Isolation, Antimicrobial Resistance, and Virulence Genes of *Pasteurella multocida* Strains from Swine in China

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A total of 233 isolates of *Pasteurella multocida* were obtained from 2,912 cases of clinical respiratory disease in pigs in China, giving an isolation rate of 8.0%. Serogroup A *P. multocida* isolates were isolated from 92 cases (39.5%), and serogroup D isolates were isolated from 128 cases (54.9%); 12 isolates (5.2%) were untypeable. *P. multocida* was the fourth most frequent pathogenic bacterium recovered from the respiratory tract, after *Streptococcus suis*, *Haemophilus parasuis*, and *Escherichia coli*. All isolates were characterized for their susceptibilities to 20 antibiotics and the presence of 19 genes for virulence factors (VFs). The frequency of antimicrobial resistance among *P. multocida* isolates from swine in China was higher than that reported among *P. multocida* isolates from swine in from other countries, and 93.1% of the isolates showed multiple-drug resistance. There was a progressive increase in the rate of multiresistance to more than seven antibiotics, from 16.2% in 2003 to 62.8% in 2007. The resistance profiles suggested that cephalosporins, florfenicol, and fluoroquinolones were the drugs most likely to be active against *P. multocida*. Use of PCR showed that colonization factors (*pfa*, *fimA*, and *hsf-2*), iron acquisition factors, sialidases (*nanH*), and outer membrane proteins occurred in most porcine strains. The VFs *pfIA*, *tadD*, *toxA*, and *pmHAS* were each present in <50% of strains. The various VFs exhibited distinctive associations with serogroups: concentrated in serogroup A, concentrated in serogroup D, or occurring jointly in serogroups A and D. These findings provide novel insights into the epidemiological characteristics of porcine *P. multocida* isolates and suggest that the potential threat of such multiresistant bacteria in food-producing animals should not be neglected.

*Pasteurella multocida* is an important cause of pneumonia and atrophic rhinitis in pigs and is responsible for significant losses on large farms worldwide (11, 16, 30). Strains of *P. multocida* are grouped into five capsular serogroups (serogroups A, B, D, E, and F) and are further classified into 16 somatic serotypes (serotypes 1 to 16), which are primarily based on lipopolysaccharide antigens (22, 31, 34). To date, only somatic serotypes (serotypes 1 to 16), which are primarily grouped into five capsular serogroups (sero-

*P. multocida* is associated with various virulence factors (VFs) (18, 20, 24). The key factors that have been identified to date include the capsule (10, 21). The recognized VFs of this organism also include diverse adhesins (e.g., filamentous hemagglutinin, type 4 fimbriae, and Flp pilin), toxins (dermonecrotic toxin), siderophores (e.g., iron acquisition proteins), sialidases (which may enhance bacterial virulence by unmasking key host receptors and/or reducing the effectiveness of host defenses), and outer

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membrane proteins (e.g., OmpA, OmpH, Oma87, and PlpB) (2, 17, 18, 23, 35). These VFs facilitate the colonization and invasion of the host, the avoidance or disruption of host defense mechanisms, injury to host tissues, and/or stimulation of a noxious host inflammatory response (20, 23). Thus, the in-formed selection of the VFs to be targeted for the prevention of P. multocida infections requires knowledge of which VFs are prevalent in specific clinical syndromes, as may be revealed by epidemiological studies. Furthermore, it was reported that there is an obvious correlation between some VFs and capsular serogroups, with the filamentous hemagglutinin gene pfhA being associated with serogroups A, B, E, and F; the iron acquisition gene tbfA being associated with serogroups A and B; and the dermonecrotroxin toxA gene being associated with serogroup D (17). Because pathogenic behavior is predicted both by the VF repertoire and by the serogroup (20), the clonal associations of VFs must be evaluated.

The current investigation is the first large study in China of the prevalence of P. multocida in clinical samples collected from 16 provinces between 2003 and 2007. To obtain more information about the epidemiology of porcine P. multocida infection and to characterize clinical isolates, we investigated a total of 233 isolates of P. multocida that were associated with clinical disease in swine for the distributions of the capsular serogroups, the phenotypic antimicrobial resistance profiles, and the presence of 19 virulence genes.

