JOURNAL OF CLINICAL MICROBIOLOGY, May 1997, p. 1199–1202 Vol. 35, No. 5
0095-1137/97/$04.00+0

Association of *Ureaplasma urealyticum* Biovars with Clinical Outcome for Neonates, Obstetric Patients, and Gynecological Patients with Pelvic Inflammatory Disease

MARIANNE ABELE-HORN,[1]* CHRISTINE WOLFF,[2] PETRA DRESSEL,[3] FRIEDERIKE PFAFF,[1] AND ANDREA ZIMMERMANN[4]

Max von Pettenkofer Institute, Ludwig Maximilian University, 80336 Munich,[1] Department of Obstetrics and Gynecology, City Hospital, Munich-Schwabing,[2] and Pediatric Hospital Munich-Schwabing, Technical University,[3] 80404 Munich, and Department of Neonatology, Klinikum rechts der Isar, Technical University, 81675 Munich,[4] Germany

Received 3 September 1996/Returned for modification 19 November 1996/Accepted 6 February 1997

In this prospective study, the prevalence of the two *Ureaplasma urealyticum* biovars, parvo and T960, was determined in pregnant women and in gynecological patients colonized by ureaplasmas. Furthermore, we investigated the association of these biovars with gynecological complications and adverse pregnancy outcome. Isolates of *U. urealyticum* from 254 women were biotyped by a PCR method recently developed. The parvo biovar was found in 81% (206 of 254) of the patients, and the T960 biovar was found in 30% (76 of 254) of the patients; 6% (14 of 254) of the women were coinfected. Identical biovars were detected in mothers and their infants. Serial isolations or cultures from different sampling sites of the same individual revealed the same biovar. T960 was dominant in patients with pelvic inflammatory disease (57%) and patients who had had a miscarriage (42%), showed a higher rate of tetracycline resistance than did parvo isolates (55 versus 18%), and seemed to have more adverse effects on pregnancy outcome with regard to birth weight (2,500 versus 1,720 g), gestational age (35 versus 30 weeks), and preterm delivery (35 versus 77%).

Ureaplasma urealyticum is considered to be a commensal in the lower genital tracts of sexually active women (4) and has been found at colonization rates of 40 to 80% (17). In some colonized pregnant women, ureaplasmas have been considered to be a cause of chorioamnionitis (9) and premature delivery (8). They are frequently transmitted from mothers to their infants either in utero or during birth (3, 8). Respiratory tract colonization of some newborn or premature infants has been associated with various diseases, including pneumonia (2), persistent pulmonary hypertension (21), chronic infection of the central nervous system (22), and bronchopulmonary dysplasia (BPD) (23).

It is not known why, despite heavy colonization of the cervix by *U. urealyticum*, only some women miscarry or deliver preterm and why some newborn infants, especially preterm infants, develop respiratory disease. Some studies (10, 11, 24)—but not all (14, 20)—have indicated that certain serotypes, especially 4 and 8, are more frequently associated with disease. These findings led to the assumption that the pathogenicity of *U. urealyticum* is linked to particular serotypes.

The 14 serotypes that have been defined (15) can be classified into two biovars according to phenotypic and genotypic differences. The parvo biovar comprises 4 serotypes, 1, 3, 6, and 14, and the T960 biovar includes 10 serotypes. An indirect immunofluorescence assay with polyclonal antisera is usually used for serotyping, but the results are often difficult to interpret. The biovars can also be discriminated by a PCR assay (16) which separates the 14 *U. urealyticum* serotypes into two biovars.

About 75% of the pregnant women tested at our hospital were colonized by *U. urealyticum*, and about 20% of them had complications during their pregnancies. The aim of this study was to determine the distribution of the two biovars among pregnant women and gynecological patients and to estimate their association with various clinical symptoms and clinical outcomes for colonized women. The study protocol excluded false-negative patients and patients with polyinfections.

