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Susceptibilities of Neonatal Respiratory Isolates of *Ureaplasma urealyticum* to Antimicrobial Agents

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Twenty-one neonatal respiratory isolates of *Ureaplasma urealyticum* were serotyped, and their susceptibilities to ciprofloxacin, gentamicin, chloramphenicol, erythromycin, azithromycin, and doxycycline were tested. Most patient strains were *Ureaplasma urealyticum* bv. parvum. Chloramphenicol, doxycycline, and azithromycin had the lowest MICs. This data may be useful when designing prophylactic or therapeutic trials of antibiotics for chronic lung disease of the newborn.

Ureaplasma urealyticum has been implicated in many infections, including neonatal sepsis, pneumonia, meningitis, and septic arthritis and in renal calculus formation (14). *U. urealyticum* has also been associated with chorioamnionitis, premature birth, and the development of chronic lung disease (CLD) of prematurity in very low birth weight infants (1, 8, 19). Despite the lack of definitive evidence for causality of CLD, patients have been treated with antibiotics, with variable results (2, 9, 10, 18).

Erythromycin has been considered the drug of choice to treat nonmeningeal neonatal *U. urealyticum* infections (16). However, in a study of 43 neonatal *U. urealyticum* isolates from Alabama, 56% of strains (24 of 43) were considered to have intermediate susceptibility to erythromycin (17). Although randomized controlled studies are required to determine the role of antibiotics in CLD, the selection of antibiotics for study will require knowledge of susceptibility patterns of *U. urealyticum*. Unfortunately, such testing is not possible in most laboratories.

The purposes of this study were to assess the susceptibilities of clinical strains of *U. urealyticum* to various antimicrobial agents by broth microdilution and to compare the susceptibilities determined from freshly prepared microtiter plates to those determined from premade frozen plates.

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Twenty-one strains of *U. urealyticum* were isolated from endotracheal aspirates of very low birth weight infants in metropolitan Toronto, Canada, in their first 2 weeks of life. Clinical specimens were cultured by standard methods (11). Isolates were serotyped by immunoperoxidase assay (12) in the Mycoplasma Laboratory at the Ontario Ministry of Health Laboratory Services Branch in Toronto, using antisera raised against the various serovars of *U. urealyticum* (13), and were stored in broth at −70°C prior to susceptibility testing.

Reference standard powders for in vitro susceptibility testing obtained from manufacturers included azithromycin (Pfizer Canada Inc., Kirkland, Quebec, Canada), ciprofloxacin (Bayer Inc., Mississauga, Ontario, Canada), doxycycline hyclate (Pfizer Canada Inc.), erythromycin base (Abbott Laboratories, Montreal, Quebec, Canada), gentamicin sulfate (Schering Canada Inc., Pointe Claire, Quebec, Canada), and chloramphenicol (Parke-Davis, Morris Plains, N.J.). A stock solution of each antibiotic dissolved in the solvent recommended by the manufacturer at a concentration of 2,048 μg/ml was prepared; the stock solution was inoculated neat into the first well and serially diluted twofold in Su broth (7) with a multichannel pipette.

Susceptibility testing was performed as per the method of Waites et al. (16) except for the following changes. Stock cultures of each organism were made in Su medium prepared in-house, with concentrations assessed by color-changing units and confirmed by 10-fold dilution of the stock culture. Su broth was chosen because, in our laboratory, it has consistently maintained viability of all serovars of ureaplasma. Each well of the assay was inoculated with 0.025 μl of antibiotic and 0.175 μl of ureaplasma culture. To verify the concentrations of organisms inoculated into the wells, stock cultures were serially diluted 10-fold in Su broth to 10^{-8}; results were considered valid only if the concentrations were 10^4 to 10^5 color-changing units/ml. Controls were the same as those used by Waites et al. with the exception of *Staphylococcus aureus* ATCC 29213.

To compare susceptibility results obtained with frozen microdilution panels to those obtained with freshly prepared panels, a batch of panels was prepared as described above, covered with acetate, and frozen at −70°C. On the day the assay with the freshly prepared panels was performed, the same number of frozen panels was thawed and both sets of panels were inoculated. Once inoculated, all panels were sealed with clear acetate, incubated at 37°C under atmospheric conditions, and observed for evidence of growth (color change after 16 to 20 h; cultures displaying growth were monitored daily until the end point was stable for 48 h). The MIC was defined as the lowest concentration of antibiotic which inhibited growth at the time the positive control tube first showed growth, which usually occurred after the first overnight incubation.

The distribution of serotypes in the 21 isolates of *U. urealyticum* is presented in Table 1. Most isolates were *U. urealyticum* bv. parvum, which includes serovars 1, 3, 6, and 14. Three

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TABLE 1. Distribution of *U. urealyticum* isolates by biovar and serotype

Biovar(s)	Serotype(s)	No. of isolates
Parvum	3	4
Parvum	6	2
Parvum	3, 14	10
Parvum	6, 14	1
T960	4	2
T960	12	1
Parvum and T960	1, 6, 13	1

patients had isolates of *U. urealyticum* bv. T960 only (two with serovar 4 and one with serovar 12). Although 12 patients had mixed serovars, only 1 patient had serovars belonging to different biovars (serovars 1, 6, and 13). This patient's data was not included in the breakdown of susceptibilities by biovar because each biovar was not tested individually, but susceptibility results from this patient were included in all other analyses.