MATERIALS AND METHODS

Clinical specimens, culture, and P. multocida screening. Over more than 4 years (from June 2003 to September 2007), 2,912 clinical samples from pigs with clinical respiratory infections which were collected by the Clinical Microbiology Laboratory of the College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, for routine pathogen identification were screened for P. multocida. The samples were plated on tryptic soy agar (Difco, Detroit, MI) containing 10 μg/ml NAD (Sigma, St. Louis, MO) and 5% bovine serum, MacConkey agar, and blood agar (5% fresh sheep blood). All plates were incubated at 37°C in air for a minimum of 48 h. After this isolation stage, the isolates were purified and cultured by standard methods for the identification of strains of bacteria, including Haemophilus parasuis, Streptococcus suis, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Escherichia coli, and Staphylococcus aureus (7, 28). Presumptive isolates of P. multocida were confirmed by a PCR assay with primers specific for the amplification of the KMT1 gene (34).

All isolates of P. multocida were subsequently characterized biochemically by using a MicroStation system (Biolog Inc.) and their capsules were serotyped. For samples in which all isolates were identical with respect to their capsular serotype, only one colony was selected. When one sample yielded colonies with different capsular serotypes, one colony of each serotype was selected for further characterization. All isolates were freeze-dried and kept at −20°C.

Capsule typing. The capsular types of the isolates were determined by multiplex capsule PCR typing with the capsule-specific primer pairs (primers specific for capA, capB, capD, capE, and capF) described by Townsend et al. (34). All oligonucleotides were synthesized with a DNA synthesizer (with finishing done by Sangon Biological Engineering Technology Inc.). The base sequences and the predicted sizes of the amplified products for the specific oligonucleotide primers used in this study are shown in Table 1. The bacterial lysates used as templates for the PCR were prepared as follows. A loopful of bacteria from a fresh overnight culture on a tryptic soy agar plate was resuspended homogeneously in 200 μl of sterile water, and the mixture was boiled at 100°C for 5 min to release the DNA and centrifuged. A 4-μl volume of the supernatant was used as a template for each 25-μl PCR mixture. The appropriate positive and negative controls for amplification were generated from clinical isolates of P. multocida by PCR carried out with a GeneAmp PCR system 9700 instrument (Applied Biosystems, Foster City, CA) and were confirmed by sequencing. The amplified products were analyzed in 0.8% agarose gels by electrophoresis, and the results were recorded with a gel documentation system. All tests were repeated three times in parallel with the relevant positive and negative controls. Discrepant results for each VF were investigated further, and samples were sequenced for gene verification.

Clinical data and statistical analysis. Clinical data were collected by retrospective analysis of the protocols. Statistical testing was performed with SPSS software (version 12.0; SPSS Inc., Chicago, IL). Comparisons of proportions were made by two-tailed Fisher’s exact test or the χ² test. Comparisons of the prevalence of different traits within the same population were made by McNemar’s test. Aggregate VF scores were compared by the Mann-Whitney U test. P values of <0.05 were considered statistically significant.

RESULTS

Prevalence of P. multocida in porcine clinical samples. We analyzed clinical specimens from diseased pigs with pneumonia or atrophic rhinitis from 16 provinces in China for the presence of P. multocida. Strains of P. multocida were detected in 233 (8.0%) of the 2,912 cases investigated, and the isolation rate at different time points (years) ranged from 6.4 to 10.2% (Table 2). Isolates of P. multocida of capsular type A were obtained from 92 cases (39.5%), capsular type D strains were isolated from 128 cases (54.9%), and 1 isolate was identified as capsular type B, whereas 12 isolates were untypeable. Capsular types E and F were not detected in the population sampled. All these isolates gave positive results by the PCR assays with primers specific for P. multocida (34).

