Mycoplasma Duo Test Versus Conventional Culture Media for Detection Of Ureaplasma In Endotracheal Aspirates From Respiratory Distressed Premature Neonates

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ABSTRACT

This work aims to evaluate the Mycoplasma Duo kit as a rapid method for detection of ureaplasma in endotracheal aspirate samples from respiratory distressed premature neonates compared to conventional culture media. Also its sensitivity and specificity were determined. This study was carried on 60 premature neonates (less than 35 gestational weeks) suffering from respiratory distress and mechanically ventilated in neonatal intensive care unit. From all cases paired endotracheal aspirate samples were collected aseptically and were transported in ureaplasma transport media to the laboratory and processed immediately. One of each pair of the collected samples was cultured in both Ureaplasma agar and broth cultures and others were cultured in Mycoplasma Duo kit. The number of ureaplasma detected with both ureaplasma agar and broth cultures are 20 cases (33.33%) while those detected by Mycoplasma DUO kit are 22 cases (36.67%). The Sensitivity of Mycoplasma Duo kit compared with both Ureaplasma agar and broth cultures is (100%) and specificity is (95%). There is a highly significant difference (P-value < 0.001) between Mycoplasma Duo kit and Ureaplasma agar and broth cultures as regards the incubation time taken to get a result by both tests.

INTRODUCTION

Perinatal bacterial colonization of urogenital tract of pregnant females has an implication in pathogenesis of both preterm labour and neonatal morbidity and mortality. Most common organisms involved are Ureaplasma and Mycoplasma species [1]. Several lines of evidence suggest that ureaplasma may cause lung injury through a number of mechanisms including the inhibition of pulmonary surfactant by phospholipase A2 produced by ureaplasma urealyticum and the production of interleukins as well as soluble intracellular adhesion molecules[2]. Ureaplasma urealyticum and mycoplasma hominis have been linked to the development of chronic lung diseases [3].

Detection of Ureaplasma species has traditionally relied on culture on Ureaplasma media. Although Culture on this medium is considered the "gold standard" for detection of ureaplasma species, it takes 2-5 days[4].

The development of a commercially available diagnostic kit (Mycoplasma Duo kit) offers a simpler and rapid alternative method for detection of ureaplasma species in urogenital and neonatal respiratory samples. With this kit identification of ureaplasma species is based on the hydrolysis of urea with the release of ammonia, signaled by a colour change of a pH indicator (phenol red), and results are read within 24-48 hours [5].

Rapid diagnosis of ureaplasma infection is mandatory for early treatment of premature neonates to avoid prematurity associated serious complications [6].

Aim of the work:

The aim of this study is to evaluate the Mycoplasma Duo kit as a rapid method for detection of ureaplasma in endotracheal aspirate samples from respiratory distressed premature neonates compared to conventional culture media. Also its sensitivity and specificity are determined.

SUBJECTS, MATERIAL & METHODS

Subjects:

This study was carried out at Microbiology & Immunology department and Neonatal intensive care unit (NICU), pediatric department, Benha University hospital- from September 2013 to March 2014. The study included 60 premature neonates (less than 35 gestational weeks) suffering from respiratory distress and mechanically ventilated. Neonates
with congenital infections, congenital anomalies, birth asphyxia and surgical problems were excluded. Consent was taken from neonate's parents before taking any samples from their neonates.

All patients were subjected to full history taking including: (gestational age in weeks, postnatal age in days, sex, mode of delivery and history of the present illness), and thorough clinical examination including general examination (weight in grams, gestational age using Ballard score, vital signs, neonatal reflexes) and systemic examinations.

**Samples:** From all cases paired endotracheal aspirate samples were collected aseptically, the trachea was suctioned at a point 0.5 cm beyond the tip of the endotracheal tube; after another 10 ventilator breaths suctioning was repeated with a new catheter. Endotracheal aspirate samples were transported in ureaplasma transport media to the laboratory and processed immediately.

