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Detection of respiratory syncytial virus & *Mycoplasma pneumoniae* in paediatric lower respiratory tract infections

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***Background & objectives*: Respiratory syncytial virus (RSV) and *Mycoplasma pneumoniae* are considered common cause of lower respiratory tract infections (LRTIs) in children. The present study was conducted to detect *M. pneumoniae* and RSV in paediatric LRTIs employing serology, polymerase chain reaction (PCR) and reverse transcriptase PCR (RT-PCR) analysis.**

***Methods*: Seventy five children aged one month to five years with acute LRTIs were investigated for *M. pneumoniae* antibodies and RSV antigen using immunochromatographic test, RT-PCR for RSV and *M. pneumoniae* by PCR on nasopharyngeal aspirates.**

***Results*: RSV infection was observed in 33 (44%) and *M. pneumoniae* was positive in 26 (35%) children. No significant difference in infection was noted between male and female children. Clinical and radiological features among RSV and *M. pneumoniae* positive and negative cases were similar. Considering RT-PCR for RSV as gold standard, RSV antigen immunochromatography was 90.90 per cent sensitive and 100 per cent specific.**

***Interpretation & conclusions*: Our study showed the presence of RSV and *M. pneumoniae* infection in 44 and 35 per cent children, respectively with community-acquired LRTIs and aged less than five years.**

Key words Children - lower respiratory tract infections - *Mycoplasma pneumoniae* - polymerase chain reaction - RSV - serology

Lower respiratory tract infections (LRTIs) are known for morbidity and mortality among children[1,2]. Viruses are the most common aetiological agents for childhood acute respiratory tract illnesses[3]. Respiratory syncytial virus (RSV) infection is a major cause of serious lower respiratory disease in infancy and early childhood[4]. Diagnosis is not possible on clinical grounds alone[5]. *Mycoplasma pneumoniae* is a common cause of respiratory tract infections in all age groups. Clinical manifestations range from mild cases of tracheobronchitis to severe atypical pneumonia and can be followed by extrapulmonary complications[6]. Serological tests are more sensitive than culture for detection of acute *M. pneumoniae* infection, but polymerase chain reaction (PCR) can detect *M. pneumoniae* earlier than serology with the potential to produce rapid, sensitive and specific results[7].

The aim of this study was to detect RSV by employing chromatographic assay and reverse transcriptase PCR (RT-PCR) and *M. pneumoniae* by

serological tests and PCR analysis in children with community-acquired LRTIs.

Material & Methods

A prospective study was designed to detect RSV and *M. pneumoniae* in 75 consecutive children aged one month to five years (57 male, 18 female) with community-acquired LRTIs presenting to the department of Pediatrics, Maulana Azad Medical College, New Delhi, India, for a period of two months (July to August 2014). Inclusion criteria were the presence of cough and fever with chest indrawing of <30 days duration, respiratory rate increase (with or without features of respiratory distress) on examination and the presence of signs of consolidation or bronchopneumonia with or without wheeze on auscultation. Exclusion criteria were hospital-acquired pneumonia *i,e.,* pneumonia that developed 72 h after hospitalization or within seven days of discharge.

Blood specimens (1 ml) were collected for enzyme-linked immunosorbent assay (ELISA) for IgM and IgG antibodies to *M. pneumoniae* (ELISA kit, Calbiotech Inc., CA, USA) and nasopharyngeal aspirates (NPAs) for RSV antigen (Binax NOW RSV Card, Alere, USA), RT-PCR for RSV and PCR for *M. pneumoniae* on admission before starting antibiotics. Convalescent serum samples were obtained for antibodies to *M. pneumoniae* 4-6 wk after enrolment. The study protocol was approved by the Institutional Ethics Committee and written informed consent was obtained from parents.

RNA was extracted from NPA specimens using RNeasy Mini Kit 120 (Qiagen GmbH, Hilden, Germany) followed by reverse transcription and PCR amplification to amplify a 287 bp fragment using the primers: 5'-GCAGCAACAATCCAACCTGCTGG-3', 5'-ATCGGAGGAGGTTGAGTGGAGGG-3'[8]. A negative and positive control was run with each batch of PCR reaction.

DNA for *M. pneumoniae* PCR was extracted from NPA using proteinase K method[9]. A 345 bp fragment on *P1* gene of *M. pneumoniae* was amplified employing the following primers[10,11]: P4A 5'-AGGCTCAGGTCAATCTGGCGTGGA-3', P4B 5'-GGATCAAACAGATCGGTGACTGGGT-3'. Negative control included all PCR components excepting DNA extract which was replaced by addition of sterile distilled water. Positive control included all PCR components and DNA extract from *M. pneumonia* (ATCC 29342).

