Infection, Genetics and Evolution xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

The clinical characteristics and genotype distribution of *Chlamydia trachomatis* infection in infants less than six months of age hospitalized with pneumonia

Yuefeng Li [a], Likuan Xiong [b,*], Yan Huang [a], Yong Xia [b], Hua Zhou [b], Fen Xu [a], Matthew V.N. O'Sullivan [c,d]

[a] Department of Neonatology, Baoan Maternal and Child Health Hospital, Shenzhen 518133, China
[b] Central Laboratory, Baoan Maternal and Child Health Hospital, Shenzhen 518133, China
[c] Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, Australia
[d] Centre for Infectious Diseases and Microbiology, Westmead Hospital, Westmead, Australia

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 10 October 2014
Accepted 2 November 2014
Available online xxxx

Keywords:
Chlamydia trachomatis
Genotyping
Reverse line blot hybridization

ABSTRACT

Background: *Chlamydia trachomatis* is a common sexually-transmitted bacterial pathogen. As no routine screening is performed during pregnancy, neonates and infants are at high risk for *C. trachomatis* infection. The objective of this study was to investigate the morbidity, clinical characteristics and genotype distribution of *C. trachomatis* pneumonia in infants less than six months of age.
Methods: Clinical manifestations and laboratory results were recorded. Respiratory sputum specimens were tested using RT-PCR targeting *C. trachomatis* cryptic plasmid. Simultaneously, respiratory virus antigens were detected by direct immunofluorescence and bacterial pathogens were examined by culture in all sputum samples. Positive *C. trachomatis* samples were further genotyped using a multiplex PCR reverse line blot assay. The relationship between genotype and pneumonia severity was explored.
Results: Of 1408 infants, 101 (7.2%) were infected with *C. trachomatis*. Sixteen of 101 (15.8%) were assessed as severe pneumonia. These severe cases had a higher proportion of viral co-infection (37.5%) compared to mild pneumonia cases (9.4%, $P < 0.05$).Infants with tachypnea (OR 9.2) and wheezing (OR 3.5) were more likely to be classified as severe pneumonia ($P < 0.05$). Amongst 66 *C. trachomatis* specimens for which a genotyping result was available, seven genotypes were detected, and 39.4% of these specimens contained two or three genotypes. Overall, genotype E (48.5%) was the most frequent, followed by genotype F (42.4%), J (31.8%), D (12.1%), K (10.6%), G (4.5%) and H (3.0%). There were no significant correlations of particular genotypes with severity of disease, although there was a weak indication that more severe pneumonia might be associated with having certain mixed genotypes of *C. trachomatis*.
Conclusions: The prevalence of *C. trachomatis* in the population of young hospitalized infants with pneumonia in Shenzhen was very high. The relationship between genotype distribution and severity of pneumonia was not clear based on this study due to small sample size. Further in-depth investigation correlating genotype and disease severity based on a larger population is needed.

1. Introduction

Chlamydia trachomatis (*C. trachomatis*) is one of the most common sexually-transmitted bacterial pathogens, and it is associated with a wide range of diseases. *C. trachomatis* infection during pregnancy can increase the risk of preterm labor, low birth weight and perinatal mortality (Silva et al., 2011). Neonatal colonization or infection with *C. trachomatis* can lead to early respiratory problems, such as pneumonia, acute respiratory distress and respiratory failure (Hon and Leung, 2013; Bellulo et al., 2012; Herieka and Dhar, 2001), which is a risk factor for chronic respiratory disease like asthma (Webley et al., 2009; Duijts, 2012; Bisgaard et al., 2007). Antimicrobial administration during the neonatal period may reduce the adverse respiratory sequelae in adulthood (Jupelli et al., 2011). Due to the absence of routine screening and treatment for C. *trachomatis* during pregnancy, this pathogen is still an important cause of lower respiratory tract

* Corresponding author. Tel.: +86 013823239989; fax: +86 0755 27820313.
E-mail address: Xionglk@sina.cn (L. Xiong).

http://dx.doi.org/10.1016/j.meegid.2014.11.004
1567-1348/

infection in infants younger than 6 months in Taiwan, China (Chen et al., 2007), which has an impact on rates of long-term chronic respiratory diseases. This might indicate that the prevalence of *C. trachomatis* infection in genital and respiratory tract in Asia is different from that in western countries. Further in-depth understanding of its prevalence in genital and respiratory tract in China and early intervention strategies are important considerations for pediatricians to prevent adverse respiratory sequelae.

Currently, *C. trachomatis* is classified into 19 genotypes, which are most commonly determined using differences in the amino acid sequence of the major outer membrane protein, encoded by the *omp*1 gene. Determining the genotype distribution is useful for epidemiologic studies and to explore differences in clinical manifestations between genotypes. Many previous studies have investigated the prevalence and genotype distribution of *C. trachomatis* in adult urogenital system infections (Gharsallah et al., 2012; Lagergård et al., 2010; Machado et al., 2011), but few studies have focused on paediatric infections. One study reported the incidence and genotype distribution of *C. trachomatis* pneumonia in infants less than 6 months of age and showed that its morbidity was 18.1% (Martinez et al., 2009). However, there is currently limited relevant data regarding prevalence and genotype distribution of *C. trachomatis* causing pneumonia among these pediatric populations. Furthermore, there are no study reports regarding the relationship between *C. trachomatis* genotype distribution and severe pneumonia.

