Rapid urinary antigen test (Binax NOW) for diagnosis of S. pneumoniae in children with upper and lower respiratory tract infections

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Abstract. To compare the prevalence of Streptococcus pneumoniae by rapid urinary test and blood culture in children with respiratory tract infection and healthy children (controls). Pneumococcal antigenuria was detected in 31.5% of CAP, 31.5% of rhinosinusitis cases and 6% of controls. Pneumococcal antigenuria was significantly more frequently detected in both CAP and rhinosinusitis cases than controls (Fishers exact test; CI 95%, p = 0.01). In no cases of non pneumococcal CAP was antigenuria detected. Compared with blood culture, the specificity of the Pneumococcal antigenuria test was 94%. Nasopharyngeal carrier states for S. pneumoniae in healthy control are very low (6%). We recommend the rapid urinary antigen test to conventional cultural methods for early diagnosis of pneumococcal respiratory infection as a basis for starting appropriate treatment. This study help to inform policy making for the mass infant immunization with PCV7 in our country to decrease incidence of invasive pneumococcal disease.

Key words: Streptococcus pneumoniae, rapid immunochromatographic test, acute rhinosinusitis, community-acquired pneumonia, nasopharyngeal carriers.

1. Introduction

Streptococcus pneumoniae is the most common cause of colonizing bacteria of the upper respiratory tract (1, 2). Streptococcus pneumoniae causes various clinical syndromes in patients and its on time and immediate diagnosis and treatment is very important and critical (3,4,5). Nasopharyngeal carriage of pneumococci is common among young children attending out-of-home care with rates of 21-59% in point prevalence estimates and 65% in longitudinal studies (4,5). Streptococcus pneumoniae is the most common cause of bacteremia; community-acquired bacterial pneumonia and acute otitis media; rhinosinusitis in children and adolescents (6-8) With global implementation of immunization with conjugated Haemophilus influenzae type b vaccines, S. pneumoniae infection increased (9-12).

In efficacy trials in the U.S., infant immunization with this vaccine decreased invasive infections by >93% and lobar pneumonias by >73%. Its administration was associated with a 6-7% decrease in otitis media, but greater reduction in complications of otitis media such as tympanostomy tube placement (11).

Most healthy individuals carry various S. pneumoniae serotypes in their upper respiratory tract, with >90% of children 6 mo to 5 yr of age
harboring *S. pneumoniae* in the nasopharynx at some point during that time. A single serotype usually is carried for extended periods (45 days to 6 mo). Carriage does not consistently induce local or systemic immunity sufficient to prevent later reacquisition of the same serotype. (1-3)

Rates of pneumococcal carriage peak during the 1st 2 yr of life and decline gradually thereafter. Carriage rates are highest in institutional settings and during the winter, and rates are lowest in summer. Although pneumococci may be found in the nose or throat of patients with pneumonia they may not be related causally to their disease and therefore nasopharyngeal cultures are not helpful for diagnosis (1-3).

The cause of community-acquired pneumonia in children is often difficult to determine because direct culture of lung tissue is invasive and rarely performed. Using "state-of-the-art" diagnostic testing, a bacterial or viral cause of pneumonia can be identified in 40-80% of children with community-acquired pneumonia (4-8).

Since licensure of the 7 valent pneumococcal conjugate vaccines (PCV7), the prevalence of carriage and infection with vaccine serotypes has declined and a shift to increased carriage or infections with nonvaccine serotypes has occurred (9-12).

The definite diagnosis of pneumococcal infection is established by recovery of *S. pneumoniae* from the site of infection or the blood. The average time to isolation of *S. pneumonia* is 14-15 hr and rarely >24 hr (1-5). Isolation of *S. pneumoniae* from blood is specific but lacks sensitivity, while isolation of *S. pneumoniae* from sputum may represent colonization. PCR test is sensitive but is very expensive and not available in our country. Recently, the rapid BinaxNOW assay has been implemented for detection the *S. pneumonia* in pneumonia cases as an adjunct to culture with a reported positive predictive value 91.3%, and a negative predictive value of 82.6% (13-19).

