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Mycoplasma and *Ureaplasma*

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INTRODUCTION

The concept that *Mycoplasma hominis* and *Ureaplasma* spp could be important pathogens that may affect pregnancy outcomes and the health of neonates was first given serious consideration in the 1960s and early 1970s when reports of postpartum endometritis with septicemia, chorioamnionitis, and low birth weight caused by these organisms began to appear and a treatment trial of pregnant women given tetracycline showed a significant beneficial effect on birth weight for their infants *(1–4)*. Since those days, a great many more case reports have been described, and numerous clinical studies have been performed in an attempt to clarify what roles, if any, these organisms play as agents responsible for invasive infections in neonates, premature labor, spontaneous abortion, stillbirth, and chronic lung disease of prematurity.

Despite more than 30 years of study, many aspects of the biology and clinical importance of genital mycoplasmas and ureaplasmas are still incompletely understood for a variety of reasons. These include (1) the high prevalence of these organisms in healthy persons; (2) poor design of many of the earlier research studies, which attempted to relate the mere presence of mycoplasmas or ureaplasmas in the lower urogenital tract to pathology in the upper tract or in offspring; (3) failure to consider multifactorial aspects of some maternal conditions and potential confounders (e.g., bacterial vaginosis); (4) unfamiliarity of clinicians and microbiologists with the complex and fastidious nutritional requirements for mycoplasmas and ureaplasmas and the methods for their proper detection; and (5) considering these organisms only as a last resort in conditions thought more likely to be caused by other micro-organisms.

An extensive review of the published literature on perinatal and neonatal aspects of mycoplasmal and ureaplasmal infections is beyond the scope of this chapter. Therefore, commentary focuses on the latest information pertinent for clinicians who need to understand the basic biology of these fascinating micro-organisms, when to consider them as possible etiologies of perinatal or neonatal conditions, and how to approach diagnosis from both clinical and laboratory standpoints. Reference texts are available for more detailed analyses of evidence for and against a pathogenic role for mycoplasmas and ureaplasmas in the conditions mentioned briefly in subsequent sections of this publication *(5,6)*.

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MOLLICUTES AS AGENTS OF HUMAN DISEASE

Classification and Cell Biology

Mycoplasmas and ureaplasmas are eubacteria classified in the class Mollicutes, which evolved from clostridium-like ancestors through the process of gene deletion. There are more than 150 species currently named in the class Mollicutes, 16 of which are known to have humans as their primary host. Mollicutes represent the smallest self-replicating organisms, both in cellular dimensions and genome, that are capable of cell-free existence. The extremely small genome, less than 600 kbp for the smallest mollicute *Mycoplasma genitalium*, and drastically limited biosynthetic capabilities explain the parasitic or saprophytic existence of these organisms, their sensitivity to environmental conditions, and their fastidious growth requirements. Mollicutes associated with humans range from coccoid cells of about 0.2–0.3 μm diameter (e.g., ureaplasmas and *Mycoplasma hominis*) to tapered rods 1–2 μm in length and 0.1–0.2 μm in width in the case of *Mycoplasma pneumoniae*. All mollicutes totally lack a cell wall barrier, making them unique among prokaryotes. Lack of a cell wall also renders these organisms insensitive to the activity of β-lactam antimicrobials, prevents them from staining by Gram stain, and is largely responsible for their pleomorphic form.

The small cellular mass also means that mollicutes cannot be detected by light microscopy, and they do not produce visible turbidity in liquid growth media. Typical colonies require examination under a stereomicroscope to visualize their morphologic features. Mollicutes have never been found as freely living organisms in nature because they depend on a host cell to supply them with the things they need for their parasitic existence. Another characteristic of most members of the class Mollicutes is the requirement for sterols in artificial growth media, supplied by the addition of serum, to provide necessary components of the triple-layer cell membrane that gives structural support to the osmotically fragile organism. Maintenance of osmotic stability is especially important in these bacteria because of the lack of a rigid cell wall. Although mycoplasmas and ureaplasmas can flourish within an osmotically stable environment in their chosen eukaryotic host, they are extremely susceptible to desiccation, a fact that greatly impacts the need for proper handling of clinical specimens in which cultural isolation is to be attempted and the need for close contact for transmission of infection from person to person. The small size, complex and fastidious nutritional requirements of mollicutes makes them challenging for detection and characterization by the microbiologist and historically has greatly hampered the ability of diagnostic laboratories to provide reliable services for their detection and identification.

Mollicute Species Pathogenic in Humans

Most research in perinatal and neonatal pathology has focused on two organisms, *M. hominis* and the bacterium formerly known as *Ureaplasma urealyticum,* because they are clearly the most significant in terms of disease-producing potential in pregnant women and neonates. However, it is relevant to mention briefly three other pathogenic mycoplasmal species that may contribute to perinatal and neonatal conditions to a lesser extent.

M. pneumoniae is well known as a major respiratory tract pathogen in older children and adults and has been detected many times in infants younger than 1 year, but it is rarely considered to be of much importance in the perinatal or neonatal period and is

believed to be uncommon. It was not detected by culture in an evaluation of more than 1500 neonates *(5)*. Nonetheless, this mycoplasma is a common cause of respiratory infections in women of childbearing age; it has been transmitted transplacentally with subsequent detection in the nasopharynx from a neonate with congenital pneumonia *(7)* and is therefore worthy of further study as a potential pathogen in this setting. More sensitive diagnostic tests such as the polymerase chain reaction (PCR) assay might yield different results if the culture-based study mentioned above was repeated.