After Streptococcus suis, Haemophilus parasuis, and Escherichia coli, P. multocida was the bacterial pathogen that was the fourth most frequently isolated from clinical porcine specimens in this study (Table 2). Streptococcus suis was isolated from 24.2% of the study samples, Haemophilus parasuis was isolated from 17.1%, Escherichia coli was isolated from 12.6%, Bordetella bronchiseptica was isolated from 7.2%, Staphylococcus aureus was isolated from 3.2%, and Actinobacillus pleuropneumoniae was isolated from 0.9%. The simultaneous detection of P. multocida and other species of bacteria pathogenic for pigs occurred in 125 specimens. Haemophilus parasuis (53.6%), Streptococcus suis (48.8%), Escherichia coli (27.2%) and Bordetella bronchiseptica (18.4%) were the agents that were the most frequently found in coinfections with P. multocida. Eleven toxigenic strains of P. multocida of serogroup D
<table>
<thead>
<tr>
<th>Gene function and gene</th>
<th>Description</th>
<th>Direction</th>
<th>Primer sequence (5’–3’)</th>
<th>Amplicon size (bp)</th>
</tr>
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<tbody>
<tr>
<td><strong>Adhesins</strong>&lt;br&gt;plfA</td>
<td>Type 4 fimbriae</td>
<td>s</td>
<td>TGTGGAATTCAGCATTTTAGTGTC&lt;br&gt;TCATGAAATTTATCGGCAAAAACCTC&lt;br&gt;GCTGG</td>
<td>488</td>
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<tr>
<td>fimA</td>
<td>Fimbriae (from Pm70)</td>
<td>s</td>
<td>CCATCGGATCTAAGCAGG&lt;br&gt;AGTTAGGCTTCAGGG</td>
<td>866</td>
</tr>
<tr>
<td>hof-1</td>
<td>Autotransporter adhesion (from Pm70)</td>
<td>s</td>
<td>TTGAGTCGGCTGTAGAGTTCG&lt;br&gt;ACTTCTTTAGCAATGGGGACACCT&lt;br&gt;GCTGG</td>
<td>654</td>
</tr>
<tr>
<td>hof-2</td>
<td>Autotransporter adhesion (from Pm70)</td>
<td>s</td>
<td>ACCGCAACACTGTCTTAC&lt;br&gt;TGACTGACATCGGCGGTAC&lt;br&gt;GCTGG</td>
<td>433</td>
</tr>
<tr>
<td>pfhA</td>
<td>Filamentous hemagglutinin</td>
<td>s</td>
<td>TCTACCCATTCTCAGCAAGGC&lt;br&gt;ATCATTTCCGGCATTTACC&lt;br&gt;GCTGG</td>
<td>416</td>
</tr>
<tr>
<td><strong>Toxins</strong>&lt;br&gt;toxA</td>
<td>Dermonecrotic toxin</td>
<td>s</td>
<td>CTTAGATGAGCGACAAGG&lt;br&gt;GAATGCCACACCTCTATAG&lt;br&gt;GCTGG</td>
<td>864</td>
</tr>
<tr>
<td><strong>Iron acquisition</strong>&lt;br&gt;exbB</td>
<td>Accessory protein Ton-dependent transport of iron compounds</td>
<td>s</td>
<td>TGGCTTGATGATGAAACCG&lt;br&gt;TGACGAAATGGCGACTAA&lt;br&gt;GCTGG</td>
<td>283</td>
</tr>
<tr>
<td>exbD</td>
<td>Accessory protein Ton-dependent transport of iron compound</td>
<td>s</td>
<td>CGTGTGGATTAGCCCTG&lt;br&gt;AAACGAAATGGCGACTAA&lt;br&gt;GCTGG</td>
<td>247</td>
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<tr>
<td>tonB</td>
<td>Iron transporters, transport ferric-siderophore complexes</td>
<td>s</td>
<td>CGAGGTGAAACCTGGACC&lt;br&gt;CCGACGATTAACGCTGAC&lt;br&gt;GCTGG</td>