MATERIALS AND METHODS

Study population. Patients included in this study were selected from women who were consecutively admitted to the Department of Obstetrics and Gynecology of the City Hospital, Munich-Schwabing for delivery, miscarriage, or pelvic inflammatory disease (PID). After admission, patients were examined clinically and vaginally and medical, sexual, and social histories were obtained. Furthermore, vaginal and cervical swabs were taken. Cases assessed were without bacterial vaginosis, *Escherichia coli* or other gram-negative bacteria, hemolytic streptococci, peptostreptococci, peptococci, *Neisseria gonorrhoeae*, *Candida albicans*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Bacteroides* sp., *Mycoplasma hominis*, and *Gardnerella vaginalis* and/or without diseases which could lead to gynecological complications, miscarriage, or preterm delivery. Only women colonized by *U. urealyticum* as the sole pathogenic microorganism were selected. The presence of bacteria like coagulase-negative staphylococci, nonhemolytic streptococci, corynebacteria, or enterococci were also analyzed. A concentration of $<10^4$ color-changing units of each of these particular microorganisms per ml was considered to be not relevant for the study and did not result in exclusion. The occurrence of preterm labor, membrane rupture, or amnionitis-chorioamnionitis was documented in detail. All women were observed from admission until discharge. Furthermore, all infants born to these women and colonized by *U. urealyticum* were included in the study protocol. Oral consent for participation was obtained from all patients. The study was approved by the Ethics Committee of our hospital.

Specimen collection. As mentioned above, cervical and vaginal swabs were routinely obtained from every patient after admission. Full clinical and microbiological examinations were repeated during birth or after miscarriage if the interval between admission and delivery or miscarriage was more than 24 h. Patients with longer hospital stays were examined twice a week. Placentas and endometrial tissues were examined histologically and microbiologically when the gestational age at delivery was <36 weeks or when a miscarriage took place. Nose and throat swabs and tracheal aspirates were collected from the infants directly after birth.

Cultures. A vaginal speculum examination was performed, and the appearance of any vaginal discharge (consistency, amount, color, odor, and pH) was recorded. One milliliter of a 0.9% saline solution was inoculated with a cotton

* Corresponding author. Mailing address: Max von Pettenkofer Institute, Ludwig Maximilian University, Pettenkoferstrasse 9a, 80336 Munich, Germany. Phone: 049/89/5160-5245. Fax: 049/89/64 270 288.

swab containing vaginal fluid for microscopic examination for *T. vaginalis*. Gram staining, and detection of bacterial vaginosis (positive amine test with 10% potassium hydroxide and microscopic examination for clue cells). A vaginal swab was obtained for the culture of *E. coli* or other gram-negative bacteria, streptococci, enterococci, coagulase-negative staphylococci, lactobacilli (all were cultured on blood agar plates with 5% sheep blood), *Haemophilus* bacteria (on chocolate agar plates), anaerobic bacteria (on Schaedler's anaerobic agar plates), *G. vaginalis* (on human blood bilayer agar plates with Tween 80 [HBT medium]), *N. gonorrhoeae* (on chocolate and Thayer Martin agar plates), and yeasts (on Sabouraud agar plates). An additional vaginal swab was placed in 2 ml of 2SP transport medium (7). An aliquot was inoculated into cycloheximide-treated McCoy cell cultures (7) for the growth of *C. trachomatis*. In addition, 0.1 ml was diluted in 0.9 ml of U9 broth (7) supplemented with arginine (0.1%) and analyzed for growth of *M. hominis* or in 0.9 ml of 10B broth (7) for growth of *U. urealyticum*. For the screening of ureaplasmas, five 10-fold dilutions were made and an aliquot (20 μl) of each dilution was inoculated onto an A8 agar plate (7). The cultures were then incubated anaerobically at 37°C. After growth in 10B broth (color change from yellow to red), subcultures to A8 agar plates were performed. On A8 agar, *U. urealyticum* organisms produce small, dark brown colonies (15- to 30-μm diameter). To confirm that *U. urealyticum* had been isolated, colonies were taken from A8 plates, inoculated into fresh 10B broth, and subcultured. Cultures in 10B broth were assayed by PCR.

