Clinical significance of airways colonization with *Ureaplasma urealyticum* in premature (<34 wk) neonates

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**Background & objectives:** *Ureaplasma urealyticum* has been implicated in various neonatal morbidities in preterm infants. Its association with chronic lung disease (CLD) remains controversial. The aim of this prospective study was to investigate colonization of *U. urealyticum* in preterm infants (with gestational age <34 wk) and to evaluate the relationship between *U. urealyticum* colonization and neonatal morbidity including CLD.

**Methods:** *U. urealyticum* was cultured from nasopharyngeal or endotracheal aspirates collected within 24 h of birth from infants ≤34 wk gestation weighing <1800 g admitted to a Neonatal Intensive Care Unit of a tertiary care hospital in north India, and PCR was performed on the DNA extracted from these samples.

**Results:** Twenty per cent of the study infants were colonized with *U. urealyticum*. The mean gestational age of the infants in the colonized group was less than that of non colonized infants (*P*<0.05). The peripheral total leukocyte counts and mortality rate were higher in infants with *U. urealyticum* colonization than in non-colonized infants (*P*<0.05). There was no significant difference between the colonized and non colonized groups with regard to the antenatal use of steroids, sex, cause of respiratory distress, use of surfactant, duration of ventilation.

**Interpretation & conclusion:** None of the 20 babies colonized with *U. urealyticum* developed CLD as compared with two (2.5%) of the non colonized group. Colonization of the airways with *U. urealyticum* had no significant role in development of CLD in Indian preterm infants.

**Key words** Chronic lung disease - culture - polymerase chain reaction - preterm infants - *Ureaplasma urealyticum*

*Ureaplasma urealyticum* is a frequent maternal colonizer. The microbe is often transmitted to newborn infants *in utero* or at the time of delivery. The rate of vertical transmission can be 66 per cent in neonates born to colonized mothers. In full term infants, *U. urealyticum* colonization is considered to be harmless.
In preterm infants *U. urealyticum* colonization is shown to be associated with perinatal mortality and morbidity including pneumonia, sepsis, meningitis and chronic lung disease (CLD) of prematurity. However, some investigators have reported contradictory results. Limited information is available from our country on the frequency of colonization of preterm infants with *U. urealyticum* and the clinical outcome of such colonization.

The present study was therefore carried out to determine the colonization rate of *U. urealyticum* in preterm infants and to evaluate its relationship with neonatal morbidity including CLD in a tertiary care hospital in north India.

**Material & Methods**

The present study was a prospective and longitudinal study performed in the Neonatal Intensive Care Unit (NICU) of the Department of Paediatrics, All India Institute of Medical Sciences, New Delhi, India, between June 2003 and June 2005. The NICU is the major tertiary care referral center catering for high-risk neonates from the northern India. All infants with gestational age <34 wk, weighing <1800 g who were admitted to the Unit within 24 h of birth and had not received prior antibiotic therapy, were included in the study after parental consent had been obtained. During the study period 130 babies were eligible for the study. Five infants died within few hours of birth. Of the remaining 125 infants, 4 were excluded from the analysis because of serious congenital malformations, 6 were lost to follow up and another 15 were not enrolled due to lack of parental consent. A total of 100 premature infants were included in the study. The study protocol was approved by the ethical committee of our institution.

**Disease definitions:** Neonates were categorized as having chronic lung disease (CLD) if there was a (i) need for supplemental oxygen at 28 days of age; and (ii) need for supplemental oxygen at 28 days of age with radiological changes consistent with CLD. Neonatal septicaemia was diagnosed on clinical signs (poor peripheral perfusion, hypotension, bradycardia, temperature instability and respiratory failure), biochemical parameters (total leukocyte count <5000/μl, immature/total neutrophil >0.2, Micro-ESR >15mm in 1st h or C-reactive protein elevation >1mg/dl) and positive blood cultures. Respiratory distress was being defined when two of the following criteria were met: respiratory rate >60/min, subcostal or intercostals recession, expiratory grunt/groaning and O₂ requirement.

**Clinical specimens:** Nasopharyngeal aspirates within 24 h of birth to exclude nosocomial transmission of infectious agents and endotracheal aspirates (if intubated) were collected. Blood and cerebrospinal fluid (CSF) samples were obtained from neonates having a clinical diagnosis of septicaemia or meningitis. Nasopharyngeal aspirates were cultured for *U. urealyticum*. Endotracheal aspirates, blood and CSF were cultured for *U. urealyticum*, aerobic and anaerobic bacteria. Polymerase chain reaction (PCR) was performed on respiratory samples. A child was considered colonized with *U. urealyticum* if any of the above mentioned samples were positive.