<td>261</td>
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<tr>
<td>hgbA</td>
<td>A hemoglobin-binding protein</td>
<td>s</td>
<td>TCAAGGGGCATACATCCAGG&lt;br&gt;GCGGAATGCGAGAAGATAG&lt;br&gt;GCTGG</td>
<td>267</td>
</tr>
<tr>
<td>fur</td>
<td>Ferric uptake regulation protein</td>
<td>s</td>
<td>GTTTACGTTATGGTAC&lt;br&gt;CATTACTACATTTGCCATAC&lt;br&gt;GCTGG</td>
<td>244</td>
</tr>
<tr>
<td><strong>Sialidases</strong>&lt;br&gt;nanB</td>
<td>Outer membrane-associated proteins, an autotransporter protein</td>
<td>s</td>
<td>CATTCACCAAGCATTG&lt;br&gt;GGACACTGATTGCCCTA&lt;br&gt;GCTGG</td>
<td>555</td>
</tr>
<tr>
<td>nanH</td>
<td>Outer membrane-associated proteins, small sialidases</td>
<td>s</td>
<td>GTGGAACGGGAAATTTGGA&lt;br&gt;ACATGGCAAGTTGCCCTA&lt;br&gt;GCTGG</td>
<td>287</td>
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<tr>
<td><strong>Hyaluronidase</strong>&lt;br&gt;pmHAS</td>
<td>Hyaluronan synthase</td>
<td>s</td>
<td>TCAATTTGTCGGATGATCGG&lt;br&gt;TGGCGAATGATCGGTGATAG&lt;br&gt;GCTGG</td>
<td>430</td>
</tr>
<tr>
<td><strong>Protectins</strong>&lt;br&gt;ompA</td>
<td>Outer membrane protein A</td>
<td>s</td>
<td>CGCATGACCTAAGTTTCTCC&lt;br&gt;CATAAACAGATGACCGAAC&lt;br&gt;GCTGG</td>
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<tr>
<td>ompH</td>
<td>Outer membrane protein H</td>
<td>s</td>
<td>CGCGTATGAGGTTTATG&lt;br&gt;TTTATGATGCGGTAC&lt;br&gt;GCTGG</td>
<td>438</td>
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<tr>
<td>oma87</td>
<td>Outer membrane protein 87</td>
<td>s</td>
<td>GGGCGACGCAAAACAGATAAGC&lt;br&gt;TGTTCCTCAAAATGCTG&lt;br&gt;GCTGG</td>
<td>838</td>
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<tr>
<td>plpB</td>
<td>Lipoprotein B</td>
<td>s</td>
<td>TTTTGTTGTTGCTTCT&lt;br&gt;AGTCATTTTAGATCGT&lt;br&gt;GCTGG</td>
<td>282</td>
</tr>
<tr>
<td><strong>Capsule serotypes</strong>&lt;br&gt;KMT1</td>
<td>Identification of all P. multocida isolates</td>
<td>s</td>
<td>ATTCGCTATTTACCCAGTG&lt;br&gt;GCTGTAACGAAACCTGCC&lt;br&gt;GCTGG</td>
<td>460</td>
</tr>
<tr>
<td>hyAL-hyaC</td>
<td>Serogroup A cap gene</td>
<td>s</td>
<td>GATGCCAAATTCGGAGCAG&lt;br&gt;TGTTGGATCATTTGCTG&lt;br&gt;GCTGG</td>
<td>1048</td>
</tr>
<tr>
<td>bchD</td>
<td>Serogroup B cap gene</td>
<td>s</td>
<td>CATTTATCCAGGCCTCACC&lt;br&gt;GCCGAGAGTTGCA&lt;br&gt;GCTGG</td>
<td>758</td>
</tr>
<tr>
<td>dcbF</td>
<td>Serogroup D cap gene</td>
<td>s</td>
<td>TTCAAATAAAGAGACTAGGAGGCC&lt;br&gt;CATCTACCTCAGACCATATG&lt;br&gt;GCTGG</td>
<td>647</td>
</tr>
<tr>
<td>ecbJ</td>
<td>Serogroup E cap gene</td>
<td>s</td>
<td>TTCCAGTAAATTCGAGT&lt;br&gt;GCTGGCTTGCTTATG&lt;br&gt;GCTGG</td>
<td>512</td>
</tr>
<tr>
<td>fcbD</td>
<td>Serogroup F cap gene</td>
<td>s</td>
<td>ATTCGGAAGAACGAAATAC&lt;br&gt;TTGCGCTGATCTG&lt;br&gt;GCTGG</td>
<td>852</td>
</tr>
</tbody>
</table>

