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USEFULNESS OF LABORATORY METHODS IN DIAGNOSIS OF PERTUSSIS IN ADULT WITH PAROXYSMAL COUGH*

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ABSTRACT

INTRODUCTION. Pertussis is an acute, highly contagious bacterial infection of respiratory system caused by *Bordetella pertussis*. Principally, disease affects young children, however, recently it is also reported in adolescents and adults. Symptoms of pertussis in adults are non-specific, i.e. dry, paroxysmal and protracted cough. Thus, it is rarely diagnosed in this group.
AIM. This paper aimed at evaluating the usefulness of the laboratory methods in diagnosis of pertussis in adults based on a case presenting with dry, paroxysmal and chronic cough.
MATERIAL AND METHODS. Sputum (collected on 25th January 2013) and paired serum samples (collected on 13th February and 19 April 2013) were tested. Pertussis diagnostics involved culture, in-house PCR, real-time PCR and ELISA.
RESULTS. Sputum culture, using commercial medium Bordetella Selective Medium by Oxoid did not reveal the presence of *B. pertussis*. Real-time PCR and PCR, however, confirmed the presence of insertion sequence IS*481* and pertussis toxin promoter sequence *ptx*-Pr, markers indicative of *B. pertussis* infection. Serological testing revealed the high titres of IgA, IgG and IgM antibodies to *B. pertussis* in the first sample. In the second sample, collected 2 months following the first one, a significant decrease in IgA antibodies was reported.
CONCLUSIONS. These data suggest a high usefulness of the laboratory methods in the diagnosis of pertussis in adults with chronic cough. Application of such methods ensures adequate diagnosis of disease, quick introduction of proper treatment and implementation of procedures preventing the spread of infection.

Key words: *pertussis in adult, Bordetella pertussis, microbial diagnostics of pertussis, paroxysmal cough*

INTRODUCTION

Pertussis is an acute, highly contagious respiratory disease, caused by *Bordetella pertussis*, a Gram-negative, aerobic coccobacillus. Pathogenic mechanism of *Bordetella pertussis* consists mainly in releasing toxins, including pertussis toxin (PT) which damage ciliated respiratory epithelial cell, leading to the onset of characteristic symptoms. Transmission of pathogen occurs via aerosolized droplets or direct contact with respiratory secretions from infected persons. Pertussis incubation period ranges from 7 to 10 days. Dependent on disease course, the following stages may be listed: catarrhal, paroxysmal and convalescent (9, 12, 16).

A list of specific symptoms of pertussis include: paroxysmal cough with characteristic inspiratory wheezing sound (whooping cough), sputum expectoration and post-tussive vomiting. These are most commonly reported in infants and young children. Currently, in many developed countries, including Poland, pertussis is more frequently reported in older age groups (2, 3, 5, 8, 12, 13). Contrary to infants, the course of pertussis is

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usually mild and less symptomatic in adolescents and adults. They may often present with only dry, persistent, protracted cough, occurring especially at night (www.who.int). It should be highlighted that early diagnosis of pertussis in adults is of importance as they may be a source of *B. pertussis* infection to infants which may be a life-threatening condition for them.

Laboratory testing for pertussis include: bacterial culture, serological and molecular methods.

According to ECDC (www.ecdc.europa.eu), in addition to clinical criteria, for pertussis case confirmation, a positive test result is required by at least one of the following three methods, i.a. culture, molecular or serological methods as well as documented epidemiological link with laboratory-confirmed pertussis case. In Poland, pertussis is subject to mandatory reporting under the Act on Preventing and Combating Human Infections and Infectious Diseases (dated as of 5th December 2008). It is mainly confirmed based on symptoms and/or serological test results.

Pertussis is still relatively prevalent infectious disease, with cyclic increase in reported pertussis cases, occurring every 3-5 years (www.cdc.gov). From the WHO data transpires that approximately 16 million pertussis cases are reported annually worldwide. Of them, nearly 195,000 die. ECDC (1) estimates suggest that a total of 19,743 (16,897 confirmed cases) pertussis cases were notified by 27 EU/EEA countries in 2011. Incidence remained at 5.57 per 100,000 population. In 2011 and 2012, 1,669 (4.33 per 100,000 population) and 4,684 (including 32%, i.e. 1,501 hospitalized cases; incidence-12.16 per 100,000 population) pertussis cases were reported in Poland, respectively (10).

This paper aimed at demonstrating the usefulness of the laboratory methods in diagnosis of pertussis in adults based on a case presenting with dry, paroxysmal and chronic cough.

