Co-infection with two different strains of *Bordetella pertussis* in an infant

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We report co-infection with two phenotypically and genotypically distinct strains of *Bordetella pertussis* in an infant male hospitalized with a 2-week history of cough, paroxysms and vomiting. Colonies from the two *B. pertussis* phenotypes were isolated and evaluated by PFGE profile analysis, gene sequence typing and PCR-RFLP of a portion of the 23S rRNA gene. These results demonstrated simultaneous infection with two different strains of *B. pertussis*.

**Introduction**

Past reports of pertussis caused by simultaneous infections of *Bordetella* spp. have primarily implicated *B. pertussis* and *B. parapertussis* strains (Bergfors et al., 1999; Eldering & Kendrick, 1938; He et al., 1998; Iwata et al., 1991; Linnemann & Perry, 1977). Anecdotal evidence of *B. pertussis* and *B. bronchiseptica* co-infection has also been reported (Brown, 1926; Kristensen & Lautrop, 1962; Shih Man, 1950). These co-infections probably reflect the concurrent circulation of strains of *Bordetella* spp. in susceptible human populations and exposure of a given individual to infectious doses of each pathogen within a limited time period. Similar conditions must also exist for transmission and concomitant infection by different strains of *B. pertussis* that are circulating at high frequency, but to our knowledge no such co-infection has previously been described. The phenotypic homogeneity among primary isolates of *B. pertussis* is a major limitation to the detection of potential simultaneous infections by multiple strains of this pathogen. However, the recent advent of a disc diffusion test to monitor *B. pertussis* isolates for erythromycin susceptibility provides a potential means of identifying co-infecting strains if they differ for this trait (Hill et al., 2000). Here we report a case from which two phenotypically and genotypically divergent strains of *B. pertussis* were isolated.

**Case report**

In September 2002, a 48-day-old Hispanic male with no previous illness or birth complications since full-term delivery by Caesarean section presented at an emergency department in Atlanta, GA, USA, with a 1-week history of cough without fever. He received nebulizer treatments during this visit but his symptoms, including post-tussive emesis and paroxysmal cough, persisted for another week, at which time he was admitted to an Atlanta metropolitan area hospital. No apnoeic episodes were noted, and no neurological signs or symptoms were reported. At admission he was treated with inhaled racemic epinephrine and albuterol, and a 5-day course of azithromycin and oral prednisolone therapy was initiated. Physical examination showed a temperature of 99.2 °F, a respiratory rate of 40–48 min⁻¹, a pulse of 144–178 min⁻¹, a blood pressure of 102/49 mmHg and room air oxygen saturation of 89–90% measured by pulse oximetry; wheezes were noted on auscultation. Respiratory secretions were obtained prior to antibiotic treatment and were cultured for bacterial agents and also tested for the presence of respiratory syncytial virus and *B. pertussis* by using fluorescent antibody staining. The white blood cell count was 3.27 mm⁻² and a chest radiograph showed interstitial opacities with peribronchial wall thickening that was interpreted as probable bronchiolitis. The patient’s hospital course was complicated by one episode of choking and apneoa, which resolved without intervention. The patient completed the azithromycin and steroid therapy and continued to improve, and was discharged home after 6 days with instructions to continue the albuterol nebulizer treatments.

No household or other contacts had a cough illness prior to this patient’s illness and he was not in day care. He did have travel history to North Carolina to visit relatives prior to illness and frequently accompanied his mother on local shopping trips.

Nasopharyngeal secretions collected from the patient prior to antibiotic treatment yielded *B. pertussis* on primary culture on Regan-Lowe agar and were positive for this agent by direct fluorescent antibody testing. Growth from...
the primary culture plate was suspended in PBS and plated on Regan-Lowe agar without cephalexin (RL-C) at the Georgia State Public Health Laboratory (GASPHL), and a 15 μg erythromycin disc was placed on this plate before incubation at 37°C. This plate showed confluent growth after 4 days of incubation with a zone of inhibition of >40 mm around the erythromycin disc, suggesting erythromycin susceptibility. One week later, three colonies were observed within the zone of inhibition on this plate. Representative growth from each phenotype was cultured on RL-C, retested for erythromycin susceptibility at the GASPHL and sent to the Centers for Disease Control and Prevention (CDC) for susceptibility confirmation and genetic analyses, where the susceptible isolate was designated D945 and the resistant isolate D946. Notably, no other cultures of *Bordetella* spp. were under investigation in either the hospital or state laboratories.

