Detection of *Chlamydophila Pneumoniae* and Typical Bacteria in Patients with Chronic Cough

A. Jama-Kmiecik, M. Frej-Madrzak, G. Gosciniak, J. Sarowska, and I. Choroszy-Krol

Abstract

The aim of research was to analyze the results of microbiological tests for typical and atypical bacteria in patients with symptoms of chronic cough. A total of 214 outpatients aged from 2 to 94 years (110 women, 64 men, and 40 children) with chronic cough were studied. Four hundred twenty eight throat swabs were examined for atypical bacteria antigen (*Chlamydophila pneumoniae*) (n = 214) and typical pathogens (n = 214). *Chl. pneumoniae* detection was performed using indirect immunofluorescence test. Classical microbiological culture was used for typical bacteria detection. *Chl. pneumoniae* antigen was detected in 55/214 (26.0 %) patients with chronic cough (in 31 (28.2 %) women, 14 (21.9 %) men, and 10 (25.0 %) children). Positive culture for typical pathogens was observed in 30 (27.3 %) women, 22 (34.4 %) men, and 21 (52.5 %) children. Simultaneous occurrence of *Chl. pneumoniae* and typical pathogens (*Staphylococcus aureus* strain MSSA, *Streptococcus pyogenes*, or *Moraxella catarrhalis*) was found in 16 (7.5 %) patients. The findings show that in patients with chronic cough *Chl. pneumoniae* infection, although less than that with typical pathogens, is rather frequent. Further, the performance of test for *Chl. pneumoniae* in throat swabs from patients with chronic cough is good and provides an efficient way to diagnose the infection and implement appropriate therapy.

Keywords

Atypical bacteria • Co-infection • Immunofluorescence • Respiratory tract • Throat swabs

A. Jama-Kmiecik, M. Frej-Madrzak (✉), J. Sarowska, and I. Choroszy-Krol
Department of Basic Sciences, Wroclaw Medical University, 4 Chwałubińskiego St., 50-368 Wroclaw, Poland
e-mail: magdalena.frej-madrzak@umed.wroc.pl

G. Gosciniak
Department of Microbiology, Wroclaw Medical University, Wroclaw, Poland

1 Introduction

Respiratory tract infections represent a heterogeneous group of common acute infectious problems and consist of various underlying

causes and symptoms which are difficult to distinguish one another. Depending on the location, infections can be divided into upper (URTI) and lower respiratory tract infections (LRTI). The most frequent URTI are common cold, tonsillitis, pharyngitis (sore throat), and sinusitis. LRTI, in turn, are acute bronchitis and pneumonia. Clinical features of different respiratory tract infections largely depend on the affected anatomical structure and on inflammation induced functional alterations.

Respiratory infections are the most common cause of outpatient counseling. They constitute about 50–60 % of all community-acquired infections and are the most common cause of fever in infants and young children (Armstrong and Pinner 1999). Infections occur at different frequencies depending on the interaction of age and risk factors. The main cause of acute respiratory tract infections are viruses; most notably rhinoviruses, followed by adenoviruses, coronaviruses, influenza and parainfluenza viruses, respiratory syncytial viruses, and enteroviruses (Alter et al. 2011; Griffin et al. 2004; Monto 2004).

Likewise, bacterial infections are of highly variable etiology. Community-acquired bacterial infections are most often caused by microorganisms included in the group of typical pathogens, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pyogenes*. Identification of typical microorganisms is possible in every microbiological laboratory, and the waiting time for the outcome does not exceed 48 h. Belonging to the group of typical bacteria, although less frequently underlying the etiology of respiratory tract infections, are also gram-negative rods, for instance *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and anaerobic bacteria.

An important role in the development of respiratory tract infections also play atypical bacteria – *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, or *Legionella pneumophila* (Käding et al. 2014). In the diagnosis of atypical bacterial infections, molecular methods, such as polymerase chain reaction (PCR), are increasingly used. Serological studies, which are widely used in this type of infection, are primarily of

epidemiological importance (Woodhead et al. 2011). The aim of the present study was to analyze the results of microbiological tests for typical and atypical bacteria in patients with symptoms of chronic cough, the most typical feature of respiratory tract infection.

2 Methods

The investigation was performed in accordance with the Declaration of Helsinki for Human Research and the study protocol was accepted by the Ethics Committee of the Medical University of Wrocław, Poland.