Table 2 presents the antibiotic susceptibility results from the freshly prepared microtiter plates. Using these results as the "gold standard," there was an exact concordance between the MICs from fresh and frozen plates for 106 of 126 (84%) of the possible drug combinations. Of the other 20 combinations, 18 (90%) had MICs 1 dilution lower in the frozen panels and 1 had a twofold-higher MIC in the frozen panel. Only one fourfold difference in MIC was detected (for gentamicin; fresh-plate MIC, 4 μg/ml; frozen-plate MIC, 1 μg/ml). The MICs at which 50 and 90% of the isolates are inhibited (MIC_{50}s and MIC_{90}s, respectively) were the same for all antibiotics. Table 3 displays the MIC_{90}s broken down by biovar; there were no significant differences.

Antimicrobial susceptibility patterns of *U. urealyticum* have been reviewed by Waites et al. (16). *U. urealyticum* is usually susceptible to agents that interfere with protein synthesis, such as tetracyclines and macrolides, and is resistant to cell wall-active drugs, like beta-lactam-containing agents. Susceptibilities to aminoglycosides and chloramphenicol in vitro are variable. Ciprofloxacin's activity against ureaplasma is poor; the MIC_{90}s of sparfloxacin and other quinolones are often lower (3, 15). Tetracyclines and fluoroquinolones are generally precluded from use in neonatal and pediatric settings due to potential toxicity; doxycycline and ciprofloxacin were included in this study because they represent two classes of antibiotic that are still used to treat mycoplasma infections in adults. Erythromycin has been the drug of choice for treating pediatric *U. urealyticum* infections, despite reports of reduced susceptibility to this drug in neonatal strains (16). Although few studies have looked at both microbiological and clinical outcomes in neonates treated with erythromycin, some studies have shown

TABLE 2. Susceptibilities to six antibiotics of 21 *U. urealyticum* isolates from respiratory specimens of neonates

Drug	MIC (μg/ml)		
	Range	50%	90%
Ciprofloxacin	0.5–8	4	4
Gentamicin	1–64	64	64
Chloramphenicol	0.125–4	1	1
Erythromycin	0.25–4	2	2
Azithromycin	0.125–2	1	1
Doxycycline	0.031–4	0.125	0.125

TABLE 3. Susceptibilities to six antibiotics of *U. urealyticum* isolates compared by biovar[a]

Drug	MIC_{90} for parvum biovar (n = 17)	MIC range for T960 biovar (n = 3)
Ciprofloxacin	4	4–8
Gentamicin	32	64
Chloramphenicol	1	0.25–2
Erythromycin	2	0.25–4
Azithromycin	1	1–2
Doxycycline	0.5	0.03–0.125

[a] Concentrations are in micrograms per milliliter.

antimicrobial efficacy. In our study, the MICs of azithromycin, chloramphenicol, and doxycycline were the lowest. We have not classified *U. urealyticum* as susceptible or resistant to the agents we studied because of the lack of established breakpoints for ureaplasma (6). The in vitro values, however, do not necessarily predict in vivo activity. Erythromycin MICs are known to be higher at lower pHs (4). In vivo, erythromycin may actually be more effective than some of the other antibiotics because at physiological pHs, MICs would be lower than in this study (performed at pH 6.0). This pH effect has not yet been accounted for in ureaplasma susceptibility testing.

The lack of standardized guidelines for susceptibility testing of *U. urealyticum* has complicated the interpretation of susceptibility test results and may explain discrepant results reported in the literature. With any method, the inoculum size for the organism, the pH of the medium, and the technique must be standardized. Agar dilution and broth microdilution are the methods commonly used in reference laboratories for susceptibility tests on *U. urealyticum* (17). Frozen microdilution panels have been recommended, but no studies have compared results obtained with frozen and freshly prepared dilution panels. In this study, fresh and frozen microdilution panels gave comparable MIC_{90}s. The ability to use frozen panels would constitute a major advantage, particularly if batch testing of isolates from a multicenter trial was considered.

In our study, most isolates were of the parvum biovar. Although there was a 1- to 2-dilution difference in MICs between biovars, validation of this difference would require further investigation with larger numbers given the small numbers of isolates representing the T960 biovar in the present study.

This study describes the antibiotic susceptibility patterns of local neonatal strains of *U. urealyticum* and provides useful information for selecting antibiotics for prophylaxis or treatment of CLD. In our study, azithromycin, chloramphenicol, and doxycycline had the lowest MICs. Chloramphenicol and doxycycline are relatively contraindicated in neonates, and data regarding the safety, tolerance, and pharmacokinetics of intravenous azithromycin is limited (5). Although the macrolides are safer than the other drugs in neonates, comprehensive pharmacokinetic and toxicity data will be required prior to including azithromycin in studies of CLD.

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