Central Asian Journal of Medicine

MICROBIOLOGICAL ASPECTS AND MODERN DIAGNOSTICS OF WHOOPING COUGH IN CHILDREN

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ABSTRACT

Despite widespread immunization efforts, whooping cough tenaciously persists as a global health concern. Frustratingly, the ideal diagnostic tool – one possessing high sensitivity and specificity, coupled with ease of reproducibility for seamless integration into clinical practice – remains elusive. The decades of the 1980s and 1990s, however, heralded a new era of understanding, yielding invaluable insights into the antigenic architecture of Bordetella pertussis and its arsenal of pathogenic weapons. This review delves into the pathogenic microbiological characteristics exhibited by isolated strains, illuminating the intricate mechanisms by which B. pertussis orchestrates the disease process. We explore the diverse pathogenic factors at play, dissecting their individual roles in disease progression and their collective contribution to the development of human immunity. Furthermore, we shed light on specific, often debated, facets of whooping cough pathogenesis. Finally, we present a critical assessment of current laboratory diagnostic methods for B. pertussis infection, carefully weighing their respective strengths and limitations, ultimately underscoring the ongoing need for improved diagnostic capabilities.

Key words: pertussis, Bordetella pertussis, pathogenic factors, pertussis toxin, laboratory diagnostics.

INTRODUCTION

The widespread adoption of mandatory vaccinations in the 1960s and 70s dramatically curtailed pertussis, slashing incidence rates and virtually eradicating mortality. However, the early 1990s witnessed a resurgence, fueled by an

unwarranted surge in medical exemptions for childhood vaccinations, igniting an epidemic that engulfed vast swathes of the nation, particularly major provinces. The crisis crested in 1994-1995, with a stark incidence rate of 32.6 per 100,000. In response, concerted efforts were launched to bolster vaccination coverage among children under four. By 1999-2000, vaccination rates had surged to 84.6%, a stark contrast to the mere 35.7% recorded in 1992. Consequently, the city's incidence rate receded, albeit lingering at 3-4 times the national average for Uzbekistan. Even now, in several Uzbek regions, including various provinces, confirmed pertussis cases remain deceptively low, often masked by delayed or inadequate examinations of suspected individuals and incomplete laboratory diagnostics. A troubling trend has emerged in recent years: pertussis cases among vaccinated children. This is a subject of intense scrutiny, particularly in the Baltic and Northern European nations, where pertussis tenaciously persists. In Norway, for example, despite a vaccination coverage exceeding 95%, the year 2000 saw a pertussis incidence rate of 76.3 per 100,000. Similarly elevated rates plague Sweden, Estonia, and other countries. These converging factors underscore the urgent need to synthesize current data on the biological nuances of Bordetella pertussis, its reliable detection, and definitive identification. This is critical to rigorously assess the efficacy of existing laboratory methods in diagnosing pertussis and adapting our strategies accordingly.

MICROBIOLOGICAL PROPERTIES OF BORDETELLA

In 1906, J. Bordet and O. Gengou meticulously isolated Bordetella pertussis, the elusive culprit behind whooping cough [1]. This bacterium resides within the Bordetella genus, a family that also shelters Bordetella bronchiseptica and Bordetella avium. The taxonomic landscape shifted in 1994-95 with the identification of two additional species: Bordetella holmesii and Bordetella hinzii. Initially considered solely a human pathogen, Bordetella pertussis was later recognized for its rare association with conditions like Alzheimer's disease and pneumonia [2], thus solidifying its place as a distinct genus. While Bordetella pertussis primarily targets humans, Bordetella bronchiseptica typically infects animals, with infrequent human cases (0.1–0.5%). Bordetella avium is exclusive to avian hosts. Bordetella holmesii, identified in 15 strains, has been predominantly found in immunocompromised individuals, alongside Bordetella avium and naphthalenic acid, particularly in intensive care unit patients [3-5]. Microscopically, Bordetella species appear as small (0.5-2.0 × 0.2-0.5 microns), Gram-negative coccobacilli, exhibiting a morphology described as smooth, glistening, and compact. Bordetella pertussis forms minute colonies (1-2 mm) after