PCR. For PCR, samples were prepared as previously described (1). Briefly, 250 μl of each sample was centrifuged (12,000 × *g* for 20 min at 4°C). The pellet was resuspended in 50 μl of solution A (10 mM Tris-HCl, pH 8.3, containing 100 mM KCl and 2.5 mM $MgCl_2$) and an equal volume of solution B (10 mM Tris-HCl, pH 8.3, containing 2.5 mM $MgCl_2$, 1% Tween 20, 1% Triton X-100, and 5 mg of proteinase K per ml). After incubation for 1 h at 60°C and heating to 95°C for 10 min, 5 μl was taken from each sample for PCR. Purified DNA of the 14 serotypes was used to evaluate the sensitivity of the amplification protocol. PCR amplification was done as described by Robertson and coworkers (16).

The positive controls were diluted lysates of *U. urealyticum* serotypes 1, 3, 6, and 14 (parvo biovar) and serotypes 2, 4, 5, and 7 to 13 (T960 biovar), which were kindly provided by G. Cassell (University of Alabama at Birmingham), and the negative control was sterile water. PCR with primers U3 and P6, which detected the parvo biovar, was named PCR-U3, and the PCR with U8 and P6 was designated PCR-U8 and detected the T960 biovar. Amplified products were 1,300-bp DNA fragments of the 16S rRNA genes of *U. urealyticum*. They were visualized under UV light after electrophoresis in a 1% agarose gel containing ethidium bromide and identified by amplicon size.

Antimicrobial susceptibility testing. Tests for susceptibility to ciprofloxacin, ofloxacin, erythromycin, clarithromycin, and doxycycline were performed once for each patient in duplicate on different days by determining the MICs. The assays were done with microtiter plates and 10B broth medium, pH 6.0, without penicillin as described by Senterfit (18). Each well contained ureaplasmas at a concentration between 5×10^3 and 5×10^4 color-changing units/ml. Controls without antibiotics were included. Before testing, the effects of the pH and the supplements in the media used for culture of the mycoplasmas on the activities of the antibiotics were determined by comparing the MICs of *Enterococcus faecalis* 7034 (A. Bauernfeind, Max von Pettenkofer Institute, Munich, Germany) obtained with Mueller-Hinton broth (pHs 7.2 to 7.4) with those obtained with 10B broth (pH 6.0). In addition, the MICs of a *U. urealyticum* serotype 2 reference strain (kindly provided by C. Bébéar, Université de Bordeaux, Bordeaux, France) were compared with the values obtained in this study. Designation of susceptibility was made according to DIN 58940 standards with the following values. For erythromycin and clarithromycin, ≤1 μg/ml was susceptible, 2 to 4 μg/ml was intermediate, and ≥8 μg/ml was resistant; for ciprofloxacin and ofloxacin, ≤1 μg/ml was susceptible, 2 μg/ml was intermediate, and ≥4 μg/ml was resistant; for doxycycline, ≤1 μg/ml was susceptible, 2 to 4 μg/ml was intermediate, and ≥8 μg/ml was resistant.

Statistical analysis. All variables were analyzed with a two-tailed chi square test or, when appropriate, Fisher's exact probability test. Continuous variables were analyzed by a two-tailed *t* test. Significance was established at 5%.

RESULTS

Study population. Of the 528 women examined, 370 (70%) were colonized by *U. urealyticum*; 116 of the colonized women were excluded because they carried microorganisms other than *U. urealyticum*. The remaining 254 patients entered the study and were categorized into three groups. Groups 1 and 2 were pregnant women admitted for delivery ($n = 174$) or miscarriage ($n = 24$); group 3 consisted of patients with PID ($n = 56$). Infants born to mothers in group 1 and colonized by *U. urealyticum* (55%; 97 of 174) were entered into the study. There were no statistically significant differences among the three groups with regard to age, social status, nationality, and state of parity. However, women in group 3 were significantly younger (24 years in group 3 versus 29 years in group 1 and 30 years in group 2; $P < 0.05$) and had significantly more previous miscarriages (50% in group 2 versus 17% in group 1 and 14% in group 3; $P < 0.001$).

