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Community-acquired Pneumonia in Children in Lambarene, Gabon

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Abstract. Community-acquired pneumonia (CAP) accounts for more than two million deaths per year in children < 5 years of age. Recognition of pathogens is vital for guiding antibiotic treatment. In Gabon, no epidemiologic data on childhood CAP were available to help guide antibiotic therapy. We conducted a prospective, hospital-based, cross-sectional survey at the Albert Schweitzer Hospital, Lambarene, Gabon, to assess the importance of atypical organisms (*Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Bordetella pertussis*, and *Legionella pneumophila*) and *Streptococcus pneumoniae* in the etiology of CAP in children by means of real-time polymerase chain reaction, cell culture, and serology. Collectively, atypical bacteria accounted for 11% of cases with a special emphasis on *B. pertussis*, accounting for 6% of cases. Clinical differentiation of atypical from typical pneumonia in children remains challenging. Molecular diagnostic methods offer fast and highly sensitive diagnostic tools and would be able to help guide antimicrobial therapy in rural areas where follow-up is difficult.

INTRODUCTION

Community-acquired pneumonia (CAP) is a disease that demands a great amount of medical resources. Pneumonia kills more children than any other illness.[1] It accounts for more than two million deaths per year in children less than five years of age, mostly in developing countries. In Africa, 21% of the 4.4 million deaths of children less than five years of age per year are caused by pneumonia.[2] Most cases of severe pneumonia are caused by bacterial pathogens and prompt treatment with effective antibiotics is life-saving.[3] Knowing the pathogens that lead to pneumonia is critical for guiding antibiotic treatment and policies.

Although *Streptococcus pneumoniae* remains the most common bacterial etiologic agent in community-acquired pneumonia in children, atypical bacteria such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Bordetella pertussis* have increasingly been identified as important causes of CAP in children.[4,5] Atypical bacteria require specific antibiotic coverage because conventional antibiotics used against typical organisms such as streptococci are not effective. Increasing recovery rates of atypical cases of CAP have been reflected in treatment guidelines favoring antibiotics with atypical coverage for certain patient populations.[6] Little is known about the relevance of atypical organisms in childhood pneumonia in Africa. A recent study in the Gambia showed that 3.1% of children less than three months of age and 15% of children three months to five years of age with acute respiratory tract infection tested positive for *C. pneumoniae* by nasopharyngeal polymerase chain reaction (PCR) or lung aspirate PCR.[7] A study from Nairobi, Kenya assessed the prevalence of *M. pneumoniae* in nasopharyngeal aspirates in children less than five years of age with pneumonia. *Mycoplasma pneumoniae* was detected in nasopharyngeal aspirates of 33.7% of the cases by PCR.[8]

Part of why so little is known about atypical pathogens in childhood pneumonia is the challenging laboratory diagnosis. The use of serology is limited by cross-reactivity, delayed or abated antibody response, and the difficulty to differentiate present from past infections. *Chlamydia pneumoniae*, *M. pneumoniae*, and *Legionella* spp. can be difficult to isolate by culture because of special growth requirements. Time required for a final result may be prolonged because of the slow growth of these organisms. This finding and the fact that clinical differentiation between atypical and typical pneumonia in children is limited explains why the precise epidemiology of childhood pneumonia remains poorly defined. Newer diagnostic methods to identify atypical organisms include molecular methods. Molecular methods are sensitive and rapid. Conventional PCR has been demonstrated to provide excellent sensitivity for detecting atypical organisms in throat swabs or respiratory secretions.[9,10] Real-time PCR has been shown to be as effective or more sensitive than culture, serologic methods, or conventional PCR for detecting these organisms.[11–16]

The Albert Schweitzer Hospital in Lambarene, Gabon, is situated on the bank of the Ogooue River in tropical rain forest in western Gabon, approximately 90 km south of the equator. It serves as a primary source of healthcare for people in Lambarene and surrounding areas. There was no microbiology laboratory present on site. Treatment of pneumonia was therefore based on clinical presentation in conjunction with vital signs and basic blood tests. No epidemiologic data were available to help clinicians decide on proper therapy. In a patient population where follow up is difficult because of lack of infrastructure and resources, data on causative organisms in CAP would be important to help guide empirical antibiotic therapy. We conducted a cross-sectional survey to assess the importance of atypical organisms in CAP in children presenting to the Albert Schweitzer Hospital in Lambarene, Gabon, using molecular methods.