M. genitalium was first detected in men with urethritis, and it has since been estimated to occur in 9–20% of men with urethritis and in up to 20% of women with urethritis or cervicitis *(8)*. This organism has the smallest genome known for any free-living micro-organism, grows very slowly, and cannot be readily detected by culture. The PCR assay has facilitated clinical studies of *M. genitalium* as evidence mounts for its role in male urethritis, pelvic inflammatory disease, and possibly cervicitis *(8)*.

Blanchard et al. *(9)* did not detect *M. genitalium* by PCR or culture in 232 amniotic fluids. Three studies have found *M. genitalium* in a very small percentage of pregnant women (3.9–6.2%) but were unable to relate the presence of this mycoplasma to preterm birth or other adverse pregnancy outcomes *(10–12)*. Vertical transmission of *M. genitalium* from mother to neonate has also been reported *(13)*, but its significance in neonates is unknown. Thus far, compelling evidence for an important role in pregnancy outcome or neonatal disease for this mycoplasma is lacking, but studies addressing this topic have been somewhat limited, and it is inappropriate to completely discount its significance based on information available at the present time.

Mycoplasma fermentans may also be pathogenic for humans in some settings, with most recent attention given to its role as an opportunist in persons with human immunodeficiency virus infection and acquired immunodeficiency syndrome and to a possible association with chronic arthritic conditions. *M. fermentans* is known to inhabit the lower and upper urogenital tracts of some adults. Furthermore, this mycoplasma has been detected by culture in placental tissue and in amniotic fluid in the presence of inflammation, but no studies have been performed to evaluate its occurrence and significance in neonates *(14)*.

Soon after ureaplasmas were first identified in the 1950s and were subsequently characterized, it became apparent that these organisms could be subclassified into several serotypes. Data obtained from 16S ribosomal ribonucleic acid sequencing has led to the further breakdown of the 14 serotypes into two biovars or clusters. The two biovars were designated as distinct species. Biovar 1 (parvo) became *Ureaplasma parvum,* whereas biovar 2 (T960) became *U. urealyticum*. Biovar 1 is the more common of the two biovars isolated in clinical specimens, especially pregnant women, but both species may occur simultaneously in the same person.

There has long been speculation that there may be differential pathogenicity of the various serotypes, biovars, and species. There was no conclusive evidence for this difference in pathogenicity for several years, and this was related to some degree to inefficient and imprecise methods for their accurate differentiation and the fact that many persons may harbor more than one serotype in their urogenital tract. The availability of the PCR assay has enabled a more rigorous assessment of whether one biovar or species is more pathogenic than the other. Kim et al. *(15)* found no difference in pregnancy outcome and magnitude of intra-amniotic inflammatory response, chorioamnionitis,

birth weight, or gestational age at delivery or neonatal morbidity in 77 women whose amniotic fluid contained ureaplasmas detected by PCR according to biovar. Zheng *(16)* suggested that the property of invasiveness for ureaplasmas is likely not limited to one or a few particular serotypes, and that perhaps antigen variability and host factors may be more important determinants for *Ureaplasma* infections than different serotypes *per se*.

However, a few limited studies have identified some differences regarding pathogenicity between the two biovars. Abele-Horn et al. *(17)* found biovar 2 to be dominant in patients with pelvic inflammatory disease as well as in women who had had miscarriages, and it seemed to have more adverse effects on pregnancy outcome regarding birth weight, gestational age, and preterm delivery than biovar 1. Others have shown that biovar 2 can be isolated more frequently from patients with a history of recurrent miscarriages than from normal pregnant women *(18)*.

Martinez *(19)* found no differences in antimicrobial susceptibilities or occurrence of the two ureaplasmal species in amniotic fluids of women with adverse pregnancy outcomes vs isolates from the lower urogenital tract of healthy pregnant women. In contrast, two other studies found more tetracycline resistance in biovar 2 than in biovar 1 *(17,20)*. The apparent contradictory results of some of these studies suggest that differencenos in antimicrobial susceptibilities, when they are observed, may reflect the history of antimicrobial exposure, the population studied, and other local environmental and host factors rather than a different capacity of the organism to acquire the *tet* (M) transposon in some instances. Based on the modest, and somewhat contentious evidence for differential pathogenicity for the two ureaplasmal species available at the present time and limitations of widely available technology for organism identification, it is neither practical nor necessary to distinguish between the two *Ureaplasma* species for clinical purposes. Therefore, diagnostic laboratories should appropriately designate cultures as positive for *Ureaplasma* spp and leave it at that.

Table 1 summarizes the major conditions of adults and infants that have been purported to be associated with or caused by *M. hominis, M. genitalium,* and *Ureaplasma* spp and the relative strengths, based on published evidence, for their roles in these conditions. The discussion of the clinical importance of these organisms has been divided into two major categories: maternal aspects and neonatal aspects.