**Materials**

1. **Ureaplasma Broth:**
   
   Ureaplasma broth was prepared according to Marmion and Harris [7] by adding 2.1gms mycoplasma broth base, 0.2 phenol red 1% to 70ml distilled water and mix well till they became completely dissolved. The PH was adjusted between 5.4 to 5.5 by 1N HCL then the medium was autoclaved for 15 min at 121°C, allowed to cool at 50°C in water bath then 10ml sterile yeast extract 25%, 20ml horse serum, 4ml sterile urea 25%, 1ml Penicillin G and 2.5µg/ml Amphotericin B were added to it. The final PH of the medium was adjusted to 6.0 before dispensing it in screw-capped tubes with sterile precaution. It was stored at 4°C till needed. The prepared broth media used within one week of preparation.

2. **Ureaplasma Agar:**

   Ureaplasma agar was prepared according to Marmion and Harris (1996) [7] by adding 1.5gms nutrient agar to ureaplasma broth.

3. **Mycoplasma DUO Kit** (Biorad ultradiagnostics)

4. **Other materials:**
   - Aerobic incubator at 37°C.
   - Plate microscope.
   - Candle jar

**Methods**

I) Ureaplasma broth tubes and urea plasma agar plates:

According to Marmion and Harris [7] one of each pair of the collected endotracheal aspirate samples were cultured in both:

- **Ureaplasma agar plates** then incubated under reduced oxygen tension in humidified candle jar at 37°C for 2-5 days and examined under plate microscope every other day for appearance of the suspected ureaplasma colonies. **Ureaplasma broth** tubes then incubated aerobically at 37°C for 2-5 days and examined daily for any colour change from yellow to red which can be considered as confirmatory test for the presence of ureaplasma (urease colour test). The positive cultures were subcultured on ureaplasma agar plates, incubated at 37°C under reduced oxygen tension in humidified candle jar for 2-5 days and examined using plate microscope to detect colony.

II) Mycoplasma Duo assay:

The second endotracheal aspirate samples were prepared under aseptic conditions by repetitive and gentle flushing of the suction catheter with 2 ml of the suspension medium provided in the Mycoplasma Duo kit. According to manufacturer's instructions a change in colour from yellow to red of microwells, without clouding of the medium indicate the presence of Ureaplasmas.

**RESULTS**

This study was carried out on 60 premature neonates (less than 35 gestational weeks) suffering from respiratory distress and mechanically ventilated in neonatal intensive care unit in Benha University Hospital. The results of the study are represented in the following tables and figures.

Twenty seven of the studied neonates were males and thirty three were females, their mean gestational age was 29.68 ± 2.37 weeks, the mean birth weight was 1407.72 ± 294.63 gm, 27 of them were delivered by cesarean section (CS) and 33 were delivered by normal vaginal delivery (NVD), the mean duration of admission in hospital was 5.7 ± 6.17 days, the diagnosis at admission was 55 of cases had respiratory distress syndrome (RDS) and 5 cases had congenital pneumonia. Table (1)
Table (1): Characteristics of the studied neonates.

<table>
<thead>
<tr>
<th>Variable</th>
<th>definition</th>
<th>N = 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male/female; (% male)</td>
<td>27/33; (45.00)</td>
</tr>
<tr>
<td>Birth weight (gm)</td>
<td>Mean ± SD; (range)</td>
<td>1407.72 ± 294.63; (800-1960)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>Mean ± SD; (range)</td>
<td>29.68 ± 2.37; (25-34)</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>CS/ NVD; (% CS)</td>
<td>27/33; (45.00)</td>
</tr>
<tr>
<td>Duration of admission in hospital (days)</td>
<td>Mean ± SD; (range)</td>
<td>5.7 ± 6.17; (1-28)</td>
</tr>
</tbody>
</table>

The number of *ureaplasma* detected with both *Ureaplasma* agar and broth cultures are 20 cases (33.33%) while those detected by *Mycoplasma* DUO kit are 22 cases (36.67%). Table (2)

Table(2): Comparison between results of *Mycoplasma* DUO kit and *Ureaplasma* agar and broth cultures.

