original article

Wien Klin Wochenschr (2013) 125:495–500
DOI 10.1007/s00508-013-0405-4

Wiener klinische Wochenschrift
The Central European Journal of Medicine

Pneumococcal urinary antigen positivity in healthy colonized children: is it age dependent?

Zuzana Vančíková · Milan Trojánek · Helena Žemličková · Zuzana Blechová · Jitka Motlová · Jana Matějková · Otakar Nyč · Jerry John · Marek Malý · Vilma Marešová

Received: 23 November 2012 / Accepted: 8 July 2013 / Published online: 9 August 2013

Abstract
Background Pneumococcal urinary antigen test is a valuable tool for diagnosing pneumococcal pneumonia and meningitis in adults. Its use in children is generally not accepted because of nonspecificity at this age. It is frequently positive in asymptomatic nasopharyngeal carriers.

The aim of our study was to assess the age limit from which the test is no longer positive in asymptomatic healthy carriers.

Methods A total of 197 children aged 36–83 months attending 9 day care centers in Prague were enrolled during February and March 2010. Nasopharyngeal swab specimens were collected from each participant and selectively cultivated. The presence of pneumococcal antigen in urine was detected by BinaxNOW® *S. pneumoniae* kit.

Results *Streptococcus pneumoniae* was cultivated in 53.3 % of healthy children with the highest colonization rate (59.3 %) in children aged 48–59 months. The most frequently colonizing serotypes were: 19F, 23F, 3, 19A, 6B and 4. The presence of pneumococcal antigen in urine decreased with age from 39.0 % in 36–47 months to 17.9 % in 72–83 months old ($p = 0.031$). The antigen positivity was serotype-dependent and more frequent in nonvaccinated children.

Conclusion We demonstrated age-dependent linear decrease of pneumococcal antigen excretion into urine in healthy children. The positivity rate of the test in children aged 72–83 months was similar to that referred in healthy adults, irrespective of colonization. To confirm this age limit for use of this test in diagnostics of pneumococcal diseases, further study in school-age children is justified.

Keywords *Streptococcus pneumoniae* · Pneumococcal C-polysaccharide · Diagnostic tests · Pneumonia · Pediatrics

M. Trojánek, MD (✉) · Z. Blechová, MD · J. John, MD · Prof. V. Marešová, MD, PhD
1st Department of Infectious Diseases, 2nd Medical Faculty and Hospital Na Bulovce, Charles University in Prague, Budinova 2, 180 81 Prague, Czech Republic
e-mail: mtrojanek@seznam.cz

Z. Vančíková, MD, PhD
Department of Paediatrics, Hořovice Hospital, NH Hospital, Hořovice, Czech Republic

H. Žemličková, MD, PhD
National Reference Laboratory for Antibiotics, National Institute of Public Health, Prague, Czech Republic

Dr. J. Motlová
National Reference Laboratory for Streptococci and Enterococci, National Institute of Public Health, Prague, Czech Republic

J. Matějková, MD · O. Nyč, MD, PhD
Institute of Medical Microbiology, 2nd Medical Faculty and Motol University Hospital, Charles University in Prague, Prague, Czech Republic

Dr. M. Malý
Centre for Biostatistics, National Institute of Public Health, Prague, Czech Republic

Die Pneumokokken-Antigen-Positivität im Urintesten bei gesunden kolonisierten Kindern – ist diese vom Alter abhängig?

Zusammenfassung
Grundlagen Ein Pneumokokken-Antigen-Urintest ist eine wertvolle Methode für die Diagnostik einer Pneumonie und Meningitis bei Erwachsenen. Die Anwendung der Methode bei Kindern ist üblicherweise nicht akzeptierbar, weil diese im Kinderalter für nicht-spezifisch gehalten wird. Der Test ist oft bei asymptomatischen nasopharyngealen Trägern positiv. Das Ziel unserer Studie war, die Altersgrenze, ab wann der Test in asymptomatischen gesunden Träger negativ ist, festzustellen.

