Patterns of Colonization With *Ureaplasma urealyticum* During Neonatal Intensive Care Unit Hospitalizations of Very Low Birth Weight Infants and the Development of Chronic Lung Disease

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**ABSTRACT.** Background. *Ureaplasma urealyticum* and its association with chronic lung disease (CLD) of prematurity has remained a controversial topic. To redress this question, we performed a longitudinal study using culture and polymerase chain reaction to detect *U urealyticum* in the respiratory tract of very low birthweight infants throughout their neonatal intensive care unit hospitalizations.

Methods. We screened 125 infants weighing <1500 g and/or <32 weeks' gestational age over a 12-month period, collecting endotracheal, nasopharyngeal, and throat specimens on days of age 1, 3, 7, and weekly thereafter. CLD was defined as dependency on supplemental oxygen at 28 days and at 36 weeks' postconceptual age.

Results. Forty infants (32%) had 1 or more positive specimens by culture or polymerase chain reaction. We identified 3 patterns of *U urealyticum* colonization: persistently positive (n = 18), early transient (n = 14), and late acquisition (n = 8). We compared the rates of CLD in each of the 3 colonized groups with the rate of CLD in the noncolonized group. We found a significantly higher rate of CLD at 28 days of age (odds ratio: 8.7; 95% confidence interval: 3.3, 23) and at 36 weeks' postconceptional age (odds ratio: 38.5; 95% confidence interval: 3.3, 374) only for infants with persistently positive colonization.

Conclusions. This study demonstrates that the risk of developing CLD varies with the pattern of *U urealyticum* colonization. Only the persistently positive colonization pattern, which accounted for 45% of the *U urealyticum*-positive infants, was associated with a significantly increased risk of development of CLD. *Pediatrics* 2002; 110(4). URL: http://www.pediatrics.org/cgi/content/full/110/4/e45; bronchopulmonary dysplasia, chronic lung disease, premature infant, polymerase chain reaction, *Ureaplasma urealyticum*, very low birth weight infant.

**ABBREVIATIONS.** CLD, chronic lung disease; PDA, patent ductus arteriosus; PCR, polymerase chain reaction; VLBW, very low birth weight; NICU, neonatal intensive care unit; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

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**MATERIALS AND METHODS**

The study was approved by the Department of Pediatrics and Columbia University Institutional Review Boards. All VLBW infants consecutively admitted to Children’s Hospital of New York between March 1999 and April 2000 were eligible for the study. Exclusion criteria were admission after 2 weeks of life, lethal anomalies, congenital heart disease, and congenital pulmonary disorders other than pulmonary disease due to prematurity. Infants who died or were transferred by 14 days of life were excluded from the final analyses. Gestational age was established based on priority by obstetrical estimates using early ultrasound (<17 weeks), last menstrual period or Ballard assessment. Clinical courses of mothers and infants were followed by ongoing chart abstraction until discharge or 36 weeks' postconceptional age.

Endotracheal (if intubated), nasopharyngeal and throat specimens were collected on days of age 1, 3, and 7, and weekly thereafter through to discharge or transfer. All specimens were
processed by culture and PCR. Study investigators processing the specimens were blinded to the identities of the infants.

**Culture Methods**

Tracheal aspirates were obtained during routine suctioning after instillation of 1 mL normal saline and collected into tracheal suction traps (Sherwood Services, Chicopee, MA). Nasopharyngeal and throat specimens were obtained using mini-tip culturettes (BBL, Starks, MD). Specimens were inoculated onto A7 agar plates (Remel, Lenexa, Kansas) and into tubes containing 2 mL of 10B broth (Remel) at the bedside, transported to the laboratory and incubated at 37°C. A7 plates were incubated in 5% CO2. Plates and broth were observed for 7 days. Broths exhibiting a color change were subcultured onto A-7 agar plates. *U urealyticum* were identified as morphotypical golden-brown colonies on days 1, 2, 5, and 7 of incubation.