Routes of Transmission and Maternal Considerations

Following puberty, colonization of the male and female lower urogenital tract by *M. hominis* and *Ureaplasma* spp usually occurs as a result of sexual activity. Up to 80% of women may harbor ureaplasmas and more than 50% may harbor *M. hominis*. These organisms are also commonly found in the lower urogenital tract of pregnant women, and they usually persist throughout pregnancy, providing a reservoir for transmission to the developing fetus and neonate *(5)*. In most healthy adults, mycoplasmas and ureaplasmas exist primarily as commensals, associated with the mucosal surfaces, and rarely cause serious invasive disease. However, in persons who are immunocompromised, especially if hypogammaglobulinemic, invasion of extragenital sites can occur. If one considers pregnant women and preterm infants to have an altered immune status, it is not surprising that these organisms can cause invasive and destructive disease when given the right circumstances.

Ureaplasma spp can be transmitted from a colonized woman to her newborn infant *in utero* either transplacentally from the mother's blood or by an ascending route sec-

Table 1
Conditions Known To Be Associated With or Caused by *Mycoplasmas* and *Ureaplasmas*

Disease	*Ureaplasma* spp	*M. hominis*	*M. genitalium*
Adults			
Male urethritis	+	–	+
Chronic prostatitis	±	–	±
Epididymitis	±	–	–
Urinary calculi	+	–	–
Pyelonephritis	±	+	–
Bacterial vaginosis	±	±	–
Cervicitis	–	–	±
Pelvic inflammatory disease	–	+	+
Infertility	±	–	–
Chorioamnionitis	+	–	–
Spontaneous abortion	±	±	–
Extragenital disease (including arthritis)	+	+	+
Infants			
Prematurity/low birth weight	+	–	–
Intrauterine growth retardation	±	–	–
Postpartum/postabortal fever	+	+	–
Congenital pneumonia	+	+	–
Chronic lung disease	±	–	–
Meningitis	+	+	–
Abscesses	+	+	–

–, no association or causal role demonstrated. In some conditions for *M. genitalium*, this may reflect the fact that no studies using appropriate techniques to detect this organism have been performed.

+, causal role.

±, significant association and/or strong suggestive evidence, but causal role not proven. (Modified from ref. *29*.)

ondary to colonization of the mother's urogenital tract, or at delivery by passage through a colonized birth canal. The rate of vertical transmission has been reported to range from 18 to 55% among full-term infants and from 29 to 55% among preterm infants *(21)*. *Ureaplasma* spp and *M. hominis* can be isolated from neonates born to mothers with intact membranes and delivered by cesarean section and from amniotic fluid during early pregnancy *(5,21)*. The rate of vertical transmission is not affected by method of delivery but is significantly increased when chorioamnionitis is present. The rate of colonization also appears to be higher in very low birth weight infants *(21)*.

The first studies attempting to make a correlation of mycoplasmas and ureaplasmas with postpartum endometritis were based on cervicovaginal cultures and caused much confusion with their inconclusive results *(5)*. However, both *M. hominis* and *Ureaplasma* spp can be detected in the bloodstream of some women with postpartum or postabortal fever, with *M. hominis* being the more common. This condition is usually self-limited, but some cases of dissemination to joints resulting in arthritis may occur.

Isolation of *Ureaplasma* spp, but not *M. hominis,* from the chorioamnion has been consistently associated with histologic chorioamnionitis and is inversely related to birth weight, even when adjusting for duration of labor, rupture of fetal membranes, and

presence of other bacteria. These organisms can invade the amniotic cavity and persist for several weeks when fetal membranes are intact and initiate an intense inflammatory reaction in the absence of labor *(22–24)*. Even though these conditions may be clinically silent, these findings are strongly supportive of a causal role for *Ureaplasma* spp in chorioamnionitis. *M. hominis* seems rarely to invade the chorioamnion and amniotic fluid in the absence of other micro-organisms, and data to support an independent role for this mycoplasma in either histologic or clinical amnionitis are modest at best. Chorioamnion colonization with *Ureaplasma* spp was associated with a threefold increased risk of post-cesarean delivery endometritis, an association that increased to eightfold in women in whom onset of labor was spontaneous *(25)*. The extent to which genital mycoplasmas may produce clinical amnionitis is unclear because some women whose placentas show significant evidence of inflammation and from whom genital mycoplasmas can be isolated from chorioamnion or amniotic fluid may not have evidence of clinical amnionitis.

Intrauterine infection is believed to be a major cause of preterm labor and can be documented in approximately one-fourth of all preterm births. The earlier the gestation age at delivery, the higher the frequency of intra-amniotic infection *(26)*. This relationship is believed to be related to the concept that uterine contractions may be induced by cytokines, prostaglandins, and phospholipases produced by micro-organisms *(27)*. *M. hominis* and *Ureaplasma* spp can be isolated from endometrial tissue of healthy, nonpregnant women, indicating they may be present at the time of implantation and might therefore be involved in early pregnancy losses *(5)*.

Studies of women from whom ureaplasmas and *M. hominis* were isolated from endometrium or placenta have shown a consistent association with spontaneous abortion, but this has not proven true for studies limited to sampling the lower genital tract *(28)*. Isolation of *Ureaplasma* spp in pure culture from amniotic fluid obtained from women with intact fetal membranes who experienced subsequent fetal loss in the presence of histological chorioamnionitis has been documented by multiple investigators, indicating that in some cases the role of this organism is causal *(22–24)*.

Other circumstantial evidence linking ureaplasmas to spontaneous abortion, low birth weight, intrauterine growth retardation, and preterm labor includes reports of successful pregnancies following antimicrobial treatment and serological studies *(5)*. Underlying problems that complicate complete understanding of any potential role for genital mycoplasmas in low birth weight are that *M. hominis* and, to a lesser extent, *Ureaplasma* spp can be components of the varied flora that occur with bacterial vaginosis, and this condition is itself associated with low birth weight *(28–31)*, problems in experimental study designs that failed to consider potential roles for organisms other than genital mycoplasmas, or use of control groups of uncertain comparability.

