*CASE REPORT*

# CULTURE-CONFIRMED *CHLAMYDIA PNEUMONIAE* INFECTION

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ABSTRACT: **A strain of *Chlamydia pneumoniae* was isolated from the throat swab of a 45 year-old female patient with tracheobronchitis who responded well to doxycycline therapy. The isolate was confirmed by fluorescent antibody staining and by polymerase chain reaction. As there are no distinct clinical features to indicate *C pneumoniae* respiratory infections, laboratory confirmation is required for definitive diagnosis.** *(JUMMEC 1996 1(2): 52-56)*

KEY WORDS: *C. pneumoniae*, culture, tracheobronchitis

## Introduction

*Chlamydia pneumoniae* is a recently recognized cause of human respiratory infections. Clinical manifestations associated with this organism resemble those caused by *Mycoplasma pneumoniae* and include pharyngitis, sinusitis, bronchitis, pneumonia and brochial asthma (1,2). Laboratory confirmation is difficult as the isolation of the bacteria requires cell culture. Non-culture methods using enzyme immunoassay (EIA), direct fluorescent antibody staining (DFA) and DNA probes which have been commonly used for the detection of *C. trachomatis* have not been adequately evaluated for *C. pneumoniae*. Polymerase chain reaction (PCR) is increasingly being used to determine the presence of the organism in clinical specimens but is still a tool largely confined to the reference laboratory. Most investigations have relied on serologic diagnosis.

In Malaysia, serodiagnosis has associated *C. pneumoniae* with 13-15% of community-acquired acute lower respiratory tract infections in adult patients (3,4) but only 1.2% among infants and children under 3 years old (5). This paper reports a culture-confirmed case of tracheobronchitis in a local patient.

## Case Study

A 45 year-old female travel consultant presented with a month's history of productive cough and wheezing which had not responded well to treatment with cephalexin and cotrimoxazole. She did not smoke cigarettes. On examination, no clinical abnormalities were found apart from an occasional wheeze. Her peak flow rate was 280 L/min. She was diagnosed as a case of tracheobronchitis.

A throat swab taken from the patient was transported to the laboratory in SPG (sucrose-phosphate glutamic acid) chlamydia transport medium kept ice-cold during transportation and cultured on the day of collection. After 3 days of incubation, typical intracytoplasmic inclusions were seen in the cell monolayer *(Figure 1)*. Two PCRs amplifying different targets in the *C. pneumoniae* genome, were carried out on the infected culture. The throat swab was also tested directly by PCR without prior culture. The respective PCR products visualized by gel electrophoresis confirmed the presence of *C. pneumoniae* in the patient's specimen *(Figure 2 and 3)*.

As the patient was allergic to macrolides, she was treated with doxycycline 100mg daily for 10 days. At the end of treatment, her symptoms had resolved and her peak flow rate had increased to 370 L/min. A repeat throat swab taken 16 days after treatment was culture and PCR negative and remained culture-negative after 2 blind passes.

## Material and Methods

### *Chlamydial culture*

Isolation of chlamydiae was done in HEp-2 cells kindly provided by Professor Akira Matsumoto of Kawasaki Medical School, Japan. The throat swab was vortexed in the SPG transport medium and the resulting suspension was sonicated at output 80 (Branson Sonifier 250) for 4 minutes. Two aliquots of 200 µL of the specimen were centrifuged at room temperature for 45 min at 2000 x g onto HEp-2 cells grown on 13mm coverslips in shell vials. The inocula were then replaced by Eagle's

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***Figure 1:*** Intracytoplasmic inclusions of *Chlamydia pneumoniae* (indicated by arrows) cultured in HEp-2 cells and stained with FITC-conjugated monoclonal antibody to *C pneumoniae* major outer membrane protein.

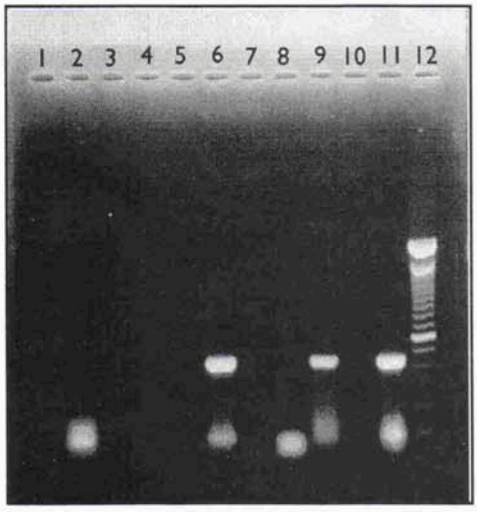


***Figure 2:*** *C pneumoniae* PCR products (463 bp amplified with CpnA-CpnB primer set) in 1.2% agarose gel electrophoresis. Lanes 1: negative control, lanes 3,4,5,7:blanks, lane 11:positive control, lanes 8,9,10:3 passes of a control *C pneumoniae* culture, lane 2:patient's throat swab, lane 6:patient's throat swab culture.