**PCR Methods**

After 24 hours, 250 μL of inoculated broths were removed, frozen at −20°C, and batched for PCR processing. Broths were thawed and centrifuged at 12,000 g at 4°C for 20 minutes. The supernatant was discarded; the pellet was resuspended in 50 μL solution A (TRIS HCL pH 8.3 10 mM, KCL 100 mM, MgCl2 2.5 mM) and an equal volume of solution B (TRIS HCL 8.3 20 mM, Tween 2%, MgCl2 5 mM, Triton-X 2%), Proteinase K [GIBCO, Gaithersburg, MD, 0.5 mg/mL], Proteinase K buffer [TRIS HCL 7.5 10 mM, CaCl2 20 mM, Glyceral 50%] and incubated at 60°C for 60 minutes then heated to 100°C for 10 minutes. Primers used were the US sense (5’-CAATCTGCTGTGAAAGTTAC-3’) and U4 antisense (5’-ACGACGTCCATAAGCACT-3’) of the urease structural genes. One positive (*U urealyticum*, ATCC, Manassas, VA) and one negative control (10B broth) were included in each PCR batch. Twenty two and a half microliters of Taq supermix (GIBCO), 2.5 μL of sample, and 5 μL of water were added to each reaction. A thermal cycler was used to process samples through 41 cycles of denaturation at 94°C for 2 minutes and 20 seconds, primer annealing at 62°C for 60 seconds, and extension at 72°C for 60 seconds. Amplified products were analyzed by electrophoresis with 2% agarose gels containing ethidium bromide and the 429 base pair DNA fragments were visualized by ultraviolet fluorescence.

**Data Analyses**

Analysis of preliminary data suggested that the rate of CLD among *U urealyticum* positive infants was approximately twice that of negative infants. Using a baseline CLD rate of 25% in the noncolonized group and the assumption that the number of *U urealyticum* positive and negative infants would be similar, we calculated that an overall sample size of 132 infants would be needed to have 80% power to detect a significant difference between CLD rates with *U urealyticum* colonization. Data analyses were performed using SPLUS, version 4.5 (Mathsoft, Inc, WA) and one negative control (10B broth) were included in each PCR batch. Twenty two and a half microliters of Taq supermix (GIBCO), 2.5 μL of sample, and 5 μL of water were added to each reaction. A thermal cycler was used to process samples through 41 cycles of denaturation at 94°C for 2 minutes and 20 seconds, primer annealing at 62°C for 60 seconds, and extension at 72°C for 60 seconds. Amplified products were analyzed by electrophoresis with 2% agarose gels containing ethidium bromide and the 429 base pair DNA fragments were visualized by ultraviolet fluorescence.

**RESULTS**

One hundred fifty-nine infants weighing <1500 g and/or <32 weeks’ gestational age were consecutively admitted within the first 2 weeks of life to the NICU during the study period. Five infants had congenital heart disease, 1 infant had an undefined neuropathy, 15 infants expired, and 13 infants were transferred to other institutions within the first 2 weeks of life. The remaining 125 infants were followed until discharge, transfer, or death. One infant in the persistently positive category and 4 infants in negative group expired beyond 2 weeks of life. Of the infants in the negative group who had recovered from acute lung disease, 3 infants died and were transferred by 28 days of age, and 2 additional infants were transferred by 36 weeks’ postconceptional age.

A total of 3720 specimens (1860 culture and 1860 PCR) were collected (mean: 29.8 and range: 6–92 per infant). Thirty-two percent (40/125) of study infants had 1 or more positive specimens by culture or PCR for *U urealyticum*. PCR identified 100% of all colonized infants versus 57.5% identified by culture. The greater sensitivity of PCR versus culture was most apparent beyond 21 days of age.