In this study, we investigated the morbidity, clinical characteristics and genotype distribution of *C. trachomatis* in cases of pneumonia among infants less than 6 months of age. We further explored the relationship between *C. trachomatis* genotype distribution and severity of pneumonia.

2. Materials and methods

2.1. Study population

This prospective study was conducted in Bao'an Maternal and Child Health Hospital of Shenzhen, Guangdong, China. Infants less than 6 months old, admitted to department of pediatrics and neonatology with pneumonia were consecutively recruited from Jun 1, 2012 to July 31, 2013 after obtaining parental consent. The demographic data, clinical, laboratory, and radiologic findings on admission of recruited infants were recorded. The study was approved by the ethics committee of Bao'an maternal and child health care hospital.

2.2. Clinical definition

Tachypnea was defined as elevated respiratory rate for age (if <2 months, $\geqslant$60 breaths per min; if 2–11 months, $\geqslant$50 breaths per min).

Infantile pneumonia was defined as an infant aged <6 months hospitalized with symptoms and signs of lower respiratory tract infection including persistent fever, cough, stridor, wheeze, tachypnea, and auscultation of crackles, bronchial breathing, wheeze sound and/or rales. Severe pneumonia was defined as an infant with cough or difficulty of breathing and at least one danger sign (i.e. chest in-drawing or nasal flaring, stridor while calm, cyanosis, convulsions, inability to drink, lethargy, unconsciousness or intractable vomiting) (Pocket book for hospital care of children, 2005; Harris and Coote, 2011).

All infants with pneumonia were confirmed by chest X-ray examination. Severity of pneumonia was assigned by hospital physicians.

2.3. Clinical specimen collection and detection of pathogens

Respiratory sputum secretions were aspirated with a suction catheter, collected in 2–3 ml of sterile normal saline and transported immediately to the laboratory, on ice, and stored at −20 °C for further studies.

2.3.1. Detection of C. trachomatis pathogen

All specimens were examined for the presence of *C. trachomatis* with a real-time PCR assay performed on the ABI Prism 7500 platform (Applied Biosystems, Singapore, USA), using a diagnostic kit for *C. trachomatis* cryptic plasmid DNA (Daan, Guangzhou, China). A 202 bp segment of *C. trachomatis* cryptic plasmid was amplified according to the manufacturer's instructions. Positive and negative controls were included in each PCR run. All precautions to prevent cross-contamination were taken. The extracted DNA was frozen at −20 °C until required for genotyping studies. Additionally, direct immunofluorescence for respiratory virus antigens and standard bacterial culture and identification were performed.

2.3.2. Nested PCR amplification

Specimens found to be positive for *C. trachomatis* were subjected to amplification of *omp*1 by nested PCR. The primary PCR reaction was performed with outer primers CTS (5′- AATATYTGGG ATCGYTTTGATGT-3′) and CTA (5′-CCRCAYTCCCASARAGCTGC-3′) (Sigma–Aldrich, St. Louis, USA). The volume of PCR mixture for primary PCR was 25 μl, containing 200 μM dNTPs, 10× PCR buffer (Mg^{2+} free) 2.5 μl, 1.6 mM $MgCl_2$, 20 μM of each primer and 1 U of DNA *Taq* polymerase (Takara, Japan). The thermal profile was an initial denaturation step of 95.0 °C for 5 min, 35 cycles of 93 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min, followed by a final elongation step of 72 °C for 5 min. Six microlitres of the primary PCR product was used for the secondary PCR, which used inner primers CTSN (5′-TTGATGTATTYTGTACAYTRGGAGC-3′) and CTAN (5′-GCTGCDCGAGCDCCNACRCT-3′) with all reagent volumes doubled to make a final volume of 50 μl, and the same cycling conditions as for the primary PCR. Amplification products were visualized by electrophoresis on a 1.5% agarose gel.

2.3.3. Reverse line blot (RLB) assay

Probe hybridization of the nested PCR products to determine *C. trachomatis* genotype was performed using a RLB assay as described previously (Xiong et al., 2006). Briefly, the channels of a Miniblotter 45 (Immunetics) were filled with 150 μl of two different concentrations of each probe solution to fix the probes on the membrane. Subsequently, PCR products were denatured by boiling and immediately chilled on ice. Hybridization of the PCR products to the probes was performed using the miniblotter, (with the membrane rotated by 90°) at 60 °C for 60 min. The washed membrane was incubated in a peroxidase-labeled streptavidin conjugate solution (Roche, Mannheim, Germany) at 42 °C for 60 min, followed by washing and incubation with a chemiluminescent substrate (ECL Direct System; Roche) for 1 min. Visualisation of chemiluminescent signal was performed by Hyperfilm X-ray film (Amersham) to the membrane for 10 min.

2.3.4. Testing of other pathogens

Respiratory virus antigens, including influenza virus type A and B, parainfluenza type 1, 2 and 3, respiratory syncytial virus and adenovirus, were detected in all sputa by D^3 Ultra™ DFA respiratory virus screening and ID kit (Diagnostic Hybrids, INC,USA) according to the manufacturer's instructions.

Bacterial pathogens were simultaneously analyzed by culture. Briefly, 10ul aliquots of respiratory sputum samples were cultured

Please cite this article in press as: Li, Y., et al. The clinical characteristics and genotype distribution of *Chlamydia trachomatis* infection in infants less than six months of age hospitalized with pneumonia. Infect. Genet. Evol. (2014), http://dx.doi.org/10.1016/j.meegid.2014.11.004