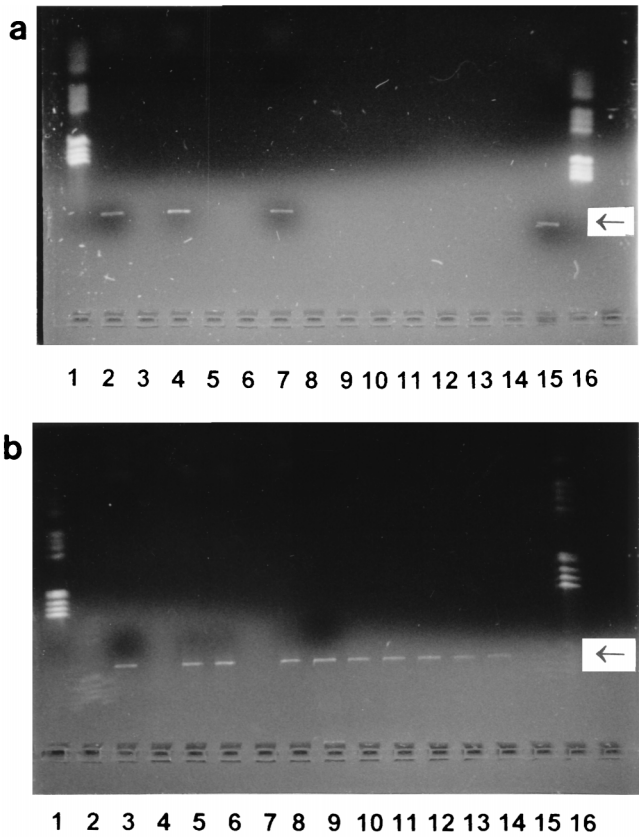

FIG. 1. Detection of *U. urealyticum* biovars by PCR. (a) DNA amplification with PCR-U3. Lanes: 2, 4, 7, and 15, *U. urealyticum* serotypes 1, 3, 6, and 14, respectively; 1 and 16, molecular size markers. (b) DNA amplification with PCR U-8. Lanes: 3, 5, 6, and 8 to 14, *U. urealyticum* serotypes 2, 4, 5, and 7 to 13, respectively; 1 and 16, molecular size markers. The position of the 1,300-bp fragment from the *U. urealyticum* biovars is indicated by the arrows.

Distribution of biovars. The amplified PCR products from *U. urealyticum* serotypes 1, 3, 6, and 14 (parvo biovar) and from serotypes 2, 4, 5, and 7 to 13 (T960 biovar) are shown in Fig. 1. Under optimal conditions, PCR amplification consistently detected 10 to 100 copies or 40 fg of DNA of each serotype. Control for polymerase inhibition was not necessary, as only positive samples were used. All strains of *U. urealyticum* that could be isolated from the 254 patients were biotyped. The parvo biovar was present in 81% (206 of 254) of the patients,

TABLE 1. Distribution of *U. urealyticum* biovars in three different groups of patients

Colonizing biovar(s)	No. (%) of patients colonized			
	Group 1 ($n = 174$)	Group 2 ($n = 24$)	Group 3 ($n = 56$)	Total ($n = 254$)
Parvo	156 (90)	18 (75)	32 (57)	206 (81)
T960	34 (20)[a]	10 (42)[b]	32 (57)[a]	76 (30)
Both	8 (5)	2 (8)	4 (7)	14 (6)

[a] $P < 0.0001$.
[b] $P < 0.05$.

TABLE 2. Influence of *U. urealyticum* biovars on pregnancies of mothers in group 1

Characteristics	Parvo biovar (n = 148)	T960 biovar (n = 26)
No. (%) of pregnancies with complications (bleeding, preterm labor)	34 (23)[a]	14 (54)[a]
No. (%) of pregnancies ending in amnionitis	40 (27)	14 (54)[b]
No. (%) of pregnancies ending in chorioamnionitis	20 (14)[c]	8 (31)[c]
No. (%) of female infants	65 (44)	12 (46)
Mean birth wt, g (SD)	2,500 (1,010)[c]	1,720 (1,345)[c]
Mean gestational age, wks (SD)	35 (7.0)[c]	30 (6.2)[c]
No. (%) of term infants	96 (65)[a]	6 (23)[a]
No. (%) of preterm infants	52 (35)[c]	20 (77)[c]
No. (%) of pregnancies lasting >30 wks	28 (19)	4 (15)
No. (%) of pregnancies lasting <30 wks	24 (16)[a]	16 (62)[a]
No. (%) of pregnancies with vertical transmission	77 (52)[c]	20 (77)[c]
No. of preterm infants/total (%)	48/52 (92)	18/20 (90)
No. of term infants/total (%)	29/96 (30)	2/6 (33)
No. of preterm infants with vertical transmission who developed BPD	12/48 (25)[c]	10/18 (56)[c]

