BIOMEDIKA
ISSN: 2085-8345, E-ISSN: 2541-2582
Vol 16, No. 1 Februari 2024
doi: 10.23917/biomedika.v16i1.2799

Detection Of Bordetella Pertussis the Cause of Whooping Cough: A Narrative Review

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ABSTRACT

Bordetella Perussis transmits pertussis disease, known as whooping cough, through airborne secretions from the respiratory tract. The bacteria then attach to the epithelial cilia of the respiratory tract of other hosts. One of the main virulence factors produced by Bordetella pertussis is pertussis toxin (PTx) which in its inactivated form is the main component of all acellular pertussis vaccines. In addition, PTx also activates several receptors and can affect various signaling pathways independent of ADP ribosylation and adenylate cyclase. PTx has been used in many research fields due to its strong ADP-ribosylation properties. To detect PTx Bordetella pertussis can use diagnostic tests such as serology test. Aim of this literary study is to determine the types of examinations for the detection of Bordetella pertussis. This study used a narrative review method sourced from searches on PubMed and ScienceDirect. The results of this search showed various types of Bordetella pertussis detection, including detection by culture, serology, and PCR. In conclusion, culture is still recommended to assess vaccine efficacy and future vaccine development, serological tests are one of the main diagnostic procedures in cases of unvaccinated adolescents and adults using specific B. pertussis proteins as antigens, in the presence of increased IgA or IgG titers against the toxin pertussis (PT) and filamentous hemmagglutinin (FHA). PCR as a diagnostic tool for pertussis in neonates and children.

KEYWORDS:
Bordetella pertussis, Whooping cough, Filamentous hemmagglutinin (FHA), Pertussis toxin

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INTRODUCTION

The respiratory infection pertussis, also referred to as whooping cough, is brought on by the bacteria *Bordetella pertussis*. Recurrent coughing fits that finish in a gasping "whoop" are the hallmark of the classic pertussis cough, which can linger for weeks.(1) According to historical accounts, pertussis first surfaced about 1000 years ago, and genetic research indicates that the *Bordetella pertussis* bacteria has been linked to humans for millions of years. There was a pertussis epidemic in Persia in the 15th century, which contributed to the numerous newborn and young child deaths in Paris in the summer of 1578. Whooping cough was also noted in early 16th-century British documents and the 1701 London Bills of Death.(2)

The bacterium that is Gram-negative The cause of whooping cough is *Bordetella pertussis*. It is a deadly and contagious respiratory illness that poses a particular hazard to premature newborns who have received vaccines or who have not completed the full course of shots.(3) The condition typically manifests as a paroxysmal cough and distinctive cry. Vomiting may come after coughing, and the symptoms may persist for several months. Pneumonia, pulmonary hypertension, febrile seizures, encephalopathy, and brain bleeding are more serious consequences. *B. pertussis* is known to produce number of virulence factors, including the *pertussis toxin* (PTx),

dermonecrotic toxin, tracheal cytotoxin, and *adenylate cyclase toxin*. Pathogenesis is also influenced by proteins that are involved in cell binding, such as *haemagglutinin* (FHA), *fimbriae* (FIM), and *pertactin* (Prn) *filaments*. A two-component signal transduction system (BvgAS) that is controlled by environmental conditions in the host, such as temperature, regulates the synthesis of these components.[4] Attachment to the tracheal epithelium is the first stage of the pathogenesis, which is followed by colonization that results in localized damage to the airways. The severity of the condition is influenced by the patient's age and immunocompetence, with newborns being the most severely impacted.[5]

Since the advent of vaccination, pertussis has been successfully controlled. However, the illness is resurfacing and is thought to be a condition that can be prevented by vaccination, even in wealthy nations with high immunization rates.[6] Since pertussis first emerged, nations all over the world have started to emphasize the necessity for sensitive and precise diagnostic techniques for *B. pertussis* diagnosis.[7]

Pertussis laboratory testing still has a lot of issues and restrictions. Because culture-based detection takes 7–10 days, it is impossible to identify *B. pertussis* infection at an early stage.[8] The limited use of serological tests in patients who have received vaccinations is due to the false positive outcomes of serological diagnostics.[9] For the diagnosis of *B. pertussis* infection, molecular-based detection methods such as real-time PCR (RT-PCR), nested PCR, and multiplex real-time PCR have been developed. This essay will go over the traits, etiology, clinical signs, pathophysiology, and tests for diagnosing Bordetella pertussis.[10]

