Appearance of Macrolide-Resistant *Bordetella pertussis* Strains in China

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In China, pertussis is a notified infectious disease, and the number of reported cases has decreased since the introduction of diphtheria-tetanus-pertussis (whooping cough) (DTwP) vaccines in the 1980s. In this country, pertussis is clinically diagnosed and laboratory methods, such as culture and PCR, are not routinely used. In China, antimicrobial susceptibility testing for *Bordetella pertussis* is rarely performed. Ou et al. tested 20 *B. pertussis* isolates collected at the Beijing Children’s Hospital from the 1970s to 2007 and found that all were susceptible to erythromycin (1). Recently, two *B. pertussis* strains isolated from healthy schoolchildren in Shandong Province, China, were found to be erythromycin resistant (2).

From January through December 2012, we conducted a study to investigate the occurrence of pertussis in young children with persistent coughing who visited the Children’s Hospital in Xi’an, the capital city of Shaanxi Province, China. This study was approved by the Institutional Review Board of Xi’an Center for Disease Control and Prevention, Xi’an, China. Informed consent was received from all children’s supervisors before the nasopharyngeal swabs were taken. The clinical case definitions recommended by the Chinese diagnostic criteria for pertussis were used. In China, antimicrobial susceptibility testing for *B. pertussis* was performed. Ou et al. tested 20 *B. pertussis* isolates collected at the Beijing Children’s Hospital from the 1970s to 2007 and found that all were susceptible to erythromycin (1). Recently, two *B. pertussis* strains isolated from healthy schoolchildren in Shandong Province, China, were found to be erythromycin resistant (2).

Nasopharyngeal (NP) swabs were immediately taken for bacterial culture and PCR (targeting IS481) when patients were admitted to the hospital. The culture plates contained charcoal agar supplemented with 20% defibrinated sheep blood. Of the 178 patients, 97 (54.5%) were PCR positive (data not shown) and 4 (2.2%) were culture positive. All four culture-positive patients were also PCR positive. Demographic and clinical characteristics of the four culture-positive patients are shown in Table 1. No epidemiological link was noticed among these four patients. Three patients had taken erythromycin for 2 to 11 days before the NP sampling (Table 1).

The susceptibilities of these 4 strains to erythromycin, clarithromycin, and azithromycin were determined with Etest strips according to the manufacturer’s instructions (bioMérieux, Marcy l’Etoile, France). The control strains included *Staphylococcus aureus* ATCC 25923 and *B. pertussis* ATCC 9797. The MICs of erythromycin, clarithromycin, and azithromycin for all four isolates were >256 mg/liter.

To determine whether macrolide resistance was caused by a mutation in domain V of the 23S rRNA gene of *B. pertussis*, PCR-based sequencing and PCR-restriction fragment length polymorphism (RFLP) analysis, as described by Barkus et al. (3), were performed. DNA sequencing results were compared to the GenBank sequences of the *B. pertussis* reference strain Tohama (accession number X68323) and the Chinese vaccine strain (CP002695). A homogeneous A2047G mutation in domain V of the 23S rRNA genes of the four isolates was identified. After the sequences were digested with BbsI, as expected, the PCR products (521 bp in length) amplified from the 23S rRNAs of the four isolates yielded two fragments, 128 bp and 383 bp, confirming the presence of the A2047G mutation in all three copies of the 23S rRNA genes of the four isolates.

### Table 1

Demographic and clinical characteristics of the four culture-confirmed patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date of sampling (day/mo/yr)</th>
<th>Age</th>
<th>Gender</th>
<th>No. of days cough had persisted at time of sampling</th>
<th>No. of days that erythromycin was used before sampling</th>
<th>Vaccination status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28/02/2012</td>
<td>84 days</td>
<td>Male</td>
<td>19</td>
<td>2</td>
<td>Not vaccinated</td>
</tr>
<tr>
<td>2</td>
<td>14/05/2012</td>
<td>86 days</td>
<td>Female</td>
<td>15</td>
<td>11</td>
<td>Not vaccinated</td>
</tr>
<tr>
<td>3</td>
<td>14/08/2012</td>
<td>2 yr</td>
<td>Male</td>
<td>19</td>
<td>Unknown*</td>
<td>Received 4 doses*</td>
</tr>
<tr>
<td>4</td>
<td>20/09/2012</td>
<td>5 mo</td>
<td>Female</td>
<td>15</td>
<td>6</td>
<td>Not vaccinated</td>
</tr>
</tbody>
</table>

*This patient had taken some antibiotics, but the details are unknown.

*In China, both the DTwP and diphtheria-tetanus-acellular-pertussis (DTaP) vaccines are administered in the 3th, 4th, and 5th months of life, and a booster dose of DTwP or DTaP is given at 18 to 24 months. This patient received the booster dose 4 months prior to the study.
All four isolates harbored the allele combination ptxA1 ptxP1, as determined by PCR with the primers and protocol recommended for genotyping (4). The allele combination observed is common in modern Chinese isolates (5). Of the four isolates, three had the pulsed-field gel electrophoresis (PFGE) profile BpFINR9 and one had the BpSR23 profile; they belonged to the same cluster (cluster III) (6).

Our finding that four isolates from young children suspected of having pertussis in Shannxi Province were resistant to macrolides confirmed the recent finding that two isolates obtained in Shandong Province, China, were resistant to erythromycin (2). Furthermore, B. pertussis strains isolated in the two provinces had identical genotypes and PFGE profiles, suggesting that B. pertussis with ptxA1 ptxP1 and BpFINR9 or BpSR23 are circulating in this country. Our data also indicate that the incidence of pertussis is most likely underestimated in China (7, 8).

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We have no conflicts of interest to declare.

REFERENCES