Methodik Insgesamt 197 Kinder im Alter von 36–83 Monaten, die 9 Tages-Betreuungszentren in Prag besuchten, wurden in Februar und März 2010 in die Studie eingeschlossen. Nasopharyngeale Abstriche wurden von jedem Teilnehmer abgenommen und selektiv kultiviert. Für die Entdeckung von Pneumokokken-Antigen im Urin wurde BinaxNOW® *S. pneumoniae* verwendet.

Ergebnisse S. pneumoniae war bei 53,3 % gesunden Kindern kultiviert, die höchste Kolonisationsrate gab es bei Kindern im Alter von 48–59 Monaten. Die am häufigsten kolonisierten Serotypen waren: 19F, 23F, 3, 19A, 6B und 4. Das Vorkommen von einem Pneumokokken-Antigen im Urin sank mit Alter – von 39,0 % in 36–47 Monaten zu 17,9 % in 72–83 Monaten ($p = 0{,}031$). Die Antigen-Positivität war vom Serotyp abhängig und fand öfter bei nicht-vakzinierten Kindern statt.

Schlussfolgerung Wir haben einen altersabhängigen linearen Abfall der Exkretion von Pneumokokken-Antigen in Urin bei gesunden Kindern bestätigt. Die Positivitätsrate des Testes bei Kindern, die 72–83 Monate alt sind, ist der Rate von gesunden Erwachsenen, ohne Zusammenhang mit der Kolonisation, ähnlich. Zur Bestätigung einer Altersgrenze für die Verwendung von diesem Test in der Diagnostik der pneumokokkalen Erkrankungen ist eine weitere Studie mit Kindern im Schulalter nötig.

Schlüsselwörter *Streptococcus pneumoniae* · Pneumokokkal C-Polysacharide · Diagnostisches Testen · Pneumonie · Pädiatrie

Introduction

Streptococcus pneumoniae is a significant cause of morbidity and mortality worldwide, affecting all age groups, especially young children and older adults. It is responsible for a wide range of community-acquired infections, ranging from superficial respiratory tract infections to potentially life-threatening invasive diseases [1]. Precise identification of pneumococcal infections, especially pneumococcal pneumonia is still problematic due to low sensitivity or specificity of routinely used diagnostic methods [2–5].

Antigens of several respiratory pathogens are filtrated into urine during infection and therefore urine could represent a suitable medium for microbiological assays aimed at these pathogens [5]. Specific methods of detection of pneumococcal capsular antigens from urine have been available since 1917 but because of insufficient sensitivity, lack of robustness, and cost, they have been overlooked in clinical practice [5–7].

Recently developed immunochromatographic membrane assay for detection of pneumococcal antigen in urine is based on detection of *S. pneumoniae* C-polysaccharide which is found on the cell wall and is common to all serotypes [8, 9]. The mechanism of excretion into urine in pneumococcal infections is explained by release of antigen fragments from macrophages during immune processing [10, 11]. Sensitivity of this immunochromatographic test in adult bacteremic pneumococcal community-acquired pneumonia (CAP) reached 82–88 % with specificity varying from 83 to 95 % [5, 12–14]. In nonbacteremic CAP of presumptive pneumococcal etiology the sensitivity was lower (27–43.7 %), but the urine antigen detection test still remained a good predictor of those cases [12, 15]. The test was approved for use in diagnosis of pneumonia in adults by Food and Drug Administration (FDA) in 1999 [16, 17]. Important benefit arising from use of this test in adults is improvement of antibiotic prescription in pneumococcal infections, especially in countries with low antibiotic resistance of pneumococci. These could be safely targeted by narrow-spectrum β-lactam treatment [18].

The diagnostic value of this test in children is still being discussed because of its low specificity. Most children are asymptomatic nasopharyngeal carriers of pneumococci [9, 16, 19, 20] and they test positive solely because of colonization. The specificity in diagnosing pneumonia is thus very low at this age.