* s, sense; a, antisense.
were isolated from 37 samples of nasal swabs and lungs lesions from pigs with typical clinical signs of atrophic rhinitis. Six strains of *Bordetella bronchiseptica* and five strains of *Pseudomonas aeruginosa* were also cultured from the same samples.

All infections with *P. multocida* detected in this study were from territorial outbreaks on pig farms, and the sources of infection were not identified in most cases. Strains of *P. multocida* were isolated throughout the year without seasonal variation, and 62.2% of the organisms were detected from growing pigs 60 to 110 days old (145 cases). Eleven toxigenic strains of *P. multocida* were collected from growing pigs 80 to 100 days old from June to August.

**Antimicrobial susceptibility.** Two hundred thirty-three isolates of *P. multocida* recovered from diseased swine were tested for resistance to 20 antibiotics (Table 3). The most prevalent phenotypes detected were resistance to lincomycin (96.6%), sulfamethazine (85.4%), amoxicillin (80.3%), clindamycin (80.3%), trimethoprim-sulfamethoxazole (74.2%), chlor-tetracycline (65.2%), and tetracycline (58.0%), followed by tilmicosin (28.3%), amikacin (14.2%), gentamicin (13.7%), kanamycin (12.8%), and spectinomycin (12.0%). Less than 10% of the isolates were resistant to erythromycin or chloramphenicol (6.0 and 2.6%, respectively). No resistance to ceftazolin, cefotiofur, fleroxacin, or ciprofloxacin could be detected. The MIC<sub>90</sub> of neomycin and polymyxin B for the *P. multocida* isolates tested were 32 μg/ml and 4 μg/ml, respectively. The proportion of *P. multocida* isolates categorized as resistant could not be evaluated in this study because the breakpoints of neomycin and polymyxin B for veterinary use have not been determined according to the CLSI criteria (29). In addition, it was observed that 98.6% of the isolates were resistant to at least one antibiotic and 93.1% were multiresistant (resistant to from 3 to 10 antibiotics). Multiresistance was predominant in isolates of serogroup D, including toxigenic *P. multocida* strains. Resistance to amoxicillin, chlor-tetracycline and tetracycline, lincomycin and clindamycin, and sulfamethazine and trimethoprim-sulfamethoxazole was the common feature of these multiresistant isolates. The percentage of isolates with resistant to at least three antimicrobials was equally high in all years from 2003 to 2007 (Fig. 1). Isolates resistant to more than five antimicrobials became more frequent over time. The prevalence increased from 47.8% in 2003, 54.1% in 2004, and 57.6% in 2005 to 81.6% in 2006 and 97.1% in 2007. It is important to note that the proportion of isolates resistant to more than seven antimicrobials increased approximately fourfold between 2003 and 2007, from 16.2% to 62.8% (*P < 0.05*).

**Distribution of virulence genes.** Among the 233 porcine *P. multocida* isolates, the 19 virulence gene regions ranged in prevalence from 4.7% (*ptoA*) to 100% (*ompA*). Multiple adhesins (including *pfpA*, *fimA*, and *hsf-2*), all iron acquisition factors (*exhB*, *exhD*, *tadB*, *hgbA*, and *fur*), *nanH*, and various outer membrane proteins (*ompA*, *ompH*, *oma87*, and *plpB*) were each found to occur in over 90% of the strains (Table 4). This shows that these virulence genes are highly prevalent in porcine isolates of *P. multocida*. Of the adhesin-encoding genes studied, *hsf-2* (99.1%) was more prevalent than *hsp-1* (67.0%; *P < 0.001, McNemar’s test), and *tadD* was more prevalent than *pfhA* (*P < 0.001, McNemar’s test). However, there was no statistically significant difference in the prevalence of *hsp-1* and *tadD* (67.0% and 43.3%, respectively; *P > 0.05*).

### Table 2. Isolation of bacterial species and the prevalence of *P. multocida* in clinical samples from China from June 2003 to September 2007

<table>
<thead>
<tr>
<th>Yr</th>
<th>No. of samples</th>
<th><em>P. multocida</em></th>
<th><em>S. suis</em></th>
<th><em>H. parasuis</em></th>
<th><em>E. coli</em></th>
<th><em>B. bronchiseptica</em></th>
<th><em>S. aureus</em></th>
<th><em>A. pleuropneumoniae</em></th>
<th>None*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>594</td>
<td>94 (15.8)</td>
<td>167 (27.9)</td>
<td>126 (21.1)</td>
<td>64 (10.8)</td>
<td>25 (4.2)</td>
<td>11 (1.8)</td>
<td>6 (1.0)</td>
<td>68 (11)</td>
</tr>
<tr>
<td>2006</td>
<td>726</td>
<td>91 (12.5)</td>
<td>156 (21.5)</td>
<td>126 (17.4)</td>
<td>64 (8.7)</td>
<td>25 (3.5)</td>
<td>11 (1.5)</td>
<td>6 (0.8)</td>
<td>61 (8.4)</td>
</tr>
<tr>
<td>2005</td>
<td>493</td>
<td>79 (16.0)</td>
<td>91 (18.4)</td>
<td>64 (12.9)</td>
<td>32 (6.5)</td>
<td>10 (2.0)</td>
<td>5 (1.0)</td>
<td>3 (0.6)</td>
<td>56 (11.4)</td>
</tr>
<tr>
<td>2004</td>
<td>502</td>
<td>76 (15.1)</td>
<td>101 (20.2)</td>
<td>64 (12.8)</td>
<td>32 (6.4)</td>
<td>10 (2.0)</td>
<td>5 (1.0)</td>
<td>3 (0.6)</td>
<td>57 (11.4)</td>
</tr>
<tr>
<td>2003</td>
<td>597</td>
<td>71 (11.9)</td>
<td>101 (16.9)</td>
<td>64 (10.7)</td>
<td>32 (5.4)</td>
<td>10 (1.7)</td>
<td>5 (0.8)</td>
<td>3 (0.5)</td>
<td>60 (10.1)</td>
</tr>
</tbody>
</table>

