Congenital pneumonia due to *Mycoplasma pneumoniae*

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Abstract

A case of probable vertical transmission of *Mycoplasma pneumoniae* is presented. The presence of *M pneumoniae* was demonstrated by the polymerase chain reaction (PCR) in the nasopharyngeal aspirate of a newborn who developed pneumonia shortly after birth. This result was confirmed by performing a second PCR, amplifying another part of the genome of *M pneumoniae*.

It is concluded that *M pneumoniae* can be added to the long list of pathogens known to cause congenital pneumonia.

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The polymerase chain reaction (PCR) allows minute amounts of a specific DNA sequence to be detected, even in the presence of an appreciable background of non-target DNA. In diagnostic microbiology PCR can demonstrate very small numbers of organisms in clinical specimens. Congenital pneumonia is caused by a number of microbiological agents. We report on the use of PCR for the detection of *M pneumoniae* in a case of congenital pneumonia.

Case report

A male, birthweight 3.550 kg, length 51 cm, was born at 38 weeks after an uneventful pregnancy. A few minutes after birth he became hypoxic; a lot of mucus and mucus plugs were aspirated. At 2 hours of age, he became cyanotic and his breathing was laboured; he was intubated and given assisted ventilation. Chest radiography showed a pleural effusion and alveolisation (fig 1). Antibiotic treatment was started (ampicillin, 175 mg every 12 hours, and netilmycin, 10 mg every 12 hours). On the second day oxygen had to be increased to 100%, and chest radiography revealed a pneumothorax and pneumomediastinum. The baby was transferred to Antwerp University Hospital.

Examination showed that his haemoglobin was 180 g/l, platelets $206 \times 10^9/l$, C-reactive protein was 17 mg/l, total bilirubin 152 $\mu\text{mol/l}$. Blood urea nitrogen, serum creatinine, and an electropherogram were within normal limits. Culture of endotracheal aspirate was negative for conventional bacteria, *M pneumoniae*, *M hominis*, *Ureaplasma urealyticum*, *Chlamydia trachomatis* and respiratory viruses. The PCR

for *M pneumoniae* was positive. Blood and urine cultures were negative. Syphilis serology was negative; antibodies to cytomegalovirus were absent. Herpes antibodies were present at a low titre; there were IgG antibodies against rubella virus and *Toxoplasma gondii*. Antibodies against *M pneumoniae* were not detected.

After the pneumothorax had been drained, chest radiography showed a reticulonodular picture indicative of generalised pneumonia and a small amount of pleural fluid. Oxygen dependency improved slowly, and the baby was extubated at the age of 6 days. Further recovery was uneventful. At the age of 1 year no further sequelae were noted.

About six weeks before this patient was born, his sister, (aged 1 year) was admitted to Antwerp University Hospital with febrile convulsions. Laboratory examination was unremarkable except for a leucocytosis of $19.7 \times 10^9/l$ with 75.3% neutrophils. A nasopharyngeal aspirate culture was negative for conventional bacteria, *M pneumoniae*, and respiratory viruses. The PCR for *M pneumoniae* on this specimen was positive. The patient was discharged without symptoms three days later, when leucocytosis had fallen to $6.1 \times 10^9/l$. Retrospectively, the mother of the two children recalled a slight upper respiratory infection (rhinitis) during the interval between the two episodes described above.

Methods

CULTURE

A 0.2 ml aliquot of nasopharyngeal aspirate was inoculated into 2.0 ml of SP-4 broth¹ without thallium acetate and supplemented with amphotericin B (0.5 g/l) and polymyxin B (5×10^5 U/l). Three serial 20-fold dilutions of this primary broth were performed in SP-4 broth. All four broths were incubated aerobically at 37°C and observed for colour change over six weeks. Quality control of the medium consisted of a titration of a low passage strain of *M pneumoniae*. The original sample and a portion of the primary SP-4 broth were stored at -80°C for PCR.

SAMPLE PREPARATION FOR PCR

For proteinase K extraction, 100 ml of nasopharyngeal aspirate diluted 1 in 10 in SP-4 broth was incubated with proteinase K and subsequently extracted with phenol:chloroform and chloroform. The nucleic acids were precipitated twice with ethanol and dissolved in 11 μl of water. Five microlitres of this

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Chest radiograph of case 1 was taken a few hours after birth. The right hemithorax is characterised by pleural effusion and signs of alveolisation.

extracted DNA were used in the PCR. One hundred microlitres of undiluted sample were freeze boiled (alternate passages in liquid nitrogen and in boiling water for 2 minutes each) five times. After centrifugation for five minutes at 14 000 rpm, 5 µl of the supernatant fluid were amplified with the PCR.