<table>
<thead>
<tr>
<th></th>
<th><em>Ureaplasma</em> agar and broth cultures</th>
<th><em>Mycoplasma</em> DUO kit</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>38</td>
<td>60</td>
</tr>
</tbody>
</table>

The Sensitivity of *Mycoplasma* Duo kit compared with both *Ureaplasma* agar and broth cultures is (100%) and the specificity is (95%). The positive predictive values of *Mycoplasma* DUO kit in relation to both *Ureaplasma* agar and broth cultures is (90.91%) and the negative predictive values is (100%). Table (3)

Table(3): Sensitivity and specificity of *Mycoplasma* Duo kit compared with *Ureaplasma* agar and broth cultures

<table>
<thead>
<tr>
<th>Measures</th>
<th><em>Mycoplasma</em> Duo kit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100.00%</td>
</tr>
<tr>
<td>Specificity</td>
<td>95.00%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>90.91%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

There is a highly significant difference (P value < 0.001) between Mycoplasma Duo kit and *Ureaplasma* agar and broth cultures as regards the incubation time taken to get positive results by both tests. Table (4)

Table (4): Comparison of average incubation time (in days) taken to get positive results with *Ureaplasma* agar and broth cultures and *Mycoplasma* Duo kit

<table>
<thead>
<tr>
<th>Test</th>
<th>Incubation time (in days)</th>
<th>Z</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ureaplasma</em> agar and broth cultures</td>
<td>Mean ± SD</td>
<td>Z</td>
<td>P-value*</td>
</tr>
<tr>
<td>(No= 20)</td>
<td>2-5 days</td>
<td>5.77</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>(3.8 ± 0.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycoplasma</em> Duo kit</td>
<td>1-2 days</td>
<td>5.77</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>(No= 22)</td>
<td>(1.14 ± 0.35)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P-value obtained using the Mann-Whitney test
** P<0.001 (Highly significant difference)

The presence of *ureaplasma* colonies on *Ureaplasma* agar plates after 2-5 days incubation appears as small dark brown colonies that lack surface growth. Different sizes of colonies are seen depending on their cultural age. Fig. (1)
Results of reading and interpretation of Mycoplasma DUO kit for the studied cases are recorded as follows:

Negative test for *Ureaplasma*: No change in color (yellow media) in U, U ≥ 10⁴ wells. Fig. (2)

2) Positive for *ureaplasma* and with high titre (≥10⁴ CCU/ml): the change in colour from yellow to red occurred in U and U ≥ 10⁴ wells. Fig. (3)

**DISCUSSION**

Respiratory distress remains the most common cause of perinatal morbidity and mortality in preterm infants despite many advances in neonatal intensive care and the introduction of artificial surfactant. It is caused by cardiopulmonary immaturity with a deficiency of surfactant in the alveolar space [9].

An appreciation for the role of inflammation as a consequence of perinatal infection emerged as an important cause in the pathogenesis of respiratory diseases, leading the way for consideration of perinatal pathogens such as *Ureaplasma* spp. as causal factors [10].

Upper respiratory colonization with *U. urealyticum* in premature neonates is often associated with clinical, radio logical and laboratory evidence of respiratory infection or frank pneumonia [11].

Li *et al.* [12] reported that there is a strong evidence that *ureaplasmas* induce proinflammatory cytokines production in utero that result in chorioamnionitis and chronic lung injury in neonates.

Kafetzis *et al.* [1] reported that using a culture based method 73% of preterm neonates colonized by *U. urealyticum* and *U. parvum* had RDS. Another study done by Kotecha *et al.* [13] showed an association of respiratory failure due to RDS with *U. urealyticum* colonization on bronchial veolar lavage samples.

In this study out of 60 respiratory distressed premature neonates *ureaplasma* is detected in 20 (33.33%) cases by both *Ureaplasma* agar and broth cultures and in 22 (36.67%) cases by *Mycoplasma* DUO kit.

This results is similar to that reported by Fook-Choe *et al.* [5] who compared *Mycoplasma* Duo assay and culture on A7 media for *ureaplasma* isolation. Out of 98 female genital swab samples that were tested by both culture on A7 media and the *Mycoplasma* Duo assay, 39 (40%) were positive by both assays and 52 (53%) were negative by both assays. The remaining seven samples were positive by *Mycoplasma* Duo but negative by culture.