a prolonged incubation period of 48-72 hours, in contrast to the faster-growing Bordetella parapertussis (24-48 hours) and Bordetella bronchiseptica (18-24 hours). Bordetella pertussis uniquely elicits enzymatic activity in eukaryotes (positive oxidase test). Bordetella parapertussis, a resourceful parasite, activates tyrosinase and urease enzymes (oxidase test). Bordetella bronchiseptica possesses a broader metabolic profile, exhibiting chiral acid, urease, oxidase, citrate utilization, and the ability to reduce nitrates and nitrites. In contrast, Bordetella parapertussis and Bordetella bronchiseptica exhibit minimal nutritional requirements, thriving even in simplified media. Further distinctions lie in their biochemical properties. Bordetella bronchiseptica and Bordetella avium possess buffer phosphatides in certain regions, potentially contributing to their infectious potential [6].

A poisonous toxin. Bordetella pertussis exerts its pathogenicity through a potent toxin (CT) found within its primary milieu. This exotoxin, a complex molecule weighing 117 kDa, comprises two functional moieties – A and B – intertwined with five tyrosine kinases. The A subunit, the engine of enzymatic activity, houses adenylate cyclase E, a pivotal enzyme that catalyzes ADP-dependent ribosylation, orchestrating a cascade of cellular events [7]. The B subunit, composed of the S2-S5 proteins, acts as the toxin's guide, binding to specific receptors and dictating its cellular target [8]. The body's immune response, specifically to serum antigens, is a key indicator, particularly pronounced in children under ten years of age exposed to CT [9-11]. While CT detection serves as a cornerstone in diagnosing and potentially treating pertussis-related lung complications, its absence in parapertussis unveils a compelling phenomenon. Despite the lack of CT's direct influence, patients with parapertussis exhibit a near-complete spectrum of symptoms, illuminating pathways beyond CT's sole dominion (as demonstrated in experiments designed to exclude mixed infections); leukocytosis [13] emerges as a prominent feature. Furthermore, the efficacy of immunization with filamentous antigens appears intricately linked to the patient's underlying immune status [12], underscoring the delicate balance between immune stimulation and potential immunodeficiency.

Agglutinogens, the sentinel surface proteins of Bordetella, orchestrate an immune response by provoking the synthesis of antibodies that bind to the bacterial cell, culminating in agglutination. Within the Bordetella genus, a repertoire of 16 distinct agglutinogens has been identified. Seven of these are shared across the entire Bordetella family, but agglutinogen 1 reigns supreme in Bordetella pertussis. Based on the presence or absence of agglutinogens 2 and 3, Bordetella pertussis is further classified into four serotypes: 2.0, 0.3, 2.3, and the

null type, 0.0. Beyond these defining factors, Bordetella pertussis also expresses agglutinogens 4, 5, 6, 13, 15, and 16, adding to its antigenic complexity. In contrast, agglutinogen 12 is the predominant type in Bordetella bronchiseptica, while agglutinogen 14 takes precedence in Bordetella parapertussis. Agglutinogens 8, 9, and 10 represent shared antigenic determinants between Bordetella bronchiseptica and Bordetella parapertussis, whereas agglutinogen 11 is uniquely exclusive to Bordetella bronchiseptica.