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

the T960 biovar was present in 30% (76 of 254) of the patients, and 6% (14 of 254) of the patients were coinfected (Table 1). The T960 biovar was dominant in women with miscarriages (42%) and PID (57%), in contrast to group 1 (20%). Identical biovars were isolated from mothers in group 1 and their newborn infants. Of 96 colonized infants, 90% (86 of 96) harbored the parvo biovar, 20% (19 of 96) harbored the T960 biovar, and 5% (8 of 96) harbored both biovars (data not shown). In addition, consecutive samples from the same patient, e.g., placenta and cervix samples in group 1 or cervix samples and endometrium tissue samples in group 2, contained the same biovars.

Clinical outcome. As mentioned above, the prevalence of the T960 biovar was significantly greater among patients with miscarriages (group 2) and PID (group 3) than in group 1. Regarding group 3, it was conspicuous that women who harbored the T960 biovar were significantly older (27 versus 21 years), had PID recurrently (14 versus 6), and had been treated repeatedly with antibiotics. In women of group 1 (Table 2), strains of the T960 biovar had more adverse effects on pregnancy outcome with regard to birth weight ($P < 0.05$) and gestational age ($P < 0.05$) and preterm delivery ($P < 0.001$), despite comparable conditions with regard to age (29 versus 30 years), socioeconomic status, nationality, and gynecological risk factors (24 versus 19%). The T960 biovar also seemed to be associated with a significantly higher rate of complications ($P < 0.05$), such as preterm labor or bleeding in early pregnancy (Table 2). Furthermore, the premature infants who were colonized by the T960 biovar had a higher rate of BPD.

Susceptibility testing. The test results of the 254 strains are shown in Table 3. Comparative testing of control strain *E. faecalis* 7034 in Mueller-Hinton medium and 10B broth showed that there was no effect on the activity of doxycycline and clarithromycin, but there was a moderate effect on the activities of ciprofloxacin (1 to 2 μg/ml in Mueller Hinton medium versus 4 to 8 μg/ml in 10B broth), ofloxacin (2 versus 4 μg/ml), and erythromycin (2 to 4 versus 4 to 8 μg/ml). The MICs for the *U. urealyticum* serotype 2 reference strain (0.5 μg/ml for erythromycin and 0.05 μg/ml for doxycycline) were in agreement with the reference values (0.5 μg/ml for erythromycin and 0.05 μg/ml for doxycycline).

TABLE 3. Susceptibility of *U. urealyticum* biovars to antibiotics

Antibiotic	No. (%) of isolates susceptible	
	Parvo biovar (n = 206)	T960 biovar (n = 76)
Erythromycin	204 (99)	76 (100)
Clarithromycin	204 (99)	76 (100)
Ciprofloxacin	91 (44)	44 (58)
Ofloxacin	89 (43)	30 (39)
Doxycycline	169 (82)[a]	34 (45)[a]

[a] $P < 0.001$.

DISCUSSION

The aim of this prospective study was to determine the distribution of the *U. urealyticum* biovars parvo and T960 among women with cervical colonization and to investigate their association with disease. As described by others (5), most (81%) of the women examined were colonized by the parvo biovar while only 30% were colonized by the T960 biovar.

Both biovars were associated with adverse effects on pregnancy outcome; therefore, diseases do not seem to be associated with specific biovars. Compared to the parvo biovar, the T960 biovar had a less pronounced effect on birth weight (2,500 versus 1,720 g), gestational age (35 versus 30 weeks), chorioamnionitis (14 versus 31%), and preterm delivery (77 versus 35%). As diseases which could lead to obstetrical complications and colonization by other pathogenic organisms causing preterm delivery were excluded, colonization by *U. urealyticum* seems to be a risk factor for adverse pregnancy outcome. This is in accordance with other, recent studies in which *U. urealyticum* was associated with chorioamnionitis (9, 11), amnionitis (10), and complications in pregnancy (12).