MATERIAL AND METHODS

Sample material. Sputum sample, collected from a non-hospitalized 43-year-old female presenting with dry, persistent cough on 25th January 2013, was subject to bacteriological and molecular testing. To confirm the diagnosis of pertussis and determine the dynamics of specific antibody titres, paired serum samples were collected from the patient (13th February and 19th April 2013).

Diagnostic methods applied:

1. **Culture.** Bacteriological testing of sputum for the presence of *Bordetella*, using commercial culture medium Bordetella Selective Medium by Oxoid.
2. **Molecular methods.** Preparation of genomic DNA from sample tested was obtained, using commercial "High Pure PCR Template Preparation Kit" (Roche), following the manufacturer's procedures.

   B. pertussis DNA was detected using commercial "Bordetella pertussis/parapertussis Real-Time Kit" by Diagenode. Real-time PCR was performed according to the manufacturer's procedures.

   In-house PCR was also used to detect the presence of *Bordetella* DNA. For identification, chromosomal markers were used, i.e. insertion sequence IS*481* and pertussis toxin promoter sequence *ptx*-Pr. Table I presents primer sequences and expected size of PCR products. The following thermal conditions were used for amplification of selected markers: initial DNA denaturation at 94°C for 10 minutes, and then 35 reaction cycles, including three stages: DNA denaturation at 94°C for 45 seconds, primer binding at 68°C for 45 seconds, DNA polymerization at 72°C for 60 seconds and final polymerization at 72°C for 5 minutes.

Table I. Diagnostics of paroxysmal cough in adult suspected of pertussis.Primer oligonucleotides used for PCR with expected size of PCR products.

Marker	Primer	Nucleotide sequence (5'-3')	Expected size of PCR products	References
ptx-Pr	BPpr-1	CGCCAAGCTGAAGTAGCA	172 bp	4
	BPpr-2	AAGGAGCGTTCATGCCG		
IS*481*	BP-1	GATTCAATAGGTTGTATGCATGGTT	180 bp	4
	BP-2	TTCAGGCACACAAACTTGATGGGCG		

ptx-Pr – pertussis toxin promoter; IS*481* – insertion sequence *IS*481, indicative of i.a. *B. pertussis*.

In PCR, DNA isolated from reference strains: *Bordetella pertussis* TohamaI (CIP 81.32=NCTC 13251=ATCC BAA-589) and *Bordetella parapertussis* (CIP12822=NCTC 13253=ATCC BAA-587) were used as positive controls.

3. **Enzyme-linked immunosorbent assay - ELISA:** Quantitation of antibodies to *B. pertussis* toxin and filamentous haemagglutinin in three immunoglobulin classes, using commerical assay - ELISA NovaLisa Bordetella (NovaTec Immunodiagnostica), according to the manufacturer's procedures.

RESULTS AND DISCUSSION

For many physicians, pertussis is still an infectious childhood disease which is rarely taken into account in

differential diagnosis of chronic cough in adults. In the light of trends in the epidemiology of pertussis observed worldwide, however, it is of importance to consider *Bordetella* as possible etiological agents of respiratory system diseases in adolescents and adults (2, 3, 5, 11).

In the case discussed, patient presented with dry, persistent, paroxysmal cough, causing abdominal pain. She experienced approximately 12 attacks in a day, occurring in the morning and night and lasting for a few or several minutes. Fever or other clinical symptoms were not present. Sputum was collected from patient about two weeks following the onset of cough. Material was sent to the Laboratory of the Department of Bacteriology of the NIPH-NIH. Medical history revealed that 9-year-old child of the patient, who completed a full course of vaccination, at the beginning of 2013 presented with dry, persistent and paroxysmal cough, leading to vomiting. Furthermore, serological test results suggested a history of infection with *Bordetella pertussis*. Therefore, sputum sample collected from patient was tested by molecular methods for the presence of atypical pathogens causing respiratory system infections and *Bordetella pertussis*.

Irrespective of the fact that isolation of *B. pertussis* by culture is recommended by the WHO and considered to be a gold standard in laboratory confirmation of pertussis, the attempts to isolate *B. pertussis* from clinical material failed. It could result from the fact that sputum sample was collected at a late stage of disease (more than two weeks following the onset of disease) as the probability of *B. pertussis* isolation rapidly decreases with time (3, 5). Another reason could be a clinical material itself. Sputum is not entirely adequate material for diagnosing pertussis, however, it is acceptable in adults (3, 14). The most reliable and recommended samples for identification of *Bordetella* are nasopharyngeal aspirates or nasopharyngeal swabs (9, 16). Additionally, patient's age could have an effect on the negative result of culture. It is thought that the probability of *B. pertussis* isolation in adults is lower compared to children (3).

