (Table 1).

**Quantification of *S. pneumoniae* using *lytA* genome equivalents.** The *lytA* target sequence was amplified from *S. pneumoniae* (ATCC 49619) using primers *LytA*-F and *LytA*-R (Table S1). The

http://jmm.sgmjournals.org
resulting amplicon was purified using a QIAquick gel extraction kit (Qiagen) and ligated into the pGEM-T Easy vector (Promega), as recommended by the manufacturer. Following transformation into *Escherichia coli* TOP10 (Life Technologies), transformants were screened by PCR. Plasmid was extracted from 1 ml overnight cultures in Luria–Bertani medium using a QIAprep Spin Miniprep kit (Qiagen) as recommended by the manufacturer, including the RNase A step. Plasmid concentration was quantified by measuring the $A_{260}$ using a NanoVue Plus spectrophotometer (GE Healthcare). Ten-fold serial dilutions of the plasmid performed in triplicate were subjected to *lytA* RT-PCR. An inverse linear relationship ($y = -3.237x + 47.52; R^2 = 0.9977$) was generated by plotting the mean $\pm$ SD $C_t$ against the plasmid concentration (log copies ml$^{-1}$). Pneumococci in bacterial dilutions or clinical specimens were expressed as copies ml$^{-1}$ by converting $C_t$ values obtained with *lytA* RT-PCR to the standard curve generated with the plasmid.

### RESULTS

#### Analytical specificity

As previously demonstrated for this target, the *lytA* RT-PCR was specific and detected only *S. pneumoniae* isolates (Table S4) (Carvalho *et al.*, 2010; Resti *et al.*, 2010; Kim *et al.*, 2012; Cvitkovic Spik *et al.*, 2013; Grijalva *et al.*, 2014; Pernica *et al.*, 2014; Straitin *et al.*, 2014). As for *cpsA* RT-PCR, the results showed it was no less specific than *lytA*. For detection and serotyping assays, no cross-reactivity occurred between serotypes or with non-pneumococcal streptococci (Table S4). In some cases, non-specific amplifications were observed in cmPCR and cmPCRmod reactions that could potentially lead to serotype misidentification (Table S3); therefore, each positive cmPCR or cmPCRmod result was confirmed by repeating with individual primer pairs for the predicted serotype (Table S1). No serotype misidentification occurred using this strategy or with rmPCRs.

#### Analytical sensitivity and inter-assay comparisons

Using Probit analysis for *S. pneumoniae* serotype 19F, the LoD for the *lytA* and *cpsA* RT-PCRs was 556 [95% confidence interval (CI): 400–762] and 948 (95% CI: 690–1284) copies ml$^{-1}$, respectively. The typing methods were less sensitive at 4833 (95% CI 3650–6318) copies ml$^{-1}$ for rmPCR and
Table 1. Correlation between lytA RT-PCR, rmPCR and cmPCR