The incidence of the T960 biovar was significantly higher among women with PID or women who had had a miscarriage (57 or 42%). Comparison of the two groups with PID showed that the women colonized by the parvo biovar were younger (mean age, 21 [range, 17 to 22] years), had not been treated previously with antibiotics, and had developed an infection for the first time. Women carrying the T960 biovar were older (mean age, 27 [range, 17 to 34] years), had PID recurrently, and had been treated repeatedly with antibiotics. According to these findings, the T960 biovar seems to be associated with chronic infections which cause or support chronic PID or recurrent unsuccessful pregnancies. This assumption has also been proposed by others (10, 12, 13). In one study (10), *U. urealyticum* strains from patients with pregnancy complications were serotyped and serotype 4, which belongs to biovar T960, was more often found in women with an irregular course of pregnancy (20.8 versus 5.1%). There have also been reports of higher isolation rates of *U. urealyticum* serotype 4 in patients with nongonococcal urethritis (6, 19). Indirect evidence for an association of *U. urealyticum* serotype 4 with pregnancy loss was described by Quinn and colleagues (13), who found higher levels of antibodies against serotypes 4 and 8 in women with unsuccessful pregnancies.

Examination of antibiotic susceptibility revealed a significant

correlation between T960 strains and doxycycline resistance. Resistant strains were isolated mainly from patients frequently using antibiotics, especially tetracyclines, for recurrent infections.

In conclusion, we found that women were more frequently colonized by the *U. urealyticum* parvo biovar than by the T960 biovar. Both biotypes were associated with unsuccessful pregnancies, the T960 biovar more often than the parvo type. Complications caused by a chronic ureaplasma infection were low birth weight, a shorter gestational period, and preterm delivery. The T960 biovar was more frequently present in patients suffering from PID. Infected women frequently had miscarriages, preterm delivery, or recurrent inflammatory diseases which often required antibiotic therapy. In these cases, the T960 biovar seemed to be an agent supporting chronic infections. In addition, T960 strains showed a strong tendency to develop tetracycline resistance. This should be considered when treating chronic PID.

ACKNOWLEDGMENTS

We thank J. Heesemann and H. Nitschko, Max von Pettenkofer Institute, and R. Boehmer, City Hospital Bogenhausen, Munich, Germany, for critical review of the manuscript.