In this study, we strive to define the age beyond which the test is no longer nonspecifically positive in asymptomatic carriers. The primary aim was to assess the influence of pneumococcal nasopharyngeal colonization and age on the presence of pneumococcal antigen in urine in healthy children. The secondary aim was to assess the serotype prevalence and the rate of antibiotic resistance of colonizing strains.

Patients, materials, and methods

A total of 197 healthy children from 9 day care centers in the Prague region were prospectively included during February and March 2010. Children were eligible to participate if their parents signed informed consent and filled in a data form (age, underlying illnesses, recent respiratory tract infections, and vaccination status). The study protocol and informed consent form were approved by the local Ethical Committee for Multicentric Clinical Trials (Reference number 78/09). Every child was clinically examined by a pediatrician (ZV). Only healthy children without clinical signs of febrile illness or respiratory tract infection were included. Collected data included age (months), date of birth, history of respiratory tract infections, and vaccination status with focus on pneumococcal vaccination (vaccine, number of doses, and date of the last dose). The vaccinated group consisted of children who received all doses of heptavalent pneumococcal conjugated vaccine (PCV7) recommended by the manufacturer according to the age.

Morning urine samples were collected into sterile plastic flasks and were immediately subjected to urinary antigen testing. Urinary antigen testing in this study was performed in accordance with manufacturer's protocol. All assays were interpreted by one person (MT). Nasopharyngeal swab specimens were collected from each participating child by one member of the study team

Table 1 Nasopharyngeal colonization with *Streptococcus pneumoniae* and urinary antigen test results

Age (months)	Number of participants	Nasopharyngeal colonization				Pneumococcal antigen test			
		Positive		Negative		Positive		Negative	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
36–47	41	18	43.9	23	56.1	16	39.0	25	61.0
48–59	59	35	59.3	24	40.7	17	28.8	42	71.2
60–71	69	38	55.1	31	44.9	17	24.6	52	75.4
72–83	28	14	50.0	14	50.0	5	17.9	23	82.1
Total	197	105	53.3	92	46.7	55	27.9	142	72.1

(ZV). Samples were obtained through nostrils using Copan Venturi Transystem® (Amies Transport Swab System). After obtaining the specimens, the swabs were immediately inserted into Amies transport medium without charcoal and transported into the laboratory at ambient temperature to be processed within 4 h of collection. The swab specimens were inoculated on Columbia blood agar with gentamicin (4 mg/l) and incubated at 37 °C for 48 h in 5 % CO2 atmospheres. *S. pneumoniae* was identified by the typical morphology of colonies, alpha-hemolysis, susceptibility to optochin, and bile-solubility testing. Pneumococci were serogrouped/serotyped using Pneumotest-Latex [21]. Specific serotypes were confirmed by the Quellung reaction using type and factor antisera (Statens Seruminstitut, Copenhagen, Denmark) [22]. Susceptibility to penicillin and erythromycin was determined using broth microdilution method of the Clinical and Laboratory Standards Institute [23], for interpretation of minimum inhibitory concentration (MIC) results the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints were used [24].

Statistical calculations of means, standard deviations (SD) for normally distributed data, medians, and interquartile ranges (IQR) for nonnormally distributed quantitative data were performed. The Mann–Whitney nonparametric test was used for comparison of continuous data. Categorical variables were presented as absolute frequencies and percentages and compared using Fisher's exact test. For evaluation of association of antigen test results with age, a logistic regression was used. The linear trend in proportions was assessed using Armitage test for trend. Mantel–Haenszel technique was used to adjust the test results for potential confounder. The significance level of $p<0.05$ was considered. Data were analyzed using Stata, release 9.2 (Stata Corp LP, College Station, USA) and STATISTICA 9.1 software (StatSoft Inc., USA).