The genetic subtypes of strains D945 and D946 were determined by PFGE profiling with XbaI as described by Hardwick et al. (2000b) and sequence analysis of the pertactin (*prn*) and pertussis toxin (*ptxS1*) genes was performed as described by Mooi et al. (2000). In addition, we used the methods of Bartkus et al. (2003) to amplify the region of the 23S rRNA gene known to confer erythromycin resistance in *B. pertussis* and PCR-RFLP to evaluate the copy number of mutant versus wild-type genes in the two *B. pertussis* strains.

Strain D945 was consistently susceptible to erythromycin on repeated testing, and no resistant colonies appeared in the zone of inhibition after prolonged incubation. Similarly, strain D946 remained resistant to erythromycin on repeated testing. PFGE analysis showed two PFGE profiles that differed in three DNA fragments. Sequence analysis of *prn* and *ptxS1* also revealed genotypic divergence. Both strains were type *ptxS1A*, but strain D945 was type *prn2* and strain D946 was type *prnL*. Sequence analysis of the three 23S rRNA gene copies showed that strain D945 was homozygous for the wild-type and that strain D946 was homozygous for the resistance mutation (Fig. 1).

**Discussion**

From previous experience, it was first suspected that the phenotypically distinct *B. pertussis* strains isolated from this infant were from the same strain exhibiting a heterogeneous phenotype for erythromycin susceptibility (Wilson et al., 2002). The heterogeneous phenotype strains described by Wilson et al. (2002) first appeared to be susceptible to erythromycin in the disc diffusion test, but after 7 days of incubation several resistant colonies appeared inside the zone of inhibition. Upon repeated erythromycin testing, colonies from inside the zone of inhibition remained resistant, whereas colonies from outside the zone of inhibition consistently reproduced the two phenotypes. The resistant and mixed phenotype colonies both produced the same profile by PFGE. One of these strains was further characterized by Bartkus et al. (2003), who reported that resistance to erythromycin in *B. pertussis* is conferred through a transitional mutation (A2047G) in the nucleotide of the 23S rRNA gene that is critical for the interaction between the ribosome and macrolide antibiotics. The 23S rRNA gene is present in three copies in *B. pertussis*, and the initial heterogeneous phenotype evaluated had one mutant copy and two wild-type copies (Bartkus et al., 2003).

The two strains of *B. pertussis* reported here did not exhibit the characteristics of heterogeneous phenotype strains as described by Wilson et al. (2002) and Bartkus et al. (2003). The resistant phenotype strain was consistently resistant in repeated erythromycin disc diffusion assays, whereas the susceptible phenotype strain was consistently susceptible, with no resistant colonies appearing in the zone of inhibition even after prolonged incubation. Typing by PFGE, a method that has been found to be reproducible and discriminatory for *B. pertussis* and that has been used for more than 10 years to characterize both sporadic and epidemiologically linked strains (Beall et al., 1995; Bisgard et al., 2001; Brennan et al., 2000; de Moissac et al., 1994; Hardwick et al., 2002a, b; Khattak & Matthews, 1993),
showed two different profiles. Likewise, sequence analysis of the \textit{prn} and \textit{ptxS1} genes, also a reproducible and discriminatory method for typing \textit{B. pertussis} strains (Cassiday et al., 2000; Mastrantonio et al., 1999; Mooi et al., 1998, 2000; van Loo et al., 2002), showed that the strains were genotypically divergent. Both strains were type \textit{ptxS1A}, but strain D945 was type \textit{prn2} and strain D946 was type \textit{prn1}. The sequences of these \textit{prn} types (formerly referred to as P.69B and P.69A, respectively) differ greatly (Mooi et al., 1998). Finally, when tested by the methods used by Bartkus et al. (2003), susceptible strain D945 was found to have three copies of the wild-type 23S rRNA gene and resistant strain D946 was found to have three copies of the mutant 23S rRNA gene. These results demonstrate sufficient phenotypic and genotypic divergence to preclude generation of strain D946 by \textit{in vitro} mutation. Moreover, our observations, together with the relative genetic homogeneity of \textit{B. pertussis}, led us to conclude that D945 and D946 were co-infecting \textit{B. pertussis} strains.

\textit{B. pertussis} strains that are resistant to erythromycin are rarely reported (Centers for Disease Control and Prevention, 1994; Lewis et al., 1995; Korgenski & Daly, 1997; Lee, 2000; Wilson et al., 2002). Although erythromycin resistance is seen infrequently, it is significant for public health. Treatment failures negatively affect patient management and efforts to interrupt secondary transmission. The persistence of resistant \textit{B. pertussis} over a wide geographical area for over 10 years challenges pertussis prevention and intervention strategies and activities. Consequently, continued monitoring for resistance in the circulating \textit{B. pertussis} population through primary culture and careful evaluation by primary care physicians and public health laboratory scientists of potential erythromycin treatment failures is warranted.

\section*{References}