*Values in parentheses are the number of samples containing other pathogenic bacteria, among 125 samples coinfected with *P. multocida.*
McNemar’s test). Of the sialidase-encoding genes studied, nanH (97.0%) was more prevalent than nanB (81.5%; P < 0.001, McNemar’s test). Of note was the substantial prevalence of the gene for hyaluronan synthase (0.001, McNemar’s test). Of the sialidase-encoding genes studied, the values, with the exception of those for ciprofloxacin, are based on CLSI standards.

The percentage of isolates resistant to at least three antimicrobials was increased approximately fourfold between the years 2003 and 2007, from 16.2% to 62.8% (P < 0.05).

For the other antimicrobials tested, breakpoint values were taken from previously used by Aarestrup et al. (1) for Danish strains of H. parasuis, and it was strictly restricted to strains belonging to serogroup D. The different capsule serogroups exhibited disparate median aggregate VF scores: serogroup A, 15.6 (range, 12 to 18); serogroup D, 15.0 (range, 9 to 17); serogroup B, 14.0 (only one strain); and the nonaligned strains (nonserogroup strains), 14.1 (range, 11 to 16). These results did not differ significantly (for all comparisons, P > 0.05, Mann-Whitney U test).

**DISCUSSION**

Pasteurellosis is one of the most common diseases of grower and finisher pigs worldwide. It is widely accepted that specific serotypes and pathotypes of *P. multocida* strains are responsible for most respiratory disease syndromes in pigs that are associated with pneumonia, atrophic rhinitis, and/or mycoplasma infection (4, 11, 30). However, the distribution and prevalence of serotypes and pathotypes can vary considerably from region to region and over time in a given region. This is the first study in China of a large collection of isolates of *P. multocida* obtained from pigs with clinical signs of respiratory infection. Our findings suggest that strains of *P. multocida* are widely prevalent on pig farms, and we have confirmed that on the Chinese mainland, infections caused by *P. multocida* strains of serogroup D are more common than those caused by strains of serogroup A (P < 0.01). Similar results were reported by Ewers et al. (17) in Germany (58.1% versus 34.9%) and Chan-
P. multocida, as reported by Gutie´rrez Martin and Rodríguez
aminoglycoside antibiotics usually showed poor activity against
quinolones (ciprofloxacin) were the most active drugs. The
cephalosporins (cefazolin, ceftiofur), florfenicol, and fluoro-
Kehrenberg et al. (25) in France, Salmon et al. (32) in North
of our antibiotic susceptibility studies, like the findings of
cludes broad-spectrum antimicrobials (5, 25, 26). The findings
should be considered when attempts are made to control dis-
secondary pathogen in herds with mixed infections, the fact
should be considered when attempts are made to control dis-
trations have confirmed the presence of atrophic rhinitis in
lack of available tests.
whether the presence of untypeable isolates is attributable to a
lack of available tests.
Atrophic rhinitis is seldom reported in China; but our inves-
tigations have confirmed the presence of atrophic rhinitis in
Henan, Shandong, Fujian, Hainan, and Hubei Provinces by the
Atrophic rhinitis is seldom reported in China; but our inves-
tigations have confirmed the presence of atrophic rhinitis in
Henan, Shandong, Fujian, Hainan, and Hubei Provinces by the
strain A. During the study period, other bacterial species, including Haemophilus parasuis
and Streptococcus suis, were often coisolated with pathogenic P. multocida strains from the same sample. Although it is not easy to distinguish whether P. multocida is a primary or a
secondary pathogen in herds with mixed infections, the fact
that various bacterial species may coexist in a given herd
should be considered when attempts are made to control dis-
ease outbreaks (7).
Treatment for infections with P. multocida commonly in-
cludes broad-spectrum antimicrobials (5, 25, 26). The findings
of our antibiotic susceptibility studies, like the findings of