Results

A total of 197 children (105 boys and 92 girls) were enrolled in the study. The median age was 59.03 (IQR 49.43–67.45) months. *S. pneumoniae* was cultivated from nasopharyngeal swabs in 105 children, which represents 53.3 % of all participants. The median age of carriers was 59.25 (IQR 49.57–66.84) months, and 58.80 (IQR 46.69–68.25) months in noncarriers ($p=0.644$). The highest proportion of carriers was in the cohort of 48–59-month-old children where the colonization rate was 59.3 % (35/59). The colonization rates in children 60–71 and 72–83 months old remained relatively high, 55.1 % (38/69) and 50.0 % (14/28), respectively (Fig. 1, Table 1).

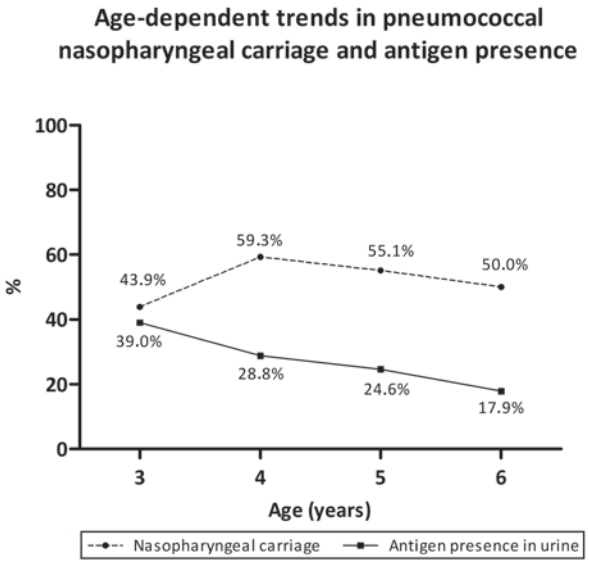

Fig. 1 Pneumococcal nasopharyngeal carriage and pneumococcal antigen presence in urine according to age

The immunochromatographic test was positive in 55/197 (27.9 %) of all included subjects. The positivity was highest among 36–47-month-old children and reached 39.0 % (16/41). The positivity of pneumococcal antigen test diminished with age and in children 72–83 months old was 17.9 %, (5/28) (Fig. 1; Table 1). The presence of a linear decreasing age-dependent trend was confirmed by the Armitage test ($p=0.043$) and remained obvious after adjustment for nasopharyngeal carriage ($p=0.031$). The trend is in contrast to the relatively stable rates of nasopharyngeal carriage ($p=0.636$).

The antigen in urine was detected in 37/105 (35.2 %) of nasopharyngeal carriers of *S. pneumoniae* with a decreasing age-dependent trend presented in Fig. 2; however, the trend was not proven to be statistically significant ($p=0.129$). The test was positive in 19.6 % (18/92) of noncolonized children. The difference of antigen

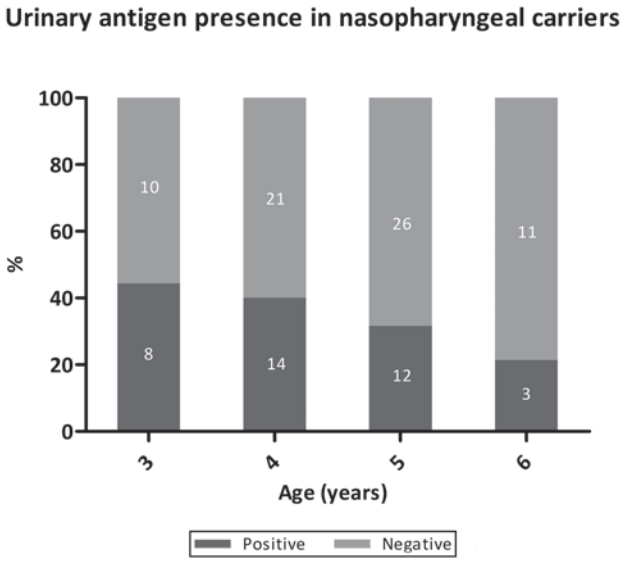

Fig. 2 Presence of pneumococcal urinary antigen in nasopharyngeal carriers of *S. pneumoniae*

Table 2 Pneumococcal serotypes among isolated strains

Serotype	Number of isolates	Percentage
19F	14	13.2
23F	12	11.3
3	12	11.3
19A	10	9.4
6B	9	8.5
4	8	7.5
22F	8	7.5
11A	7	6.6
35F	5	4.7
17F	3	2.8
6A	3	2.8
10A	2	1.9
15B	2	1.9
18C	1	0.9
7F	1	0.9
9V	1	0.9
37	1	0.9
31	1	0.9
1	1	0.9
ND	5	4.7

positivity between the two groups was statistically significant ($p=0.017$).