Hannaford *et al.* [8] isolated *ureaplasma* from 39 (27%) aspirates out of 143 ventilated premature neonates by culture on *Ureaplasma* broth and A8 agar and Bayraktar *et al.* [14] detected *ureaplasma* in 27 (27%) cases out of 100 neonatal aspirate samples by culture on A7 agar medium. Panero *et al.* [15] also detected *ureaplasma* in 16 (29 %) cases out of 55 endotracheal aspirate samples with culture on differential agar medium A7.
Biernat-Sudolska et al.\cite{16} found a significant difference in the isolation rate of *ureaplasmal* by culturing on PPLO agar plates and a commercial kit similar to Mycoplasma DUO kit (Mycoplasma IST2). Out of 500 neonates ureaplasmas were detected in 68 (13.6%) cases by culture and 77 (15.4%) cases by Mycoplasma IST2 test. Cultera et al.\cite{19} also detected *ureaplasmal* by conventional culture method in 5 (10%) out of 50 newborns, three of them had RDS.

In this study there are 2 cases positive for *ureaplasmal* by Mycoplasma DUO kit but negative by Ureaplasma agar and broth cultures. Biernat-Sudolska et al.\cite{16} stated that the differences between both tests may be due to differences in methodology of endotracheal samples processing.

In this study the sensitivity and specificity of *ureaplasmal* detection by Mycoplasma DUO kit versus Ureaplasma agar and broth cultures are 100% and 95% respectively and the positive and negative predictive values are 90.91% and 100% respectively. The differences between values are due to the number of positive *ureaplasmal* detected by each method. These results are in line with that reported Fook-Choe et al.\cite{5} who found that there was 96% agreement between Mycoplasma Duo and culture on A7 media for detection of *ureaplasmal spp.* in female genital swab samples.

Results of this study are in consistent with that recorded by Clegg et al.\cite{17} and Saed\cite{18} who reported high sensitivity by using a similar commercial kit (Mycoplasma IST2) when validated against culture with vaginal specimens.

Luki et al.\cite{19} also reported that the cultivation method is less sensitive. This relatively low frequency of *ureaplasmal* detection by means of culturing may be attributed in part to the difficulties in growing and isolation of these microorganisms.

Biernat-Sudolska et al.\cite{16} also reported that the specificity and sensitivity of *ureaplasmal* detection by culturing in PPLO agar and broth were slightly worse (both 97%) than those of the a similar commercial kit (Mycoplasma IST2).

Regarding the incubation time taken to get positive results by both tests it was found that the incubation time taken to get positive results by both *Ureaplasmal* agar and broth cultures was (2-5) days with mean ± SD (3.8 ± 0.95) while incubation time taken to get positive results by Mycoplasma Duo kit was (1-2) days with mean ± SD (1.14 ± 0.35). There is a highly significant difference (P-value < 0.001) between both tests as regards incubation time taken to get positive results.

We agree with Fook-Choe et al.\cite{5} who reported that Mycoplasma Duo assay is a commercially available kit that is simple to use, rapid with the results available in (24-48 hrs). Rapid diagnosis of *ureaplasmal* infection is mandatory for early treatment of premature neonates to avoid prematurity associated serious complications, also it allows laboratories to culture, identify, differentially titrate *ureaplasmal* and prepare a standardized inoculum for antibiotic susceptibility testing.

Waites and Canupp\cite{50} and Biernat-Sudolska et al.\cite{16} stated that although culture is considered the gold standard for detection of *ureaplasmal spp.*, it is expensive and requires specialized media and expertise. From in vitro cultivation of *ureaplasmal* it showed that they are difficult to cultivate "fastidious", slow growing, produces small colonies that may be missed unless examined under a stereomicroscope. Furthermore, it takes 2 to 5 days to obtain a result.

**CONCLUSIONS**

The development of a commercially available diagnostic kit (Mycoplasma Duo kit) offers a simpler and rapid alternative method for detection of *ureaplasmal spp.* in neonatal respiratory samples. The Mycoplasma Duo assay allows laboratories to culture, identify, differentially titrate *ureaplasmal* and Prepare a standardized inoculum for antibiotic susceptibility testing. It is a commercially available kit that is simple to use, rapid with the results available in (24-48 hrs). Rapid diagnosis of *ureaplasmal* infection is mandatory for early treatment of premature neonates to avoid prematurity associated serious complications. Mycoplasma Duo assay has also a sensitivity and specificity comparable to conventional culture methods for detection of *ureaplasmal spp.* These characteristics make this test suitable for use in diagnostic laboratories that do not currently test for *ureaplasmal spp.*