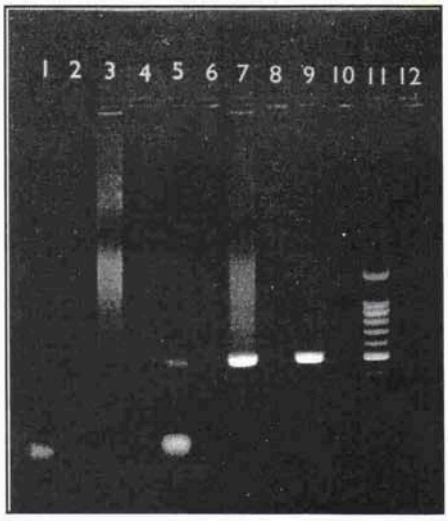


***Figure 3 :*** *C pneumoniae* PCR products (499 bp amplified with 53.1-53.2 primer set) in 1.2% agaros gel electrophoresis. Lanes 1-4,6,8,10,12: negativ control and blanks, lane 9:positive control, lan 5:patient's throat swab culture diluted 1:5, lan 7:patient's throat swab *culture neat.*

MEM (Gibco Laboratories, Chicago, USA) supplemented by 10% fetal calf serum, 1000 µg/ml gentamicin, 0.2 ug/ml streptomycin, 250 µg/ml amphotericin B and 1 µg/ml cycloheximide and the cultures incubated in 5% $CO_2$ at 35° C for 3 days. The infected monolayers were then fixed with acetone and stained with FITC-conjugated monoclonal antibody to *C pneumoniae* major outer membrane protein (MOMP) (DAKO A/S Denmark). *C. pneumoniae* appeared as apple-green intracytoplasmic inclusion bodies under the epifluorescence microscope.

### *Chlamydial PCR*

The 2 primer sets used for the PCR were CpnA-CpnB (6) which amplified a target on the *C pneumoniae* MOMP gene and 53.1-53.2 (7) which amplified the gene encording a *C pneumoniae*-specific immunodominant protein. CpnA-CpnB was a gift from Dr Charlotte Gaydos, The Johns Hopkins University, USA. The patient's specimen was incubated with proteinase K (100 µg/ml) and Nonidet P40/Tween 20 (0.5% v/v) at 55°C for 1 h and then boiled for 5 min. Five µl of this sample was used for the PCR in a 50 µl reaction volume containing PCR buffer (10 mM Tris pH 8.3, 50 mM EDTA), 200 µM concentrations of each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 0.5 µM concentrations of each primer and 1 unit of Taq DNA polymerase (Promega, U.K.). Amplifications were carried out in a Perkin-Elmer 480 thermal cycler with 35 cycles of 94° C x 1 min, 55° C x 1 min and 72° C x 2 min. The positive control used was a suspension of *C. pneumoniae* elementary bodies processed as for the patient's specimen while the negative control consisted of the PCR reaction mixture without DNA. At the completion of amplification, PCR products were analyzed by 1.2% agarose gel electrophoresis. The presence of *C. pneumoniae* was indicated by a 463 bp band in the gel with CpnA-CpnB primers and a 499 bp band with the 53.1-53.2 primer set. The specificity of the bands were not determined by hybridization with specific probes but the use of 2 PCRs amplifying different targets in the *C. pneumoniae* genome helped to reduce the possibility of non-specific amplification.

## Discussion

The routine diagnosis of *C. pneumoniae* infections by serology is not entirely satisfactory as serologic results are difficult to interpret. Antibody response may be delayed or absent altogether, especially in children. Cross-reactions between *C. trachomatis* and *C.. pneumoniae* are not infrequent and diagnostic titres are often found in healthy asymptomatic persons (8).

The isolation of *C. pneumoniae* is confirmatory but this organism has been reported to grow poorly in tissue culture, often requiring multiple passages for positive isolation from clinical specimens. The inclusion bodies produced tend to be smaller and fewer than for other chlamydial species (1). Many laboratories experienced in *C. trachomatis* culture had had difficulty isolating *C. pneumoniae* and even in the best laboratories, the sensitivity of culture compared to non-culture methods is less than 90% (8). Hence, false culture-negatives are common. On the other hand, a positive culture does not always indicate disease as there is evidence that the organism can be carried in the apparently healthy respiratory tract. Isolation rates of 2% and 5% respectively have been found among asymptomatic adults and children (8) and in a Finnish outbreak, it was noted that only 1 in 10 infections resulted in clinical disease (9).

PCR is the most promising non-culture method for the diagnosis of *C .pneumoniae* infection although it can also give false negatives due to the presence of polymerase inhibitors and nucleases in clinical specimens as well as false positives due to amplicon contamination in the laboratory. Compared to culture and serology, it can certainly provide a more rapid diagnosis for the administration of appropriate antibiotic treatment.

In the presence of clinical features which are known to be associated with *C. pneumoniae* infection, especially the prolonged symptoms which did not respond well to treatment with antibiotics not totally effective against chlamydia but disappeared on treatment with doxycycline, it is most likely that the *C .pneumoniae* isolated from the patient was the cause of her illness. *Mycoplasma pneumoniae* and *Legionella pneumophila* can also cause similar clinical presentations and co-infection with *C. pneumoniae* is possible. A multiplex PCR for the detection of all 3 pathogens would greatly facilitate the diagnosis of respiratory infections caused by these organisms.

Most studies on *C. pneumoniae* infection in Malaysia and many other parts of the world have not had culture-proven cases. This report documents the role of this organism as a cause of airway infection in Malaysia.

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