Three patterns of infant colonization were identified: Persistently positive colonization (n = 18) defined as having positive specimens by culture or PCR throughout their hospitalization, early transient colonization (n = 14) defined as having at least 1 specimen positive by culture or PCR at ≤21 days of age with all subsequent specimens negative and late acquisition (n = 8) defined as negative cultures and PCR specimens until day of age 21 with subsequent positive specimens. Eighty-five infants had all culture and PCR specimens negative for *U urealyticum*. The overall incidences of CLD at 28 days and at 36 weeks’ postconceptional age were 24.2% (29/120) and 6.8% (8/118), respectively. Comparison of positive and negative colonization pattern with their rates in the negative colonization pattern. Remaining 125 infants were transferred by 36 weeks’ postconceptional age.

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Table 2 displays measures of association between infant characteristics and each pattern of *U urealyticum* colonization. We found significant differences in mean birth weight, mean gestational age, birth weight distribution, gender, inborn (vs outborn),
need for delivery room resuscitation, low Apgar scores at 5 minutes of life, and need for conventional ventilation among infants with persistently positive U. urealyticum colonization compared with infants with negative colonization. Early transient and late acquisition colonization rates compared with those for infants in the negative colonization group did not differ.

Maternal characteristics considered possible risk factors for U. urealyticum colonization were also analyzed. In addition, although this was not part of the study design, 71 placentas were examined for clinical indications: 52 from the negative group, 6 from early transient, 4 from late acquisition, and 9 from persistently positive colonization patterns. Compared with the pattern of negative colonization, persistently positive U. urealyticum colonization was significantly associated with histologic evidence of placental chorioamnionitis (OR: 19.0; 95% CI: 5.64, 64.00), clinical chorioamnionitis defined as maternal evidence of infection including fever, leukocytosis, maternal and fetal tachycardia, uterine tenderness or malodorous vaginal discharge (OR: 7.40; 95% CI: 1.03, 53.2), prolonged rupture of membranes defined as rupture of membranes for >24 hours (OR: 13.0; 95% CI: 4.16, 40.4), premature rupture of membranes defined as rupture of membranes before the onset of labor (OR: 19.2; 95% CI: 7.10, 52.1), and preterm labor (OR: 34.6; 95% CI: 15.2, 78.9).

There was no significant association between these factors and the other 2 U. urealyticum-positive colonization patterns.

Table 3 displays ORs for the strength of association between the CLD outcomes (at 28 days of age and 36 weeks' postconceptional age) and risk factors for the development of CLD. Birth weight <750 g, gestational age <26 weeks, gender, PDA, inborn (vs outborn), sepsis defined as a symptomatic infant prompting a work up, and yielding a positive blood culture, surfactant administration, and persistently positive U. urealyticum colonization were significantly associated with CLD at 28 days of age. Risk factors significantly associated with CLD at 36 weeks' postconceptional age were birth weight <750 g, gestational age <26 weeks, PDA, surfactant administration, and persistently positive U. urealyticum colonization.

Table 3 displays the adjusted ORs and CIs for factors associated with CLD in the 2 multiple logistic regression models (at 28 days of age and 36 weeks' postconceptional age) produced after stepwise removal of nonsignificant predictor terms. In the first model, birth weight <750 g (AOR: 5.03; 95% CI: 1.29, 19.7), gestational age <26 weeks (AOR: 5.58; 95% CI: 1.48, 21), gender (AOR: 4.03; 95% CI: 1.08, 15.1), PDA (AOR: 14.1; 95% CI: 2.97, 67), and persistently positive U. urealyticum colonization (AOR: 5.33; 95% CI: 1.05, 27) were associated with CLD at 28 days of age. The adjusted ORs for each of these terms corresponds in magnitude to the unadjusted OR. In the second model only 2 variables, sepsis (AOR: 10.3; 95% CI: 1.53, 69) and persistently positive colonization (AOR: 33.6; 95% CI: 4.78, 237), were associated with CLD at 36 weeks' postconceptional age. The AOR for persistently positive colonization in this model is similar in magnitude to the unadjusted OR (OR: 24.5; 95% CI: 4.44, 135). The wide CIs for these ORs is most likely attributable to the small number of infants (8, of whom 6 had persistently positive colonization) with CLD at 36 weeks' postconceptional age in our sample.