In the study group there were 35 children fully vaccinated with PCV7 and 162 nonvaccinated. The overall rate of nasopharyngeal colonization was 45.7 % (16/35) in vaccinated and 54.9 % (89/162) in nonvaccinated ($p=0.354$). A total of 19 serotypes were recognized among 101 strains available for serotyping (five isolates were not viable and one child carried two strains of different serotypes). The most frequently isolated serotypes of *S. pneumoniae* are listed in Table 2. The resistance to tested antibiotics was rare, six (5.9 %) isolates of serotypes 19F ($n=5$) and 23F ($n=1$) were intermediary resistant to penicillin (MIC's of penicillin 0.125–2 mg/l) and two (2 %) isolates (serotypes 11A and 19F) were resistant to erythromycin. Serotypes covered by PCV7 (4, 6B, 9V, 14, 18C, 19F, and 23F) represented 37.5 % (6/16) and 43.8 % (39/89) of isolates within vaccinated and nonvaccinated children, respectively ($p=0.786$). In nonvaccinated children serotypes 3, 19F (both 11/89), and 23F (10/89) and in vaccinated children serotypes 19A (4/16) and 19F (3/16) were dominant. The urinary antigen test was positive in 6/35 (17.1 %) vaccinated children (2 colonized and 4 noncolonized), and in 49/162 (30.2 %) nonvaccinated children (35 colonized and 14 noncolonized). The differences did not achieve the levels of statistical significance ($p=0.147$).

The urinary antigen positivity differed among the serotypes and ranged from 14.3 % (1/7) and 16.7 % (2/12) in serotypes 11A and 23F, respectively, to 57.4 % (8/14) and 50.0 % (6/12) in serotypes 19F and 3, respectively; however, the statistically significant difference was not proved ($p=0.331$). For other serotypes see Table 3.

Discussion

We proved that pneumococcal nasopharyngeal colonization in our children was frequent, and the rates remained relatively stable in all age cohorts involved. We observed that the positivity of urinary antigen test decreased significantly with age. Penetration of pneumococcal antigen into urine is not dependent solely on nasopharyngeal carriage but it is significantly influenced by age. In 72–83-month-old children the positivity of this test is low even in colonized children and is similar to that described in adults.

The immunochromatographic test for detection of pneumococcal antigen in urine is established as a sensitive and specific diagnostic tool for pneumonia caused by *S. pneumoniae* in adults [16, 17]. In children, however, the utility of this test is still a matter of discussion. The test is considered to be insufficiently specific, especially due to frequent positivity in nasopharyngeal carriers of *S. pneumoniae* in the upper-respiratory tract [9, 16, 19]. This has been evaluated in various previously published studies originating mostly from developing countries with partially discrepant results. Nevertheless, age limit for the use of this test has not been determined.

Previously published study originating from Beijing [2] compared test results among 88 children (aged 2–60 months) with radiologically confirmed CAP and 198 control subjects with dermatitis or diarrhea, and found that there was no statistically significant difference in test positivity between those two groups (35 vs 34 %, $p=$ not significant (NS)), but the test positivity was strongly associated with pneumococcal colonization (61 vs. 13 %, $p<0.001$ in patients with CAP and 54 vs. 21 %, $p<0.001$ in control group). The study concludes that this test is not useful for detection of pneumococcal CAP in infants and

Table 3 Presence of pneumococcal antigen in urine of carriers of the most frequently isolated serotypes