<table>
<thead>
<tr>
<th>rmPCR</th>
<th>Serotype</th>
<th>Cₜ value ranges (sd)</th>
<th>Equation of lytA versus rmPCR plot (y = mx + b, R²)</th>
<th>Estimated fold increase in sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>lytA</td>
<td>rmPCR</td>
<td>lytA versus cmPCR</td>
</tr>
<tr>
<td>1</td>
<td>19A</td>
<td>19.48–38.66 (0.06–0.22)</td>
<td>17.43–36.50 (0.03–0.73)</td>
<td>y = 0.98x + 1.33; 0.9988</td>
</tr>
<tr>
<td>3</td>
<td>20.66–37.34 (0.02–0.26)</td>
<td>17.60–34.58 (0.19–0.78)</td>
<td>y = 1.00x–2.60; 0.9974</td>
<td>Equivalent</td>
</tr>
<tr>
<td>7F</td>
<td>27.11–37.65 (0.01–0.08)</td>
<td>25.07–35.88 (0.04–0.11)</td>
<td>y = 1.03x–2.90; 0.9998</td>
<td>Equivalent</td>
</tr>
<tr>
<td>2</td>
<td>23.40–37.25 (0.07–0.26)</td>
<td>20.28–36.49 (0.28–0.90)</td>
<td>y = 1.00x–2.60; 0.9915</td>
<td>Equivalent</td>
</tr>
<tr>
<td>6C</td>
<td>27.53–37.87 (0.09–0.20)</td>
<td>27.88–38.15 (0.15–1.73)</td>
<td>y = 0.97x + 1.49; 0.9932</td>
<td>Equivalent</td>
</tr>
<tr>
<td>12F</td>
<td>19.71–35.49 (0.02–0.40)</td>
<td>18.48–36.94 (0.03–2.03)</td>
<td>y = 1.12x–3.17; 0.9955</td>
<td>Equivalent</td>
</tr>
<tr>
<td>3</td>
<td>18.36–34.83 (0.09–0.36)</td>
<td>15.91–32.85 (0.05–0.17)</td>
<td>y = 1.01x–2.42; 0.9989</td>
<td>Equivalent</td>
</tr>
<tr>
<td>33F</td>
<td>18.31–34.64 (0.08–0.33)</td>
<td>18.93–37.88 (0.08–1.49)</td>
<td>y = 1.14x–2.19; 0.9963</td>
<td>10</td>
</tr>
<tr>
<td>23A</td>
<td>20.49–33.76 (0.01–0.12)</td>
<td>19.36–32.72 (0.06–0.39)</td>
<td>y = 1.00x–0.64; 0.9915</td>
<td>Equivalent</td>
</tr>
<tr>
<td>4</td>
<td>21.39–36.33 (0.03–0.68)</td>
<td>19.70–37.68 (0.18–0.71)</td>
<td>y = 1.15x–5.20; 0.9916</td>
<td>Equivalent</td>
</tr>
<tr>
<td>16F</td>
<td>26.46–36.93 (0.09–0.20)</td>
<td>23.08–31.76 (0.35–0.66)</td>
<td>y = 0.84x + 1.19; 0.9839</td>
<td>Equivalent</td>
</tr>
<tr>
<td>11A</td>
<td>20.40–32.00 (0.18–0.30)</td>
<td>19.00–33.09 (0.04–0.66)</td>
<td>y = 1.15x–3.86; 0.9907</td>
<td>Equivalent</td>
</tr>
<tr>
<td>5</td>
<td>23.50–31.73 (0.05–0.18)</td>
<td>19.09–33.11 (0.36–1.40)</td>
<td>y = 1.20x–8.55; 0.9936</td>
<td>10</td>
</tr>
<tr>
<td>9V</td>
<td>26.53–36.74 (0.05–0.88)</td>
<td>23.45–36.35 (0.08–1.38)</td>
<td>y = 1.31x–12.06; 0.9817</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>21.98–35.01 (0.01–0.51)</td>
<td>20.40–36.71 (0.42–1.10)</td>
<td>y = 1.21x–6.71; 0.9811</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>18.60–36.78 (0.03–0.84)</td>
<td>17.29–34.62 (0.01–0.28)</td>
<td>y = 0.97x–0.67; 0.9980</td>
<td>Equivalent</td>
</tr>
<tr>
<td>14</td>
<td>19.55–36.87 (0.06–0.56)</td>
<td>17.55–35.66 (0.56–1.36)</td>
<td>y = 1.05x–2.77; 0.9974</td>
<td>Equivalent</td>
</tr>
<tr>
<td>18C</td>
<td>20.22–37.48 (0.06–0.21)</td>
<td>17.34–35.50 (0.08–0.69)</td>
<td>y = 1.019x–2.38; 0.9931</td>
<td>Equivalent</td>
</tr>
<tr>
<td>19F</td>
<td>22.61–36.61 (0.02–0.40)</td>
<td>14.87–29.05 (0.13–0.42)</td>
<td>y = 1.01x–7.38; 0.9915</td>
<td>Equivalent</td>
</tr>
<tr>
<td>7</td>
<td>17.56–30.39 (0.06–0.42)</td>
<td>18.93–34.69 (0.05–1.22)</td>
<td>y = 1.28x–3.73; 0.9949</td>
<td>100</td>
</tr>
<tr>
<td>23F</td>
<td>20.43–34.01 (0.05–0.12)</td>
<td>16.33–30.56 (0.35–0.49)</td>
<td>y = 0.10x–4.9955</td>
<td>10</td>
</tr>
</tbody>
</table>