GENUS *Bordetella pertussis*

There are six species of *Bordetella pertussis* that can be seen in a biological overview of the genus *Bordetella* (Table 1). Humans are susceptible to *Bordetella pertussis* infection, particularly in the respiratory system. No longer a parasite of humans, *Bordetella parapertussis*. Since being isolated from pneumonia-free sheep in Scotland and New Zealand.[11] There is no proof of *B. parapertussis* transmission between humans and sheep because recent genetic research have revealed that the human and sheep strains belong to separate populations. Although there is a chance of animal-to-human transfer, *Bordetella bronchiseptica* rarely affects people when it is isolated from other animals or birds. It is a significant respiratory tract pathogen for many different animals and birds. It seems to be more of an opportunistic and respiratory commensal pathogen in humans, occasionally being linked to septicemia in people with impaired immune systems. The respiratory illness *Bordetella avium* affects young turkeys and other birds.[12]

Table 1. Host Specificity of *Bordetella* Species

Spesies	Host	Site of isolation (Man)
B. pertusiss	Man	Respiratory tract
B. parapertussis	Man, sheep	Respiratory tract
B.bronchisepta	Animals, bird, man	Respiratory tract,blood
B.avium	Birds	
B.hinziibird,	Birds, man	Respiratory tract, blood
B.holmesii	Man	blood

The respiratory ciliated epithelium is a favorite target for the obligatory, non-invasive parasite *Bordetella*, which affects mammals including birds. Recent observations have cast doubt on this definition. For instance, *Bordetella* species' capacity to control the expression of virulence factors in response to environmental cues strongly suggests that they have habitats elsewhere besides the surface of the respiratory tract where such regulation would take place. This environment raises the possibility that *B. pertussis* and other species may penetrate and persist inside the host, or that they may be present at various locations simultaneously or on other hosts. As was previously indicated, human blood has isolated a number of species. primarily from immunocompromised hosts, albeit there isn't any conclusive data to support this. It's possible that the frequency of these isolates will rise.[13]

CHARACTERIZATION of *Bordetella pertussis*

A tiny, encapsulated, non-motile coccobacillus with exterior pili, *Bordetella pertussis* is Gram negative and non-motile. The typical size range of *Bordetella pertussis* is 0.5 to 1.0 nm. Although *Bordetella pertussis* is aerobic and develops best around 35°C to 37°C, it also needs nicotinamide supplementation, and the presence of fatty acids, metal ions, sulfides, and peroxides in the media can limit growth. The production of extracellular toxins like invasive adenylate cyclase, tracheal cytotoxins, and pertussis toxin (PT) is a major factor in the pathogenicity of this organism.[14]

Because *Bordetella* species' morphology differs greatly from that of other pathogenic species', it is simpler to distinguish between them using their morphological traits. Usually seen in clinical samples, *Bordetella pertussis* cells can occasionally be filamentous and can reach lengths of several millimeters. Since *Bordetella pertussis* lacks flagella, it can be distinguished from other bacterial species including *B. bronchoseptica* and *B. avium*. A mucous sheath encases or surrounds the cells. Typically, newly isolated species show capsules, but in vitro slime production takes the form of biofilms. The expression conditions for the polysaccharides that make up the mucosal membrane and capsule remain unknown. Fimbriae are 110–250 nm long and 3-5 mm broad. Lipopolysaccharides, which are kept together by lipid units and linked by lengthy sugar units, make up the outer membrane. With a different phosphate (can be seen in (Figure 1).[15]