Serotype	Number of isolates	Presence of pneumococcal antigen in urine		Mean age (months)	SD (months)
		Number	Percentage		
19F	14	8	57.4	55.25	8.46
23F	12	2	16.7	60.25	12.99
3	12	6	50.0	62.11	12.74
19A	10	3	30.0	58.59	11.88
6B	9	3	33.3	59.43	12.35
4	8	4	50.0	62.45	8.52
22F	8	4	50.0	58.85	15.39
11A	7	1	14.3	55.94	9.54

SD standard deviations

children, and the authors consider colonization as the main factor influencing the presence of antigen in urine in children.

In the study from Quito, Ecuador [14] including healthy children aged 2–60 months, the test was more frequently positive in carriers (21.7 %) than in noncarriers (4.2 %, $p < 0.001$). Having taken nasopharyngeal colonization in consideration, the authors found no significant association with age and false-positive results. The authors conclude that the test could be used with caution for diagnosis of pneumococcal pneumonia or bacteremia in young children in populations with low nasopharyngeal carriage, such as in developed countries [14]. Unlike Hamer et al. we included children aged 36–83 months and confirmed that even in developed countries the colonization rate in preschool children is high as it reached 53.3 % and was relatively stable disregard of the age [25–27]. This result is comparable to those reported in other European countries [28, 29] and is slightly higher than in previous Czech study (38.1 % in 425 children) from day care centers [27].

In a Spanish study [30], which included 40 healthy carriers of *S. pneumoniae* aged 2–168 months, a correlation between the age of pneumococcal carriers and false positivity of antigen test was found. The statistically significant difference between the median age of carriers with positive result of the urinary antigen test (29 months) and carriers with negative result (52 months) was demonstrated, $p < 0.001$ [30]. Our findings among 197 children even in narrower age cohort (36–83 months) support these results. Owing to higher number of participating children we were able to assess the pneumococcal antigen positivity rates in different age cohorts separately. We found that the false positivity rate of pneumococcal antigen test linearly decreases with age and varies from 39.0 % in 36–47-month-old to 17.9 % in 72–83-month-old children. The false positivity rate in the last age cohort (72–83-month-old children) is similar to the adult rates [12, 13, 17]. In this cohort, the test was positive in 21.4 and 14.3 % of colonized and noncolonized children, respectively. Positivity of the urine antigen test in children who did not carry pneumococcus at the time of sampling could be explained by either low load of colonizing bacteria which remained undetected by routine cultivation or by persisting urinary antigen excretion after a recent infection [31].

Among children vaccinated by PCV7, the colonization rate differed between nonvaccinated and vaccinated children, and the urinary antigen positivity was more frequent in nonvaccinated children. We have noticed that during colonization with certain serotypes, particularly 19F and 3, the C-polysaccharide penetrates to the urine more frequently than with others (11A, 23F). The reason for this observation remains unclear, but several mechanisms, such as bacterial load, duration of colonization, and immunological response to specific capsular antigens, should be taken into account [32].

In conclusion, we found that in our country the nasopharyngeal colonization rate of day care center attendees, who constitute a majority of preschool children, is relatively high and comparable to developing countries. We also conclude that the penetration of pneumococcal antigen into urine is dependent not only on nasopharyngeal colonization but also on age. In healthy children of 72–83 months old the nonspecific positivity rate is comparable to results from previously published studies in adults.

Pathogen detection in childhood pneumonia is still problematic due to the lack of sensitive and specific methods. Definition of the age limits of pneumococcal urinary antigen test is therefore desirable and could improve the management of these infections and improve antibiotic policy by avoidance of unnecessary broad-spectrum antibiotic treatment, especially in countries with low pneumococcal resistance rates. For further evaluation of the test utility in children, the study should be continued in school-aged children.