The study group consisted of 214 outpatients (110 women, 64 men, and 40 children) aged from 2 to 94 years. The main criterion for inclusion in the study was persisting cough and hoarseness due to respiratory tract infection. The study covered the period September 2013 to September 2014. The analyzed material were pharyngeal swabs collected in the morning, after overnight fasting and before performing routine oral hygiene. Pharyngeal swabs were examined for atypical bacteria – *Chl. pneumoniae* antigen and for typical pathogens. *Chl. pneumoniae* antigen was detected using indirect immunofluorescence test (Chlamydia Cel PN-IFT Kit; Cellabs Pty Ltd, Sydney, Australia). Specimens were stained in two sequential steps; the first one using suspension with monoclonal antibodies which bind specifically to *Chl. pneumoniae* antigen and the second using FITC- conjugated goat anti-mouse antibodies to visualize *Chl. pneumoniae* microorganisms. Visualization of 4 or more chlamydial elementary bodies among epithelial cells was taken as the criterion of positive diagnosis.

Typical bacteria were detected using a classical microbiological throat swab culture. All swab specimens were taken before the onset of antibiotic therapy.

3 Results

Table 1 presents the results of pharyngeal swabs examination for *Chl. pneumoniae* and typical

Table 1 Distribution of pathogens found in throat swabs in patients with respiratory tract infection

	No. of patients	<i>Chl. pneumoniae</i>	Typical pathogens ^a	Co-infection
Women	110	31 (28.2 %)	30 (27.3 %)	9 (8.2 %)
Men	64	14 (21.9 %)	22 (34.4 %)	3 (4.7 %)
Children	40	10 (25.0 %)	21 (52.5 %)	4 (10.0 %)
Total	214	55 (25.7 %)	73 (34.1 %)	16 (7.5 %)

^a*Staphylococcus aureus* strain MSSA, *Streptococcus pyogenes*, *Moraxella* sp.

Fig. 1 Distribution of typical and atypical pathogens underlying respiratory tract infections

bacteria in 214 patients with chronic cough and hoarseness. *Chl. pneumoniae* antigen was found in 55/214 (25.7 %) patients; including 31 (28.2 %) women, 14 (21.9 %) men, and 10 (25 %) children. Typical pathogens were detected in 73/214 (34.1 %) patients; including 30/110 (27.3 %) women, 22/64 (34.4 %) men, and 21/40 (52.5 %) children. The most frequently occurring typical pathogen was *Staphylococcus aureus* MSSA, followed by *S. pyogenes* and *Moraxella catarrhalis*. Figure 1 presents the distribution of typical and atypical bacteria pathogens underlying respiratory tract infections manifesting with cough and hoarseness as well as the prevalence of co-infections with both types of pathogens. Co-infections occurred in 11 % of the patients in whom positive test results were found.

4 Discussion

The most common etiological agents of bacterial community-acquired respiratory tract infections include *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and atypical bacteria such as *Chlamydomphila pneumoniae*,

Mycoplasma pneumoniae, and *Legionella pneumophila*. Infections caused by atypical pathogens are not characterized by specific clinical course; therefore they are difficult to diagnose empirically. In addition, recommendations do not point to a single standardized diagnostic test which could distinguish between typical and atypical pathogens. A high percentage of specific antibodies to *Chl. pneumoniae* in the general population indicate the appreciable prevalence of infections with this microorganism. In a study of Miyashita et al. (2001), the occurrence of antibodies against *Chl. pneumoniae*, indicative of previous infection, was 58.8 % in men and 39.6 % in women, although the acute signs of infection were manifest in just 10.6 % of these cases. Acute phase of infection often leads to cough and hoarseness lasting many weeks, whereas chronic phase of infection is generally asymptomatic.

Epidemiological data on the incidence of infection caused by *Chl. pneumoniae* are variable, depending on the diagnostic methods used. Direct methods, such as PCR or cell culture, demonstrate that *Chl. pneumoniae* infection also affects young children (She et al. 2010; Michelow et al. 2004). Modern molecular techniques allow detecting the presence of pathogens in infants, where the widely used serologic tests usually fail to confirm the occurrence of infection caused by this pathogens (Verkooyen et al. 1998). Interestingly, publications of many authors point to large differences regarding the prevalence of *Chl. pneumoniae* in respiratory tract infections in children; ranging from about 10 % in hospitalized patients and 21–43 % in outpatients. However, the prevalence of *Chl. pneumoniae* may be underestimated due to a relatively low rate of acute infection requiring outright medical intervention (Choroszy-Krol et al. 2010, 2014;