Neonatal Infections

M. hominis and *Ureaplasma* spp can be isolated from organs of aborted fetuses and stillborn infants in pure culture and in the presence of an inflammatory response *(5)*. Ureaplasmas are more commonly detected in products of early abortions and midtrimester pregnancy losses than from induced abortions, and they are more commonly detected in endometrium of habitual aborters and from placentas of aborted fetuses than from controls *(5)*. Several studies have reported an association between

isolation of *Ureaplasma* spp from the chorioamnion and perinatal morbidity and mortality *(5,32,33)*. Because these studies did not attempt to detect the organisms directly in the infants, it is uncertain whether the problems experienced by the infants were caused by infection or complications of prematurity. However, when investigations were designed to culture directly the lower respiratory tract, blood, and cerebrospinal fluid (CSF), it became evident that both *Ureaplasma* spp and *M. hominis* can cause a variety of clinically significant infections in neonates.

Retrospective as well as prospective studies and well-documented case reports indicated *Ureaplasma* spp can cause congenital pneumonia *(5)*. Proof for causality includes isolation of the organism in pure culture from affected lungs of neonates and from the chorioamnion, demonstration of a specific immunoglobulin M response, presence of histologic pneumonia and chorioamnionitis, clinical manifestations of respiratory distress, and demonstration of the organisms in lung tissue by immunofluorescence and electron microscopy. In some instances, ureaplasmas have been detected from multiple sites in neonates before and after death. Although individual case reports suggested *M. hominis* may cause pneumonia in newborns, it has not been implicated as a common cause in prospective studies *(5)*. No convincing evidence exists to support a significant role for *Ureaplasma* spp or *M. hominis* as common independent causes of pneumonia in otherwise healthy infants beyond the neonatal period.

In the late 1980s, bacteremia and progression to chronic lung disease of prematurity and death were described in very low birth weight infants who were infected with *Ureaplasma* spp in the lower respiratory tract *(34–37)*. Presence of ureaplasmas in the lower respiratory tract has also been significantly associated with radiographic evidence of pneumonia when compared with uninfected infants, and precocious dysplastic changes were also significantly associated with the presence of these organisms *(38)*. Further studies have shown that infants from whom ureaplasmas were isolated from endotracheal secretions had significantly more neutrophils in endotracheal secretions, attesting to their inflammatory potential *(39)*.

An explanation for the association of perinatal infections caused by *Ureaplasma* spp and development of chronic lung disease relates to intrauterine exposure to proinflammatory cytokines that are released in response to infection, which persists because antimicrobials commonly used are not active against this organism. Chronic inflammation then increases the requirement for supplementary oxygen, which can lead to dysplastic changes in the airways as a result of oxygen toxicity or a synergistic effect between the ureaplasmas and hyperoxia. It is also speculated that this cytokine cascade may induce both preterm labor and inflammation in the airways, which triggers the lung injury sequence before birth.

Several additional studies performed during the 1990s confirmed that there was a significant association between chronic lung disease of prematurity and the presence of *Ureaplasma* spp in the lower respiratory tract of preterm neonates *(40)*. This association was also detected in studies performed after introduction of exogenous surfactant *(39)*. This relationship has not been shown consistently in all investigations performed to date *(41–44)*, but two studies from Europe supported a role for *Ureaplasma* spp in chronic lung disease of prematurity even as technological advances in neonatology continue to improve survival of very low birth weight infants *(45,46)*. A summary and review of all published studies on the role of ureaplasmas in neonatal lung disease through 2004 was published by Waites *(47)*.

There are also data to suggest that infants with perinatal ureaplasmal infections have a significantly greater need for hospital care during the first year of postnatal life *(48)*. Further evidence of the inflammatory potential for this organism comes from animal models that indicate *Ureaplasma* spp can induce pneumonia and chronic inflammation *(49–51)*.

Despite numerous studies and considerable data supporting an association, demonstration of a cause-and-effect relationship between *Ureaplasma* spp and chronic lung disease or prematurity has not been conclusively proven. Even though treatment with intravenous erythromycin may eradicate ureaplasmas from the lower respiratory tract of neonates, at least temporarily *(52)*, small randomized trials of erythromycin treatment initiated early in the neonatal period have failed to show a benefit in reducing chronic lung disease *(47)*. A larger treatment trial would provide greater insights regarding whether targeted antimicrobial therapy can reduce the incidence of morbidity and mortality associated with chronic lung disease.

Both *M. hominis* and *Ureaplasma* spp have been isolated from maternal and umbilical cord blood and the blood of neonates. Both species can also invade the CSF *(53,54)*, resulting in either mild, subclinical meningitis without sequelae or neurological damage with permanent handicaps. Mononuclear or polymorphonuclear pleocytosis and elevated protein have been reported, but in some cases inflammation is minimal or absent. CSF glucose concentrations are usually normal. Most reported cases have involved preterm infants; full-term infants with neurological defects, including meningomyelocele; or older children with ventriculoperitoneal shunts, but infections in otherwise normal full-term infants have also been described.