**RECOMMENDATIONS**

- A study is required to compare results of Mycoplasma Duo Test with PCR for detection of *ureaplasmal* Species in endotracheal aspirates samples from premature neonates for better evaluation of the performance of these different methods in the diagnosis of *ureaplasmal* infections.
REFERENCES


اختبار الميكوبلازم ديو مقابلة بالمزرعة التقليدية للتعرف على الورايايلزما في عينات الأبوية الحنجرية للأطفال المبتسرين متعثر التنفس

أ/ أحمد الجزار، أ/ أمال صبر، أ/ سوسن عبد الرحمن، د/ نقيب توفيق عادل، د/ حنان عبد الفتاح

استاذ ميكروبيولوجيا الطبية والمناعة ، وأستاذ مساعد ميكروبيولوجيا الطبية والمناعة

مدرس طب الأطفال، *** معة، *** بكية الطب/ جامعة بنها

يعتبر الاختبار البكيري المجريي البولي التناسلي في السيدات الحوامل سبيبا في الولادة المبكرة وارتفاع نسبة الوفيات في الأطفال المبتسرين ومن أكمل الميكروبات التي تسبب ذلك فصائل الوريايلزما. تتواجد فصائل الوريايلزما في أفراوات الجهاز التنفسي للأطفال المبتسرين متعثر التنفس والتي تشير إلى احتمال وجود دورها في حدوث التهاب التنفسي. يعتمد التعرف على الوريايلزما على طريقة المزرعة التقليدية والتي تعتبر مجمدة، استهلاك لوقت وتحتاج أيضاً للخبرة الخاصة.

وجد اختبارات تشخيص موجاختة تجاريةً مثل الميكوبلازم ديو 0.2 كت يقد طرق وبسيطة وسريعه للتعرف على الوريايلزما في أفراوات الجهاز التنفسي للأطفال حديثي الولادة. لذلك كان هدف هذا البحث هو تقسيم فئة الميكوبلازم ديو كت في التعرف السريع على الوريايلزما في عينات الحولية الحنجرية للأطفال المبتسرين وتعثير التنفس مقارنة بالمزرعة التقليدية وكذلك تحديد مدى التخصيص والحساسية، وقد تم أجراء هذا البحث على 100 حالة من الأطفال مبتسر دينيي التنفس والمحتاجين على جهاز التنفس الصناعي بحجة العناية المركزة حديثي الولادة بمستشفى بنها الجامعي. وتم أخذ عينة من كل حالة تم تفوقها في السطح الحقيقي للوريايلزما إلى كتل لعمل الاختبارات الألمانية. تم زرع العينة الأولي في المزرعة التقليدية الخاصة بالوريايلزما ورغمها في الحوض تحت ظروف تلف بسبب هوية عند درجة حرارة 37.2 درجة مئوية.

أيام تم التعرف على مستعمرات الوريايلزما بناء على الطرق الفطرية الخاصة بها، تم اختبار العينة الثانية لوجود الوريايلزما باستخدام الميكوبلازم ديو كت على حسب التعليمات المصاحبة لها ومن نتائج البحث التي ميتي: أظهرت نتائج هذه الدراسة أن عدد الحالات الإيجابية للوريايلزما في الأطفال مبتسر دينيي التنفس وتقسيم الدم بطريقة المزرعة التقليدية الخاصة بالوريايلزما كان 23% (3.6%) حالة بينما استخدام اختبار ميكوبلازم ديو كت كان 22% (3.3%) حالة. وبالنسبه لدرجة الحساسية والحساسية الميكوبلازم ديو كت مقارنة بالمزرعة التقليدية للوريايلزما وجد أن درجة الحساسية (4.0%)، أما بالنسبة للدرجة التقليدية للوريايلزما وجد أنها (3.4%) أياً بالعلاقة التقليدية للوريايلزما. بينما كانت (201.3) يوم باستخدام الميكوبلازم ديو كت، وبهذا يكون هناك فرق إحصائي على بين الطرقين في سرعة التعرف على الوريايلزما في العينات الميكوبلازم ديو كت موجاختة تجارية.

وتقدم طرق بسيطة وسريعة خلال (30) يوم للتعرف على الوريايلزما ويعتبر التشخيص السريع للوريايلزما في الأطفال المبتسرين هام للبدء المبكر في العلاج وتجنب الشاكلات الخطيره المصاحبة للمرض.