Schmidt et al. 2003; Normann et al. 1998). Concerning the etiological factors of respiratory tract infections, it is important to consider co-infections, which are quite common. In the course of *Chl. Pneumoniae*-induced acute pharyngitis in children, this pathogen was found in 3 % of patients, while other co-infecting bacterial pathogens were isolated in 13 % of cases (Esposito et al. 2004). Schmidt et al. (2003) have demonstrated a much higher proportion of infections caused by *Chl. pneumoniae*, amounting to 28 %, in which the antigen was found in 25 %. The present results are generally in line with those findings, although we found a lower co-infection rate with other bacterial pathogens, which may be related to a different patient population studied or different diagnostic methods.

We conclude that prevalence of infection with *Chl. pneumoniae* is high, as it nears about two thirds of infections with typical pathogens, *Chl. pneumoniae* infection occurs frequently in children, and is often associated with co-infections.

Acknowledgments This research was supported by the Medical University in Wrocław (grant Pbm/60).

Conflicts of Interest The authors declare no conflicts of interest in relations to this article.

References

- Alter SJ, Vidwan NK, Sobande PO, Omoloja A, Bennett JS (2011) Common childhood bacterial infections. *Curr Probl Pediatr Adolesc Health Care* 41:256–283
- Armstrong G, Pinner R (1999) Outpatients visits for infectious diseases in the United States, 1980 through 1996. *Arch Intern Med* 159:2531–2536
- Choroszy-Krol I, Frej-Mądrzak M, Teryks-Wołynec D, Jama-Kmiecik A, Gosciniak G, Pirogowicz I, Pokorski M (2010) Respiratory infection caused by *Chlamydomphila pneumoniae* in children and adolescents in the Lower Silesia Region of Poland. *Eur J Med Res* 15:112–114
- Choroszy-Krol I, Frej-Mądrzak M, Sarowska J, Jama-Kmiecik A, Gosciniak G (2014) Detection of *Chlamydomphila pneumoniae* antigens in children in the Lower Silesia Region in 2011. *Adv Clin Exp Med* 23:411–414
- Esposito S, Blasi F, Bosis S et al (2004) Aetiology of acute pharyngitis: the role of atypical bacteria. *J Med Microbiol* 53:645–651
- Griffin M, Walker F, Ivane M et al (2004) Epidemiology of respiratory infections in young children. Insight from New Vaccine Surveillance Network. *Pediatr Infect Dis J* 23(Suppl 11):S188–S192
- Käding N, Szasz M, Rupp J (2014) Imaging of *Chlamydia* and host cell metabolism. *Future Microbiol* 9:509–521
- Michelow I, Olsen K, Lozano J, Rollins NK, Duffy LB, Ziegler T, Kauppila J, Leinonen M, McCracken GH (2004) Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 113:701–707
- Miyashita N, Niki Y, Nakajima M, Fukano H, Matsushima T (2001) Prevalence of asymptomatic infection with *Chlamydomphila pneumoniae* in subjectively healthy adults. *Chest* 119:1416–1419
- Monto A (2004) Occurrence of respiratory virus: time, place and person. *Pediatr Infect Dis J* 23:58–64
- Normann E, Gnärpe J, Gnärpe H, Wettergren B (1998) *Chlamydomphila pneumoniae* in children with acute respiratory tract infections. *Acta Paediatr* 87:23–27
- Schmidt SM, Muller CM, Krechting M, Wiersbitzky H, Gürtler R, Wiersbitzky SH (2003) *Chlamydomphila pneumoniae* carriage and infection in hospitalized children with respiratory tract diseases. *Infection* 28:410–416
- She RC, Thurber A, Hymas WC, Stevenson J, Langer J, Litwin CM, Petti CA (2010) Limited utility of culture for *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae* for diagnosis of respiratory tract infections. *J Clin Microbiol* 48:3380–3382
- Verkooyen R, Willemsse D, Hiep-van Casteren S, Mousavi Joulandan S, Snijder R, van den Bosch J, van Helden H, Peeters M, Verbrugh H (1998) Evaluation of PCR, culture and serology for diagnosis of *Chlamydomphila pneumoniae* respiratory infections. *J Clin Microb* 36:2301–2307
- Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Ieven M, Ortgvist A, Schaberg T, Torres A, van der Heijden G, Read R, Verheij TJ (2011) Guidelines for the management of adult lower respiratory tract infections. *Clin Microbiol Infect* 17:E1–E59