Genotypic Characterization of Ureaplasma Serovars from Clinical Isolates by Pulsed-Field Gel Electrophoresis


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Genetic relationships within ureaplasma serovars were investigated by pulsed-field gel electrophoresis (PFGE). One hundred thirteen Ureaplasma parvum isolates and 78 Ureaplasma urealyticum isolates were different from their ATCC serovar type strains and different within the same serovars. The organisms were geographically widespread. No unique patterns were associated with invasive disease.

Two Ureaplasma species infect humans. There are 14 serovars in the two species, four serovars in Ureaplasma parvum (serovars 1, 3, 6, and 14) and the remaining 10 serovars in Ureaplasma urealyticum (8). Although found in many healthy persons, Ureaplasma spp. are sometimes implicated in clinically significant infections, such as nongonococcal urethritis (NGU), chorioamnionitis, bacteremia, meningitis, and bronchopulmonary dysplasia (BPD) in neonates (11). Whether differential pathogenicity exists in Ureaplasma at the species or serovar level has not been resolved, and little is known regarding the genetic relatedness of clinical isolates within a serovar.

Pulsed-field gel electrophoresis (PFGE) is a widely accepted reference standard for genotyping bacteria. PFGE has been used to differentiate the two Ureaplasma species and to distinguish among the 14 serovars and a limited number of clinical isolates of the same serovar (6). However, this technique is time-consuming and somewhat tedious to perform.

To improve understanding of the differential pathogenicity and characterize the genetic relatedness of ureaplasmas within the same serovar, we used a modified PFGE method to analyze clinical isolates from asymptomatic controls and persons with various clinical diseases from the United States and Canada. The species and serovar designations were determined by real-time PCR (12). One hundred thirteen unique U. parvum isolates and 78 unique U. urealyticum isolates containing a single serovar or that were nontypeable were evaluated (Table 1).

The genomes of U. parvum serovar 3 (ATCC 27815) and U. urealyticum serovar 10 (ATCC 33699) were analyzed for restriction sites, and BssHII and BamHI were selected based on fragment numbers and sizes that allowed for separation by in silico PFGE. A simplified PFGE method that shortened the experimental time by 2 or 3 days while producing similar results was developed based on the previous procedures (6). Briefly, 2 mg/ml of lysozyme and 10 mg/ml of proteinase K were added to the washed bacterial pellets, and agarose plugs were made. Lysis and digestion of the plugs were combined by incubating at 55°C for 90 min in EC buffer (6) containing 0.1 mg/ml proteinase K. The plugs were then quickly digested by 40 U BssHII at 50°C for 2 h or 30 U BamHI-HF (New England BioLabs, Ipswich, MA) at 37°C for 30 min. PFGE was performed on a CHEF II mapper (Bio-Rad, Hercules, CA). BssHII-digested products were run at 200 V (6 V/cm) and 14°C on a 1.0% agarose gel with a switch time of 1 to 40 s for 20 h. BamHI-digested products were run at 200 V and 14°C on a 1.5% agarose gel with a switch time of 1 to 12 s for 28 h. Gel images were analyzed with BioNumerics. A cutoff of ≥80% similarity was used to designate related isolates (10).

The 14 ATCC serovar type strains were distinguished into two major groups, U. parvum and U. urealyticum, in both BssHII and BamHI patterns, and the patterns were stable after 10 serial passages (Fig. 1A and B). BssHII patterns were similar to the previous ones (6), and 4 U. parvum serovars were completely separated. BamHI showed better discrimination, and all serovars were separated except serovars 10 and 12. Finally, BssHII was used to analyze U. parvum isolates, and BamHI was used to analyze U. urealyticum isolates.

Among 113 U. parvum isolates, the 31 serovar 1 isolates formed 11 different patterns (Fig. 1C). Isolates from asymptomatic controls and from diseased patients were clustered together in the 5 patterns and even in the 7 subgroups showing 100% similarities. No clinical isolate grouped together with the ATCC type strain. Similar trends were observed in serovars 3 and 6 (see Fig. S1 in the supplemental material). The serovar 14 ATCC strain grouped with 1 isolate (Fig. S1). There were 2 U. parvum isolates negative for the any of the 4 known U. parvum serovars. Compared with all 14 ATCC type strains, both were clustered with the U. parvum group with low similarities (43% and 25%), although 1 isolate carried a marker of U. urealyticum serovar 9 (Fig. S1).

Among 78 U. urealyticum isolates, BamHI patterns showed wide genetic diversity in each of the U. urealyticum serovars. Serovar 10 was the best represented U. urealyticum serovar with 15 isolates. Six patterns were formed (Fig. 1D). The larg-
est one included 4 isolates from Canadian NGU patients; another pattern had 3 isolates from symptomatic patients from the United States, indicating a possible geographic association. Isolates from both control and symptomatic groups were clustered together in 3 patterns, and one group contained the serovar 10 ATCC type strain. Similarly, in serovar 7 which had 11 isolates, the largest pattern contained 4 isolates, 2 highly similar ones from infants without BPD (97% similarity) and 2 from the symptomatic adult patients (see Fig. S2 in the supplemental material). Other serovars contained fewer than 10 isolates. However, the trends in pattern components were similar to those in serovars 10 and 7 (Fig. S2).