Agglutinogens are inextricably linked to fimbriae, sculpted into the shape of Fim proteins. A diverse array of these fimbrial proteins exists, including Fim2, Fim3, and FimX. Serotyping Bordetella pertussis hinges on the agglutination reaction, a dance between bacterial cells and monoclonal antibodies targeting these fimbrial proteins. The precise epitopes of Fim2 and Fim3, the very sites responsible for antibody binding, have been mapped, revealing that maximum activity of immunoglobulins A and G is orchestrated by distinct epitopes. Fimbriae themselves are constructed from both larger (Fim2 or Fim3) and smaller (FimD) subunits. The diminutive subunit finds purchase on Vla-5 receptors (integrins) gracing the surface of monocytes. Through the expansive reach of these fimbriae, pertussis bacteria seize sulfated sugars – chondroitin sulfate, heparan sulfate, and dextran sulfate among them – ubiquitous on the epithelial landscape lining the human respiratory tract. A comparative study of the heparin-binding region of Fim2, alongside its homologous counterparts in Fim3 and FimX, unveils a striking correspondence to basic amino acids and tyrosine residues.

Upon breaching the human body's defenses, bacteria unleash a sophisticated arsenal of genetically encoded adhesins, with the outer membrane protein pertactin playing a crucial role in this initial adhesion process. These adhesins, much like PHA, employ diverse mechanisms to secure their hold [14]. The lipopolysaccharide of Bordetella stands apart from its enterobacterial counterparts, distinguished by its unique composition of Lipid A and Lipid X. It is Lipid X that dictates the lipopolysaccharide's biological activity, while Lipid A, though exhibiting low pyrogenicity and non-toxicity, paradoxically retains antiviral capabilities, adjuvant properties, and the potential for broad-spectrum, nonspecific protection against certain bacterial invaders.

Further contributing to Bordetella's arsenal is tracheal cytotoxin, a fragment cleaved from the bacterial cell wall's peptidoglycan. This molecule, a member of the muramylpeptide family, exerts a multifaceted biological influence, acting as a pyrogen, adjuvant, and even exhibiting arthritogenic potential.

The dermonecrotizing toxin, a sinister agent, exerts a vasoconstrictive grip, insidiously diminishes body weight in experimental subjects, and leaves behind the

grim hallmarks of splenic atrophy and the blighted landscapes of ischemic or necrotic skin. Yet, its precise role in the unfolding drama of the disease remains veiled in uncertainty.

These pathogenic actors are all present in freshly isolated strains of the pertussis bacillus, a testament to their primal virulence. However, the artificial embrace of nutrient media proves a taming influence, rendering their pathogenicity capricious. As revealed by the discerning eyes of P. Leslie and A. Gardner, the pertussis bacillus undergoes a metamorphic journey through four distinct phases during saprophytization. The newly awakened microbe, a smooth (S) strain, bursts forth in Phase I, a paragon of virulence and immunogenic potency. But as it descends into the twilight of Phase IV, its immunogenicity and virulence wane, its cultural and biological essence transformed, leaving behind a mere shadow of its former self.

The production and release of antigens by microbial cells are intrinsically linked to the microorganism's growth phase. Our experiment focused on quantifying the pertussis toxin produced by Bordetella pertussis in a liquid culture. Intriguingly, during the lag and log phases, the toxin remained elusive. However, as the culture transitioned into the stationary maximum phase, a dramatic surge in toxin production occurred, only to be followed by a swift decline [15]. Further analysis, aimed at discerning free and cell-bound antigens, revealed that both pertussis toxin and pertactin were detectable in the liquid broth even at the nascent stages of growth. Moreover, the amount of cell-bound PHA exhibited a gradual decrease concurrent with culture growth, suggesting its liberation from the cell. Conversely, the concentration of the 92 kDa protein within the cell exhibited an inverse relationship, steadily increasing as the culture progressed.