Cases of *M. hominis* CSF infection in infants are more numerous than those that can be attributed to *Ureaplasma* spp because the former is much more readily detected without specialized methods. There appears to be an association with hydrocephalus and intraventricular hemorrhage in preterm infants with *Ureaplasma* CSF infections *(54,55)*. A report of a brain abscess in a neonate in which both *M. hominis* and *Ureaplasma* spp were isolated concomitantly proved these organisms can cause focal central nervous system infection as well as meningitis *(56)*. Relatively little is known about the long-term prognosis and neurodevelopmental outcomes for infections of the central nervous system caused by *M. hominis* or *Ureaplasma* spp because most available information comes from individual case reports, many of which provided scant information beyond the immediate period following infection and short-term prospective studies.

In addition to the above examples, occasional cases have appeared in the literature describing other conditions that bear consideration by clinicians. These include infections of pericardial fluid causing cardiac tamponade *(57)* and subcutaneous abscesses associated with forceps delivery *(58)* or an internal heart monitor *(59)*. Two studies have described isolation of *Ureaplasma* spp from the respiratory tract and blood in infants with persistent pulmonary hypertension of the newborn and suggested there may be a possible association or interaction between the ureaplasmas and the vascular events that characterize this syndrome *(60,61)*. Other isolations of these organisms from urine and conjunctiva are more difficult to evaluate because of the uncertain contribution of these bacteria to illness in these cases.

DIAGNOSTIC APPROACH

Because of the frequency with which genital mycoplasmas inhabit the lower urogenital tract of healthy adult women, there is no justification for performance of screening cultures. However, because they can produce invasive infections in a subpopulation of those who are colonized in the lower urogenital tract, cultures using methods that circumvent cervical contamination may be useful in some cases to confirm a microbiological diagnosis in conditions such as pelvic inflammatory disease.

Formulating recommendations for performing diagnostic evaluations for genital mycoplasmas in pregnant women is both complex and problematic. Many women who have significant histologic evidence of chorioamnionitis do not have clinical manifestations of infection, and only half of those who have a culture-positive chorioamnion will have positive amniotic fluids that may be readily obtainable for culture prior to delivery. Blood and amnionitic fluid cultures for mycoplasmas can be useful in women who have clinical evidence of amnionitis. Cultures may also be obtained in women with postpartum fever and endometritis if a microbiological diagnosis is desired. However, because most cases of postpartum fever caused by genital mycoplasmas resolve without sequelae, often without specific treatment, and the fact that chemotherapy with broad-spectrum antimicrobials to cover a wide array of possible micro-organisms is usually successful, the cost of mycoplasmal cultures may not always be justified.

Routine screening of neonates is not clinically justified based on the available evidence that many healthy neonates may be colonized without consequence. However, if there is clinical, radiologic, or laboratory evidence of pneumonia, meningitis, or overall instability suggestive of sepsis, particularly in preterm neonates in whom there are no obvious alternative etiologies, infection with *M. hominis* or *Ureaplasma* spp should be considered, and appropriate diagnostic studies should be obtained. It may also be useful to assess preterm neonates whose birth weight is less than 1250 g for the presence of ureaplasmas if they have respiratory distress that lasts more than a few hours after birth. Obtaining mycoplasmal cultures is particularly important if routine bacteriological studies fail to yield an etiologic agent within 2–3 days.

There are no specific clinical features that will provide clues to the clinician that these organisms may be involved in a particular condition. Thus, continued vigilance and an awareness of the possible contribution of genital mycoplasmas should always be considered earlier rather than later in ill neonates, particularly those born preterm. It should be noted that many serious, invasive, neonatal infections caused by the genital mycoplasmas have been detected after several days have passed without identification of a microbial etiology using conventional means or if the neonate fails to improve after institution of antimicrobial therapy with β-lactams and aminoglycosides. Unless specific diagnostic tests are requested, it is very unlikely that a mycoplasmal etiology will be identified. Possible exceptions may occasionally occur because *M. hominis* will sometimes grow after 3 or more days on routine bacteriological media, but this should not be assumed. Treatment of mycoplasmal infections and the value of antimicrobial susceptibility testing are discussed in reference texts and reviews *(14,47)*.

DIAGNOSTIC TESTING

Stains and Culture-Based Tests

Culture is still the most widely used means for detection and identification of genital mycoplasmas in clinical specimens, and it remains the accepted reference standard *(14,62)*. The relative rapidity of their growth will allow cultural detection and presumptive identification of these organisms within 2–5 days with an analytical sensitivity comparable to that of the PCR assay. The main challenges that may be encountered by clinicians are finding an experienced laboratory capable of performing and interpreting the results of mycoplasmal cultures and the careful attention required for proper specimen collection and transport to ensure that viable organisms are obtained and preserved until received in the diagnostic laboratory.

Stains

Mycoplasmal and ureaplasmal cells are too small to be visualized in Gram-stained preparations of clinical specimens or cultures, and the lack of a cell wall precludes uptake of crystal violet or safranin. However, the Gram stain may prove useful to exclude contaminating bacteria. Giemsa stains may be used, but the results can be difficult to interpret because of debris and artifacts in clinical specimens that can be confused with mycoplasmas because of their small size. Deoxyribonucleic acid (DNA) fluorochrome stains may be useful to determine whether micro-organisms are present in a clinical specimen or culture, but they do not distinguish mycoplasmas from other bacteria *(62)*.