**Culture methods:** For isolation of *U. urealyticum* samples were inoculated into Pleuropneumonia Like Organism broth (Becton, Dickinson & Co., USA) (PPLO broth) and immediately transported to the laboratory. Samples were filtered through a 0.45μm filter and 0.2 ml was inoculated into PPLO broth containing urea. Serial dilutions were made and the broths were incubated at 37°C under 5 per cent CO₂. The broths were inspected twice daily and sub cultured onto PPLO agar plates containing urea when a colour change occurred. *U. urealyticum* was identified by its characteristic morphologic colonies and urease production. Blood cultures, endotracheal aspirates and CSF were also cultured for aerobic and anaerobic bacteria. Isolates were identified by standard biochemical procedures.

**PCR methods:** After 24 h, 300 μl of the broth was stored at -70°C for further analysis by PCR. DNA extraction from nasopharyngeal/endotracheal aspirates was done
by using Phenol: chloroform: isoamylalcohol (PCI) method\textsuperscript{6}. The oligonucleotide primers U5 sense (5’-CAA TCT GCT CGT GAA GTA TTA C-3’) and U4 antisense (5’-ACG ACG TCC ATA AGC AAC T-3’) used to amplify a 429 bp region of the \textit{U. urealyticum} were chosen from published nucleotide sequence of urease structural gene\textsuperscript{3}. One positive control (\textit{U. urealyticum}, NCTC10177, National Collection of Type Cultures, London, UK) and a negative control (PPLO broth) were included in each PCR batch. The 25 µl amplification reaction mixture containing 2.5 µl of 10x PCR buffer [1x PCR buffer is 10mM Tris-HCl (pH 8.8 at 25° C), 1.5 mM MgCl\textsubscript{2}, 50 mM KCl, and 0.1% Triton X-100], 1 unit of Taq polymerase (Banglore Genei), 200 uM (each) deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP) (Banglore Genei), 10 pmol of each primer, 5 µl of sample DNA, and ultra pure sterile water added to 25 µl. A thermal cycler (MJ Research Inc. USA) was used to process the samples through 35 cycles with initial denaturation at 94° C for 5 min; cyclic denaturation at 94°C for 1 min, annealing at 56° C for 1 min, extension at 72°C for 1 min followed by final extension at 72°C for 5 min. PCR product was analyzed by electrophoresis on 2.0 percent agarose gels.

\textbf{Data analysis:} The statistical analysis was done using Fisher’s exact test, independent t-test, Mann-Whitney test and Chi-squared test.

\textbf{Results}

Of the 100 infants, 20 (20\%) were colonized with \textit{U. urealyticum} by culture or PCR for \textit{U. urealyticum} (Table I). All the blood (54 samples) and CSF (14 samples) cultures were negative for \textit{U. urealyticum} and anaerobes. Five infants had blood culture positive for other bacterial pathogens viz., \textit{Citrobacter} spp., \textit{Acinetobacter} spp., \textit{Pseudomonas aeruginosa} and methicillin resistant \textit{Staphylococcus aureus} (MRSA).

Of the 100 neonates, nine were twins, three were triplets and one was a quadruplet with a mean gestational age of 31.0, 30.5 and 27.8 wk, respectively. Birth weight ranged from 600 to 1768 g (median=1260 g) and gestational age from 26.5 to 33.8 wk (mean=30.7 wk). The Apgar score at 1 min ranged from 2 to 9 (mean=6.2) and at 5 min ranged from 2 to 9 (mean=7.4) respectively. Male babies dominated among the neonates (62 vs. 38, ~2:1). Eight of the 27 infants (29.6\%) weighing less than 1000 g at birth were colonized compared with only twelve of the 73 infants (16.4\%) weighing more than 1000 g. Although 7 of 20 colonized infants (35\%) were delivered vaginally compared with 16 (20\%) of 80 non colonized babies.