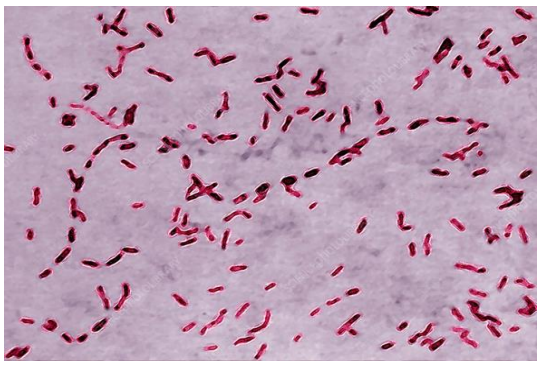

Figure 1. *Bordetella pertussis* on Gram stain

PATHOGENESIS and VIRULENCE OF *Bordetella pertussis*

Filamentous haemagglutinin

It is crucial for *B. pertussis* to produce filamentous haemagglutinin (FHA), which is produced as a preprotein (FHAB) and then transformed into a mature FHA molecule. FHA reduces airway inflammation by acting as an anti-inflammatory. This immunomodulatory function may hamper the efficacy of the acellular pertussis vaccination as FHA is a component of it. In contrast, the N-terminal segment of 80 kDa FHA stimulated the secretion of various cytokines but not interleukin (IL)-10 in dendritic cell-derived human monocytes exposed to completely FHA. This was discovered by Locht et al. in 2016. Consequently, the FHA fragment might function as a better vaccine antigen than the full-sized molecule.(16). The FHA molecule stimulates responses through pattern recognition receptor Toll-like receptor (TLR)2 but not TLR4 or TLR5, as determined by Villarino et al. in 2017; the TLR2 stimulation region of FHA is located in the central C-terminal fragment of the known domain. Since this domain is absent from the 80 kDa N-terminal fragment of FHA, this TLR2-stimulatory activity may be responsible for FHA's ability to elicit IL-10 production in its fulllength form. Nonetheless, Sebo et al. (2017) reported that the activity of FHA preparations that elicit cytokines and stimulate TLR2 is due to endotoxin contamination, making the findings regarding FHA's immunostimulant properties questionable. Cotter *et al.* (2016) studied the closely related pathogen *Bordetella bronchiseptica* in a mouse model of respiratory infection and discovered that the preprotein FHAB appears to play a role in bacterial persistence in the airways. Deletion of two C-terminal subdomains of FHAB had no effect on mature FHA production, adherence, or suppression of inflammation, but accelerated clearance of the mutant strain from infected mice's airways. They hypothesize that the transmembrane signaling of this FHAB subdomain aids bacterial resistance to the initial immune response of the host, and determining the mechanism underlying this activity will be an intriguing challenge. This indicates that Fha is essential for pathogenesis and vaccine considerations, whereas IL-10 is an important stimulatory activity to comprehend because it may be a cause of immunosuppression in pertussis infection or vaccination.[17]

Fimbriae

The pathogen Bordetella produces fimbriae (Fim), which is thought to be an adherent factor. Guevara et al. studied *B. pertussis* adhesion to

human bronchial epithelial cells in 2016. Mutations in the major fim2 and Fim3 fim D subunits and the adhesin minor fim D subunit significantly reduced bacterial adhesion to cells, and the addition of fimbrial subunits could competitively inhibit bacterial adhesion.[18] Cotter *et al.* discovered *B.bronchiseptica* in 2017 Fim mediates bacterial attachment to the airway epithelium and, in conjunction with Fha, can inhibit the airway inflammatory response. If this also holds true for *B.pertussis*, then Fim in the pertussis vaccine may be beneficial in reducing respiratory tract bacterial colonization, while avoiding the immunosuppressive property to the greatest extent possible.[19]

Adenylate Cyclase Toxin

Adenylate cyclase toxin (Act) targets phagocytic cells via binding to αMβ2 complement receptor integrin 3 (CR3, also known as CD11b/CD18), enters cells to increase *cyclic adenosine monophosphate* (cAMP) levels via the adenylate cyclase domain and forms cation-selective pores in the cell membrane via the hemolysin/repeats in toxin (RTX) domain. Hewlett *et al.* in 2016 found that Act inhibits apoptosis and neutrophil formation by increasing cAMP and inhibiting oxidative stress and contributing to neutrophils. Sebo (2016) found Act binding to the C-terminal site of CD11b by N-glycosylation of multiple residues in this CR3 site. Furthermore, Act will bind to an integrin that is different from the integrin's ligand-binding domain. Binding does not result in signaling from CR3, and cAMP elevation mediated by inhibition of CR3 signaling is induced by other ligands. Sebo (2016) also found Act-mediated cAMP signaling via protein kinase A which activates the tyrosine phosphatase protein Srchomology 2 domain protein tyrosine phosphatase 1, which can suppress TLR4-stimulated nitric oxide synthase (NOS) gene expression and bactericidal production of nitric oxide thereby increasing survival of *B. pertussis* in macrophages. In addition, cAMP signaling mediated by dendritic cells can reduce the capacity and enhance T cell stimulation of immunosuppressive IL-10 production.[20]

