PREVALENCE OF *CHLAMYDIA TRACHOMATIS* IN CLINICAL SAMPLES BY POLYMERASE CHAIN REACTION AND CELL CULTURE

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Abstract

Clinical samples in transport media from forty-nine patients exhibiting pathologies potentially caused by *Chlamydia trachomatis* infections were analyze for amplification, including the plasmid and major outer membranes protein (MOMP) and the resulted were reported with the those of cell culture. *Chlamydia trachomatis* was performed by shaking vigorously on a vortex mixer for releasing elementary bodies from intact host cells and inoculated in monolayer of McCoy cells. By using procedures, plasmid-PCR and MOMP-PCR primers were 10 µM. Cell culture, MOMP-PCR and Plasmid-PCR based assays detected 4/49 (8%), 5/49 (10%) and 6/49 (12%), respectively. The data may indicated an unrecognized process in *Chlamydia trachomatis* infection or that these patients were infected by a variant strains of *Chlamydia trachomatis* which lacks the *Chlamydia trachomatis*-specific MOMP. The plasmid-based PCR are more sensitive than MOMP-PCR for detecting *Chlamydia trachomatis*.

Introduction

*Chlamydiae* are obligate intracellular bacteria that have associated with a wide spectrum of human diseases. Nowadays, they can be divided into four groups; *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci* and *Chlamydid pecorum*. *Chlamydia trachomatis* has been recognized as a pathogen of nongonococcal urethritis (NGU), salphingitis, endocervicitis, pelvic inflammatory disease (PID), inclusion conjunctivitis of neonates, follicular conjunctivitis of adults, infantile pneumonia and associated conditions. (1) The developmental cycle of Chalmydiae is unique infections extracellular form, but metabolically inactive elementary bodies (EB), attach to the host cell and are taken up by endocytosis. Within six to eight hours EB become noninfections, metabolically active reticulate bodies (RB) which has been replicated by binary fission. (2) Fifteen prototypic serovars labeled A to K and L₁, L₂ and L₃ were initially recognized by polyclonal antibodies, and additional immunovariants (Ba, Da, Ia), which in some publications are referred to as distinct serovars, have been identified by monoclonal antibodies. (3-5) Serovars A, B and C have been found usually associated with trachoma, D to K with urogenital infections and L₁, L₂ and L₃ with lymphogranuloma venereum (LGV), a systemic disease. The urogenital serovars are found
all regions of the world, whereas the LGV and trachoma serovars are found primary in tropical and subtropical regions (6).

*Chlamydia trachomatis* infections have been diagnosed by cell culture, immunofluorescence (IF), enzyme immunoassay (EIA), direct DNA hybridization and more recently by polymerase chain reaction (PCR). The gold standard for diagnosis of Chlamydia infections is based on isolation of the organism in McCoy cells. The specification of cell culture methods are high; however, the sensitivities of assays are typically 60-70% which it depends on factors such as the status of patient symptom, specimen collection techniques, specimen transport and storages. Several laboratories have demonstrated that the polymerase chain reaction is more sensitivity than culture or enzyme immunoassay for detecting *Chlamydia trachomatis* (7). In our examination of a urogenital specimens obtained from patients with suspected *Chlamydia trachomatis* infections. We have also noted between polymerase chain reaction assays and cultures. This study described a DNA targeted against the MOMP gene, yielding product of 871 base pairs (8) and the *Chlamydia trachomatis* specific plasmid, yielding product of 279 base pairs. (9)

**Material and Methods**

**Patient specimens and cell culture:** samples were submitted to our laboratory from the Phramongkutkloa hospital and other hospitals in Bangkok between on May 2005 to on Jan 2006. For female urogenital samples, mucous was removed from the cervix and male urethral specimens, a swab was inserted into the urethral. Both of all were placed immediately into 2 ml of 0.2 M sucrose-phosphate medium containing appropriate antibiotic (amphotericin B 2.5 µg/ml, streptomycin 0.2 mg/ml, gentamycin 0.5 mg/ml and vancomycin 0.05 mg/ml) and 10% fetal bovine serum and were stored at -70°C until used. Specimens were thawed and 200 µl were inoculated in duplicate onto culture of McCoy cells seeds on 24 well-plate. The culture were centrifuged for 1 hour at 2400xg at 25°C and incubated at 35°C in 5% CO₂ incubator for 2 hours. Add 2 ml growth medium supplemented with 2 µg/ml of cycloheximide and 0.6 mg/ml of glucose and in cubate at 35°C in 5% CO₂ incubator for 2 hours. The monolayers are examined by Jone’s iodine staining to visualize the cytoplasmic inclusions under the inverted microscope.