Chi-square and the Fischer's exact tests were used for testing the difference of proportion between the qualitative variables.

Results & Discussion

RSV is the most common viral pathogen causing LRTIs in young children[11,12]. In the present study, RSV infection was positive in 20 (60.60%) children aged up to one year and 13 (39.40%) children aged 2-5 yr. No significant difference in RSV positivity was observed between male and female children (Table I). Fattouh *et al*[13] also reported similar findings with 64 per cent RSV-positive patients less than six months of age. The clinical and radiological profiles were similar in RSV-positive and RSV-negative children (Table II) in agreement with a previous study which reported no significant difference among RSV-positive and RSV-negative cases in LRTIs[14]. Considering RT-PCR as a gold standard, the sensitivity of RSV antigen by immunochromatography was 90.90 per cent, specificity

Table I. Age and sex distribution in respiratory syncytial virus (RSV) and *Mycoplasma pneumonia* infections in children with lower respiratory tract infections

Characteristics	Total (n=75), n (%)	RSV		*M. pneumoniae*	
		Positive (n=33), n (%)	Negative (n=42), n (%)	Positive (n=26), n (%)	Negative (n=49), n (%)
Age					
1 month - 1 year	45 (60)	20 (60.60)	25 (59.52)	15 (57.69)	30 (61.22)
2-5 year	30 (40)	13 (39.39)	17 (40.47)	11 (42.30)	19 (38.77)
Sex					
Male	57 (76)	23 (69.69)	34 (80.95)	18 (69.23)	39 (79.59)
Female	18 (24)	10 (30.30)	8 (19.04)	8 (30.76)	10 (20.40)

Table II. Clinical and radiological findings in respiratory syncytial virus (RSV) and *Mycoplasma pneumoniae* infection in children with lower respiratory tract infections

Clinical findings	RSV		*M. pneumoniae*		Total (n=75), n (%)
	Positive (n=33), n (%)	Negative (n=42), n (%)	Positive (n=26), n (%)	Negative (n=49), n (%)	
Wheeze	20 (60.60)	30 (71.42)	18 (69.23)	32 (65.30)	50 (66.66)
Rhonchi	22 (66.67)	33 (78.57)	20 (76.92)	35 (71.42)	55 (73.33)
Crepitations	19 (52.57)	32 (76.19)	17 (65.38)	34 (69.38)	51 (68)
Radiological profile					
Bronchopneumonia	3 (9.09)	3 (7.14)	2 (7.69)	4 (8.16)	6 (8)
Interstitial infiltrates	4 (12.12)	6 (14.28)	8 (30.76)	14 (28.57)	10 (13.33)
Consolidation	5 (15.15)	6 (14.28)	4 (15.38)	7 (14.28)	11 (14.67)
Hyperinflation	8 (24.24)	12 (28.57)	7 (26.92)	13 (26.53)	20 (26.67)
Pleural effusion with collapse	5 (15.15)	5 (11.90)	4 (15.38)	6 (12.24)	10 (13.33)
Within normal limits	8 (24.24)	10 (23.80)	6 (23.07)	12 (24.48)	18 (24)

100 per cent, positive predictive value 100 per cent and negative predictive value 93.3 per cent.

M. pneumoniae infection was documented in 15 (57.69%) children aged up to one year and in 11 (42.4%) children aged 2-5 yr; the difference was insignificant. The difference in the presence of *M. pneumoniae* among male and female children with LRTIs was insignificant (Table I). Kashyap *et al*[15] reported no association between sex of the patient and the incidence of *M. pneumoniae* infection. Clinical and radiological profiles across *M. pneumoniae* positive and negative children were comparable (Table II). An earlier report has cast doubts on the specificity of clinical and radiological features in predicting the microbial cause of LRTIs[16]. Serological evidence of *M. pneumoniae* infection was observed in 24 (32%) children. *M. pneumoniae* PCR was positive in eight (10.66%) patients - six with serologically proven and two serological unproven for *M. pneumoniae* infections. Kumar *et al*[16] reported 10 per cent PCR positivity in children with acute LRTIs. Together, serology and PCR detected *M. pneumoniae* in 26 (34.66%) children in concordance with earlier studies which reported 34 and 30 per cent *M. pneumoniae* infection in children[16,17]. Considering PCR as a diagnostic standard, serology sensitivity was 75 per cent, specificity 73.3 per cent, positive predictive value 25 per cent and a negative predictive value 96 per cent.