REFERENCES

1. **Blanchard, A., J. Hentschel, L. Duffy, and K. Baldus.** 1993. Detection of *Ureaplasma urealyticum* by polymerase chain reaction in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. Clin. Infect. Dis. **17:**S148–S153.
2. **Brus, F., W. M. van Waarde, C. Schoots, and S. Bambang Oetomo.** 1991. Fatal ureaplasmal pneumonia and sepsis in a newborn infant. Eur. J. Pediatr. **150:**782–783.
3. **Cassell, G. H., D. T. Crouse, K. C. Canupp, G. R. Cutter, K. B. Waites, P. T. Rudd, and S. Stagno.** 1988. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very low birth weight infants. Lancet **i:**240–244.
4. **Cassell, G. H., K. B. Waites, and D. Taylor-Robinson.** 1990. Genital mycoplasmas, p. 7.2–7.15. *In* S. H. Morse, A. A. Moreland, and S. E. Thompson (ed.), Atlas of sexually transmitted diseases. Lippincott, Philadelphia, Pa.
5. **Clerc, M. T., H. Renaudin, B. de Barbeyrac, J. Robertson, G. W. Stemke, and C. Bébéar.** 1994. PCR, a useful tool for biovar determination in *Ureaplasma urealyticum*. IOM Lett. **3:**326–327.
6. **Cracea, E., S. Constantinescu, and M. Lazar.** 1985. Serotypes of *Ureaplasma urealyticum* isolated from patients with nongonococcal urethritis and gonorrhoea from asymptomatic urethral carriers. Sex. Transm. Dis. **12:**219–223.
7. **Deutsche Gesellschaft für Hygiene und Mikrobiologie.** 1989. Isolierung und Identifizierung von Mykoplasmen und Chlamydien. Zentralbl. Bakteriol. Hyg. A **270:**470–486.
8. **Gravett, M. G., D. Hummel, D. A. Eschenbach, and K. K. Holmes.** 1986. Preterm labor associated with subclinical amniotic infection and with bacterial vaginosis. Obstet. Gynecol. **6:**229–237.
9. **Hillier, S. L., J. Martius, M. Krohn, M. Kiviat, K. K. Holmes, and D. A. Eschenbach.** 1988. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N. Engl. J. Med. **19:**972–978.
10. **Horowitz, S., M. Mazor, R. Romero, J. Horowitz, and M. Glezerman.** 1995. Infection of the amniotic cavity with *Ureaplasma urealyticum* in the midtrimester of pregnancy. J. Reprod. Med. **40:**375–379.
11. **Mahler, C. F., M. V. Haran, D. J. Farrell, and D. G. Cave.** 1994. *Ureaplasma urealyticum* chorioamnionitis. Aust. N. Z. J. Obstet. Gynecol. **34:**477–479.
12. **Naessens, A., W. Foulon, J. Breynaert, and S. Lauwers.** 1988. Serotypes of *Ureaplasma urealyticum* isolated from normal pregnant women and patients with pregnancy complications. J. Clin. Microbiol. **26:**319–322.
13. **Quinn, P. A., A. B. Shewchuk, J. Shuber, K. I. Lie, E. Ryan, M. Sheu, and M. L. Chipman.** 1983. Serologic evidence of *Ureaplasma urealyticum* infection in women with spontaneous pregnancy loss. Am. J. Obstet. Gynecol. **145:**245–250.
14. **Robertson, J. A., L. H. Honore, and G. W. Stemke.** 1986. Serotypes of *Ureaplasma urealyticum* in spontaneous abortion. Pediatr. J. Infect. Dis. **5:**S270–S272.
15. **Robertson, J. A., and W. G. Stemke.** 1982. Expanded serotyping scheme for *Ureaplasma urealyticum* strains isolated from humans. J. Clin. Microbiol. **15:**873–878.
16. **Robertson, J. A., A. Vekris, C. Bébéar, and W. G. Stemke.** 1993. Polymerase chain reaction using 16S rRNA gene sequences distinguishes the two biovars of *Ureaplasma urealyticum*. J. Clin. Microbiol. **31:**824–830.
17. **Sanchez, P., and J. A. Regan.** 1987. Vertical transmission of *Ureaplasma urealyticum* in full term infants. Pediatr. Infect. Dis. J. **6:**825–828.
18. **Senterfit, L. B.** 1983. Antibiotic susceptibility testing of mycoplasmas, p. 397–401. *In* J. G. Tully and S. Razin (ed.), Methods in mycoplasmology, vol. 2. Academic Press, Inc., New York, N.Y.
19. **Shepard, M. C., and C. D. Lunceford.** 1985. Serological typing of *Ureaplasma urealyticum* isolates from urethritis patients by an agar growth inhibition method. J. Clin. Microbiol. **8:**566–573.
20. **Stemke, G. W., and J. A. Robertson.** 1985. Problems associated with serotyping strains of *Ureaplasma urealyticum*. Diagn. Microbiol. Infect. Dis. **3:**311–320.
21. **Waites, K. B., D. T. Crouse, J. B. Philips, K. C. Canupp, and G. H. Cassell.** 1989. Ureaplasma pneumonia and sepsis associated with persistent pulmonary hypertension of the newborn. Pediatrics **83:**79–85.
22. **Waites, K. B., P. T. Rudd, D. T. Crouse, K. C. Canupp, K. G. Nelson, and C. Ramsey.** 1988. Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* infections of central nervous system in preterm infants. Lancet **i:**17–21.
23. **Wang, E. L., H. Fraya, J. Watts, O. Hammerberg, J. B. Mahony, and G. H. Cassell.** 1988. Role of *Ureaplasma urealyticum* and other pathogens in the development of chronic lung disease of prematurity. Pediatr. Infect. Dis. J. **7:**547–551.
24. **Wiley, C. A., and P. A. Quinn.** 1984. Enzyme-linked immunosorbent assay for detection of specific antibodies to *Ureaplasma urealyticum* serotypes. J. Clin. Microbiol. **19:**421–426.