POLYMERASE CHAIN REACTION

Two primer pairs (P1-1/P1-3 and P1-4/P1-5 (table) were selected from RepMP2/3 and RepMP4, which are sequences present in at least 10 and eight copies, respectively, throughout the *M pneumoniae* genome, one copy being part of the P1 gene.² For both primer sets, specificity was confirmed by failure to amplify

DNA from several Gram negative and Gram positive conventional bacteria and from several human mycoplasmas: *M fermentans*, *M primatum*, *M salivarium*, *M orale* and *M genitalium*. Only *M pneumoniae* DNA gave a positive amplification signal.

Primer pair P1-1 and P1-3 were used for PCR-1 and produced an amplicon of 209 base pairs. Primer pair P1-4 and P1-5 were used for PCR-2 and produced an amplicon of 259 base pairs. Amplifications were performed in a Techne PHC-2 thermocycler in 50 µl containing 10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 µM dNTP, 1 unit of Taq polymerase (Perkin-Elmer, Norwalk, Connecticut, USA) and 0.1 µM (PCR-1) or 0.2 µM (PCR-2) of each primer. Forty (PCR-1) or 50 (PCR-2) cycles of 1 minute of denaturation at 94°C, 1 minute of hybridisation at 65°C, and 2 minutes of elongation at 72°C were performed. The final elongation step lasted for 10 minutes.

For PCR-1, a modified amplicon containing a piece of foreign DNA was used as an internal control,³ to detect inhibitors present in the samples. After amplification, Southern blotting was performed and hybridisation with ³²P-labelled oligonucleotide P1-2 (table) confirmed the specificity of the amplification product.

Results and discussion

In both cases the nasopharyngeal aspirate produced an amplicon of 209 base pairs with PCR-1 both after proteinase K treatment and freeze boiling. The specificity of the amplification product was confirmed by a successful hybridisation with labelled oligonucleotide P1-2 after Southern blotting. Although all necessary precautions were taken to avoid contamination,⁴ and although all negative controls included in the extraction and amplification runs were negative, an independent confirmation was necessary. Therefore, the specimens were extracted again by freeze boiling and amplified with PCR-2 targeted at another DNA sequence of *M pneumoniae*. PCR-2 has been used sparingly as a confirmatory test in our laboratory to avoid amplicon buildup. PCR-2 detected a 259 base pair amplicon in both specimens, thus confirming the results of PCR-1. Contamination by amplicon carry-over, which represents by far the highest contamination risk, was excluded in this way. Contamination with *M pneumoniae* DNA during sample taking is unlikely because the two samples were taken independently in different hospitals and at intervals of several weeks. For both samples to have been contaminated during their processing in the laboratory without any of the negative controls having become positive, seems unlikely.

The amplification of specimens from these patients was part of a study on the usefulness of PCR for the diagnosis of *M pneumoniae* infections. Collection of serum specimens was not a part of the study design, and the results of the amplification were not known until several months after specimen collection. Therefore,

Sequence of oligonucleotides used for amplification and detection of *M pneumoniae*

Name	Sequence	Base pairs*
P1-1	5'-GCC ACC CTC GGG GGC AGT CAG-3'	2599-2619
P1-2	5'-CTG AAC GGG GGC GGG GTG AAG G-3'	2676-2696
P1-3	5'-GAG TCG GGA TTC CCC GCG GAG G-3'	2786-2807
P1-4	5'-GGC CAT GAC CGC GAA CTA TCC G-3'	1191-1212
P1-5	5'-CGG GAG GGC GAT CGG GGC CG-3'	1430-1449

*Nucleotide number in the P1 gene as described in ⁶.

serology was not available. Culture for *M pneumoniae* was negative in both cases. However, the low sensitivity of culture is well known; we therefore tried to validate the results technically with a second PCR.

To our knowledge, the presence of *M pneumoniae* in the respiratory tract of a newborn with congenital pneumonia has not been described before. Miller and Enbom isolated *M pneumoniae* from a newborn whose mother had had a culture confirmed *M pneumoniae* infection three months before delivery.⁵ Interestingly, this child had no signs or symptoms of respiratory tract infection until the age of 4 months. At that time he developed lower respiratory tract infection, but unfortunately *M pneumoniae* was not searched for. In a larger study on prenatal infections these authors showed a possible association between neonatal respiratory tract disease and the presence of complement fixing antibodies to *M pneumoniae* in cord blood.⁵

A second issue is whether the mycoplasmas were merely colonising the patients or causing disease. In both patients an extensive search for pathogens was carried out and *M pneumoniae* was the sole pathogen demonstrated. The short interval between birth and specimen taking for the boy in this study points to a prenatal infection. The fact that pneumonia was

present at birth provides no alternative to intrauterine infection. Finally, the history of a mycoplasma associated respiratory infection in the household and the mild respiratory illness of the mother shortly before delivery as the only anomaly during pregnancy are perfectly compatible with transplacental infection of the fetus by *M pneumoniae*.

We conclude that *M pneumoniae* was most probably the cause of congenital pneumonia in this patient, meaning that it can be added to the long list of pathogens known to cause this condition.

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