Bacterial meningitis has become much less common in developed countries since the introduction of universal immunization against *S. pneumoniae* and H. influenzae type b beginning at 2mo of age. Mass immunization of children with *H.influenzae* and pneumococcal vaccines is not used in Iran. *S. pneumonia* is the second most common cause of bacterial meningitis in our children with high morbidity and mortality among unvaccinated children in Iran (20, 24).

The nasopharyngeal carrier state for pneumococci in our country (Iran) is very lower than other developed countries (3 -7%) (20-24) Khotaei et al showed that rapid detection of penicillin-nonsusceptible NP pneumococcal isolates during antibiotic treatment is common (22). Results of 3 studies in Tehran showed the increasing rate of resistant *S.pneumonia* in our country during recent years (20,23,24). This may contribute to the spread of resistant pneumococci.

Bacterial rhinosinusitis is diagnosed in many febrile children. Some of those cases are admitted in our center in cold weather annually. Severe complication of bacterial rhinosinusitis including periorbital and orbital cellulitis, brain abscess, subdural empyema etc are common in our centers (25).

Diagnosis and isolation of this bacteria from blood of patients with bacterial pneumonia, is about 10-30% in most of studies but unfortunately, in our condition, the access to etiology of bacterial pneumonia is very difficult and restricted (23-25). The role of *S. pneumoniae* in bacterial pneumonia in our children is unknown. Definite and rapid diagnosis of bacterial pneumonia is needed in our children. Due to difficulty in diagnosis of *S. pneumoniae* and other common organisms in community-acquired pneumonia (negative blood or other body fluids in pneumonia cases), we decided to evaluate the role of *S. pneumoniae* in bacterial pneumonia and rhinosinusitis in children by rapid urinary antigen detection kit.

Goal of study: To compare the incidence of *S.pneumoniae* by rapid urinary test and blood culture in children with respiratory tract infection and in controls (only by rapid urinary test).

2. Materials and methods

This case control study was done (2006 – 2007) in 3 tertiary care centers in Tehran (Rasul, Mofid, and the Medical Children Hospital). This study was approved by the Ethical Committee in the Ear Nose, Throat & Neck Research Center; and the Research Center of Pediatric Infectious Diseases affiliated by Iran University of Medical Sciences (see the Ethical Considerations in end).

Study subjects included 54 children with CAP, 56 children with acute rhinosinusitis and 50 healthy children who were candidates for elective surgery on the general surgery ward (i.e., appendectomy and herniorrhaphy). All cases and controls were less than 14 years old.

Inclusion criteria for acute rhinosinusitis: All cases had an acute febrile illness with clinical diagnosis based on Guidelines of American Academy of Pediatrics and Subcommittee on Management of Sinusitis confirmed by CT scan (3).
Table 1. Demographic characteristics in cases and controls

<table>
<thead>
<tr>
<th>Cases</th>
<th>Characteristic</th>
<th>Age Range; (Mean ±SD) months</th>
<th>&lt; 5 years old (89%)</th>
<th>Male/female</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP (54)</td>
<td></td>
<td>1-168 (63.5±64)</td>
<td>50</td>
<td>33/21</td>
</tr>
<tr>
<td>Rhinosinusitis(56)</td>
<td></td>
<td>6-36 (27±43)</td>
<td>26 (46.5%)</td>
<td>35/21</td>
</tr>
<tr>
<td>Healthy (50)</td>
<td></td>
<td>-</td>
<td>21(42%)</td>
<td>22/28</td>
</tr>
</tbody>
</table>

Table 2. Season for antigenuria test in cases and controls

<table>
<thead>
<tr>
<th>Cases</th>
<th>Season</th>
<th>Spring (83.3%)</th>
<th>Summer (5.6%)</th>
<th>Winter/autumn (11%)</th>
<th>autumn (0.01)</th>
<th>autumn (0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia (54)</td>
<td></td>
<td>45</td>
<td>3</td>
<td>6</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Rhinosinusitis (56)</td>
<td></td>
<td>11 (19.5%)</td>
<td>8 (16.5%)</td>
<td>35 (64%)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Healthy (50)</td>
<td></td>
<td>14 (28%)</td>
<td>32 (64%)</td>
<td>4 (8%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The diagnostic parameters for bacterial and community-acquired pneumonia (CAP) were based on Nelson JD Guidelines for Community-acquired pneumonia in children (4). Bacterial pneumonia: typically begins suddenly with a shaking chill followed by a high fever, cough, and chest pain. A large pleural effusion, lobar consolidation, and a high fever at the onset of the illness are also suggestive of a bacterial etiology (4).