Specimen Collection and Transport

Cultures of nasopharyngeal, throat, and endotracheal secretions of neonates are appropriate to evaluate respiratory infection. Gastric aspirates and throat and nasal swabs are less desirable because they may not always accurately reflect the microbiology of the lower respiratory tract because some infants may be colonized in other locations without ill effects. If urogenital specimens from adults are of interest, urethral swabs, urine, and cervical or vaginal swabs are acceptable. Urine samples from females are most meaningful when obtained by catheter or suprapubic aspiration and if numbers of organisms are quantitated. Endometrial tissue, tubal samples, or pouch of Douglas fluid can be obtained to confirm mycoplasmal etiology of postpartum fever. For women with clinical amnionitis, amniotic fluid, blood, and placenta should be cultured. Other sterile fluids from neonates, such as CSF and blood, are suitable for culture, as are wound aspirates, abscess fluid, and tissue collected by biopsy or autopsy.

Mollicutes are extremely sensitive to adverse environmental conditions, particularly to drying and heat, so great care must be taken to ensure proper specimen collection and transport. Clinical specimens from neonates will usually be of very small volumes or quantities. Therefore, they should always be collected and placed in appropriate transport medium immediately to prevent desiccation and loss of organism viability. If larger samples, such as lung tissue collected at autopsy or placenta, can be placed in a sterile screw-capped container and sent to the laboratory immediately, no additional transport medium is necessary. However, if there is any delay anticipated or if specimens have to be shipped to a laboratory off site, addition of transport medium is essential.

Transport medium such as Shepard's 10B broth is acceptable for transport of *M. hominis* as well as *Ureaplasma* spp. This medium is available commercially (Remel

Laboratories, Lenexa, KS) or can be prepared locally *(62)*. 2 SP (10% v/v heat-inactivated fetal calf serum with 0.2*M* sucrose in 0.02*M* phosphate buffer, pH 7.2), which is also used for transport of specimens for chlamydial cultures, is also acceptable. Other media available commercially for transport and storage of specimens include Stuart's medium, A3B, and arginine broth. From a practical standpoint, transport media can be kept frozen in small volumes in a freezer located in a clinical unit so that it can be rapidly thawed and used to inoculate specimens at bedside.

When swabs are obtained, care must be taken to sample the desired site vigorously to obtain as many cells as possible because mycoplasmas are cell associated. Calcium alginate, Dacron, or polyester swabs with aluminum or plastic shafts are preferred. Wooden shaft cotton swabs should be avoided because of potential inhibitory effects. Swabs should be vigorously swirled in the appropriate transport broth, pressed against the side of the tube to express as much fluid as possible, and discarded.

Endotracheal secretions from neonates who are intubated can be collected using a small-bore suction catheter connected to a vacuum outlet. The catheter is passed through the endotracheal tube, and suction is applied. The tip of the suction catheter is cut with a sterile scalpel blade. Then, 1 mL of 10B broth is drawn into a 3-mL syringe to which a 21-gage needle is attached. The tip of the suction catheter is placed into the tube from which the broth was drawn, and the needle is used to flush the catheter with broth, forcing the respiratory secretions into the tube, which is then transported to the laboratory.

Fluids such as CSF or urine should be obtained according to standard clinical procedures and inoculated immediately into transport media in an approx 1:10 ratio. Mycoplasmas and ureaplasmas are inhibited by sodium polyanethol sulfonate present in most commercial blood culture media, so this is not an acceptable means for detection. Commercial blood culture media designed for use in automated instruments may support growth of *M. hominis*, but the instruments usually do not flag the bottles containing this organism as positive *(63)*. Successful isolation of *M. hominis* and *Ureaplasma* spp from blood can be achieved by inoculating blood directly into liquid mycoplasmal growth media such as 10 B broth in at least a 1:10 ratio.

Specimens should be refrigerated if immediate transportation to the laboratory is not possible. If specimens must be shipped or if the storage time is likely to exceed 24 hours prior to processing, the specimen in transport medium should be frozen to prevent loss of viability. Specimens can be stored for long periods in appropriate growth or transport media at −70°C or in liquid nitrogen. Storage at −20°C for even short periods will result in loss of viability. Frozen specimens may be shipped with dry ice to a reference laboratory if necessary.

Growth Media

There are a variety of commercial and nonproprietary culture medium formulations that have been used to detect *M. hominis* and *Ureaplasma* spp. Their merits and relative disadvantages have been reviewed *(62)*. Shepard's 10B broth is an ideal choice for general use because it can be used for cultivation of both *M. hominis* and *Ureaplasma* spp with A8 agar *(62)* as the corresponding solid medium. In the event that other mycoplasmal species are to be sought, alternative methods such as the PCR assay should be utilized because culture techniques are not well established for other species. Even though 10B broth and A8 agar are available commercially, the comparability of the

commercially prepared products to nonproprietary formulations in their ability to support the growth of these fastidious organisms has not been documented, so internal quality control should be practiced *(62)*. There are a number of complete diagnostic kits for detection and identification of *M. hominis* and *Ureaplasma* spp; these are sold commercially in several European countries, but none is available in the United States. A comprehensive discussion and description of these products is available elsewhere *(64)*.

Specimen Processing and Interpretation

It is beyond the scope of this chapter to describe in depth the procedures that must be undertaken in a diagnostic laboratory to recover mycoplasmas and ureaplasmas from clinical specimens. Refer to reference microbiology texts *(14,62,64)* that deal with this topic for specific information, and only general comments are provided here.