Methods for detecting the pathogen and its antigens. Among the methods for detecting the causative agent of whooping cough, the main one was and remains bacteriological. The material for the study was taken from the mucous membrane of the upper respiratory tract. For the first time, J. Bordet and O. Gengou isolated Bordetella by culturing sputum collected at the end of a spasmodic cough attack. Later, Shiewitz and Meyer proposed the use of the "cough plate" method, but for this it was necessary to wait for a cough attack [1]. To eliminate this inconvenience, methods of active sampling of the material using a swab from the back of the throat through the lower nasal passages or through the mouth were proposed. J. Bordet and O. Gengou isolated the culture on potato-glycerol agar with the addition of 50% fresh blood (later the amount of blood was reduced to 20%). Then another medium was proposed - milk-blood agar, which gave good results, but at the same time it was necessary to add a large amount of blood. At

the same time, the production of vaccines required the growth of the pathogen in a medium that did not contain foreign proteins. For these reasons, research moved to the use of semi-synthetic media without the addition of blood or with a minimal amount of blood. Other work was aimed at eliminating the effect of foreign proteins on the growth of pertussis bacilli, which was achieved by adding activated charcoal to the medium [4]. To eliminate the growth of foreign microflora added during contamination, penicillin was added to the nutrient medium.

In medical practice, specimen collection involves swabbing the posterior pharynx, while in some countries, nasopharyngeal swabs are preferred. The latter technique is often deemed simpler and less prone to contamination. Direct inoculation follows, either onto a nutrient agar plate or into transport media. For short transport durations, under two hours, a 1% casamino acid solution suffices. If transport extends to a day, blood-free charcoal medium or Steiner-Scholte cyclodextrin broth is employed. For longer journeys, exceeding a day, charcoal-blood agar becomes the medium of choice. Comparative studies indicate that maintaining a temperature of 4°C is paramount. Pre-incubation in transport medium, specifically 50% charcoal-blood agar, at 35°C for one to two days may enhance culture growth, according to some researchers. Transport at 25°C, however, demonstrably diminishes positive test outcomes.

The primary nutrient medium for Bordetella isolation remains charcoal-blood agar (Regen-Lowe medium). This refined medium, derived from casein-charcoal agar, notably enhanced the method's efficacy and permitted the incorporation of only a minimal amount of blood [1.2]. While horse or sheep blood are considered optimal supplements, human blood yields comparatively inferior results. Bovine serum (5%) or medium No. 199 (1%) can serve as alternatives or adjuncts to blood.

Within the sterile halls of international laboratories, cephalexin, at a concentration of 40 mg/L, stands as a vigilant sentinel against bacterial interlopers, while amphotericin B (50 mg/L) is occasionally invoked to quell the uprising of fungal colonies. Cephalexin, in contrast to the blunter instrument of penicillin, delicately sidesteps the intricate choreography of Bordetella pertussis and B. parapertussis, displaying instead a decisive hand against the associated, less welcome microflora. Incubation unfolds at a constant 35°C, within an atmosphere of ambient air, nurtured only by a generous humidity. The time-honored Bordeaux-Jangue medium persists in its utility, offering results that rival the opulent Regen-Lowe (coal blood agar). Yet, the bacteriological method, possessing a theoretical potency of 80%, often finds itself mired in practical realities, achieving a mere 10-20% success rate. This frustrating divergence arises from the pathogen's inherent

capriciousness, its agonizingly slow pace of proliferation, the ever-looming threat of contamination, infrequent forays into testing, imperfect sampling methodologies, protracted patient assessments, and the fluctuating quality of the very sustenance provided by the medium. The lingering shadow of prior antibacterial exposure further diminishes the test's diagnostic promise.

It's worth noting a certain observational bias: clinicians often direct less scrutiny towards the precise epicenter of infection. However, targeted investigations, laser-focused on the specific nidus of the disease, demonstrably amplify pathogen detection rates, employing not only traditional cultures but also a broader spectrum of diagnostic techniques. This phenomenon stems, in part, from widespread vaccination efforts, which, while beneficial, often cloak infections in asymptomatic or minimally symptomatic guises, particularly within the 4-6 year-old demographic where post-vaccination immunity often peaks. The corroboration arrives via a collaborative study spanning Finland and Switzerland, revealing a higher incidence of pertussis among Swiss children aged 1-6, as compared to their Finnish counterparts, who benefit from a booster dose at age 2. Further highlighting this nuance, Finland sees a greater prevalence of subclinical infections in children under 7, painting a picture of a thriving, albeit silent, reservoir of carriers. In adults, pertussis frequently slips under the diagnostic radar, likely due to its attenuated symptomology, absence of the characteristic leukocytosis, and the ingrained perception of pertussis as a purely pediatric ailment. A Japanese study cast a revealing light: in 11% of familial pertussis cases, the adult population emerged as the unsuspected source. Moreover, a striking 61.3% of adults exhibiting clinical pertussis saw their diagnosis cemented through bacteriological or serological confirmation. Even more intriguingly, 25% of asymptomatic adults, upon closer examination, were revealed to harbor subclinical infections.