9568 (95% CI: 7345–12 321) copies ml⁻¹ for cmPCR. No differences were seen between cmPCR and cmPCRmod. For other serotypes, a similar trend was observed where the lytA screening assay was either equivalent or ∼10-fold more sensitive than rmPCR and cmPCR was approximately 10- to 100-fold more sensitive than cmPCR (Table 1). For serotypes only detected by cmPCR (Table S2), lytA screening was also more sensitive than cmPCR at values of ∼10-fold for serotypes 9N, 10A, 13, 15C, 21, 34 and 35A or ∼100-fold more sensitive for serotypes 10F, 20, 23B, 31, 17F, 35F and 39 (data not shown). For serotypes 2, 4 and 23F, no differences were seen between rmPCR and cmPCR.

**Serotyping of S. pneumoniae isolates**

Of the 87 S. pneumoniae isolates characterized by molecular typing, all were positive using cmPCR alone or a combination of rmPCR and cmPCRmod (Figs 2 and 3). The serotypes deduced by the PCR methods were concordant to those serotyped by Quellung. The distribution of S. pneumoniae serotypes showed a predominance of serotypes 19A, 7F and 3, and a smaller number of various serotypes (Fig. 3).

**Detection and typing from NP swabs**

Of the 1770 NP swabs processed by lytA RT-PCR (Table 2), 132 (7.5%) were positive for S. pneumoniae, 122 of which could be confirmed by cpsA RT-PCR. There was good correlation for Cₜ values between the two targets, and the lytA CT values for the 10 cpsA-negative specimens were all below the LoD and were thus considered indeterminate (Table 2). Of the 122 lytA- and cpsA-positive specimens, 52 (42.6%) were non-typable and a serotype could be assigned in 70 (57.4%) using a combination of rmPCR and cmPCR (Table 2). All serotypes predicted by cmPCR were also confirmed by cmPCRmod and individual primer pairs. Of the 71 typable results, 35 were concordant between rmPCR and cmPCR (or cmPCRmod), which were represented serotypes detectable by both methods. However, the cmPCR (and cmPCRmod) reactions detected the presence of serotypes in 16 samples that were negative when tested using rmPCR. These serotypes were not detectable in the rmPCRs: serotypes 8 (3), 9N/L, 10F, 15B/C, 23B (5), 22F/A, 34 (2), 7F/A, 11A/D, 12F, 15A/F, 16F, 19A (2), 19F, 22F/A (2) and 23A (2).

**DISCUSSION**

Ongoing surveillance for CAP and IPD and S. pneumoniae serotype distribution is crucial to evaluate the impact of new vaccine programs and help guide future vaccine formulations. With many laboratories and networks...
performing influenza virus surveillance, this study demonstrated the proof-of-principle that archived NP swabs in UTM collected for respiratory virus diagnostics could also be used for serotyping of *S. pneumoniae* in specimens that were screened positive for pneumococci by PCR. Since antibiotic susceptibilities are performed following isolation of *S. pneumoniae*, and other bacteria could be causing CAP, NP swabs testing positive for *S. pneumoniae* does not preclude the use culture for in respiratory specimens for the diagnosis of CAP but provides another tool for pneumococcal surveillance. UTM contains antimicrobial agents that prevent culture of *S. pneumoniae*, but this medium supports the recovery of nucleic acids and thus allows a single specimen to be used for several studies.

Compared with culture, *lytA*-based PCR has previously been shown to be highly sensitive for the detection of *S. pneumoniae* (Carvalho et al., 2010; Resti et al., 2010; Kim et al., 2012; Cvitkovic Spik et al., 2013; Grijalva et al., 2014; Pernica et al., 2014; Strälin et al., 2014). In addition, the *lytA* gene target was shown to be specific for the detection of *S. pneumoniae* (Carvalho et al., 2007, 2013; Abdeldaim et al., 2010; Greve & Møller, 2012; Strälin et al., 2014). In this study, *S. pneumoniae* detection in NP swabs was performed using two pneumococcal-specific RT-PCR targets (*lytA* and *cpsA*) to reduce the possibility of false-positive results with other streptococci found in the respiratory tract. It should be noted that *lytA* Ct values did not differ following a freeze/thaw cycle (Fig. S1), suggesting that screening for pneumococci can be done in batches and DNA can be frozen for subsequent molecular serotyping.

