

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 hominus* 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 departament, 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

I) *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.

II) *Ureaplasma* Agar:

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

III) *Mycoplasma* DUO Kit (*Biorad ultradiagnostics*)

IV) 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.

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

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.

<i>Ureaplasma</i> agar and broth cultures	<i>Mycoplasma</i> Duo kit		Total
	Positive	Negative	
Positive	20	0	20
Negative	2	38	40
Total	22	38	60

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

Measures	<i>Mycoplasma</i> Duo kit (%)
Sensitivity	100.00%
Specificity	95.00%
Positive predictive value	90.91%
Negative predictive value	100.00%

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

Test	Incubation time (in days) Mean \pm SD	Z	P-value*
<i>Ureaplasma</i> agar and broth cultures (No= 20)	2-5 days (3.8 \pm 0.95)	5.77	<0.001**
<i>Mycoplasma</i> Duo kit (No= 22)	1-2 days (1.14 \pm 0.35)		

* 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)

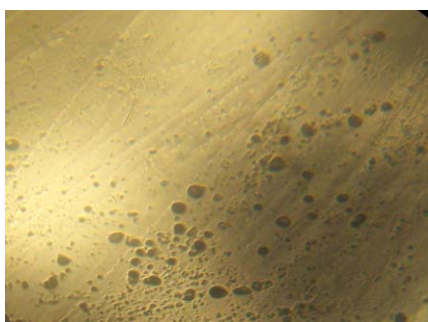


Fig.(1): *Ureaplasma* colonies on *Ureaplasma* agar plate (100 X)

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, $U \geq 10^4$ wells. Fig. (2)



Fig.(2): Negative *Mycoplasma* DUO kit

2) Positive for *ureaplasma* and with high titre ($\geq 10^4$ CCU/ml): the change in colour from yellow to red occurred in U and $U \geq 10^4$ wells. Fig.(3)



Fig. (3): Positive *Mycoplasma* DUO kit

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, radiological 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.^[16] found a significant difference in the isolation rate of *ureaplasmas* 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. **Cultrera et al.**^[9] also detected *ureaplasma* 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 *ureaplasma* by *Mycoplasma* DUO kit but negative by *Ureaplasma* agar and broth cultures. **Biernat-Sudolska et al.**^[16] stated that the differences between both tests may be due to differences in methodology of endotracheal aspirate samples processing.

In this study the sensitivity and specificity of *ureaplasma* 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 *ureaplasmas* detected by each method. These results are in line with that reported **Fook-Choe et al.**^[5] who found that there was 96% agreement between *Mycoplasma* Duo and culture on A7 media for detection of *ureaplasma* spp. in female genital swab samples.

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

Luki et al.^[19] also reported that the cultivation method is less sensitive.

This relatively low frequency of *ureaplasma* detection by means of culturing may be attributed in part to the difficulties in growing and isolation of these microorganisms.

Biernat-Sudolska et al.^[16] also reported that the specificity and sensitivity of *ureaplasma* 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 *Ureaplasma* agar and broth cultures was (2-5) days with mean \pm SD (3.8 ± 0.95) while incubation time taken to get positive results by *Mycoplasma* Duo kit was (1-2) days with mean \pm 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.**^[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 *ureaplasma* infection is mandatory for early treatment of premature neonates to avoid prematurity associated serious complications, also it allows laboratories to culture, identify, differentially titrate *ureaplasma* and prepare a standardized inoculum for antibiotic susceptibility testing.

Waites and Canupp^[20] and **Biernat-Sudolska et al.**^[16] stated that although culture is considered the gold standard for detection of *ureaplasma* spp., it is expensive and requires specialized media and expertise. From in vitro cultivation of *ureaplasmas* 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 *ureaplasma* spp. in neonatal respiratory samples. The *Mycoplasma* Duo assay allows laboratories to culture, identify, differentially titrate *ureaplasma* 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 (24-48hrs). Rapid diagnosis of *ureaplasma* 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 *ureaplasma* spp. These characteristics make this test suitable for use in diagnostic laboratories that do not currently test for *ureaplasma* spp.