Acknowledgments
The first two authors contributed equally to this study. The authors thank Alena Manhartova from Medial Ltd. for donating BinaxNOW® *Streptococcus pneumoniae* kits, Jarmila Dlaskova, MD, and Nadeje Kocnarova, MD, for participation in nasopharyngeal swab taking, and teachers from day care centers, especially Lenka Bartova, Zdenka Dohnalova, Eva Fulinova, Vlasta Gregorova, Dana Hrotkova, Hana Chmelarova, Eliska Janeckova, Hana Kosova, and Eva Libalova.

Conflict of interest
This study was kindly supported by Medial Ltd. by providing the kits for detection of pneumococcal antigen in urine. The authors did not receive any funding or financial assistance from the aforementioned company, neither was the sponsor involved in the design of the study, collection, analysis, interpretation of data, as well as in compilation or in decision to submit the manuscript. The study was partially supported by research grant A/CZ0046/2/0007 from Iceland, Liechtenstein and Norway via the EEA financial mechanism and by research grant IGA 9643–4 from the Internal Grant Agency, Czech Ministry of Health.

References

1. Ortqvist A, Hedlund J, Kalin M. *Streptococcus pneumoniae*: epidemiology, risk factors, and clinical features. Semin Respir Crit Care Med. 2005;26(6):563–74.
2. Dowell SF, Garman RL, Liu G, Levine OS, Yang YH. Evaluation of BinaxNOW, an assay for the detection of pneumococcal antigen in urine samples. performed among pediatric patients. Clin Infect Dis. 2001;32(5):824–5.
3. Fine MJ, Orloff JJ, Rihs JD, Vickers RM, Kominos S, Kapoor WN, et al. Evaluation of housestaff physicians' preparation and interpretation of sputum Gram stains for community-acquired pneumonia. J Gen Intern Med. 1991;6(3):189–98.
4. Hickey RW, Bowman MJ, Smith GA. Utility of blood cultures in pediatric patients found to have pneumonia in the emergency department. Ann Emerg Med. 1996;27(6):721–5.
5. Stralin K, Kaltoft MS, Konradsen HB, Olcen P, Holmberg H. Comparison of two urinary antigen tests for establishment of pneumococcal etiology of adult community-acquired pneumonia. J Clin Microbiol. 2004;42(8):3620–5.
6. Dochez AR, Avery OT. The elaboration of specific soluble substance by Pneumococcus during Growth. J Exp Med. 1917;26(4):477–93.
7. Farrington M, Rubenstein D. Antigen detection in pneumococcal pneumonia. J Infect. 1991;23(2):109–16.
8. Jenning HJ, Lugoeski C, Young NM. Structure of the complex polysaccharide C substance from *Streptococcus pneumoniae* type 1. Biochemistry. 1980;19:4712–9.
9. Marcos MA, Jimenez de Anta MT, de la Bellacasa JP, Gonzalez J, Martinez E, Garcia E, et al. Rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia in adults. Eur Respir J. 2003;21(2):209–14.
10. Coonrod JD. Urine as an antigen reservoir for diagnosis of infectious diseases. Am J Med. 1983;75(1B):85–92.
11. Coonrod JD. Evidence of breakdown of pneumococcal polysaccharides in vivo. Proc Soc Exp Biol Med. 1979;162(2):249–53.
12. Dominguez J, Gali N, Blanco S, Pedroso P, Prat C, Matas L, et al. Detection of *Streptococcus pneumoniae* antigen by a rapid immunochromatographic assay in urine samples. Chest. 2001;119(1):243–9.
13. Yu VLKJ, Plouffe JF, et al. Evaluation of the Binax urinary, Gram stain and sputum culture for *Streptococcus pneumoniae* in patients with community-acquired pneumonia. In: Program abstracts of the 38th Annual Meeting of the Infectious Disease Society of America (New Orleans); 2000.
14. Hamer DH, Egas J, Estrella B, MacLeod WB, Griffiths JK, Sempertegui F. Assessment of the Binax NOW *Streptococcus pneumoniae* urinary antigen test in children with nasopharyngeal pneumococcal carriage. Clin Infect Dis. 2002;34(7):1025–8.