The mean gestational age of the infants in the colonized group (30.1 ± 1.7 wk) was significantly less than that of non colonized infants (31.2 ± 2.0 wk) (\(P<0.05\)). The peripheral total leukocyte counts were higher in infants with \textit{U.urealyticum} colonization (Quantil Q25: 12000; Quantil Q75: 20000; Interquartile range: 8000) than in non colonized infants (Quantil Q25: 9600; Quantil Q75: 16500; Interquartile range: 6900) (\(P<0.05\)). Apgar score (<3) at 5 min and mortality of the colonized babies were significantly higher (\(P<0.05\)) as compared to the non-colonized babies. None of the 20 neonates colonized with \textit{U. urealyticum} developed CLD as compared with two (2.5\%) of the non colonized group who developed CLD (Table II). Pregnancy induced hypertension and premature rupture of membranes (PROM) were found to be 28.7 per cent each in preterm deliveries.

\textbf{Discussion}

Over recent years, several studies have investigated the association between \textit{U. urealyticum} colonization and neonatal morbidities. The present

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Specimen tested & Culture positive (%) & PCR positive (%) \\
\hline
NPA 100 & 8 (8) & 20 (20) \\
ETA 7 & 2 (28.57) & 3 (81.8) \\
NPA, nasopharyngeal aspirate; ETA, endotracheal aspirate & & \\
\hline
\end{tabular}
\caption{Results of cultures and PCR for \textit{U. urealyticum} in preterm infants}
\end{table}
study defined colonization based on detection of organisms in nasopharyngeal aspirates and/or endotracheal aspirates. The reported prevalence of U. urealyticum colonization in the newborn varies from 13 to 47 per cent. A study conducted by Sethi et al showed colonization rate to be 14 per cent. In the present study, U. urealyticum colonization rate was found to be 20 per cent.

The present study also evaluated the diagnostic efficacy of PCR in neonatal respiratory samples. Many Western studies have evaluated different PCR systems, commercial as well as in-house PCR assays, and many of these have been shown to be highly sensitive for the detection of U. urealyticum in neonatal respiratory samples. The sensitivity, specificity, positive predictive and negative predictive values of the PCR were 100.0, 87.0, 40.0 and 100.0 per cent respectively, taking culture as a gold standard.

The effect of mode of delivery on rate of colonization is controversial. Iwasaka et al observed that vertical transmission of U. urealyticum was significantly higher among infants delivered vaginally than those born by caesarean section. In contrast, Sanchez found no effect of the mode of delivery on the vertical transmission rate of U. urealyticum. A similar observation was made in the present study. Saxen et al showed that the rate of premature rupture of membranes (PROM) (64%) was higher in mothers of colonized babies. In the present study also, infants

### Table II. Comparison between colonized & non-colonized preterm infants

<table>
<thead>
<tr>
<th>Profile characteristics</th>
<th>Colonized (n=20)</th>
<th>Non-colonized (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean birth weight ± SD (g)</td>
<td>1142.45 ± 279</td>
<td>1242.34 ± 302</td>
</tr>
<tr>
<td>Mean gestational age ± SD (wk)</td>
<td>30.1 ± 1.7*</td>
<td>31.2 ± 2.0</td>
</tr>
<tr>
<td>SGA</td>
<td>2 (10)</td>
<td>7 (8.75)</td>
</tr>
<tr>
<td>AGA</td>
<td>18 (90)</td>
<td>73 (91.25)</td>
</tr>
<tr>
<td>M : F ratio</td>
<td>1 : 1</td>
<td>2 : 1</td>
</tr>
<tr>
<td>Birth HC (cm) ± SD</td>
<td>27.8 ± 1.0</td>
<td>27.7 ± 1.3</td>
</tr>
</tbody>
</table>

Apgar score at 1 min:

- <3 | 4 (20) | 08 (10) |
- 4-6 | 5 (25) | 25 (31.25) |

Apgar score at 5 min:

- <3 | 1 (5)* | 0 (0) |
- 4-6 | 5 (25) | 12 (15) |

Ventilatory support:

- CPAP alone | 8 (40) | 36 (45) |
- CPAP + O2 by hood | 2 (10) | 9 (11.25) |
- CPAP + intubation | 3 (15) | 4 (5) |
- CPAP + SIMV | 1 (5) | 2 (2.5) |
- Ceftazidime/amikacin | 17 (85) | 64 (80.0) |
- Duration of ventilation (interquartile range) | 6 | 7 |

Neonatal outcome:

- TLC (cell/mm³) (interquartile range) | 8000* | 6900 |
- Septicaemia | 1 (5.0) | 7 (8.75) |
- NEC | 1 (5.0) | 3 (3.75) |
- IVH | 1 (5.0) | 0 (0) |
- Mean days ± SD (hospital stay) | 15.0 ± 6.9 | 18.64 ± 12.8 |
- Mortality | 2 (10) | 1 (1.2) |
- PROM | 7 (35) | 18 (22.5) |

Values in parentheses are percentages

PROM, premature rupture of membranes; NEC, necrotizing enterocolitis; CLD, chronic lung disease; IVH, intraventricular haemorrhage; LSCS, lower segment caesarean section; SVD, spontaneous vaginal delivery; TLC, total leucocyte count; HC, head circumference; CPAP, continuous positive airway pressure; SIMV, synchronized intermittent mandatory ventilation; TTNB, transient tachypnoea of the newborn; HMD, hyaline membrane disease; SGA, small for gestational age; AGA, appropriate for gestational age

The present study also evaluated the diagnostic efficacy of PCR in neonatal respiratory samples. Many Western studies have evaluated different PCR systems, commercial as well as in-house PCR assays, and many of these have been shown to be highly sensitive for the detection of U. urealyticum in neonatal respiratory samples. The sensitivity, specificity, positive predictive and negative predictive values of the PCR were 100.0, 87.0, 40.0 and 100.0 per cent respectively, taking culture as a gold standard.
born to mothers with PROM (35%) showed *U. urealyticum* growth in the airways more frequently than did those born to mothers without PROM (22.5%).

The gestational age of the infants in the colonized group was less than that of non colonized infants. In a study conducted by Agarwal *et al*\(^\text{17}\) on Asian neonates, the gestational age of the colonized infants was less than that of the non colonized group.

The peripheral total leukocyte counts were higher in infants with *U. urealyticum* colonization than cell counts in non colonized infants and the difference was statistically significant. Viscardi *et al*\(^\text{18}\) also observed the similar findings showing the raised TLC as a marker of inflammation.

In the present study, presence of respiratory distress and duration of ventilation in colonized group did not show any difference from the non colonized group. The mean Apgar score at 1 min as well as at 5 min were slightly lower in the colonized infants as compared to the non colonized infants, but this did not reach statistical significance.

Cassell *et al*\(^\text{19}\) emphasized that endotracheal aspirates are the most valuable markers of lower respiratory tract infection. In the present study, only a few endotracheal aspirates could be obtained. This is because a milder form of ventilatory support i.e., nasal prong continuous positive airway pressure was used in our NICU. It has been proven that prolonged mechanical ventilation works in synergy with antenatal or postnatal infection to increase the risk of CLD\(^\text{20}\). In this study none of the survivors in the colonized group developed CLD compared to the non colonized group. Saxen *et al*\(^\text{16}\) were the first to negate an association between *U. urealyticum* and CLD. Other studies\(^\text{21,22}\) also failed to demonstrate an association between *U. urealyticum* isolation and respiratory disease in new born babies. In our study CLD appeared to be associated with prematurity rather than *U. urealyticum* colonization. A similar observation was also made by Jonsson *et al*\(^\text{23}\). It has been shown that postnatal sepsis increases the risk of CLD\(^\text{20}\). In the present study, one of the preterm infants in the non colonized group who had sepsis, later developed CLD, thereby suggesting the multifactorial aetiology of CLD\(^\text{20}\). *U. urealyticum* was neither isolated from blood nor from CSF specimens. Their role, as pathogens of meningitis and sepsicaemia is questionable\(^\text{24}\).

Our findings showed that *U. urealyticum* is not a direct risk factor for CLD. It is obvious that the smallest and youngest infants are most susceptible to opportunistic micro-organisms, such as *Ureaplasma* due to their immature defence system and vulnerable airways. Therefore, these differences may indicate an association between *Ureaplasma* colonization and gestational age rather than a causal relationship between *Ureaplasma* and CLD.

In conclusion, the present study showed the better diagnostic potential of PCR compared to culture in the detection of *U. urealyticum* in neonatal respiratory samples. The enhanced sensitivity of *Ureaplasma* detection with PCR is consistent with the findings reported in literature\(^\text{25-27}\). Colonization of the airways with *U. urealyticum* had no significant role in the development of CLD in preterm infants.

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**References**


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