Currently, culture is replaced by polymerase chain reaction (PCR) in a number of laboratories involved in diagnosis of pertussis worldwide. PCR enables to detect *Bordetella* DNA from the clinical material collected in the period ranging from early stage of disease to the week 5 of its duration. Compared to culture, PCR is of considerably higher sensitivity (especially in the late stage of disease or following the initiation of antimicrobial therapy) (7, 14). Furthermore, PCR provides test results in a short period of time, i.e. 1-2 days which is of importance as proper therapy may be initiated (7). Real – time PCR is a method which is recommended and applied to the largest extent by reference centers. There is a number of commercial kits for real – time PCR which are available on the market and these include primers which amplify one target sequence, i.e. insertion sequence *IS*481 of *B. pertussis*. According to the latest literature data, if exclusively sequence *IS*481 is to be used, false positive PCR results may be obtained. It was concluded that *IS*481 may be present in the genome of other *Bordetella* species, i.e. *B. bronchiseptica* and *B. holmesii* (4, 14). Therefore, specialist from reference centres recommend to use additional target sequence, i.e. pertussis toxin promoter region to increase the sensitivity of *B. pertussis* identification.

In the case discussed, *B. pertussis* DNA from the patient's sputum was detected by both commercial real – time PCR and own-developed PCR which confirmed also the presence of the second characteristic marker of *B. pertussis,* i.e. pertussis toxin promoter.

Based on molecular test results, the patients was subject to 7-day therapy with clarithromycin, administered in a dosage of 500 mg two times a day and then 250 mg two times a day.

In Poland, serological testing is predominantly applied in the diagnostics of pertussis. These tests detect specific antibodies in the serum of infected patients. Commercial immunoenzymatic test – ELISA is the most commonly used test in routine serum diagnostics. It allows for determining antibodies to *B. pertussis* toxin and/or filamentous haemagglutinin in immunoglobulin classes. Contrary to molecular tests, which allow for confirmation of infection at its early stage, serological testing provides etiology at later stage, usually 2-3 weeks following the onset of symptoms. In addition, interpretation of serological test results of persons suspected of pertussis is hindered by the presence of vaccine-induced antibodies, especially of IgG class. Therefore, serological diagnostics of pertussis should aimed at detecting seroconversion, i.e. diagnostically significant increase in antibody titres in at least two serum samples collected at 2-4 week intervals (6). It should not be forgotten, however, that if the first sample is collected at a later stage of disease, antibody titres may be so high that significant increase in antibody titres should not be expected in the second sample. It is assumed that an increase in antibody titres by 100% or their decrease by 50% in the next sample is the best possible evidence of active infection with *B. pertussis* (6). In the case analyzed, the first serum sample collected from the patient on 13th February, diagnostically significant increase in antibody titres for *B. pertussis* (> 11 NTU) was detected in three immunoglobulin classes, i.e. IgA (20.26), IgG (17.52) and IgM (20.08). Second serum sample, collected on 19th April, revealed diagnostically significant decrease in antibody titres of IgA class (9.87 NTU). Furthermore, a decrease in antibody titers was also noted in IgG and IgM classes (13.44 and 19.65, respectively).

SUMMARY

Having considered an increase in the number of pertussis cases, which is observed in many countries, including Poland, this infection should be referred to as re-emerging threat for public health, not only in the population of children, but also adolescents and adults. Increasing number of pertussis cases in older age groups is an important risk factor of *B. pertussis* transmission to unvaccinated or partially vaccinated infants and young children for whom this infection may be a life-threatening condition. Furthermore, failure to detect and diagnose pertussis in adolescents and adults may lead to underestimation of epidemiological data (15). Adults, presenting with protracted cough, which is a predominant and frequently the only symptom of disease, are rarely tested for infection with *B. pertussis*. It may be affected by difficulties associated with pertussis diagnostics, i.e. the cost of diagnostic tests or the possibility to perform exclusively serological testing. Such situation is also determined by the prevalent use of empiric antimicrobial therapy in respiratory system diseases which eliminates the possibility to detect etiological agent. Case discussed presents the usefulness of microbial laboratory methods in pertussis diagnosis, especially in adults. Performance of diagnostic tests and proper selection of methods, considering disease stage, enable to initiate adequate therapy and implement procedures preventing the transmission of infection.

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