

Urogenital Mycoplasmas Detection in New Born Child's with Respiratory Complications

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Abstract: Among premature infants, the bacterial colonization of the respiratory tract has been associated with the development of pneumonia, chronic lung disease, acute respiratory insufficiency, and even death. Genital mycoplasmas, particularly *Mycoplasma hominis*, *M. genitalium*, and *Ureaplasma* spp., have been associated with these pathological conditions, and also with neonatal pneumonia and respiratory distress syndrome. The objective of this study was to determine the frequency of *M. hominis*, *M. genitalium*, *U. parvum* and *U. urealyticum* in newborn child's, and to determine whether its presence is associated with the development of respiratory complications in these patients. Were analyzed 62 respiratory secretion samples (27 nasal swabs and 35 endotracheal tubes) obtained from 34 mechanical ventilated infants. Was realized the bacteriological culture for the isolation of *Ureaplasma* and *Mycoplasma* spp., and the PCR for the Mollicutes Class and Multiplex-PCR for the identification of *U. urealyticum*, *U. parvum*, *M. hominis* and *M. genitalium* species. The bacteriological culture was positive in one sample to mycoplasmas, while with the PCR for the Mollicutes Class was obtained 25 positive samples. From these, with the Multiplex-PCR were identified three positive samples to *U. parvum*, two positive to *U. urealyticum* and one positive to *M. hominis*. These six samples showed coinfection with other bacteria. Were identified mycoplasmas and ureaplasma species in new born ventilated child's. Statistical association was not demonstrated between the mycoplasmas or ureaplasmas infection and respiratory complications in the infant.

Keywords: Urogenital mycoplasmas, new born child, respiratory complications

Introduction

Preterm birth and low birth weight are the leading causes of neonatal mortality and morbidity in the developed world. More than 60% of mortality among infants without anatomical or chromosomal defects can be attributed to underweight. Ascending genital infections contribute to over 50% of premature births, particularly those that occur before 30 weeks of gestation (1, 2).

Bronchopulmonary dysplasia (BPD) is a common disorder among infants with extremely low birth weight, with an incidence ranging from 30% to 75% (3). This is one of the major causes of mortality and morbidity in preterm infants and has been associated with lung function abnormalities in the long term, prolonged oxygen therapy and respiratory infections frequent requirement for hospitalization (4).

It has been suggested that infants with patterns of persistent infection/colonization breathing are more susceptible to developing DPB than those with transient colonization (5), and is the most common form of chronic lung disease in preterm and resulting in a significant morbidity during early childhood (6).

Between premature infants, bacterial colonization of the respiratory tract has been associated with the development of pneumonia, chronic obstructive pulmonary disease (COPD), acute respiratory failure and death. Urogenital mycoplasmas, including *Mycoplasma hominis* and *M. genitalium* and *Ureaplasma* spp., are some of the microorganisms associated with these diseases. Moreover, among preterm infants, *Ureaplasma* infection can cause bacteremia, meningitis, intraventricular hemorrhage and persistent fetal circulation (7). The respiratory tract colonization by *Ureaplasma* spp., it has been associated with respiratory distress, persistent

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pulmonary hypertension, neonatal pneumonia and the development of BPD (8, 9) syndrome. The objective of this study was to determine the frequency of *M. hominis*, *M. genitalium*, *U. urealyticum* and *U. parvum* in newborn infants ventilated, identified by bacterial culture techniques and polymerase chain reaction (PCR) kind-specific, and determine whether the presence of these species is associated with the development of BPD and/or COPD.

Material and Methods

Study design

A prospective study was conducted at the Department of Microbiology of the Hospital "Eusebio Hernandez", and the National Reference Laboratory Mycoplasma Institute of Tropical Medicine "Pedro Kouri" in the period from February 2010 to July 2011. Sixty-two respiratory samples, of which 27 nasal swabs and endotracheal tubes 35, obtained from 34 children mechanically ventilated through tracheal intubation, were analyzed for detection of urogenital mycoplasmas. The research protocol was approved by the hospital ethics committee, and written informed consent was obtained from parents through medical assistance before being included in the study.