Kehrenberg et al. (25) in France, Salmon et al. (32) in North
America, and Yoshimura et al. (38) in Japan, indicated that
cephalosporins (cefazolin, ceftiofur), florfenicol, and fluoro-
quinoles (ciprofloxacin) were the most active drugs. The
aminoglycoside antibiotics usually showed poor activity against
P. multocida, as reported by Gutiérrez Martin and Rodríguez
Ferri (19) in Spain and Yoshimura et al. (38) in Japan; how-
ever, in the present study, spectinomycin, kanamycin, genta-
micin, and amikacin exhibited moderate activity against all
strains tested. The average prevalence of resistance to conven-
tional antibiotics, including amoxicillin, lincomycin, clindamy-
cin, chlorotetracycline, tetracycline, sulfamethazine, and tri-
methoprim-sulfamethoxazole, among the P. multocida isolates
was found to be in excess of 60% for each antibiotic. There-
fore, preventive and therapeutic effects on porcine P. multocida
strains should no longer be expected from these antibiotics.
Furthermore, the increased incidence of multidrug-resistant patho-
genic bacteria has been widely reported in the last decade (8, 35, 37). This is presumably attributable at least in part to the use
of antibiotic additives in animal feed and the extensive use of
antimicrobial agents in veterinary medicine. Here we have
shown that P. multocida exhibited a rapid increase in the rate
of resistance to a large number of antimicrobial agents. This
revealed that a high prevalence of multiple-drug resistance
exists among isolates of P. multocida from pigs. If this situation
continues, there will be no effective antibiotic therapeutic re-
serve for some bacterial infections. The implications of a large
reservoir of multiresistant organisms, particularly P. multocida,
which is not host specific, with resistance that is potentially
transferable among livestock species are obvious (3, 12, 24).
Therefore, the use of antimicrobial agents in food animals in
ways that minimize the emergence of resistance not only in
target pathogens but also in zoonotic bacteria is warranted
in the future for the protection of public health.
Although the molecular basis of the pathogenicity and host
specificity of P. multocida is not well understood, several stud-
ies have reported that a number of VFs are correlated with the
pathogenic mechanisms (20, 23). The present study has pro-
vided novel epidemiological information on the prevalence and distribution of the various VFs of porcine strains of P.

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TABLE 4. Distribution of VFs according to capsule serotypes among 233 porcine isolates of P. multocida

<table>
<thead>
<tr>
<th>VF gene</th>
<th>Total no. (% of 233)</th>
<th>No. (%) of VFs with the following capsule serotypes:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>capA (n = 92)</td>
</tr>
<tr>
<td>ptfA</td>
<td>218 (93.6)</td>
<td>82 (89.1)</td>
</tr>
<tr>
<td>fimA</td>
<td>231 (99.1)</td>
<td>90 (97.8)</td>
</tr>
<tr>
<td>hsf-1</td>
<td>156 (67.0)</td>
<td>35 (38.0)</td>
</tr>
<tr>
<td>hsf-2</td>
<td>231 (99.1)</td>
<td>91 (98.9)</td>
</tr>
<tr>
<td>pfhA</td>
<td>35 (15.0)</td>
<td>23 (25.0)</td>
</tr>
<tr>
<td>tadD</td>
<td>101 (43.3)</td>
<td>85 (92.4)</td>
</tr>
<tr>
<td>tacA</td>
<td>11 (4.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>exbA</td>
<td>231 (99.1)</td>
<td>91 (98.9)</td>
</tr>
<tr>
<td>exbD</td>
<td>231 (99.1)</td>
<td>91 (98.9)</td>
</tr>
<tr>
<td>hgbA</td>
<td>228 (97.9)</td>
<td>87 (94.6)</td>
</tr>
<tr>
<td>fur</td>
<td>225 (96.6)</td>
<td>90 (97.8)</td>
</tr>
<tr>
<td>nanB</td>
<td>190 (81.5)</td>
<td>89 (96.7)</td>
</tr>
<tr>
<td>nanH</td>
<td>226 (97.0)</td>
<td>91 (98.9)</td>
</tr>
<tr>
<td>pmHAS</td>
<td>105 (45.1)</td>
<td>77 (83.7)</td>
</tr>
<tr>
<td>ompA</td>
<td>233 (100)</td>
<td>92 (100)</td>
</tr>
<tr>
<td>ompH</td>
<td>217 (93.1)</td>
<td>88 (95.7)</td>
</tr>
<tr>
<td>oma87</td>
<td>220 (94.4)</td>
<td>83 (90.2)</td>
</tr>
<tr>
<td>plpB</td>
<td>231 (99.1)</td>
<td>91 (98.9)</td>
</tr>
</tbody>
</table>

