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Appearance of Multidrug-Resistant Virulent *Rhodococcus equi* Clinical Isolates Obtained in China

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Rhodococcus equi is a major cause of pneumonia in domestic animals, especially foals, and an opportunistic pathogen of immunocompromised humans (1). Control of *R. equi* infections in horses has relied on early detection of subclinical pulmonary disease and treatment with antimicrobial drugs before development of clinical signs (2). However, the onset of clinical signs of *R. equi* pneumonia in foals is often insidious, and delay in diagnosis and treatment tends to increase the morbidity and mortality. The combination of erythromycin and rifampin has been the treatment of choice since the 1980s and has improved survival rates considerably (3, 4). In China, data on the antimicrobial sensitivity of *R. equi* are sparse. In the present study, an *R. equi* strain isolated from a racehorse in China was found to be multidrug resistant.

From June through August 2013, 108 (20%) of 903 horses showed symptoms of pneumonia on an English thoroughbred racehorse breeding farm in Beijing, China. They were treated by injection with erythromycin, ampicillin, and ceftiofur for 1 month, but the treatment was unsuccessful. Thirty-eight horses (35.2%) aged 1.5 to 3 months died of pneumonia, and postmortem examination revealed multiple pyogranulomas in all regions of the lungs. The 152 samples, including trachea secretions and tissues of lung, liver, and intestine, were collected under aseptic conditions and plated on Trypticase soy agar supplemented with 5% sheep blood. *R. equi* was isolated from 76 (50%) of 152 samples and identified by the Phoenix 100 identification system (BD, USA).

According to a nucleotide-by-nucleotide GenBank search by using BLAST (http://blast.ncbi.nlm.nih.gov/), the 16S rRNA gene sequence of the isolated strain was 100% identical to the sequences of 16S rRNA genes of *R. equi*. Further confirmation of *R. equi* was accomplished by amplification of the *choE* gene and detection of the virulence plasmid by amplification of the *vapA* gene by using multiplex PCR (5).

The susceptibilities of the strain to erythromycin, clarithromycin, azithromycin, and rifampin were determined with the Phoenix 100 antimicrobial susceptibility testing system (BD, USA). In accordance with CLSI guidelines, the virulent *R. equi* strain was considered resistant to macrolides and rifampin, with MIC values of ≥25 μg/ml (azithromycin, clarithromycin, and erythromycin) and ≥128 μg/ml (rifampin).

To determine whether macrolide and rifampin resistance was caused by mutations in the V domain, we amplified and sequenced the 23S rRNA gene and *rpoB* gene of the *R. equi* isolate. A mutation in the *rpoB* gene (codon 526, His526Asp) associated with rifampin resistance was confirmed by partial sequencing (6). An A-to-G mutation at position 2063 in domain V of the 23S rRNA gene of *R. equi* resulted in the high-level macrolide resistance of the isolated strains (7). DNA sequencing results were compared to the GenBank sequences of the *R. equi* reference strain ATCC 33701.

Our finding that the isolated strain from a racehorse breeding farm was resistant to macrolides and rifampin confirmed the recent finding that the resistant *R. equi* isolates were obtained in the United States (8). Our data indicate that the incidence of multidrug-resistant *R. equi* is most likely underestimated in China, and this finding raises serious questions regarding the effective treatment of *R. equi* in the future.

Nucleotide sequence accession number. The nucleotide sequence of the *R. equi* isolate was submitted to GenBank under accession number KF612274.

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We have no conflicts of interest to declare.

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