Of the 1770 NP swabs evaluated in this study, 122 (6.9%) were positive for both *lytA* and *cpsA* targets. Of these, a serotype could be assigned in 70 (57.4%), but only 35 serotypes were identified by both cmPCR and rmPCR (Table 2). cmPCR (and cmPCRmod) detected the presence of a serotype in 16 samples that were negative when tested using rmPCRs and, with the exception of one serotype identified as 22F/22A, represented serotypes not targeted in rmPCRs (Table S2). For serotypes detectable by rmPCR, Ct values correlated with a high degree ($R^2 = 0.8367$) with those obtained during *lytA* RT-PCR (Fig. 4b), suggesting that some untypable pneumococci may be attributed to a low bacterial load. In fact, rmPCR detected the presence of a serotype in 19 samples that were negative by cmPCR (and cmPCRmod) (Table 1, Fig. 4a). Magomani *et al.* (2014) recently showed that samples with a high *lytA* Ct value were less likely to be serotyped, and this study demonstrated that differences in

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**Fig. 2.** Algorithm used for the molecular detection and serotyping of *S. pneumoniae* from NP swabs and isolates. Dashed lines indicate processes applicable to only one sample type (NP swab or isolate). *For NP swabs, all PCR-based serotyping assays are performed as co-colonization if possible. Isolates with PCR results that were not typable or sufficiently discriminatory were subjected to Quellung serotyping. NEG, negative; IND, indeterminate; POS, positive.
Table 2. Summary of PCR detection and serotype results obtained from NP swabs

<table>
<thead>
<tr>
<th>Assay</th>
<th>n (%)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Screening assays</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lytA&lt;sup&gt;-&lt;/sup&gt;cpsA&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1638/1770 (92.5)</td>
<td>No pneumococcus detected*</td>
</tr>
<tr>
<td>lytA&lt;sup&gt;+&lt;/sup&gt;cpsA&lt;sup&gt;-&lt;/sup&gt;</td>
<td>122/1770 (6.9)</td>
<td>Pneumococcus detected</td>
</tr>
<tr>
<td>lytA&lt;sup&gt;+&lt;/sup&gt;cpsA&lt;sup&gt;-&lt;/sup&gt;</td>
<td>10/1770 (0.56)</td>
<td>Indeterminate result</td>
</tr>
<tr>
<td><strong>Serotyping assays</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-tybable</td>
<td>52/122 (42.6)</td>
<td>Proportion of positive specimens where a serotype could not be assigned</td>
</tr>
<tr>
<td>Typable, cmPCR and rmPCR</td>
<td>70/122 (57.4)</td>
<td>Proportion of positive specimens where a serotype could be deduced with cmPCR and rmPCR</td>
</tr>
<tr>
<td>Typable, cmPCR*</td>
<td>51/70 (72.8)</td>
<td>Proportion of typable specimens detected by cmPCR</td>
</tr>
<tr>
<td>Typable, rmPCR</td>
<td>54/70 (77.1)</td>
<td>Proportion of typable specimens detected by rmPCR</td>
</tr>
<tr>
<td>Typable, cmPCR only</td>
<td>16/70 (22.9)</td>
<td>Proportion of serotypes only detected by cmPCR; likely reflecting serotypes that are not included in rmPCRs</td>
</tr>
<tr>
<td>Typable, rmPCR only</td>
<td>19/70 (27.1)</td>
<td>Proportion of serotypes only detected by rmPCR; likely attributed to increased sensitivity</td>
</tr>
</tbody>
</table>

*Identical results obtained with cmPCR and cmPCRmod.