MATERIAL AND METHODS

Study design. A prospective, hospital-based, cross-sectional survey was conducted at the Albert Schweitzer Hospital, Lambarene, Gabon from September 2003 through June 2004. The study period included Gabon's rainy season, which is from September to March. The study was reviewed and ap-

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proved by the Albert Schweitzer Foundation ethics committee and the University of Tuebingen, Germany, ethics committee. Written consent embodied the elements of informed consent as described in the Declaration of Helsinki and also complied with local regulations. Information was given in both oral and written form. Consent was given in French and was documented by the use of a written consent form signed by the person's parent or guardian. A form of oral consent was provided for illiterate parents/guardians.

Patients. Patients were recruited in the pediatric ward and the pediatric outpatient department of the Albert Schweitzer Hospital in Lambarene, Gabon. Outpatients and hospitalized patients with CAP were included in the study. Male and female patients > 2 months of age and < 15 years of age with clinical and radiological signs of CAP were eligible for inclusion. Three or more of the following clinical symptoms were present: cough, fever, tachypnea, intercostal retractions or nasal flaring, wheezing, gastrointestinal symptoms, or malaise. Radiologic diagnosis was defined as a new infiltrate on a chest radiograph that could not be attributed to some other etiology. Refused consent, and prior hospitalization within two weeks before consultation were reasons for exclusion. Children diagnosed with severe malaria were excluded from the study. All patients were treated with a sequential parenteral and oral antibiotic regimen for presumed bacterial lower respiratory tract infection if hospitalized. Patients were treated with oral antibiotics alone if treated as an outpatient. Antibiotic use prior to study enrollment was defined as antibiotic use within the week before presentation. No patient had received the pneumococcal conjugate or polysaccharide vaccines or the *Haemophilus influenzae* type b conjugate vaccine. Two nasopharyngeal swabs, a urine sample, and a blood sample were obtained from patients at presentation. Demographic data was entered in a clinical record form.

Microbiologic testing. Two nasopharyngeal swabs were obtained from each study participant for isolation of *C. pneumoniae* by means of cell culture as described recently[17] and for detection of *C. pneumoniae*, *M. pneumoniae*, *B. pertussis*, *L. pneumophila*, and *S. pneumoniae* DNA by means of real-time PCR. DNA from nasopharyngeal swabs was extracted by means of the QIAamp DNA Mini Kit (body fluid protocol) (Qiagen, Hilden, Germany) and eluted in a volume of 70 μL. For every two specimens, one extraction negative control consisting of 200 μL of phosphate-buffered saline was processed. Quantification and determination of quality of DNAs was performed by means of spectrophotometric analysis (VersaFluor; Bio-Rad, London, United Kingdom). DNA was diluted in double-distilled water to a concentration of 0.125 μg/5 μL and was used as template for subsequent PCR analyses.

With reference to all five pathogens, two replicates of each specimen were analyzed. Specific sequences of primers and probes for the TaqMan-based real-time PCR assays were selected from the genes encoding for the major outer membrane protein of *C. pneumoniae*,[18] the P1 adhesion protein of *M. pneumoniae*,[19] the 16S ribosomal RNA of *L. pneumophila,* pneumolysin,[13] the sulfhydryl-activated toxin of *S. pneumoniae*,[20] and the IS481 insertion sequence of *B. pertussis*.[21] Data in context with sensitivity, specificity, and reliability as well as a comparison with the four conventional PCRs most widely used for the detection of *C. pneumoniae* were recently reported.[18] For the other four real-time PCR assays, dilution series of purified DNA (range corresponding to 10^4–10^{-1} colony-forming units/mL) of *M. pneumoniae*, *S. pneumoniae*, *L. pneumophila*, and *B. pertussis* were used to test published forward and reverse primers in every possible combination between 50 nM and 500 nM/PCR. Concentrations were chosen that gave the greatest yield of amplification product without any non-specific signals. Preliminary experiments for evaluation of the performance of the various PCR assays, if applied on nasopharyngeal specimens, were conducted as recently described in detail.[18]