The polymerase chain reaction (PCR) stands as a modern beacon in diagnostic methodology. After the initial unveiling of DNA, the method deftly amplifies the desired genetic fragment of the microorganism, magnifying it until detectable. Howard et al illuminated the path for PCR's application in B. pertussis detection. Its sensitivity pierces through to reveal even a few bacteria lurking within a sample, while its specificity approaches near perfection. Results emerge with remarkable swiftness, often within a single day. PCR elegantly confirms positive culture results in a striking 73-100% of cases, mirroring the truth in a substantial 71% of instances. Intriguingly, in a small fraction of cases (6-11%), PCR unveils the pathogen's presence when traditional bacteriological examinations and enzyme immunoassays remain silent, hinting at its superior resolving power.

SEROLOGICAL METHODS

Serological methods for the laboratory diagnosis of pertussis hinge on discerning the levels of specific antibodies targeting specific antigens, or groups thereof, stemming from the pertussis bacillus. The time-honored serological approach, the agglutination reaction, a veteran of over half a century, serves to detect antibodies provoked by phase I agglutinogens of the pathogen. This reaction has been a mainstay in evaluating both post-infection and post-vaccination immunity.Its findings correlate with ELISA determinations of IgG and IgA concentrations, particularly at antibody titers exceeding 1:320 [10]. However, this method suffers from limitations, including a modest sensitivity and the absence of a standardized protocol.

The indirect hemagglutination reaction (IHA), employing erythrocytes from vaccinated animals sensitized with a complex of pertussis bacillus antigens, boasts a higher sensitivity than its agglutination counterpart. Yet, the diagnostic landscape is variegated, with differing sensitization and stabilization techniques for erythrocytes. The adsorption of antigen, whether whole pathogen cells or their components, has been executed via diverse methodologies, resulting in varying degrees of informational yield. Consequently, IHA remains relegated to a less prominent role in practical application. A notable variant of the agglutination reaction is the latex microagglutination reaction. This method operates on the principle of sensitizing polystyrene latex with components of pertussis bacillus cells, followed by mixing with serially diluted test substrate on a glass plate. Readout is facilitated by staining, leading to facile interpretation and enabling the detection of pertussis antibodies in the nascent stages of the disease. Nevertheless, the reliance on pathogen cell breakdown products as antigens renders this method susceptible to generating spurious results.

The enzyme-linked immunosorbent assay (ELISA) revolutionized pertussis antibody detection in the 1980s, swiftly eclipsing the traditional agglutination reaction with its superior sensitivity and ease. Early iterations of the ELISA utilized whole bacterial cells, antigen complexes, or partially purified individual antigens derived from the pathogen. However, the inherent cross-reactivity of several B. pertussis antigens with those of other gram-negative bacteria meant that the method's very sensitivity could be a double-edged sword, prone to yielding false positives.

The advent of purified B. pertussis antigens in ELISA test systems marked a pivotal advancement, immediately raising crucial questions about the individual diagnostic value of each antigen.

One pivotal study directly compared the immune response elicited by whole microbial cells to that induced by purified pertussis toxin (CT), filamentous hemagglutinin (PHA), pertactin, and a complex comprised of these three antigens [10]. The sensitivity varied significantly depending on the antigen employed: 54% for whole cells, 85% for PHA, 92% for CT, 62% for pertactin, and 85% for the CT-PHA-pertactin complex, across IgG, IgA, and IgM isotypes.