Bacteriological culture

Samples of nasal discharge and endotracheal tube were cultured for isolation of *Ureaplasma* spp. and *Mycoplasma* spp. Each sample was inoculated into glass tubes containing 2 mL of liquid culture medium U/N and Friis previously and specific for the detection of these agents described respectively (10). The inoculated tubes were incubated at 37 in an atmosphere of 5% CO₂ for 7 days (primary culture). The cultures showing color change were seeded on Petri dishes containing solid medium U/N and Friis, which were incubated under the same conditions, to display daily by checking specific mycoplasma colonies and/or ureaplasmas.

PCR-Mollicutes Class

PCR assay for the identification of the Class Mollicutes was made to all primary cultures. For this test 1 mL aliquot thereof was centrifuged at 12 000 rpm for 15 minutes at room temperature. The pellet was resuspended in 1 mL of PBS solution and centrifuged for 15 minutes, and the supernatant was discarded. A volume of 10 mL of deionized water was added, and the solution was boiled for 10 minutes and kept in ice quickly. 5 mL of the supernatant for PCR assay was taken. The PCR reaction was performed as described by Fernandez *et al.*, 1998 (11). Positive samples were analyzed for the identification of species of mycoplasma and ureaplasma.

Multiple-PCR for identification of *U. urealyticum* and *U. parvum*

The PCR technique for the identification of *U. parvum* and *U. urealyticum* was performed on all samples positive for the Class Mollicutes. Reference strains *U. parvum* (ATTC 2815) and *U. urealyticum* (ATCC 27618) were used as positive controls for the reaction. Specific to a region of 418 bp of the urease gene of *Ureaplasma urealyticum*, and specific to a region of 812 bp spacer region between the 16S rRNA gene and *U. parvum* 23S primers were used.

The PCR was performed in a total volume of 50 µL, containing 32.25 µL of sterile water (Molecular Biology Reagent), 1.25 µL of each primer (10 pmol/µl), 5 µl of 10X PCR buffer, 1.25 µL of each dNTP (20mM/µL), 0.25 µL (1.25 U) of Taq polymerase and 5 µL of DNA. Amplification was performed in a thermocycler "personal MastercyclerR" (Eppendorf, Germany), with an amplification program consisted of 94°C for 4 minutes, 39 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds extension 72°C for 1 minute and a final extension step of 72°C for 10 minutes. The PCR products were separated by electrophoresis in an agarose gel of 2%, at 110 V for 30 minutes and visualized by staining with ethidium bromide.

Multiple PCR-for identification of *M. hominis* and *M. genitalium*

The PCR technique for the identification of *M. hominis* and *M. genitalium* was performed on all samples positive for the Mollicutes Class. They were used reference strains *M. hominis* (ATCC 23114) and *M. genitalium* (R32G) as positive controls for the reaction. Specific to a region 280 bp rRNA gene of *M. hominis* and 72 bp region of the adhesive protein of *M. genitalium* primers were used. PCR was performed as previously described for ureaplasma species. The PCR products were separated by electrophoresis on agarose gel 4% at 110 V for 30 minutes visualized by staining with ethidium bromide.

Detection of other microorganisms

Endotracheal tube samples were analyzed by bacterial culture for the detection of other bacterial species associated with respiratory infections in infants.

All children were followed up during their stay in the hospital after removing the ventilator to see if they developed BPD, defined as cellular changes that affect the bronchioles (small airways) and alveoli, preventing causing breathing problems and lung function; and/or COPD, characterized by a permanent

airflow limitation caused by abnormalities of the airways and lung parenchyma as emphysema.

Results

The method of bacterial culture resulted in one positive sample mycoplasma, representing 3.7% of the 27 samples of nasal discharge, and was negative for 35 samples of endotracheal tube.

Through Mollicutes Class PCR 25 positive samples, of which 10 (37%) nasal swabs and 15 (42.9%) were obtained endotracheal tubes. The frequency of mycoplasma and ureaplasma species obtained in each type of sample using PCR-Multiple conducted at 25 samples positive for the Mollicutes Class (Table 1).

Patients with positive samples to Mollicutes Class had coinfection with other microorganisms. Endotracheal tube cultures showed growth of *Staphylococcus haemolyticus*, *S. capitis capitis sub*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli* and *Candida albicans*. To one of the samples it was detected coinfection with *U. parvum* and *Morganella morganis*, and another sample was detected coinfection with *M. hominis* and *S. auricularis*.