Further preliminary experiments were performed to determine a consensus cycling protocol allowing for amplification of all five pathogens in one PCR. Finally, cycling conditions were chosen as follows: after 2 minutes at 50°C and 10 minutes at 95°C, the samples were submitted to 45 cycles, each consisting of a step at 95°C for 1 minute, followed by a step at 60°C for 1 minute. Real-time PCR was performed in 96-well MicroAmp optical plates (Applied Biosystems, Foster City, CA), and optical adhesive covers with reaction volumes of 25 μL were used. Amplification by means of the Brilliant Plus Quantitative PCR Core Reagent Kit including dUTP/AmpErase UNG as anti-contamination strategy (Stratagene, La Jolla, CA), and detection of the PCR product were performed with an ABI Prism 7700 sequence detection instrument (Applied Biosystems), as suggested by the manufacturer using all default program settings. The PCR results were considered negative for the respective pathogen if the cycle threshold value exceeded 45 cycles.

In addition, urine samples were obtained from the patients. The Binax NOW *S. pneumoniae* urinary antigen test and the Binax NOW *Legionella pneumophila* O1 urinary antigen test (Binax, Portland, ME) were performed according to the manufacturer's recommendations. Results were available within 15 minutes: one pink line was considered a negative result, and two pink lines were considered a positive result. At the same time blood was obtained for clinical and diagnostic purposes, an additional blood sample was drawn. *Mycoplasma pneumoniae* serologic analysis was performed by a hemagglutination technique (Serodia-Myco II; Fujirebio Diagnostics, Tokyo, Japan). *Chlamydia pneumoniae* IgM, IgA, and IgG antibodies were detected by means of an enzyme-linked immunosorbent assay (Labsystems, Helsinki, Finland). Microbiologic diagnostic criteria for infection are shown in Table 1.

Statistical analysis. Statistical analysis was performed with SPSS for Windows version 14.0 (SPSS Inc., Chicago, IL). Categorical variables are summarized as number (%) and compared using Fisher's exact test. Continuous variables are summarized as median and 25th and 75th quartiles (Q1 and Q3) and compared using the Wilcoxon rank sum test. Differences were considered to be significant if a two-tailed P was < 0.05.

RESULTS

Patient demographics. Of 103 children with clinical and radiologic signs of CAP who came to the Pediatric Department of the Albert Schweitzer Hospital from September 2003 through June 2004, four were excluded because they refused consent. Ninety-nine patients were enrolled in the study. Most of the children were hospitalized for treatment; only 16 patients were treated as outpatients. All children were from Lambarene or within 50 km of Lambarene. Fifty-three study

TABLE 1
Diagnostic criteria for infection with *Streptococcus pneumoniae*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila**

Bacteria	Diagnostic criteria for infection
S. pneumoniae	Nasopharyngeal swab real-time PCR positive and positive urine antigen test result
B. pertussis	Nasopharyngeal swab real-time PCR positive
C. pneumoniae	Nasopharyngeal swab real-time PCR positive and/or positive cell culture and/or IgM serology > 1.1. EIU
M. pneumoniae	Nasopharyngeal swab real-time PCR positive and/or antibody titer ≥ 1:640
L. pneumophila	Nasopharyngeal swab real-time PCR positive and/or positive urine antigen test result
	Diagnostic criteria for possible infection with *C. pneumoniae* and *M. pneumoniae*
C. pneumoniae	IgG > 45 EIU or IgA > 12 EIU
M. pneumoniae	Antibody titer 1:320

* PCR = polymerase chain reaction; EIU = enzyme immunounits.

participants were male. Median age was 21 months (Q1 = 11, Q3 = 49) months. The diphtheria-tetanus-pertussis vaccination rate was 71%. Demographic information is summarized in Table 2. There were no statistical significant differences in initial presentation between children diagnosed with atypical infections (*M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*) and the rest of the study participants (Table 3). Thirty-eight percent of the patients had received antibiotic therapy prior to study enrollment. Antibiotics most commonly used were amoxicillin and trimethoprim/sulfamethoxazole. Seven of the eleven patients in whom atypical pathogens were isolated received antibiotics prior to presentation.