15. Smith MD, Sheppard CL, Hogan A, Harrison TG, Dance DA, Derrington P, et al. Diagnosis of *Streptococcus pneumoniae* infections in adults with bacteremia and community-acquired pneumonia: clinical comparison of pneumococcal PCR and urinary antigen detection. J Clin Microbiol. 2009;47(4):1046–9.
16. Smith MD, Derrington P, Evans R, Creek M, Morris R, Dance DA, et al. Rapid diagnosis of bacteremic pneumococcal infections in adults by using the Binax NOW *Streptococcus pneumoniae* urinary antigen test: a prospective, controlled clinical evaluation. J Clin Microbiol. 2003;41(7):2810–3.
17. Stralin K, Holmberg H. Usefulness of the *Streptococcus pneumoniae* urinary antigen test in the treatment of community-acquired pneumonia. Clin Infect Dis. 2005;41(8):1209–10.
18. Stralin K. Usefulness of aetiological tests for guiding antibiotic therapy in community-acquired pneumonia. Int J Antimicrob Agents. 2008;31(1):3–11.
19. Pesola GR. The urinary antigen test for the diagnosis of pneumococcal pneumonia. Chest. 2001;119(1):9–11.
20. Gray BM, Converse GM 3rd, Dillon HC Jr. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis. 1980;142(6):923–33.
21. Slotved HC, Kaltoft M, Skovsted IC, Kerrn MB, Espersen F. Simple, rapid latex agglutination test for serotyping of pneumococci (Pneumotest-Latex). J Clin Microbiol. 2004;42(6):2518–22.
22. Sorensen UB. Typing of pneumococci by using 12 pooled antisera. J Clin Microbiol. 1993;31(8):2097–100.
23. Performance standards for antimicrobial susceptibility testing; 21st informational supplement. 21st ed. Wayne, Pa.: Clinical and Laboratory Standards Institute; 2011.
24. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters (version 1.3). Volume 2011; 2011.
25. Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. J Antimicrob Chemother. 2002;50(Suppl 2):59–73.
26. Leiberman A, Dagan R, Leibovitz E, Yagupsky P, Fliss DM. The bacteriology of the nasopharynx in childhood. Int J Pediatr Otorhinolaryngol. 1999;49(Suppl 1):151–3.
27. Zemlickova H, Urbaskova P, Adamkova V, Motlova J, Lebedova V, Prochazka B. Characteristics of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* isolated from the nasopharynx of healthy children attending day-care centres in the Czech Republic. Epidemiol Infect. 2006;134(6):1179–87.
28. Dunais B, Pradier C, Carsenti H, Sabah M, Mancini G, Fontas E, et al. Influence of child care on nasopharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae*. Pediatr Infect Dis J. 2003;22(7):589–92.
29. Marchisio P, Claut L, Rognoni A, Esposito S, Passali D, Bellussi L, et al. Differences in nasopharyngeal bacterial flora in children with nonsevere recurrent acute otitis media and chronic otitis media with effusion: implications for management. Pediatr Infect Dis J. 2003;22(3):262–8.
30. Dominguez J, Blanco S, Rodrigo C, Azuara M, Gali N, Mainou A, et al. Usefulness of urinary antigen detection by an immunochromatographic test for diagnosis of pneumococcal pneumonia in children. J Clin Microbiol. 2003;41(5):2161–63.
31. Tateda K, Kusano E, Matsumoto T, Kimura K, Uchida K, Nakata K, et al. Semi-quantitative analysis of *Streptococcus pneumoniae* urinary antigen: kinetics of antigen titers and severity of diseases. Scand J Infect Dis. 2006;38(3):166–71.
32. Sa-Leao R, Nunes S, Brito-Avo A, Alves CR, Carrico JA, Saldanha J, et al. High rates of transmission of and colonization by *Streptococcus pneumoniae* and *Haemophilus influenzae* within a day care center revealed in a longitudinal study. J Clin Microbiol. 2008;46(1):225–34.