To date, this is the largest and most comprehensive study performed using PFGE to investigate the genetic relatedness of U. parvum and U. urealyticum. Great genetic variation of strains within the same serovar exists in most of the 14 serovars and is observed between type strains and the clinical isolates and among the clinical isolates themselves. Most of the BssHII and BamHI sites in type strains are in genes that encode housekeeping enzymes or putative proteins. The great genetic variability of the clinical isolates indicates that they have either acquired mutations in the restriction sites or large deletions or insertions have occurred between the restriction sites. Horizontal gene transfer (HGT) has been observed between U. parvum and Mycoplasma hominis (7). HGT also occurs among Ureaplasma species and serovars (13) and can generate chimeric isolates containing markers and/or genes from two or more serovars. This genetic heterogeneity should then be reflected by different PFGE patterns. Another possible mechanism is the frequent occurrence of repetitive elements in the mollicute genomes that may generate polymorphisms and is considered to be an indication of intense selection pressure (9). Additionally, the existence of multiple phase-variable gene systems in ureaplasmas that operate by chromosome rearrangements will complicate the banding patterns for some PFGE conditions (14, 15). The high degree of heterogeneity of Ureaplasma isolates may reflect a rapid evolution as demonstrated in several other mycoplasmal species (2). The 14 type strains of Ureaplasma exhibited stable PFGE patterns after 10 serial passages, indicating the stability of the genomes, whereas isolates from different patients exhibited substantial variation, suggesting adaptation to the host environment by selective evolutionary pressure.

The fact that closely related PFGE patterns within each serovar contained organisms from varied geographic regions and included invasive and commensal isolates suggests that these clonal organisms were widespread geographically and that there were no unique patterns associated with invasive

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**TABLE 1. Description of Ureaplasma clinical isolates**

<table>
<thead>
<tr>
<th>Group and specimen type</th>
<th>Group description</th>
<th>No. of isolates belonging to the following serovar:</th>
<th>No. of isolates*</th>
<th>Total no. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 3 6 14 2 4 5 7 8 9 10 11 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal swabs</td>
<td>Healthy pregnant females (female control [FCtrl])</td>
<td>13 12 11 1</td>
<td>1 1 39</td>
<td></td>
</tr>
<tr>
<td>Placental tissue samples collected at Cesarean section</td>
<td>Females without histologic chorioamnionitis (Chor Neg)</td>
<td>3 5 1 1 3 1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Catheterized urine samples</td>
<td>Males with neurogenic bladder without urethritis (male control [MCtrl])</td>
<td>1 1 2 1 1 1</td>
<td>1 9</td>
<td></td>
</tr>
<tr>
<td>Endotracheal aspirate samples</td>
<td>Preterm infants without bronchopulmonary dysplasia (BPD Neg)</td>
<td>1 2 3 1 1</td>
<td>2 10</td>
<td></td>
</tr>
<tr>
<td>Diseased groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial biopsy tissue samples</td>
<td>Females with pelvic inflammatory disease (PID) and/or postpartum endometritis (ENDO)</td>
<td>6 6 6 1 3 1 4</td>
<td>1 3 31</td>
<td></td>
</tr>
<tr>
<td>Placental tissue samples collected at Cesarean section</td>
<td>Females with histologic chorioamnionitis (Chor Pos)</td>
<td>1 2 2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Endotracheal aspirate samples</td>
<td>Preterm infants with bronchopulmonary dysplasia (BPD Pos)</td>
<td>1 5 2 1</td>
<td>2 13</td>
<td></td>
</tr>
<tr>
<td>Urethral swabs or urine samples</td>
<td>Males with nongonococcal urethritis from Canada (CaNGU)</td>
<td>3 3 6 1 2 1</td>
<td>9 43</td>
<td></td>
</tr>
<tr>
<td>Urethral swabs or urine samples</td>
<td>Males with nongonococcal urethritis from the United States (US NGU)</td>
<td>1 7 3 2 1</td>
<td>4 22</td>
<td></td>
</tr>
<tr>
<td>Blood, cerebrospinal fluid, synovial fluid, pleural fluid, or lung tissue samples</td>
<td>Invasive isolates from various patient groups including adults and children (IVIS)</td>
<td>1 1</td>
<td>2 5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31 43 34 3 4 2 2 11 3 6 15 9 1 1</td>
<td>2 24 191</td>
<td></td>
</tr>
</tbody>
</table>

* NT, nontypeable; Up, Ureaplasma parvum; Uu, Ureaplasma urealyticum.
disease. This is in agreement with results of a previous randomly amplified polymorphic DNA (RAPD) PCR typing study (3).

We have shown that PFGE has the potential to discriminate and assess genetic relatedness of the Ureaplasma clinical isolates within the same serovar, which could not be completely achieved by other molecular typing methods (3–5, 12). The RAPD PCR may provide resolution similar to that of PFGE and is quicker and less technically demanding (3). Though widely used as a genotyping tool, PFGE is a very imprecise form of whole-genome sequence analysis. Next-generation DNA sequencing technologies, such as 454 pyrosequencing or Illumina sequencing by synthesis are comparable in cost, speed, and requirements for starting material to PFGE while offering much higher resolution (1).

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FIG. 1. PFGE banding patterns and dendrograms for 14 ATCC serovars and clinical isolates of serovar 1 and serovar 10. (A) BssHII restriction patterns of U. parvum ATCC strains (serovars 1, 3, 6, and 14) at passages 1 and 10 (Pass 1 and Pass 10). (B) BamHI restriction patterns of U. urealyticum (serovars 2, 4, 5, and 7 to 13) at passages 1 and 10. (C) BssHII patterns of 31 U. parvum serovar 1 isolates. (D) BamHI patterns of 15 U. urealyticum serovar 10 isolates. The serovar types are given to the right of the PFGE banding pattern. Control and diseased group designations are defined in Table 1 in the Group description column.

REFERENCES