Specimens should be mixed, and fluids should be centrifuged and the pellet serially diluted to at least 10^{-3} and inoculated into liquid and solid medium. Tissues should be minced in broth prior to diluting. Subculture of each dilution onto agar is an extremely important step in the cultivation process because it will help overcome possible interference by antibiotics, antibodies, and other inhibitors, including bacteria that may be present in clinical specimens. Omission of this critical dilution step can be one reason why some laboratories have difficulty in recovering the organisms. Dilution also helps to overcome the problem of rapid decline in culture viability, which is particularly common with ureaplasmas, and it also provides information about the number of organisms present in the specimen. Colonies develop best when agar plates are incubated in an atmosphere of 95% N_2 plus 5% CO_2 *(14)*, but successful isolation is also possible using an atmosphere of room air plus 5% CO_2 or in a candle jar.

Growth in 10B broth is suggested by an alkaline shift and change in color of the medium from yellow to pink because of urea hydrolysis by *Ureaplasma* spp or arginine hydrolysis by *M. hominis*. Such changes may be evident in 24 hours or less in the case of *Ureaplasma* spp and in 24–28 hours for *M. hominis*. Turbidity in broth cultures indicates bacterial contamination. Positive broths should always be subcultured to agar immediately because the primary inoculum does not always grow. Subcultures must be performed soon after the color change occurs, particularly if the organism is *Ureaplasma,* because the culture can lose viability within hours. Occurrence of pinpoint colonies on bacteriological media such as Columbia agar that do not produce a recognizable Gram reaction warrants subculture to mycoplasma media because of the possibility they may be *M. hominis*.

Colonies of *Ureaplasma* spp and *M. hominis* growing on A8 agar are shown in Figs. 1 and 2. Except for hydrolysis of urea and development of characteristic colonies on A8 agar that are unique for ureaplasmas, biochemical and colonial features are insufficient for definitive species distinction. However, colony morphology in conjunction with the biochemical profile, body site of origin, and rate of growth will often allow presumptive identification of the most common clinically significant species of large colony mycoplasmas. An arginine-hydrolyzing, urease-negative organism that produces fried egg colonies within 3–4 days of incubation may be presumptively identified as *M. hominis*, and in most instances no further work-up is required. Mycoplasmas that require more definitive identification can be submitted to a reference laboratory for characterization by PCR or other immunologic procedures in the event that this is

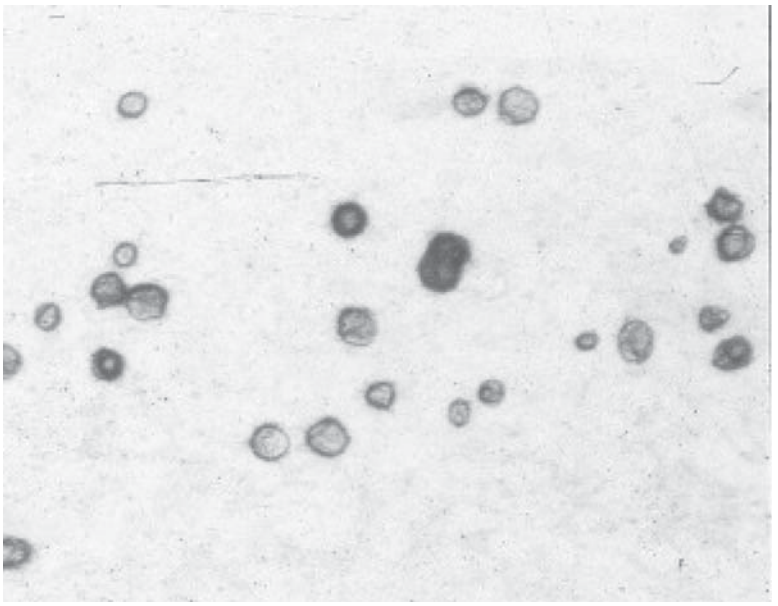

Fig. 1. Colonies of *Ureaplasma* spp growing on A8 agar after 48 hours of incubation as they appear under 126× magnification using a stereomicroscope. Colonies are typically 15–30 μm in diameter and have a brownish granular appearance caused by urease activity in the presence of the $CaCl_2$ indicator contained in the agar.

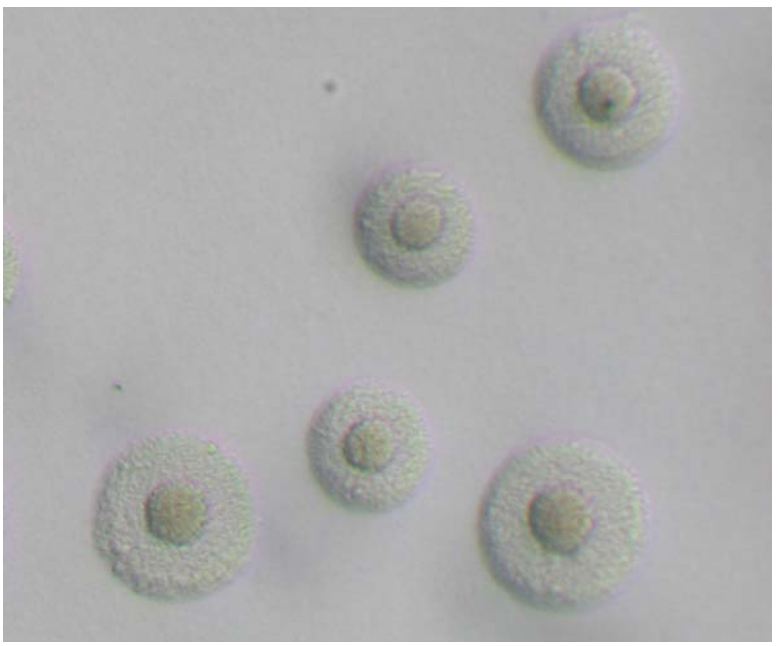

Fig. 2. Colonies of *Mycoplasma hominis* growing on A8 agar after 72 hours of incubation as they appear under 126× magnification using a stereomicroscope. Colonies are typically 200–300 μm in diameter and demonstrate the fried egg appearance characteristic of this organism.