### DISCUSSION

This prospective longitudinal study demonstrates that the pattern of persistently positive U. urealyticum colonization is associated with CLD at 28 days and 36 weeks' postconceptional age. Neither early transient colonization nor late acquisition of U. urealyticum was associated with CLD. Recognition that different patterns of U. urealyticum colonization exist and have different risks of CLD may help clarify the conflicting earlier reports regarding the association between U. urealyticum and CLD.

Although this work demonstrates a strong associ-

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**Table 1. Outcome Data and Colonization Patterns of Ureaplasma urealyticum in Preterm VLBW Infants**

<table>
<thead>
<tr>
<th>Outcome*</th>
<th>Persistently Positive</th>
<th>Early Transient</th>
<th>Late Acquisition</th>
<th>Negative</th>
<th>N Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 18</td>
<td>n/N (%)</td>
<td>n/N (%)</td>
<td>n/N (%)</td>
<td>n/N (%)</td>
<td>P Value†</td>
</tr>
<tr>
<td>CLD at 28 d of age</td>
<td>12/18 (67)</td>
<td>1/14 (7)</td>
<td>1/8 (12.5)</td>
<td>15/80 (19)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>CLD at 36 wk postconceptional age</td>
<td>8.7 (3.3, 23)‡</td>
<td>0.33 (0.2, 5.6)</td>
<td>0.62 (0.4, 10.5)</td>
<td>1/8 (12.5)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Radiographic diagnosis of CLD</td>
<td>38.5 (4.0, 74)‡</td>
<td>0/14 (0)</td>
<td>1.0 (0.4, 3.3)</td>
<td>1/78 (1.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>Mean length of stay</td>
<td>9.17 (53)</td>
<td>0/14 (0)</td>
<td>2/25</td>
<td>13/84 (15)</td>
<td>.002</td>
</tr>
</tbody>
</table>

* One infant in persistently positive category and 4 infants in negative group expired beyond 2 weeks of life. Of the infants in the negative group who had recovered from acute lung disease, 3 infants died and 2 were transferred by 28 days of age, and 2 additional infants were transferred by 36 weeks' postconceptional age.

† P value based on global χ² test or Fisher exact test on 3 degrees of freedom, or on 1-way analysis of variance F test (for length of stay).

‡ P value <.05 when 95% CI for OR excludes 1.

§ P <.05 by post-hoc Dunnett test, using negative U urealyticum colonization status as the reference level (control).

∥ Radiographs were obtained within a 7-day window from the CLD definitions (day of life 28 and 36 weeks' postconceptional age). The radiographs were read independently by pediatric radiologists who were blinded to the U urealyticum colonization patterns. Radiographic results were not available for 1 infant in the persistently positive and 1 infant in the negative categories.