RECOMMENDATIONS

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

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اختبار الميكوبلازما ديو مقابل المزرعة التقليدية للتعرف علي اليوريا بلازما في عينات الأنبوية الحنجرية للأطفال المبتسرين متعثري التنفس

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يعتبر الاستعمار البكتيري للمجري البولي التناسلي في السيدات الحوامل سبب في الولادة المبكرة وارتفاع نسبة الوفيات في الأطفال المبتسرين. ومن أكثر الميكروبات التي تسبب ذلك فصائل اليوريا بلازما. تتواجد فصائل اليوريا بلازما في افرازات الجهاز التنفسي للأطفال المبتسرين متعثري التنفس والتي تشير الي احتمال وجود دور لها في حدوث التعثر التنفسي. يعتمد التعرف علي اليوريا بلازما علي طريقة المزرعة التقليدية والتي تعتبر مجهددة، استهلاك للوقت وتحتاج ايضا الي خبرة خاصة.

وجود اختبارات تشخيص متاحة تجاريا" مثل الميكوبلازما ديو كت يقدم طرق بسيطة وسريعة للتعرف علي اليوريا بلازما في افرازات الجهاز التنفسي للأطفال حديثي الولادة. لذلك كان هدف هذا البحث هو تقييم قدرة الميكوبلازما ديو كت في التعرف السريع علي اليوريا بلازما في عينات الأنبوية الحنجرية للأطفال المبتسرين ومتعثري التنفس مقارنة بالمزرعة التقليدية وكذلك تحديد مدى التخصص والحساسية. وقد تم اجراء هذا البحث علي ٦٠ حالة من الأطفال متعثري التنفس والمحجوزين علي جهاز التنفس الصناعي بوحدة العناية المركزة لحديثي الولادة بمستشفى بنها الجامعي. وتم أخذ ٢ عينة من كل حالة تم نقلها في الوسط الناقل لليوريا بلازما الي المعمل لعمل الاختبارات اللازمة. تم زرع العينة الأولى في المزرعة التقليدية الخاصة باليوريا بلازما وحفظها في الحضانة تحت ظروف شبه هوائية عند درجة حرارة ٣٧° لمدة ٢-٧ أيام ثم تم التعرف علي مستعمرات اليوريا بلازما بالطرق المعملية الخاصة بها وتم اختبار العينة الثانية لوجود اليوريا بلازما باستخدام الميكوبلازما ديو كت علي حسب التعليمات المصاحبة لها. ومن نتائج البحث تبين ما يلي:

أظهرت نتائج هذه الدراسة أن عدد الحالات الإيجابية لليوريا بلازما في الأطفال متعثري التنفس وناقصي النمو بطريقة المزرعة التقليدية الخاصة باليوريا بلازما كان ٢٠ (٣٣.٣٣%) حالة بينما باستخدام اختبار ميكوبلازما ديو كت كان ٢٢ (٣٦.٦٧%) حالة. وبالنسبة لدرجة الحساسية والتخصص للميكوبلازما ديو كت مقارنة بالمزرعة التقليدية لليوريا بلازما وجد ان درجة الحساسية (١٠٠%) ودرجة التخصص (٩٥%). أما بالنسبة لفترة التحضين للحصول علي نتائج اليوريا بلازما وجد أنها (٥-٢) أيام بالمزرعة التقليدية لليوريا بلازما بينما كانت (١-٢) يوم باستخدام الميكوبلازما ديو كت. وبهذا يكون هناك فرق إحصائي عال بين الطريقتين في سرعة التعرف علي اليوريا بلازما في العينات. الميكوبلازما ديو كت متاحة تجاريا" وتقدم طرق بسيطة وسريعة خلال (١-٢) يوم للتعرف علي اليوريا بلازما. ويعد التشخيص السريع لليوريا بلازما في الأطفال المبتسرين هام للبدء المبكر في العلاج لتجنب المشاكل الخطيرة المصاحبة