Infections with *M. pneumoniae*, *C. pneumoniae*, *B. pertussis*, and *L. pneumophila*. Results are summarized in Table 4. Eleven patients had proven infections with atypical pathogens (*M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*). Additional patients were possibly infected with atypical organisms based on serologic analysis. No follow-up serologic analysis was conducted for a definitive diagnosis. Cases occurred throughout the study period, suggesting an endemic pattern. All children who tested positive for *Bordetella* spp. were not or only partially immunized. Patient demographics for patients with *Bordetella* app. infection are summarized in Table 5.

Infections with *S. pneumonia*. Infection with *S. pneumoniae* was thought to be likely in study participants with positive urine antigen test results for *Streptococcus* spp. in conjunction with positive nasopharyngeal PCR results. Thirty-five patients had positive *S. pneumoniae* urine antigen test results and positive streptococcal nasopharyngeal PCR results and were therefore thought to have infections with *S. pneumoniae*.

Mixed infections. No patient had an infection with more than one atypical organism. Four patients with *B. pertussis*, two patients with *M. pneumoniae* and one patient with *C. pneumoniae* also had infections with *S. pneumoniae.*

TABLE 2
Patient demographics*

Characteristic	No. (%)
Male sex, no. (%)	53 (53.3)
Age, months, median (Q1, Q3)	20.7 (10.8, 48.9)
People living in the same household, median (Q1, Q3)	7 (4, 10)
Smoking in same household, no. (%)	49 (49.9)
Families who cooked with fire, no. (%)	20 (20.2)
Families who used water from the local pump, no. (%)	62 (62.6)
Treated with antibiotics prior to presentation, no. (%)	38 (38.4)

* Categorical variables are summarized as no. (%). Continuous variables are summarized as median (25th quartile [Q1] and 75th quartile [Q3]).

DISCUSSION

Clinical differentiation of atypical from typical organisms in the etiology of childhood CAP remains challenging. This problem shows the importance of rapid diagnostic tests to help identify causative organisms to guide proper antibiotic therapy, especially in a patient population where follow up is difficult as reflected by the study population in rural Gabon. Despite limitations, results of this study show the importance of atypical respiratory pathogens including *B. pertussis* in the etiology of CAP in children in central Africa. Collectively, atypical pathogens accounted for 11% of cases of CAP with *B. pertussis*, accounting for 6% of the cases. An additional 18% of the study participants had possible infections with *C. pneumoniae* or *M. pneumoniae* based on serologic analysis. Except for one child, all children in our study who tested positive for atypical pathogens were less than five years of age. This finding is consistent with findings of previous studies[22] and does not support the suggestion that atypical infections should mainly be considered in children more than five years of age.

The role of *S. pneumoniae* as an etiologic agent in CAP in our patient population is more difficult to interpret. Eighty-seven of the study participants tested positive for *S. pneumoniae* by nasopharyngeal PCR. Nasopharyngeal carriage of streptococci does indicate invasive streptococcal infection because carriage can also be caused by colonization. Feikin and others[23] showed that 84% of children less than five years of age who came to a rural health clinic in Malawi had nasopharyngeal colonization with *S. pneumoniae*. Asymptomatic carriage is reported to be higher in children at orphanages or in children living together in large households. In the area of Gabon where our study was conducted, patients lived in households with a mean of seven people. We conclude that the usage of nasopharyngeal PCR alone to identify children with streptococcal lung infection is insufficient in rural Gabon. A positive result might reflect carrier state. Although there are reports that indicate that a positive *S. pneumoniae* urine antigen test result might reflect colonization, it seems to be more precise in diagnosing streptococcal pneumonia. However, even if *S. pneumoniae* is identified, further microbiologic testing must be conducted to exclude co-infection with atypical pathogens, including *B. pertussis*, to provide adequate antimicrobial coverage.