¶ Infants transferred to other institutions were not included in the analysis.
It is possible that persistent colonization and CLD, it does not establish a causal relationship. It is possible that persistent *Ureaplasma urealyticum* colonization is a marker of other multifactorial factors that lead to CLD.Persistently colonized infants were smaller, younger, and sicker than the culture-negative, early transient, and late acquisition groups. However, controlling for these factors in the multivariate analyses, this association between persistently positive colonization and CLD was similar. Inborn 12 (67) Vaginal delivery 10 (56) Death beyond 2 weeks of life 1 (6) 11 (79) 0 (0) 6 (7) .52 Paten ductus arteriosus 12 (67)‡ 4 (29) 0 (0) 6 (7) .52 Any intraventricular hemorrhage 2 (11) — — — 2 (25) 14 (17) .23 Any episode of sepsis 5 (28) 4 (29) 2 (25) 14 (17) .23 Necrotizing enterocolitis 1.6 (0.4, 7.0) 0.4 (0.02, 7.1) 1.8 (0.2, 14) 6 (7) .52 Surface tension administration 6 (4, 22) — 1 (7) 2 (25) 13 (15) .55 Pneumothorax 2 (11) 0 (0) 0 (0) 6 (7) .52 Late postnatal steroid use 1 (6) 0 (0) 0 (0) 1 (1) .56 Any intraventricular hemorrhage 2 (11) — — — 2 (25) 14 (17) .23 Any episode of sepsis 5 (28) 4 (29) 2 (25) 14 (17) .23 Necrotizing enterocolitis 1.6 (0.2, 13) — — — 1.7 (0.2, 13) .08 Patent ductus arteriosus 12 (67)‡ 4 (29) 0 (0) 6 (7) .52 Death beyond 2 weeks of life 1 (6) 0 (0) 0 (0) 6 (7) .52 Surfactant administration 5.5 (2.3, 13) 0.4 (0.08, 2.3) 1.6 (0.4, 6.7) 3 (4) .34 High-frequency oscillatory ventilation 6.4 (1.6, 26) — 1.8 (0.1, 33) 6 (7) .006 Conventional ventilation 14 (78) 3 (21) 4 (50) 33 (39) .006 Univariate analysis of variance F test (for continuous variables). Average of the three Apgar scores. Delivery room resuscitation defined as need for positive pressure ventilation with bag and mask or intubation with positive pressure ventilation. All other infants were placed immediately onto nasal continuous positive airway pressure. Use of conventional ventilation defined as being required at any time during hospitalization. Sepsis defined as a symptomatic infant prompting a work up and yielding a positive blood culture. The organisms identified from blood cultures included: 1 Escherichia coli, 1 group D Streptococcus, 1 Group B Streptococcus, 15 Staphylococcus epidermidis, 5 Staphylococcus aureus, 3 Staphylococcus hominis, 2 Klebsiella pneumoniae, 3 Enterobacter cloacae, 1 Acinetobacter, and 4 Candida. Retinopathy of prematurity defined as stage 1 or greater during screening examination at any age during hospitalization. Patent ductus arteriosus defined as a symptomatic infant with a murmur and/or bounding pulses with echocardiographic confirmation. **P value based on global \(R^2\) test or Fisher exact test on 3 degrees of freedom, or on 1-way analysis of variance F test (for continuous variables). † † P value <.05 when 95% CI for OR excludes 1. § Delivery room resuscitation defined as need for positive pressure ventilation with bag and mask or intubation with positive pressure ventilation. All other infants were placed immediately onto nasal continuous positive airway pressure. Use of conventional ventilation defined as being required at any time during hospitalization. Sepsis defined as a symptomatic infant prompting a work up and yielding a positive blood culture. The organisms identified from blood cultures included: 1 Escherichia coli, 1 group D Streptococcus, 1 Group B Streptococcus, 15 Staphylococcus epidermidis, 5 Staphylococcus aureus, 3 Staphylococcus hominis, 2 Klebsiella pneumoniae, 3 Enterobacter cloacae, 1 Acinetobacter, and 4 Candida. Retinopathy of prematurity defined as stage 1 or greater during screening examination at any age during hospitalization. Patent ductus arteriosus defined as a symptomatic infant with a murmur and/or bounding pulses with echocardiographic confirmation.

