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Pertussis Outbreak, Southeastern Minnesota, 2012

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Abstract

Objective: To describe clinical and laboratory findings from the 2012 southeastern Minnesota pertussis outbreak.

Patients and Methods: Patients were selected for 2 parts of the study. In the first part, nasopharyngeal swabs from a convenience sample of 265 unique patients were used for both the clinician-requested polymerase chain reaction (PCR) test and culture. *B pertussis* isolates were tested for macrolide susceptibility and typed using whole genome sequencing and pulsed-field gel electrophoresis. Pertactin gene sequences were analyzed to identify pertactin-deficient *B pertussis*. In the second part, all patients seen at Mayo Clinic in Rochester, Minnesota, who had PCR results positive for *Bordetella pertussis* or *Bordetella parapertussis* between January 1, 2012, and December 31, 2012, were analyzed for patient demographic features and vaccination records.

Results: One hundred sixty patients had results positive for *B pertussis*, and 21 patients had results positive for *B parapertussis*. Among the 265 swabs cultured, *B pertussis* was detected by both culture and PCR in 11. One swab was positive for *B pertussis* by culture alone, and 13 were positive by PCR alone. Polymerase chain reaction detected *B pertussis* more frequently than did culture (P=.001). No macrolide resistance was detected. All 12 isolates tested had an altered pertactin gene, including 9 with a signal sequence deletion, 2 with insertion sequence disruptions, and 1 with a premature stop codon. Nine and 3 isolates were pertactin types *prn1* and *prn2*, respectively. Whole genome sequencing and pulsed-field gel electrophoresis detected the presence of multiple *B pertussis* strains. The mean age of patients with pertussis was younger than that of those without pertussis (15.6 and 25.5 years, respectively; P=.002). Compared with those whose test results were negative for *B pertussis*, fewer patients with positive results had received whole-cell pertussis vaccine (P=.02). In the subgroup who had received acellular vaccine exclusively, the time since the most recent pertussis vaccination in those with results positive for *B pertussis* was longer than that in those with negative results (1363 vs 1010 days; P=.004).

Conclusion: The 2012 pertussis outbreak in southeastern Minnesota included multiple strains of *B pertussis*, all putatively lacking pertactin. Our findings may indicate decreased efficacy of (and waning immunity from) acellular vaccines as contributors to the outbreak.

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Pertussis is a respiratory illness that begins with upper respiratory tract symptoms, progresses to severe paroxysms of cough, and evolves into a convalescent stage. Although infection can be associated with mild symptoms in adults and older children, neonatal infections may be deadly. The etiologic agent is *Bordetella pertussis*. Other less common *Bordetella* species (*Bordetella parapertussis*, *Bordetella bronchiseptica*, and *Bordetella holmesii*) have been implicated in similar illness. *B pertussis* is found exclusively in humans, with adolescents and adults likely serving as a source of infection of younger children and infants. Since the introduction of vaccines against *B pertussis*, the incidence of pertussis has declined, reaching a nadir in 1976.[1,2] The yearly incidence of pertussis has steadily increased since the 1980s, and communities throughout the United States have experienced a resurgence of pertussis in recent years.[2-5]

Whole-cell pertussis vaccines (thermally or chemically inactivated *B pertussis* cells) were introduced in the 1940s and later combined with diphtheria and tetanus toxoids to form the "DTP" (diphtheria and tetanus toxoids and pertussis) vaccine.[1] Although the vaccines

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were efficacious and immunogenic, tolerability was limited by vaccine reactions, including local reactions, fever, and febrile seizures. Acellular vaccines, composed of proteins purified from *B pertussis* cell lysates, were introduced in the 1990s. Compared with whole-cell vaccines, acellular vaccines have fewer adverse events.[1,6] Several acellular pertussis vaccines have been used, all of which have contained pertussis toxin, with or without pertactin, filamentous hemagglutinin, and/or fimbrial proteins.[1]

Our medical center is a large, tertiary/quaternary referral center in Rochester, Minnesota, where polymerase chain reaction (PCR) has been used to diagnose pertussis since 1995. Rochester is a city of approximately 109,000 residents located in Olmsted County. In 2012, southeastern Minnesota experienced its largest pertussis outbreak in recent history. That year, Olmsted County reported 237 cases of pertussis (compared with 19 and 28 in 2011 and 2010, respectively). The outbreak occurred in a region with a relatively high vaccination rate compared with that reported in other studies[7]; children in Olmsted County have an 88% rate of acellular pertussis vaccination, higher than the state average of 77%.[8] Herein, we report the epidemiology and clinical and microbiological characteristics of the 2012 pertussis outbreak in southeastern Minnesota and examine possible contributing factors.

PATIENTS AND METHODS

This study was approved by the Mayo Clinic Institutional Review Board. Patients were selected for 2 parts of the study (Figure 1). In the first part, detailed subsequently, a convenience sample of submitted nasopharyngeal swabs was used for both a clinician requested PCR test and an additional culture as part of the study. In the second part, the electronic charts of all patients seen at the Mayo Clinic in Rochester who had PCR results positive for *B pertussis* or *B parapertussis* between January 1, 2012, and December 31, 2012, were analyzed for patient demographic features and vaccination records. We defined a pertussis or parapertussis case as any patient with PCR or culture positive for *B pertussis* or *B parapertussis*. All patients were cross-referenced with the Minnesota research authorization status database and excluded if records indicated a request to be excluded from research studies.

Culture and *B pertussis* Identification

Nasopharyngeal swabs received for *Bordetella* PCR testing were cultured on Regan-Lowe charcoal media with cephalexin (Hardy Diagnostics). Colonies suspected to represent *B pertussis* were subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry,[9] with identification confirmed phenotypically. *B pertussis* isolates were frozen in *Brucella* broth on freezer beads (Hardy Diagnostics).

Real-time PCR for *B pertussis* and *B parapertussis*

Nasopharyngeal swab samples were placed into LightCycler Advanced lysis tubes (Roche Molecular Diagnostics) and subjected to heat lysis on a Thermomixer R (Eppendorf AG) for 6 minutes at 99°C and 1400 rpm, followed by centrifugation for 20 seconds at 20,800*g*. Then 5 μL of the supernatant was combined with 15 μL of PCR master mix and tested using a previously described duplex PCR assay targeting IS*481* and IS*1001* of *B pertussis* and *B parapertussis*, respectively.[10]

Macrolide Resistance Detection in *B pertussis*

Isolates of *B pertussis* were tested for phenotypic and genotypic macrolide resistance. 0.5 McFarland suspensions of each isolate were prepared in normal saline. Using the prepared suspensions, 2 Regan-Lowe agar plates without cephalexin (Hardy Diagnostics) were inoculated for a lawn of growth and allowed to acclimate. A 15-μg erythromycin disk (Becton Dickinson and Company) was placed on one plate and an erythromycin Etest strip (bioMérieux, Inc) on the other. Plates were incubated for 5 days at 35°C in room air. Disk inhibition zone diameters were measured with a micrometer, and Etest minimum inhibitory concentration values were determined following the manufacturer's recommendations. Polymerase chain reaction targeted to the 23S ribosomal RNA gene followed by bidirectional sequencing of the amplified product was performed to detect the A-to-G sequence variation at position 2058 (*Escherichia coli* numbering) associated with macrolide resistance in *B pertussis*.[11] For resistance studies, American Type Culture Collection strains BAA-1335 and 9797 were included as positive and negative controls, respectively.