Detection of *Bordetella pertussis*

Culture, storage and antimicrobial susceptibility test

B. pertussis is a finicky bacterium; therefore, NPS/NPA should be conveyed to the microbiology laboratory at room temperature within four hours of sampling. Transport times of up to 48 hours are permissible when an appropriate transport medium (such as Reagan Lowe's medium) is utilized. Therefore, the culture-based diagnosis of pertussis in infants and young children is extremely useful. A positive diagnosis is typically possible within two to three weeks of wheezing onset. After transport, NPS or NSA was streaked onto freshly supplemented Regan-Lowe (RL) or Bordet-Gengou (BG) media containing 15% defibrillated sheep or horse blood. The sluggish growth rate of *B. pertussis* necessitates the use of selective media to inhibit the growth of

the normal flora. For this purpose, Regan-Lowe's medium contains cephalexin, and cephalexin (40 g/ml) can also be added to Bordet-Gengou medium incubated for 7 days at 35–36°C and 60% humidity with aerobic conditions. After seven days of incubation, *B. pertussis* grew more slowly on BG media than on RL, but there is a difference between BG and RL: the ability to visualize hemolysis. You can use a specific B. pertussis agglutination test or PCR as a confirmatory test. Two distinct storage media can be utilized: 1) Trypticase Soy Broth (TSB) - 10-20% Glycerol vial or 2) Bovie Serum albumin–saccharose/ phosphate/ glutamate (SPG) buffer.[21] In the antimicrobial susceptibility test using Macrolides [erythromycin (ERY) and azithromycin (AZT)], which are first-line medications for treating *B. pertussis* infection. To date, macrolide-resistant isolates have been discovered primarily in China, but also occasionally in the EU/EEA, the Middle East, and North and South America. The prevalence of macrolide-resistant isolates has increased, particularly in China. According to studies conducted in Beijing (2013–2014), Zhejiang province (2016), and Shanghai (2016–2017) by Chinese researchers, the prevalence of *B. pertussis* macrolide resistance ranges from 60% to 92%. These isolates were discovered in various regions of China, indicating that they did not spread clonally in a single area. Recently, macrolide-resistant *B. pertussis* isolates have emerged in China's neighboring countries of Japan and Vietnam. A point mutation change of the A to G nucleotide at position 2047 (A2047G) in the V domain of the 23S rRNA gene of B. pertussis is the only identified mechanism for macrolide resistance to date. It remains to be determined whether additional mutations or molecular alterations in the genome contribute to macrolide resistance. Consequently, the antimicrobial susceptibility test (AST) based on culture is the gold standard for determining macrolide resistance. As measured by the minimum inhibition concentration (MIC) test, all resistant B. pertussis isolates were extremely resistant (>256 g/mL) to macrolides (both ERY and AZT), whereas the sensitive isolates exhibited no tolerance to macrolides (both ERY and AZT). ERY and AZT levels 0.250 g/mL).[22]

Blood sampling to detect anti-PT IgG antibodies

Serological assays are used to determine the validity of serum; therefore, the use of serum tubes without additives is recommended. Many different manufacturers produce serum containers. If the serological test of preference has been validated, plasma heparin or plasma in ethylenediaminetetraacetic acid (EDTA) tubes may also be used. After two to three weeks of coughing, a serologic diagnosis consists of the detection of specific anti-PT antibodies in the sera of an infected individual. However, serological testing is not recommended for infants because their immature immune systems can lead to false positives or

negatives, or for patients who have been vaccinated within the past year. One year after vaccination, serology cannot be used for diagnosis because it cannot distinguish between vaccine-induced antibodies and infection.[23]

Sampling of oral fluids for detection of anti-PT IgG antibodies

The serological diagnosis of pertussis is most accurate in coughing patients. Antibodies against PT are targets in serology, and only B. pertussis produces PT. Serology is particularly beneficial for diagnosing children as young as two, adolescents, and adults. Pertussis is more likely to be diagnosed in its early phases in infants and young children. ELISA or multiplex immunoassays (MIA) can be used to perform pertussis serology by detecting specific B. pertussis antibodies to PT.[24]