### TABLE 2. Patient Characteristics by *Ureaplasma urealyticum* Colonization Pattern in Preterm VLBW Infants

| Characteristics | Persistently Positive
text| Early Transient
text | Late Acquisition
text | Negative
text | P Values* |
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Continuous Variables</td>
<td>Mean ± Standard Error, Range</td>
<td>Mean ± Standard Error, Range</td>
<td>Mean ± Standard Error, Range</td>
<td>Mean ± Standard Error, Range</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>755 ± 9.0†</td>
<td>1192 ± 19.9 (530–1170)</td>
<td>1068 ± 25.1 (900-1580)</td>
<td>1028 ± 3.6 (510–1350) &lt;.001</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>25.2 ± 0.10†</td>
<td>29.4 ± 0.15 (23–29)</td>
<td>28.0 ± 0.28 (25–32)</td>
<td>28.2 ± 0.03 (23–35) &lt;.001</td>
<td></td>
</tr>
<tr>
<td>Categorical Variables</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Birth weight distribution (g)</td>
<td>10 (56)</td>
<td>1 (7)</td>
<td>0 (0)</td>
<td>22 (26)</td>
<td>.004</td>
</tr>
<tr>
<td>&lt;750</td>
<td>3.58 (1.31, 9.8)</td>
<td>0.22 (0.01, 3.7)</td>
<td>—</td>
<td>18 (21)</td>
<td>.33</td>
</tr>
<tr>
<td>750–999</td>
<td>2.37 (0.74, 7.6)</td>
<td>0.62 (0.08, 4.7)</td>
<td>0.53 (0.03, 8.9)</td>
<td>45 (53) &lt;.001</td>
<td></td>
</tr>
<tr>
<td>≥1000</td>
<td>0.05 (0, 0.86)</td>
<td>3.26 (1.28, 8.3)</td>
<td>6.22 (2.01, 19.3)</td>
<td>48 (56) .03</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>10 (56)</td>
<td>2 (14) †</td>
<td>4 (50)</td>
<td>50 (53)</td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>10 (56) †</td>
<td>4 (29)</td>
<td>3 (37.5)</td>
<td>20 (24) .06</td>
<td></td>
</tr>
<tr>
<td>Inborn</td>
<td>4.1 (1.5, 11.2)</td>
<td>1.3 (3.3.7)</td>
<td>2.0 (4, 10.4)</td>
<td>79 (93) .003</td>
<td></td>
</tr>
<tr>
<td>Multiple birth</td>
<td>12 (67)‡</td>
<td>14 (100)</td>
<td>6 (75) §</td>
<td>26 (31) &lt;.001</td>
<td></td>
</tr>
<tr>
<td>Resuscitation at delivery</td>
<td>11.4 (4.8, 27)</td>
<td>0.91 (0.2, 3.9)</td>
<td>0.32 (0.2, 5.4)</td>
<td>14 (17) .07</td>
<td></td>
</tr>
<tr>
<td>Apgar score &lt;3, 1 min</td>
<td>3.1 (0.9, 10)</td>
<td>0.82 (0.16, 3.3)</td>
<td>0 (0)</td>
<td>6 (7) .006</td>
<td></td>
</tr>
<tr>
<td>Apgar score &lt;6, 5 min</td>
<td>6 (33)</td>
<td>0 (0)</td>
<td>1 (12.5)</td>
<td>24 (28) .26</td>
<td></td>
</tr>
<tr>
<td>Conventional ventilation</td>
<td>14 (78)</td>
<td>3 (21)</td>
<td>4 (50)</td>
<td>33 (39) .006</td>
<td></td>
</tr>
<tr>
<td>High-frequency oscillatory ventilation</td>
<td>5.5 (2.3, 13)</td>
<td>0.4 (0.08, 2.3)</td>
<td>1.6 (0.4, 6.7)</td>
<td>3 (4) .34</td>
<td></td>
</tr>
<tr>
<td>Surfactant administration</td>
<td>4 (22)</td>
<td>1 (7)</td>
<td>2 (25)</td>
<td>13 (15) .55</td>
<td></td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>2 (11)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (7) .52</td>
<td></td>
</tr>
<tr>
<td>Late postnatal steroid use</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1) .56</td>
<td></td>
</tr>
<tr>
<td>Any intraventricular hemorrhage</td>
<td>2 (11)</td>
<td>0 (0)</td>
<td>2 (25)</td>
<td>14 (17) .23</td>
<td></td>
</tr>
</tbody>
</table>