TABLE 3
Presenting symptoms of patients with atypical infection (*Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*) and all other study participants*

Symptom	Atypical infection (n = 11)	Others (n = 88)	P†
Cough, no. (%)	11 (100)	85 (96.6)	1.0
Temperature (°C), median (Q1, Q3)	38.0 (37.9, 38.3)	38.2 (37.8, 39.5)	0.31
Tachypnea, retractions, or nasal flaring, no. (%)	10 (90.9)	64 (72.7)	0.28
Wheezing, no. (%)	1 (9.1)	5 (5.7)	0.52
Diarrhea, no. (%)	6 (54.5)	40 (45.5)	0.75
Prior antibiotic use, no. (%)	7 (63.6)	31 (35.2)	0.11
Age, months, median (Q1, Q3)	14.5 (11.2, 49.2)	20.7 (9.9, 47.9)	0.82
Leukocyte count, median (Q1, Q3)	10.1 (8.3, 20.2)	13.0 (9.8, 20.3)	0.45
Hematocrit, median (Q1, Q3)	28 (25.1, 29.9)	27.3 (22.9, 31.4)	0.95
Platelet count, median (Q1, Q3)	393 (250, 471)	312 (219, 444)	0.36

* Categorical variables are summarized as no. (%) and compared using Fisher's exact test. Continuous variables are summarized as median (25th quartile [Q1] and 75th quartile [Q3]) and compared using the Mann-Whitney U test.
† Significance was defined as $P < 0.05$ (two-tailed).

The use of real-time PCR to detect *C. pneumoniae* is reported to be equal or superior to conventional PCR, culture, or serologic analysis.[13,15,24] Different studies showed that the rate of isolation of *C. pneumoniae* from acute lower respiratory tract infections in children ranges from 0% to 18%.[25–28] This variation may be caused by different methods used for diagnosis. A recent study performed in 12 medical centers in Asia demonstrated that on the basis of PCR, 2% of children included in that study tested positive for *C. pneumoniae*.[29] This result is consistent with findings in our study.

Molecular methods are also believed to be superior in the detection of *M. pneumoniae*.[16] The role of *M. pneumoniae* as an atypical pathogen in CAP in children was expected to be higher when compared with other studies. Michelow and others[22] reported infections with *M. pneumoniae* in 14% of children hospitalized with CAP. A recent study performed in 12 medical centers in Asia demonstrated that on the basis of PCR, 4–16% of pediatric patients tested positive for *M. pneumoniae*.[29] In a review of *M. pneumoniae* pneumonia, Hammerschlag[30] reported that the rate of pneumonia caused by this organism was highest in children 5–9 years of age. This finding could explain the lower rate of *M. pneumoniae* infection in our patient population because the median age of study participants was 21 months. The ages of the two children in our study with *M. pneumoniae* infections were four and eight years, respectively.

No child tested positive for *L. pneumophila* O1 by urine antigen test or by nasopharyngeal real-time PCR. We conclude that there is no need for routine testing for this organism in children in Lambarene.

Results of this study show the importance of pneumonia as a complication of *B. pertussis* infection. The diphtheria-tetanus-pertussis vaccination rate in the study population was 71%. All children with proven *Bordetella* infections were not immunized or only partially immunized. Empiric antibiotic coverage in all these patients consisted of amoxicillin or ampicillin and gentamicin. Because these antibiotics are not effective against *B. pertussis*, the expected treatment failure rate in the non-immunized group or the partially immunized group is 18%. These results lead to two conclusions: 1) the importance of universal *B. pertussis* vaccination coverage and 2) the use of empiric antibiotic therapy for infections with *B. pertussis* in children with lower respiratory tract infections who are not immunized or only partially immunized. There is a vaccination program at the Albert Schweitzer Hospital in Lambarene that offers routine vaccinations as recommended by the World Health Organization on a free basis. Nevertheless, many of the children, especially those living in small villages around Lambarene with more difficult access to medical care, are not immunized or only partially immunized. The diagnosis of *B. pertussis*-associated pneumonia in children less than five years of age is complicated by the fact that these children do have the typical whooping cough. Dragsted and others[31] showed that a PCR assay for detecting *B. pertussis* was highly sensitive, especially in 0.5–3-year-old children, and was thought to be superior to culture. Other studies confirmed this finding in showing a greater sensitivity in detecting *Bordetella* spp. by real-time PCR than by culture.[11,12] *Bordetella* spp. serology is difficult to interpret especially in immunized or partially immunized persons. Clinicians should be aware of *B. pertussis* as a possible etiologic agent of CAP, especially in children who have been treated with appropriate antibiotics, but who have not received a full series of vaccinations against this organism.