In general, a range of 100 or 125 IU/mL serves as an indicator of recent infection within the past year, and a range of 50/62.5 IU/mL to 100 IU/mL serves as an indicator of recent infection within the past few years. A comparison of data from Denmark, the Netherlands, and the United Kingdom suggests that the optimal sensitivity and specificity for anti-PT IgG could be between 60 and 75 IU/ml. The diagnosis is primarily dependent on a single serum sample from a patient. If the diagnosis cannot be confirmed from a single sample and a second sample is deemed necessary based on the clinical presentation, a second (convalescent) serum sample should be obtained two to four weeks after the initial sample. If the second sample yields anti-PT IgG concentrations above the cutoff, or if a 100% increase or 50% decrease in values above or below the diagnostic quantification level in anti-PT IgG is observed between the first and second samples, the results can be interpreted as confirming the diagnosis. Because recent pertussis vaccination will result in elevated concentrations of anti-PT IgG antibodies, serological diagnosis of pertussis is not advised for at least a year after vaccination.[25]

PCR for *Bordetella* Nucleic Acid Detection

Because B. pertussis predominantly adheres to the ciliated epithelium of the airways, it is preferable to collect a diagnostic sample from the nasopharynx (rather than the nose or throat). Aspirates and swabs may be utilized, with rayon, nylon, or dacron swabs being preferred over cotton or calcium alginate. Prior to PCR analysis, DNA must be extracted using one of several available commercial products. There are multiple plausible genetic targets for the detection of Bordetella species. Due to the fact that several of these targets are present in the genomes of multiple Bordetella species, the choice of target and interpretation of the results is crucial. Insertion elements (IS) are utilized most frequently, particularly IS 481 and SI 1001. IS 481 is found in *B.pertussis, B. holmesii*, and several *B.bronchiseptic* isolates, whereas SI 1001 is found in *B.parapertussis* and several *B.bronchiseptic* isolates.

Insertion elements (IS) occur in a high number of copies within the genome, thereby increasing the sensitivity of detection. Numerous laboratories and test kits have interpreted a positive result for IS-481 as confirming *B. pertussis* and a positive result for IS-1001 as confirming *B. parapertussis.*[26] For definitive confirmation of *B. pertussis*, specific *B. pertussis* assays targeting the pertussis toxin promoter (ptxP) for *B. pertussis* or the IS 1001-like element for *B. holmesii* are recommended as additional targets. For optimal sensitivity and specificity, it is highly recommended to use controls, including controls for extraction, internal amplification, and positive and negative controls for the PCR step. PCR is particularly useful in diagnosing pertussis in infants and young children because they are more likely to be diagnosed in the early phases of infection, whereas adults tend to wait longer before seeking medical attention. Because the disease is extremely contagious, it is recommended to use serology (anti-PT IgG) or PCR in secondary cases if the cough lasts for two to three weeks or more. Quantitative PCR has several advantages over block-based PCR, as qPCR provides quantitative results and is quicker. It is possible to monitor the results in real time, and product-amplified agarose gel-based PCR detection is superfluous. In order to avoid the possibility of false positive results due to the high number of PCR cycles, block-based PCR or qPCR typically utilizes no more than 40 cycles. As a number of respiratory pathogens can be detected simultaneously, multitarget PCR combination analysis is widely used for the diagnosis of pertussis in clinical microbiology laboratories today. Although this method is useful for the detection, identification, and differentiation of respiratory pathogens and increases the number of pertussis diagnostics performed, the sensitivity of the kits developed is not as high as that of the PCR designed specifically for pertussis diagnostics. According to research and information from reagent manufacturers, this multi-target PCR can detect up to 22 distinct pathogens. In clinically confirmed cases of pertussis, multiplex PCR demonstrated a lower rate of pertussis confirmation positivity (67%) when compared to single-target pertussis-specific PCR. Therefore, the sensitivity of multi-target PCR for diagnosing pertussis in samples with minimal DNA/bacterial content was diminished. IS element pocesses high sensitivity but low specificity, whereas ptxP PCR possesses low sensitivity but high specificity. IS elements (IS 481 and SI 1001) and ptxP as targets constitute the optimal analysis for PCR-based confirmation of pertussis.[27]

CONCLUSION

The diagnosis of whooping cough is predominantly based on the direct detection of *B. pertussis* nucleic acid (PCR), the culture of nasopharyngeal samples, and the recognition of specific antibodies (serology) in the serum of

patients suspected of having pertussis. Although the use of culture has decreased from year to year, it is still advised because isolates can be used for molecular monitoring of bacterial population changes. Monitoring variants of circulating isolates is essential for assessing vaccine efficacy and future vaccine development. In addition, *B. pertussis* isolates will be essential for macrolide resistance monitoring and epidemiology. For the diagnosis of pertussis in neonates and young children, polymerase chain reaction (PCR) is highly recommended, while serology is the method most commonly used in adolescents and adults.

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