None of the children involved in this study with positive samples ureaplasmas mycoplasma and developed bronchopulmonary dysplasia or COPD.

Discussion

Respiratory diseases are still the most common cause of perinatal morbidity and mortality, especially in preterm infants. *Ureaplasma* spp., and *Mycoplasma hominis* are known as congenital pneumonia pathogens, although there is little information indicating the long-term consequences that can result from neonatal infection by these microorganisms. They have also been associated with preterm birth, low birth weight, and intrauterine growth retardation, and are thought to be infected or colonized up to 37% of newborns (12).

The technique of polymerase chain reaction showed a higher sensitivity than bacterial culture for the detection of *Ureaplasma* spp. Although cultivation technique is considered the gold identifying mycoplasma and ureaplasma, this only allows the detection of live cells multiplying in artificial media. When living organisms are not present in the sample or due to different reasons, are not able to survive outside the host, the results obtained by this method can be false negative. This relatively low frequency of ureaplasmas detected by culture can be attributed in part to difficulties in the growth and isolation of this organism (13). It may also be impossible to detect by the method ureaplasmas culture when

another rapidly growing bacteria is present in the sample. In our study we found co-infection with other bacteria in ureaplasmas positive samples, which could have interfered with the growth of mycoplasmas and its detection by culture (14).

In this study, we identified *Ureaplasma* spp. 20% of the infants studied, *U. parvum* being the most common species. Previous studies reported at *U. parvum* as the most common of the two species detected in the lungs of preterm infants requiring intubation and mechanical ventilation in early neonatal periods (15, 16). *M. hominis* has also been identified in clinical samples of preterm infants under ventilation, although it has been reported at a low percent, matching the results obtained (13).

Numerous studies have attempted to relate *U. urealyticum* colonization with the development of respiratory disease in high risk infants (gestational age <28 weeks) and most chronic lung disease, but the effective role of *U. urealyticum* and *U. parvum* in the pathogenesis of respiratory distress syndrome when associated with prematurity remains controversial (17).

In this study samples belonging to the Mollicutes Class which were not identified were detected. *Mycoplasma penetrans* seems to have the potential to be a human pathogen, but have not provided conclusive evidence to prove this fact. *M. fermentans* has been isolated from the throat of the 16% of children with community-acquired pneumonia, some of which had no other identified causative agent, but the frequency of their occurrence in healthy children is not known. No prospective studies have been reported in neonates to determine whether *Mycoplasma fermentans* can be important as an agent of disease in this population. The species of mycoplasmas most recently recognized *Mycoplasma amphoriforme*, is a body that has been detected in the lower respiratory tract in many immunocompromised persons in association with chronic bronchitis, and therefore investigations are now underway to determine their role in human disease and can I remain firmly established (18). Detection of these species was not performed in this study, and may be present in the samples analyzed.

Previous studies have identified other bacterial species besides *Ureaplasma* spp., in endotracheal aspirates and nasogastric of preterm infants. These species include *S. epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacteroides* spp., *Lactobacillus* spp., *Fusobacterium nucleatum* and *Prevotella bivia* among others, suggesting that if the postnatal infection is involved in part in causing lung

damage and subsequent dysplasia bronchopulmonary, this may be the result of a process of polymicrobial infectious disease, as described for chronic lung conditions such as cystic fibrosis (19-21). In our study we found coinfection in positive ureaplasmas and mycoplasma samples *Morganella morganis* and *Staphylococcus auricularis* respectively, which could confirm this theory, and direct further studies to determine the pathogenic role of different microorganisms related to respiratory complications in newborn infants.

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Table 1: Frequency mycoplasma and ureaplasmas species in respiratory samples in ventilated newborns.

Respiratory samples	<i>U. parvum</i>	<i>U. urealyticum</i>	<i>M. hominis</i>	<i>M. genitalium</i>
Nasal swab	20% (2/10)	10% (1/10)	10% (1/10)	0% (0/10)
Endotracheal tube	6.7% (1/15)	6.7% (1/15)	0% (0/15)	0% (0/15)