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High Rates of Genital Mycoplasma Infection in the Highlands of Papua New Guinea Determined Both by Culture and by a Commercial Detection Kit

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Duplicate vaginal swabs were collected from 100 women, and comparisons were made between an in-house broth-agar culture system and a commercially available kit, the Mycoplasma IST kit (bioMérieux), for the detection of *Mycoplasma hominis* and *Ureaplasma urealyticum*. There was good agreement between the two systems for detection of the genital mycoplasmas in terms of sensitivity, with values of >92% being obtained. In terms of specificity, the mutual comparisons were less favorable, though specificity values of >72% were obtained. Statistically there was no significant difference in the performance of the two tests ($P > 0.1$ for both *M. hominis* and *U. urealyticum*). While the broth-agar culture system was considerably less expensive than the kit, the Mycoplasma IST kit provided additional information on antibiotic susceptibilities and had the advantages of a shelf life of up to 12 months and not requiring the preparation of culture media. The prevalences of colonization obtained for *M. hominis* and *U. urealyticum* were extremely high in this randomly selected group of women from periurban and rural settlements in the Eastern Highlands of Papua New Guinea, being ≥70% for *M. hominis* and ≥78% for *U. urealyticum*. Colonization with both genital mycoplasmas simultaneously was also very common, with ≥60% of women being colonized by both *M. hominis* and *U. urealyticum*.

The genital mycoplasmas, *Mycoplasma hominis* and *Ureaplasma urealyticum*, have been frequently isolated from the female genital tract with reported colonization values of between 20 to 30% for *M. hominis* and 60 to 80% for *U. urealyticum* (3, 4). Higher colonization rates have been found among women from lower socioeconomic backgrounds and increase in association with sexual experience and larger numbers of sex partners (2, 3, 17). The presence of genital mycoplasmas in a large proportion of healthy women complicates the assessment of the pathogenic roles of these organisms, but several studies have indicated that vaginal colonization with the genital mycoplasmas can be associated with an increased risk of developing certain pathogenic conditions and pregnancy abnormalities, e.g., bacterial vaginosis, pelvic inflammatory disease, postpartum septicemia, premature rupture of membranes, and preterm labor and birth (7–9, 12, 14). In addition, *U. urealyticum* has been identified as a possible etiological agent of systemic neonatal infections, particularly in preterm and low-birth-weight infants (2, 19).

With an increasing body of evidence suggesting that under certain circumstances, the genital mycoplasmas may be clinically significant, a reliable and sensitive laboratory procedure for the detection of these organisms is a prerequisite to undertaking any research aimed at elucidating their pathogenic roles. The mycoplasmas are a unique group of bacteria, characterized by their small cell size (0.3 to 0.8 μm), lack of a bacterial cell wall (the cytoplasm being bound by a plasma membrane), and small genome size (4). To culture this group of nutritionally fastidious bacteria in the laboratory requires the use of a complex growth medium containing supplements, cofactors, and vitamins, in addition to a well-controlled physicochemical environment. For these reasons, routine culture for the genital mycoplasmas is performed by relatively few laboratories.

The first aim of this study was to compare an in-house broth-agar culture system with a commercially available system, the Mycoplasma IST kit (bioMérieux), for the detection of the genital mycoplasmas. The second aim of the study was to determine the prevalence of *M. hominis* and *U. urealyticum* colonization in a group of women in the Eastern Highlands Province of Papua New Guinea, who had been selected to participate in a community-based study of sexually transmitted diseases.

MATERIALS AND METHODS

Patient population. Specimens were collected from 100 women aged between 15 and 45 years, who had been randomly selected from rural and periurban settlements in the Asaro Valley, Eastern Highlands Province, of Papua New Guinea for participation in a community-based study of sexually transmitted diseases (18). This sample is considered to be representative of the general population of women living in this area.