Fig. 3. *S. pneumoniae* serotype distribution in respiratory isolates and NP swabs. Serotyping was performed by PCR on NP swabs and isolates were characterized by Quellung serotyping. Detectable serotypes for Quellung and PCRs are depicted in the columns on the left.
respectively. (b) For each positive rmPCR, correlations were
tured isolates of could be argued that cmPCR alone may be sufficient for cul-
mPCR and cmPCRmod was beneficial for NP swabs, it
detected (Fig. 1, Table S2). Whilst the combined use of
reactions reduced the number of cmPCRs required from
cimens were subjected to serotyping using both rmPCR and
serotype coverage of cmPCR, a new testing algorithm was
methods such as sequencing could be performed.

It should be noted that 52 (42.6%) of the S. pneumoniae-
positive NP swabs remained non-typable. Failure to assign
a serotype was likely attributed to lack of serotype coverage
in the PCRs or to the higher sensitivity of the detection
compared with typing methods for some serotypes (Fig. 4a,
Table 1). With the proposed algorithm (Fig. 2), serotypes
targeted by more sensitive rmPCR over those targeted by
cmPCR only might be favoured; however, the extent of this
bias would be identified by the proportion of results with
lower C_{t} values in the screen assays (Fig. 4 and Results).
Further refinement of rmPCR is required to enhance sensi-
tivity and expand their spectrum for the detection and dis-
 crimination of all serotypes. Secondly, this study is limited
from a lack of direct comparison of PCR with swabs used
for S. pneumoniae culture, and as such the clinical sensitivity
and specificity of the detection and serotyping methods
could not be assessed.

Overall, PCR-based detection and serotyping of pneumo-
cocci directly from NP swabs provides a powerful epidemi-
ological tool to assess rates and serotypes distribution of
NP colonization without the requirement for culture
and isolation of the organism. For large surveillance net-
works like the CIRN SOS Network, the ability to use a
shared specimen (and its derived nucleic acid) for
S. pneumoniae and respiratory virus surveillance provides
a cost-effective strategy that could easily be expanded for
the molecular detection of other pathogens and vaccine-
preventable diseases.

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|Fig. 4. Distribution of ltyA C_{t} values. (a) C_{t} values for ltyA
obtained with each positive specimen are illustrated, and separated
by serotype detection method and typable and non-typable S. pneumoniae. For the screening methods, RT-PCR for ltyA and
cpsA have been abbreviated to ltyA qPCR and cpsA qPCR, respectively. (b) For each positive rmPCR, correlations were
made between ltyA and serotype-specific C_{t} values.|

LoD are serotype-specific (Table 1). For specimens with low
S. pneumoniae DNA concentrations, alternative serotyping
methods such as sequencing could be performed.

To take advantage of the enhanced sensitivity of rmPCR and
serotype coverage of cmPCR, a new testing algorithm was
implemented for NP swabs where pneumococci-positive spec-
cimens were subjected to serotyping using both rmPCR and
cmPCRmod reactions (Fig. 2, Table S2). The cmPCRmod
reactions reduced the number of cmPCRs required from
eight to four, without compromising the number of serotypes
detected (Fig. 1, Table S2). Whilst the combined use of
rmPCR and cmPCRmod was beneficial for NP swabs, it
could be argued that cmPCR alone may be sufficient for cul-
tured isolates of S. pneumoniae as higher DNA concentrations
are achievable for specimen processing. Regardless, the mol-
ecular testing algorithm used for NP swabs mirrored trends in serotype distribution obtained with Quellung
serotyping of cultured S. pneumoniae (Fig. 3). In both cases,
there was a predominance of serotypes 19A, 7F/A and 3.
For NP swabs, all cmPCR and rmPCRs should be performed

on pneumococci-positive specimens as co-colonization with
different S. pneumoniae serotypes is possible in the nasopharyn-
(Pasinato et al., 2014; Saha et al., 2015). It is possible that
some co-colonization serotypes may be overlooked with
competition in multiplex, but this hypothesis would require
further testing.

Overall, PCR-based detection and serotyping of pneumo-
cocci directly from NP swabs provides a powerful epidemi-
ological tool to assess rates and serotypes distribution of
NP colonization without the requirement for culture
and isolation of the organism. For large surveillance net-
works like the CIRN SOS Network, the ability to use a
shared specimen (and its derived nucleic acid) for
S. pneumoniae and respiratory virus surveillance provides
a cost-effective strategy that could easily be expanded for
the molecular detection of other pathogens and vaccine-
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REFERENCES