*a* All genes were detected by PCR.

*b* P < 0.05 for the indicated group compared with the results for all other strains (negative association).

*c* P < 0.01 for the indicated group compared with the results for all other strains (negative association).

*d* P < 0.001 for the indicated group compared with the results for all other strains.

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The treatment of infections with P. multocida commonly in-
cludes broad-spectrum antimicrobials (5, 25, 26). The findings
of our antibiotic susceptibility studies, like the findings of
Kehrenberg et al. (25) in France, Salmon et al. (32) in North
America, and Yoshimura et al. (38) in Japan, indicated that
cephalosporins (cefazolin, ceftiofur), florfenicol, and fluoro-
quinoles (ciprofloxacin) were the most active drugs. The
aminoglycoside antibiotics usually showed poor activity against
P. multocida, as reported by Gutiérrez Martin and Rodríguez
multocida. Consistent with previous observations (17), the prevalence of 11 of the 19 VF genes examined, which encode colonization factors (ptfA, fimA, and hsf-2), iron acquisition factors, sialidases (nanH), and outer membrane proteins, were broadly characteristic of the three categories of isolates (serogroup A, serogroup D, and others [1 serogroup B isolate and 12 untypeable isolates]). These distribution patterns would support some lines of evidence that suggest that factors involved in cross-protection may potentially serve as vaccine candidates that can elicit homologous protective immunity against all serotypes of P. multocida (2, 20, 35). However, certain VFs varied significantly among the different serogroups. For example, hsf-1, which has been described to be an autotransporter adhesin in a common avian clone, Pm70 (27), was concentrated significantly in serogroup A. Protein D in Pm70 (27), was more frequently seen in serogroup D, whereas tadD, which has been described as putative nonspecific tight adherence protein D in Pm70 (27), was concentrated significantly in serogroup A. pftA, which governs the adherence of Bordetella pertussis to host cells and which plays a role in the virulence of P. multocida (18, 20, 27), showed a low prevalence in strains of serogroup D compared with its prevalence in serogroup A or untypeable isolates. Various hyaluronan synthases have been described in the last 5 years (13, 14). Preliminary data from a Southern blot analysis suggested that the P. multocida serogroup A hyaluronan synthase PmHAS and the P. multocida serogroup D hyaluronan synthase PmHS1 were not similar at the DNA level (13). However, our study showed that PmHAS not only was prevalent in serogroup A strains but also was found in other serogroups of porcine P. multocida. It seems probable that different VFs have entered P. multocida strains independently at multiple different times in the evolutionary history of the species and at multiple positions within the phylogenetic tree. Moreover, the observed distribution pattern suggests that it is likely that the acquisition of certain VFs has led to divergent patterns of vertical inheritance and horizontal transmission (via pathogenicity-associated islands, plasmids, and transposons) within the P. multocida population.

In conclusion, given that it is a pathogenic microorganism that is not host specific, we believe that the occurrence of P. multocida in food-producing animals should not be forgotten. In China, as in many other countries, strains of P. multocida have frequently been isolated from pigs, and they represent a significant cause of territorial outbreaks of respiratory infections. The high prevalence of multiresistant strains of P. multocida in pigs and the association of such strains with serious disease strongly suggest that more attention should be paid to the prudent use of antimicrobials and to vaccination. Nowadays, many key VFs of P. multocida are slowly being identified. Further work is required to elucidate the mechanisms of pathogenesis and to determine unequivocally the role of these factors in immunity to pasteurellosis.

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REFERENCES