* P value based on global \(R^2\) test or Fisher exact test on 3 degrees of freedom, or on 1-way analysis of variance F test (for continuous variables). † † P value <.05 when 95% CI for OR excludes 1. § Delivery room resuscitation defined as need for positive pressure ventilation with bag and mask or intubation with positive pressure ventilation. All other infants were placed immediately onto nasal continuous positive airway pressure. Use of conventional ventilation defined as being required at any time during hospitalization. Sepsis defined as a symptomatic infant prompting a work up and yielding a positive blood culture. The organisms identified from blood cultures included: 1 Escherichia coli, 1 group D Streptococcus, 1 Group B Streptococcus, 15 Staphylococcus epidermidis, 5 Staphylococcus aureus, 3 Staphylococcus hominis, 2 Klebsiella pneumoniae, 3 Enterobacter cloacae, 1 Acinetobacter, and 4 Candida. Retinopathy of prematurity defined as stage 1 or greater during screening examination at any age during hospitalization. Patent ductus arteriosus defined as a symptomatic infant with a murmur and/or bounding pulses with echocardiographic confirmation.
in magnitude to that obtained in the univariate analysis.

Although causality was not addressed in this study, a substantial body of literature including human, animal, and in vitro studies argues that *U urealyticum* may cause lung injury. At least 3 mechanisms of lung damage to the developing lung have been proposed.

Cassell et al\(^ {14} \) first suggested that *U urealyticum* infection produces acute pulmonary inflammation with histologic evidence of bronchopneumonia. This finding was demonstrated in a murine model,\(^ {27} \) but not confirmed in human autopsy specimens.\(^ {38} \) A second hypothesis is that phospholipase A2, which is produced by *U urealyticum*,\(^ {39} \) causes inhibition of pulmonary surfactant,\(^ {40} \) thereby worsening acute respiratory disease and leading to CLD.

The most recently suggested mechanism is that proinflammatory cytokines found in tracheal aspirates of VLBW infants who are colonized with *U urealyticum* injure the lung, resulting in the development of CLD. Patterson et al\(^ {41} \) found significantly higher levels of interleukin-1\( \beta \) and tumor necrosis factor-\( \alpha \) and significantly lower levels of interleukin-6 when exposed to *U urealyticum* on days 1 and 7 in comparison to noncolonized infants. Infants who ultimately developed CLD had significantly higher interleukin-1\( \beta \) and interleukin-1\( \beta \): interleukin-6 ratios.

An unresolved question regarding the role of cytokines in the development of CLD is whether these cytokines are merely aspirated from amniotic fluid in a setting of choorioamnionitis or are produced by the infant in response to ongoing inflammatory stimuli.\(^ {42} - {44} \)

Several investigators have demonstrated that elevated cytokine levels are associated with *U urealyticum* colonization and that the highest levels of cytokines are induced in the presence of increased ambient oxygen concentrations.\(^ {45} - {46} \) Li et al\(^ {47} \) have recently reported in an in vitro study demonstrating that macrophages from tracheal aspirates of VLBW infants produce high levels of tumor necrosis factor-\( \alpha \) and interleukin-6 when exposed to *U urealyticum*. We speculate that persistent colonization with *U urealyticum* results in ongoing cytokine production leading to prolonged inflammation and lung injury.

Aside from persistent *U urealyticum* colonization, risk factors for oxygen dependency at 28 days and 36 weeks’ postconceptional age differed. At 28 days of age these included lower birth weight, shorter gestation, and the presence of a symptomatic PDA, long considered “traditional” risk factors for CLD. At 36 weeks’ postconceptional age the influence of these traditional risk factors disappeared.