clinically indicated. This may be relevant when the organism is present in a clinically significant infection of a normally sterile site.

Nonamplified Probes and Antigen Detection Systems

In the early 1980s, there was some interest in development of DNA probes and antigen detection systems for *M. pneumoniae*, but this technology was largely abandoned because of the low sensitivity of these assays and the more widespread development of nucleic acid amplification assays that were superior in many aspects. In view of the rapidity and relative ease for detection of *M. hominis* and *Ureaplasma* spp by culture, alternative methods based on nonamplified probes or antigen detection systems have never generated much interest.

Nucleic Acid Amplification

Nucleic acid amplification assays, particularly PCR, have been described for all mycoplasmas and ureaplasmas that are known to be significant pathogens for humans. Theoretically, PCR can detect a single organism or a single copy of the targeted gene when purified DNA is used, greatly exceeding the detection threshold of culture, which is approx 100–1000 cells under optimum conditions. PCR is also a very good tool for identification of an unknown mycoplasma previously obtained by culture, and it can be used for characterization of strains within a species.

Practically, PCR technology is less valuable for routine diagnostic purposes in the case of the more rapidly growing and relatively easily cultivable organisms, such as *M. hominis* and *Ureaplasma* spp, except in specific cases when isolation by culture could be difficult, such as fixed tissue samples. For slow-growing organisms, such as *M. pneumoniae*, and especially for extremely fastidious species for which optimum cultivation techniques are not established, such as *M. genitalium*, the use of PCR assays may be the only practical means of detecting their presence *(14)*.

Presently, PCR detection for mycoplasmas is still too labor intensive, expensive, and complex to be carried out routinely in most diagnostic laboratories and is not offered. Some drawbacks must still be corrected, such as the presence of inhibitors in the specimens and laboratory contamination. The possible development of commercial PCR kits in the future should bring about better standardization of the technique, and if available at a reasonable cost, PCR could become a major method for the diagnosis of mycoplasmal and ureaplasmal infections because it can theoretically provide extremely rapid turnaround time.

Serologic Diagnosis

The ubiquity of most genital mycoplasmas in adults makes interpretation of antibody titers difficult, and the mere existence of antibodies alone cannot be considered significant. However, infants with systemic infections of the central nervous system caused by *M. hominis* may mount a measurable serologic response. It has also been suggested that increases in titers of type-specific antibodies against certain ureaplasmal serovars may occur in women with pregnancy wastage and in infants with respiratory disease compared with titers in control patients *(65)*. More comparative data from well-characterized and carefully matched control populations are needed to fully assess the value of serologic diagnosis in these settings *(5)*. The relative hypogammaglobulinemic

state of preterm neonates, those who are most likely to experience significant invasive disease caused by these organisms, adds to the complexity of interpreting serologic findings.

Serologic assays that have been described for *M. hominis, M. genitalium,* and *Ureaplasma* spp include indirect immunofluorescence assays, enzyme-linked immunoassays, microimmunofluorescence, and metabolic inhibition tests *(14)*. At the present time, no assays designed for genital mycoplasmas have been standardized and sold commercially in the United States, and serology cannot be recommended for routine diagnosis of neonatal or perinatal mycoplasmal or ureaplasmal infections.

CONCLUSIONS

As evidence mounts for a role for *M. hominis* and *Ureaplasma* spp in neonatal and perinatal diseases, the need for their accurate laboratory detection and identification becomes more important. The lack of diagnostic services has been a contributing factor to the general unfamiliarity with these organisms that is widespread among clinical microbiologists as well as physicians. Greater availability of commercially prepared media and reagents for use in mycoplasmal detection and identification has helped the situation somewhat, but it is important to remember that most of these products have not been compared rigorously to traditional nonproprietary methods for mycoplasma detection, and therefore their analytical sensitivities are largely unknown.

Several microbiology reference laboratories offer detection of mycoplasmas and ureaplasmas by culture. The Diagnostic Mycoplasma Laboratory at the University of Alabama at Birmingham offers specialized testing that includes culture and PCR. Cultures for mycoplasmas and ureaplasmas are also becoming more common in hospital microbiology laboratories located in major medical centers. Should a clinician feel it necessary to obtain diagnostic specimens for detection and identification of genital mycoplasmas, the most important factors to consider are how to collect and transport the specimen to ensure that any mycoplasmas that may be present can be preserved for cultural isolation.

Understanding the role of mycoplasmas and ureaplasmas in neonatal and perinatal disease will be facilitated if more physicians attempt to make microbiological diagnoses when their presence is suspected, and patients themselves may benefit when the etiology of such infections is established so that appropriate treatment can be rendered.

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