**DNA extraction:** Swabs transport medium 500 µl were prepared by centrifugation at 6,000xg for 20 seconds. Discard the supernatant and resuspend the cells in 150 µl lysis buffer add 200 µl GB buffer to the tube and mixed by vortexing. Incubate at 70°C for 10 minutes until the sample lysate is clear (During incubation, invert the tube every 3 minutes). Total nucleic acids were precipitated by the addition of 100% ethanol. Following centrifugation for 2 minutes at 6000xg, the pellet was washed in 500 µl of wash buffer by centrifugation (Repeat step). Nucleic acids were dissolved in 100 µl of sterile water by centrifugation and aliquot each 50 µl for until used (10).
**Primer:** The oligonucleotide primers were selected from the published sequences of the MOMP gene of Chlamydia trachomatis. The sequences from 5' to 3' are represented sense: CtF TGA CTT TGT TTT CGA CCG TGT TTT (309-333) Antisense: Ct-R TTT TCT AGA TTT CAT CTT GTT CAA T/CTG (1179-1153) (8). These primers generate a 871 base pairs product. Another primers were selected from plasmid gene of Chlamydia trachomatis. The sequences from 5' to 3' are represented sense: CtP F AGG TAA ACG CTC CTC TGA. Antisense: CtP R CGT TTG TAC TCC GTC ACA (7048-7345) (9). These primers generate a 297 bp product following amplification of the Chlamydia trachomatis plasmid gene.

**Polymerase chain reaction:** The final concentrations in the reaction mixture has been contained 20 µl of 1X of 10X buffer with 2 mM MgCl₂, 5 mM each deoxynucleoside triphosphates (dNTP), 2 unit Tag DNA polymerase (Yea DNA), 10 µM each primers and 5 µl DNA extract, Sterile distilled water as a negative control, 5X10⁴ McCoy cells infected with L2 as a positive control. DNA was amplified by one cycle of 95°C for 3 minutes, 95°C for 40 seconds, 58°C for 50 seconds and 72°C for 1 minute followed by 39 cycles. The PCR products were analysed by electrophoresis through 1.5% agarose gels in parallel with a 100 bp ladder (Biorad).

**Results**

Known about Chlamydiae are the intracellular bacteria or else detection of this microorganism has to keep infected epithelial cells. In our study, we used β actin primers which are represented internal control for showing of effective of samples. Forty-nine samples are positive for β actin primers and DNA amplified 400 base-pair as shown in Figure 1. The PCR assay directed against the Chlamydia trachomatis MOMP gene gave rise to the predicted 871-bp product, while the PCR assays directed against the Chlamydia trachomatis-specific Plasmid generated the predicted 297-bp product. Four samples were positive by cell culture techniques. Five and six samples were positive by MOMP gene primers and plasmid primers, respectively. All four of the Chlamydia trachomatis culture-positive clinical samples were positive by MOMP-PCR and plasmid-PCR.
**Discussion**

We tested samples submitted for diagnosis of chlamydial infection using several different nucleic acid-based tests. Other factors may play role in determining the level of sensitivity of various primers. Sensitivity is linked to specificity including primer length and concentration, GC
content, 3’-terminal base, annealing temperature, MgCl$_2$, deoxynucleoside triphosphate and Tag polymerase concentrations affect the sensitivity of an individual PCR (11). Although most reported have shown that polymerase chain reaction is more sensitive than culture, some reports were not (12). Other factors such as prevalence of infection, number and type of specimens tested and method of analysis have affected performance.

References

10. Genomic DNA extraction commercial Kit, Taiwan. (Cat no YGB 100)

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