One reason for the difference in risk factors for CLD at these 2 endpoints might be the long recovery period between 28 days chronologic age and 36 weeks’ postconceptional age experienced by most of the tiny infants with CLD in our sample.

Respiratory management at our center differs significantly from other NICUs.\(^ {48} - {49} \) We use more nasal prong continuous positive airway pressure, less intubation, less surfactant administration, and less mechanical ventilation. In this setting of “gentler ventilation,” only sepsis and persistently positive *U urealyticum* colonization emerged as significant risk factors for this outcome. Additional investigation of the association between CLD and *U urealyticum* colonization in different respiratory management settings would be valuable.

Our observations support the concept of the “new BPD,”\(^ {50} \) which unlike classic BPD is not thought to be primarily related to barotrauma and oxygen toxicity, but rather to ongoing injury that interferes with normal parenchymal development and alveolarization. We suggest that our findings of increased risk of CLD with long-term *U urealyticum* colonization are consistent with the concept of the “new BPD.”

A clearer understanding of the factors that impact on lung development and disrupt normal alveolarization is needed. The interaction between *U urealyticum* colonization, phospholipase production and cytokine activity in the developing lung should be elucidated before large scale *U urealyticum* targeted treatment trials aimed at reducing the incidence of CLD are undertaken. The results of clinical trials of interventions to prevent CLD should include consid-

### Table 3

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>AOR (95% CI)</td>
<td>AOR (95% CI)</td>
</tr>
<tr>
<td>Birth weight &lt;750 g g</td>
<td>7.38 (2.89, 18.9)*</td>
<td>6.67 (1.48, 30.1)*</td>
<td>5.03 (1.29, 19.7)*</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age &lt;26 wk</td>
<td>13.3 (4.89, 35.9)*</td>
<td>7.06 (1.56, 31.9)*</td>
<td>5.58 (1.48, 21)*</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender</td>
<td>2.83 (1.16, 6.89)*</td>
<td>1.73 (0.39, 7.59)</td>
<td>4.03 (1.08, 15.1)*</td>
<td>NS</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>17.9 (5.01, 64)*</td>
<td>9.23 (1.10, 77.6)*</td>
<td>14.1 (2.97, 67)*</td>
<td>NS</td>
</tr>
<tr>
<td>Inborn</td>
<td>0.27 (0.08, 9.3)*</td>
<td>0.24 (0.04, 1.36)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>1.12 (0.45, 2.79)*</td>
<td>2.35 (0.66, 10.8)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>3.66 (0.80, 16.8)</td>
<td>0.67 (0.13, 3.55)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Any episode of sepsis</td>
<td>3.03 (1.22, 7.52)*</td>
<td>4.11 (0.88, 19.1)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Surfactant administration</td>
<td>3.24 (1.13, 9.26)*</td>
<td>24.5 (4.44, 135)*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Persistently positive <em>U urealyticum</em> colonization</td>
<td>10.0 (3.30, 30.3)*</td>
<td>24.5 (4.44, 135)*</td>
<td>5.33 (1.05, 27)*</td>
<td>33.6 (4.78, 237)*</td>
</tr>
</tbody>
</table>

NS indicates not significant.

* \( P \)-value <.05 when 95% CI for OR excludes 1.
eration of persistent U urealyticum colonization in the pathogenesis of CLD.

CONCLUSION

This is the first study to demonstrate that different patterns of U urealyticum colonization relate to the development of CLD among VLBW infants. Although early transient and late acquisition colonization accounted for 55% of the U urealyticum-positive infants in our NICU population, only persistently positive colonization was associated with the development of CLD. Failure to distinguish between the patterns of U urealyticum colonization among VLBW infants may mask the association between U urealyticum colonization and CLD.

REFERENCES

Patterns of Colonization With *Ureaplasma urealyticum* During Neonatal Intensive Care Unit Hospitalizations of Very Low Birth Weight Infants and the Development of Chronic Lung Disease

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