In conclusion, our study showed the presence of RSV and *M. pneumoniae* infection in community-acquired LRTIs in children aged less than five years.

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***Conflicts of Interest*:** None.

References

1. Kumar S. *Mycoplasma pneumoniae*: A significant but underrated pathogen in paediatric community-acquired lower respiratory tract infections. *Indian J Med Res* 2018; *147* : 23-31.
2. Heiskanen-Kosma T, Korppi M, Jokinen C, Kurki S, Heiskanen L, Juvonen H, *et al.* Etiology of childhood pneumonia: Serologic results of a prospective, population-based study. *Pediatr Infect Dis J* 1998; *17* : 986-91.
3. Kesson AM. Respiratory virus infections. *Paediatr Respir Rev* 2007; *8* : 240-8.
4. Chu HY, Kuypers J, Renaud C, Wald A, Martin E, Fairchok M, *et al.* Molecular epidemiology of respiratory syncytial virus transmission in childcare. *J Clin Virol* 2013; *57* : 343-50.
5. Kumar S, Mehra B, Sethi GR, Saigal SR. Rapid detection of respiratory syncytial virus in community-acquired lower respiratory tract infections in children by chromatographic assay. *Indian J Pathol Microbiol* 2018; *61* : 236-60.
6. Dumke R, Schnee C, Pletz MW, Rupp J, Jacobs E, Sachse K, *et al. Mycoplasma pneumoniae* and *Chlamydia* spp. infection in community-acquired pneumonia, Germany, 2011-2012. *Emerg Infect Dis* 2015; *21* : 426-34.
7. Kumar S, Bala I, Sethi GR. *Mycoplasma pneumoniae* in community-acquired lower respiratory tract infections. *Indian J Pediatr* 2018; *85* : 415-9.

8. Yadav K, Ramachandran VG, Das S, Shah D. One step RT-PCR for detection of respiratory syncytial virus in children with lower respiratory tract infection. *Res Rev J Med Health Sci* 2013; *2* : 49-53.
9. Williamson J, Marmion BP, Worswick DA, Kok TW, Tannock G, Herd R, *et al.* Laboratory diagnosis of *Mycoplasma pneumoniae* infection 4. Antigen capture and PCR-gene amplification for detection of the mycoplasma: Problems of clinical correlation. *Epidemiol Infect* 1992; *109* : 519-37.
10. Waring AL, Halse TA, Csiza CK, Carlyn CJ, Arruda Musser K, Limberger RJ. Development of a genomics-based PCR assay for detection of *Mycoplasma pneumoniae* in a large outbreak in New York state. *J Clin Microbiol* 2001; *39* : 1385-90.
11. Nour M, Trabelsi A, Maatouk N, Hammami M. Amplification of *P1* and *16S rRNA* genes by nested PCR for detection of *Mycoplasma pneumoniae* in paediatric patients. *Pathol Biol (Paris)* 2005; *53* : 9-14.
12. Nokes DJ, Okiro EA, Ngama M, Ochola R, White LJ, Scott PD. Respiratory syncytial virus infection and disease in infants and young children observed from birth in Kilifi district, Kenya. *Clin Infect Dis* 2008; *46* : 50-7.
13. Fattouh AM, Mansi YA, El-Anany MG, El-Kholy AA, El-Karaksy HM. Acute lower respiratory tract infection due to respiratory syncytial virus in a group of Egyptian children under 5 years of age. *Ital J Pediatr* 2011; *37* : 14.
14. Sutmöller F, Ferro ZP, Asensi MD, Ferreira V, Mazzei IS, Cunha BL. Etiology of acute respiratory tract infections among children in a combined community and hospital study in Rio De Janeiro. *Clin Infect Dis* 1995; *20* : 854-60.
15. Kashyap B, Kumar S, Sethi GR, Das BC, Saigal SR. Comparison of PCR, culture & serological tests for the diagnosis of *Mycoplasma pneumoniae* in community-acquired lower respiratory tract infections in children. *Indian J Med Res* 2008; *128* : 134-9.
16. Kumar S, Saigal SR, Sethi GR. Rapid diagnosis of *Mycoplasma pneumoniae* by polymerase chain reaction in community-acquired lower respiratory tract infections. *Trop Doct* 2011; *41* : 160-2.
17. Kumar S, Garg IB, Sethi GR, Kumar S, Saigal SR. Detection of immunoglobulin M and immunoglobulin G antibodies to *Mycoplasma pneumoniae* in children with community-acquired lower respiratory tract infections. *Indian J Pathol Microbiol* 2018; *61* : 214-8.

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