The study was limited by the fact that no testing for human immunodeficiency virus (HIV) was performed for study participants. There is currently no routine testing for HIV for children in Lambarene, and there was no routine antiretroviral treatment available at the time of the study. In most countries in central Africa, there are large differences in HIV prevalence between rural and urban areas, and the number of HIV-infected persons in and near Lambarene is not known. As reported by Makuwa and others,[32] HIV prevalence in the main urban areas in Gabon (Libreville and Port Gentil) was 4% in 1998. The United Nations Joint Program on HIV/AIDS estimates that there were 1,400–8900 children < 1 to 14 years of age infected with HIV in Gabon in 2005,[33] which indicates that 0.2–1.4% of all children less than 15 years of age

TABLE 4
Number of patients with isolated bacteria

Bacteria	Proven infection, no. (%)	Possible infection, no. (%)	Mixed infections,* no. (%)
Streptococcus pneumoniae	35 (35)	Not applicable	7 (7)
Bordetella pertussis	6 (6)	Not applicable	4 (4)
Chlamydia pneumoniae	3 (3)	15 (15)	1 (1)
Mycoplasma pneumoniae	2 (2)	3 (3)	2 (2)
Legionella pneumophila	0	Not applicable	0

* Mixed infections reflect infection with *S. pneumoniae* and *B. pertussis* or *C. pneumoniae* or *M. pneumoniae*.

TABLE 5
Characteristics of six patients with proven *Bordetella pertussis* infection

Patient ID	1	2	3	4	5	6
Sex	M	M	M	F	F	M
Age	6 months	12 years	13 months	14 months	11 months	12 months
Bordetella pertussis immunization	Incomplete	No	Incomplete	No	Incomplete	Incomplete
Breastfeeding at time of diagnosis	Yes	No	Yes	Yes	Yes	No
Leukocytes/μL	23.7	32.5	11.8	9.3	6.4	10.1
Smoking in same household	No	No	No	Yes	Yes	No
Co-infection with *S. pneumoniae*	Yes	Yes	No	Yes	Yes	No
Temperature (°C) at presentation	38.2	38.0	38.0	38.0	38.3	38.5
Antibiotics prior to presentation	Yes	No	No	Yes	Yes	Yes
Empiric antibiotic therapy	Ampicillin plus gentamicin	Amoxicillin	Ampicillin plus gentamicin	Ampicillin plus gentamicin	Ampicillin plus gentamicin	Ampicillin plus gentamicin

are infected. Another limiting fact was that patients were not tested for other pathogens, e.g., *H. influenzae* b or viruses, to assess the clinical significance of the atypical organisms detected and to assess mixed infections. However, even if atypical pathogens are part of a mixed lower respiratory tract infection, they should be treated with specific antibiotics with atypical coverage.

Molecular methods for detection of atypical organisms proved highly sensitive and rapid. Although the start-up costs for real-time PCR are high, the test reagents are inexpensive and interpretative subjectivity is eliminated. This method may not be usable in remote field areas where electricity would not be available, but could be valuable for rapid diagnosis in regional clinics such as the Albert Schweitzer Hospital in Lambarene. Rapid diagnosis would be particularly valuable in a patient population where follow up is difficult, as in rural areas of Gabon where this study was conducted.

Results of the study show the relevance of atypical organisms in CAP in children in central Africa. Atypical pathogens (*M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*) accounted for 11% of infections, mainly in pre-school children. The clinical differentiation of atypical pneumonia from typical pneumonia in children of all ages remains poor. The number of children with confirmed atypical pathogens in this study is small, thus limiting conclusions. However, 6 of the 11 cases of atypical infections were caused by *B. pertussis,* emphasizing the importance of routine vaccination programs. We conclude that empirical coverage of CAP in children in similar settings with an agent effective against *B. pertussis* would be justified if the child did not receive full diphtheria-tetanus-pertussis immunization. Molecular diagnostic methods such as real-time PCR offer fast and highly sensitive diagnostic tools and would be able to help guide antimicrobial therapy in rural areas where follow up is difficult.

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