A comparative analysis of paired sera from patients of varying ages with confirmed pertussis revealed that CT elicited the highest rate of seroconversion and positive results in the initial serum sample. Notably, seroconversion rates were highest in infants aged 3 months and older adults over 15 years However, the potential influence of widespread vaccination on IgG levels to CT in children cannot be discounted. Consequently, a positive result may reflect either a high absolute level of IgG (relative to control sera from healthy children of the same age) or a demonstrable seroconversion (a significant increase in immunoglobulin levels). M. Viljanen et al. demonstrated that detecting IgM and IgA antibodies in serum, using whole cells as antigens, offered a rapid diagnostic tool, particularly for atypical pertussis cases where culture results were often negative without paired sera testing. When employing purified antigens, the most informative serological assessment involves quantifying IgG and IgA antibodies, particularly IgA to CT. Recognizing that pertussis immunity develops both locally and systemically, researchers have posited a crucial role for secretory IgA (sIgA). Studies have shown that artificial immunization does not induce an increase in sIgA levels. Therefore, detection of sIgA in nasopharyngeal mucosa may be indicative of active disease, even with negative bacteriological findings. In ELISA assays using purified antigens, sIgA to PHA was detected in 70% of nasopharyngeal secretions, while sIgA to CT was detected in 54% of cases.

In many countries, the diagnostic prowess of ELISA, bacteriological methods, and PCR—the stalwarts of pertussis diagnosis—has been rigorously compared. Yet, a crucial caveat emerges: ELISA's effectiveness blossoms only by the fourth week of the disease. Thus, while ELISA can corroborate the diagnosis amidst classic clinical presentations, it truly shines as a pivotal tool in unveiling elusive, atypical forms of infection. The efficiency of ELISA fluctuates dramatically, a chameleon adapting to its context: it dips to a mere 23% during broad screenings of children in schools, kindergartens, and health centers amidst a pertussis epidemic, yet soars to 68% during focused examinations of patients under the shadow of suspected pertussis.

In the tempestuous course of whooping cough, bacteriological and PCR methods exhibit their zenith in the initial weeks, peaking around week two with

positive results in 32% and 46% of cases, respectively. Conversely, ELISA crests later, at six weeks or beyond, revealing its presence in 54% of cases. One study unveiled that a mere 47% of examined cases were ultimately confirmed as pertussis. Another investigation, probing children afflicted with respiratory tract infections, discovered at least one positive test result in 37% of cases, a figure further refined to 26% by bacteriological validation and a more substantial 87% through serological confirmation.

Therefore, to navigate the intricate landscape of pertussis diagnosis in children, a dual-pronged approach is paramount: simultaneous deployment of nasopharyngeal culture and the meticulous determination of G, A, and M antibody levels against two key toxins, CT and PHA. While recent strides have undeniably augmented the diagnostic acuity of bacteriological methods, the field yearns for laboratory diagnostic tools that are not only more sensitive and specific but also readily reproducible, thus empowering practical healthcare with unwavering certainty.

CONCLUSION

1. Working conditions of seamstresses-motorists belong to class 3, 3rd degree of harm, they can cause professional risk of developing diseases in working women.

2. According to the "microclimate" factor, there is a risk of developing general somatic pathology of the respiratory system, circulatory system, digestion, skin, visual and auditory analyzers, musculoskeletal, genitourinary systems, blood disorders in the cold season and the risk of getting finger injuries due to sweaty palms in the warm period.

3. According to the noise factor, professionally caused 2nd degree of hearing loss is possible with 40 years of experience, and according to the vibration factor, there is a risk of developing vascular spasm of the hands.

4. According to the "severity of the work process" factor, there is a probability of developing functional and pathological disorders of the shoulder girdle muscles.

5. The intense nature of the work process of seamstresses indicates the likelihood of a professional risk of developing neurotic disorders, hypertension and ischemic heart disease.

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