Inclusion criteria for CAP: All children had an acute febrile illness with clinical features of community-acquired pneumonia (CAP) confirmed by chest X-ray and was neither preexisting nor due to some other known causes defined (Tuberculosis, nosocomial infection, VAP). Exclusion criteria: Patients were excluded from study when pneumonia/or sinusitis was not the principal reason for admission; proved cases with nosocomial organisms.

Controls were excluded if they had abnormal physical exams or respiratory infection during the previous 10 days; or had positive urine culture.

Initially, a questionnaire was completed by an authorized physician for each case and control, followed by a complete clinical exam. Fresh urine samples (2 ml) were collected soon after admission from cases and in controls during routine tests before scheduled surgery respectively. A swab was dipped into the urine sample and then inserted into the test device. Testing was blinded and read at 15 min. Urine samples were tested for pneumococcal antigen using the immunochromatographic test (BinaxNOW Inc., USA) according to the manufacturer's instructions. Conventional urine culture was done upon urine samples in cases and controls.

Blood cultures were collected at the time of admission from cases (not in controls) and processed using the BACTEC Ped Plus medium (Becton Dickenson company) and automated system was used (BioMerieux). S. pneumoniae was identified as gram-positive, lancet-shaped diplococci. Other isolates were identified using standard techniques (4). After definition of other isolated organisms (from blood culture; urine culture; other body fluid in cases); classification of CAP or nosocomial infection was done. We compared the S.pneumoniae antigenuria test between cases and normal children (controls). The rapid S.pneumoniae antigenuria test was compared with the results of blood culture (gold standard) between CAP; HAP and rhinosinusitis cases.

Statistical analysis: All analyses were conducted using SPSS, version 11.5 (China software). The Student’s t test was used to determine significant differences between means for all continuous variables. The significant Chi-square values (CI 95%, p<0.05) were calculated for all categorical. Comparison between qualitative variable and test results was assessed by chi-square (or Fisher exact test if proper). P value <0.05 was considered statistically significant.
Table 3. Antigenuria in cases and controls

<table>
<thead>
<tr>
<th>Antigenuria</th>
<th>Frequency</th>
<th>Percent</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP</td>
<td>17/54</td>
<td>31.5%</td>
<td>0.01(significant)</td>
</tr>
<tr>
<td>Rhinosinusitis</td>
<td>17/54</td>
<td>31.5%</td>
<td>0.01(significant)</td>
</tr>
<tr>
<td>Healthy</td>
<td>3/50</td>
<td>6%</td>
<td></td>
</tr>
</tbody>
</table>

P value <0.05 considered statistically significant.

Table 4. Antigenuria in CAP and HAP cases

<table>
<thead>
<tr>
<th>Pneumonia cases Result</th>
<th>Positive S.pneumoniae in blood culture</th>
<th>Positive non-S.pneumoniae in blood culture</th>
<th>Positive S.pneumoniae Antigenuria</th>
<th>Negative S.pneumoniae Antigenuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP</td>
<td>0/54</td>
<td>4/54</td>
<td>17/54</td>
<td>37/54</td>
</tr>
<tr>
<td>HAP</td>
<td>0/25</td>
<td>15/25</td>
<td>0/25</td>
<td>25/25</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>19</td>
<td>17</td>
<td>62</td>
</tr>
</tbody>
</table>

3. Results

The demographic characteristics of cases and controls showed in tables 1 & 2. Most pneumonia cases (83.3%) were admitted and studied in spring but cases with rhinosinusitis (64%) were diagnosed in cold weather. Antigenuria in cases and controls is shown in Table- 3. All cases with positive antigenuria were observed in younger children (<5 years) (Table- 3).

S.pneumoniae antigenuria test was significantly higher in rhinosinusitis cases than controls (p = 0.01) (Table-3). 1/54 rhinosinusitis cases had positive blood culture for H. influenza. 17/54 (31.5 %) of rhinosinusitis cases had positive results for S.pneumoniae antigenuria (negative blood culture).

