Reviewers report

Title: Molecular evidence of Ureaplasma urealyticum and Ureaplasma parvum colonization in preterm infants during respiratory distress syndrome.

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Reviewer: Robert Schelonka

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General
The manuscript entitled “Molecular evidence of Ureaplasma urealyticum and Ureaplasma parvum colonization in preterm infants during respiratory distress syndrome† by Rosario Cultrera, Silva Seraceni, Rossella Germani, Carlo Contini aims â€œto detect by molecular techniques the role of Mycoplasma spp. and Ureaplasma spp. in respiratory secretion [sic] and blood specimens of preterm newborns with or without RDS and to evaluate the prevalence of perinatal U. urealyticum or U. parvum infection.â€ The study is a prospective, cohort analysis of 24 infants with the respiratory distress syndrome and 26 infants requiring assisted ventilation who did not have features of the respiratory distress syndrome. The investigators determined Ureaplasma spp. and mycoplasma colonization/infection of the respiratory tract by culture and by an â€œin houseâ€ polymerase chain reaction (PCR) amplification of ureaplasmal DNA. PCR amplicons were directly sequenced and the DNA sequences were aligned with published genomic sequences of U. urealyticum, U. parvum for subspecies identification. The major finding was that infants with the respiratory distress syndrome were more likely to be colonized in the lower respiratory tract with Ureaplasma spp. and particularly with U. parvum. The authors conclude in the abstract that â€œthe routine use of molecular methods could be useful to screen candidate babies to etiologic therapy.â€ The authors further conclude in the body of the manuscript that â€œThe routinely [sic] use of this technique for NICU patients could have a role in more accurately diagnosing infection by U. parvum and U. urealyticum especially in newborns at risk to develop RDS.

Still complicating the interpretation of the results is the lack of a true â€œgold standardâ€ technique to which the PCR results can be compared. Detection by PCR does appear to be more sensitive than culture since more specimens were positive by PCR than by culture; however, the possibility remains that there could have been DNA â€œcontaminationâ€ in the PCR amplifications. This is a common problem in all PCR amplification strategies and the authors indicate in the methods that they have taken measures to reduce the possibility of DNA contamination. Without a referent â€œgold standard, usual calculations of sensitivity of the PCR technique are not possible.

As treatment of infected infants with clarithromycin was uncontrolled, the statement: â€œThese patients did improve clinically,â€ while this may be true, the improvement may or may not be related to treatment with clarithromycin. The statement â€œrespiratory secretions did result negative with laboratory assays.â€ I think the authors are saying that treatment led to eradication of Ureaplasma spp. in the airways or nasopharynx by culture and DNA detection by PCR. If that is the authorsâ€™ intent, it should be so stated. In addition, it should be clearly stated what proportion of the treated infants were tested for â€œproof of cureâ€ (microbe eradication). The authors do state in the methods that PCR positive TA or NF specimens were PCR negative after treatmentâ€”but do not indicate a specific time point or the proportion of infants sampled. Nor do the authors state that the TA positive specimens were negative by a post treatment TA specimen or was a nasopharyngeal aspirate acceptable for babies who were no longer intubated and receiving mechanical ventilation. It is plausible that site cultured or assayed for Ureaplasma spp. DNA may impact yield. Have the authors determined that matched TA and NF samples are concordant for DNA detection?

An important flaw in the study design was that the RDS+ infants (group 1) and RDS- infants (group 2) were not birth weight or gestational age matched. This calls to question the results of the univariate analysis. To control for the differences in birth weight (and perhaps other confounding variables), a multivariable analysis would strengthen or perhaps refute the Ureaplasma-RDS and PDA associations. I do not believe the sample size is sufficiently robust to perform a multivariable analysis, however.

The manuscript still suffers from spelling and grammatical errors and unclear sentence structure.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

See above

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.