S.pneumoniae antigenuria test was significantly higher in pneumonia cases than controls (p = 0.01). Table- 3 Comparing of blood culture and urinary test in cases with pneumonia presents in Table- 4. 19 pneumonia cases with negative blood culture for S.pneumoniae (14 HAP; 4 CAP) had negative antigenuria. 54 children was diagnosed as CAP. Positive blood culture obtained in 4 CAP cases included S.aureus: 2; Streptococcal infection: 2; E coli: 1; S.pneumoniae; not detected in all CAP. 31.5 % (17/54) of CAP cases had positive urinary test for S.pneumoniae (Table- 4) 15 hospital acquired pneumonia (VAP; nosocomial pneumonia) was diagnosed. Positive blood culture in HAP included; Acinetobacter: 7; K. pneumonia: 4; P.aeruginosa : 2; Enterobacter: 2. S.pneumoniae not detected in all. None of HAP cases had positive urinary test for S.pneumoniae (Table- 4).

Due to the low sensitivity of blood culture for S.pneumoniae detection, sensitivity of the BinaxNOW assay was incalculable. 94% specificity was calculated for S.pneumoniae antigenuria test in differentiation of CAP and rhinosinusitis cases from normal children.

4. Discussion

In the present study, pneumococcal antigenuria was observed in 6 % of younger controls (<5 years). These results are similar to 3% in school children reported in Tehran, 8.7% in Mashhad city (East of Iran) (20-22) and 3 % in children in a day care center (20).
Probably, with increasing the age (≥ 5 year), the carrier state in our children will decrease. Antibody production might be the cause for this changes (25). The carrier state for *S. pneumoniae* in Iran is much lower than its rate (21-59%) in young children attending out-of-home care in developed countries (1-3). Most febrile children with rhinosinusitis are treated by antibiotics in our country (25). The high rate of Group G streptococci detected in Iranian studies, due to frequent antibiotic usage is in Iran (20,21); exposure at different ages; different seasonalities could be the reason for this wide difference; (1-5) Most pneumonia cases (83.3%) are admitted and studied in spring but cases with rhinosinusitis (64%) are diagnosed in cold weather.

In the present study, positive antigenuria in both CAP and rhinosinusitis cases was 5 times more than in healthy controls (p = 0.01) and frequently seen in younger children (< 5 years). The incidence of pneumococcal pneumonia (31.5%) is lower than expected (4-5). It is probable that other causes of bacterial pneumonia (*Haemophilus influenzae*, streptococci etc) cause a major proportion of CAP in the lower age range (<6 years) in Iran (22).

In contrast, 31.5% of rhinosinusitis cases had antigenuria; which is very close to 30% *S. pneumoniae* reported in studies of acute sinusitis in children (6-8). Specificity for the pneumococcal antigenuria test is high (94%). We found similar specificity of the assay to the 2 other studies (13-16). Sato *et al* established a sensitivity of 72% and a specificity of 94% (13). Gutiérrez *et al* reported a sensitivity of 70.4% and a specificity of 89.7% (14). Weatherall *et al* concluded the Binax now sensitivity was at least equal to conventional culture methods (16). Many difficulties in conventional bacteriologic methods in our country are present. The BinaxNOW test is easy to perform and interpret, presenting a solution to overcome the difficulties encountered with diagnosis of *S. pneumoniae*. (17) We agree with Ishida *et al* that diagnostic yield of pneumococcal pneumonia increases by using Binax now combined with conventional methods (18).

Bacterial rhino sinusitis and severe complication of bacterial rhinosinusitis are common in our centers (25). This study is the first description for *S. pneumoniae* urinary antigen detection in rhinosinusitis cases.

5. Conclusions

Nasopharyngeal carrier states for *S. pneumoniae* in healthy control are very low (6%). We recommend the rapid urinary antigen test to conventional cultural methods for early diagnosis of pneumococcal respiratory infection as a basis for starting appropriate treatment. This study help to inform policy making for the mass infant immunization with PCV7 in our country to decrease incidence of invasive pneumococcal disease.

Acknowledgments

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References