Specimens. Two Dacron swabs were used simultaneously to collect vaginal specimens from each woman. One swab was placed in mycoplasma transport medium (tryptose phosphate broth, 10% bovine serum albumin, 100,000 U of penicillin per ml), and the other swab was placed in bioMérieux transport medium R1, provided with the Mycoplasma IST kit. Both swabs were stored in the transport media at 4°C until transported to the laboratory.

Laboratory processing and interpretation. All swabs were processed in the laboratory within 16 h of collection, with the majority being processed within 4 h. The in-house mycoplasma transport medium containing one swab was vortexed for 30 s. A 200-μl volume was inoculated into 1.8 ml of ureaplasma 10C broth (U10C) (16) for culture of *U. urealyticum*, and another 200-μl volume was inoculated into 1.8 ml of arginine broth (1) for culture of *M. hominis*. The U10C and arginine broths were incubated at 37°C overnight and subcultured the following morning by spreading 100 μl of each broth across the surfaces of separate A7 agar plates (16). This was done irrespective of whether there were signs of growth in the broth (pH change indicated by phenol red; pale yellow to amber for U10C broth and amber to red for arginine broth) and is subsequently referred to as blind subculture. In addition, broths which had not shown a pH change after incubation overnight were observed twice daily for up to 5 days and subcultured onto A7 agar as described above, if and when any pH change was detected. This is referred to hereafter as indicated subculture. Once inoculated, the A7 agar plates were incubated anaerobically in a gas jar with a GasPak (BBL 70305) at 37°C for 48 h. Following incubation, the plates were examined with a binocular microscope (×40 magnification) for characteristic colonies of *M. hominis* which

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TABLE 1. Detection of *M. hominis* and *U. urealyticum* from vaginal specimens by using the broth-agar culture method and the Mycoplasma IST kit

Broth-agar culture result	Mycoplasma IST result			
	M. hominis (n = 100)		*U. urealyticum* (n = 100)	
	Positive	Negative	Positive	Negative
Positive	65	5	76	2
Negative	4	25	1	15
Unknown[a]	0	1	5	1

[a] For statistical analyses, unknown results from culture were regarded as negative.

are described as having a "fried-egg" appearance (30- to 300-μm diameter) and for the tiny (10- to 50-μm diameter), dark-brown colonies of *U. urealyticum*. A positive result was recorded when the characteristic colonies were seen on A7 agar. In cases where the agar was overgrown with other organisms, it was not possible to determine whether *M. hominis* or *U. urealyticum* was present or absent and the result was recorded as unknown.

The swab in R1 transport medium was processed according to the manufacturer's instructions. In summary, the swab in R1 transport medium was vortexed for 30 s and 3 ml of R1 broth was used to rehydrate the lyophilized growth medium, R2 (provided with the Mycoplasma IST kit). A Mycoplasma IST strip, consisting of 16 wells, was then inoculated with the rehydrated R2 growth medium (50 μl per well, overlaid with mineral oil). The remainder of the broth and the inoculated strip were incubated at 37°C and observed for color changes, and the results were interpreted after 24 and 48 h of incubation. The strips provided information on the presence or absence of *M. hominis* and *U. urealyticum*, an estimate of the density of each organism ($\geq 10^4$ CFU) and antimicrobial susceptibilities to doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, and pristinamycin.

Statistical analysis. Relative sensitivities and specificities were calculated for each method by using the other method as the "gold standard." As the specimens were matched, statistically significant differences in the performance of the two methods were tested by McNemar's test. Unknown results obtained from broth-agar culture were considered as negative for all analyses.

RESULTS

The results of the in-house broth-agar culture system versus the Mycoplasma IST kit for the detection of *M. hominis* and *U. urealyticum* are shown in Table 1. *M. hominis*, was isolated on A7 agar from 70 specimens and was detected in 69 specimens by the Mycoplasma IST. A pH change was seen in 69 of the 70 specimens in arginine broth which were positive for *M. hominis* on A7 agar. A pH change was also noted in five specimens in arginine broth where *M. hominis* was not isolated on A7 agar. On subculture from one of the latter five specimens in arginine broth, the A7 agar was overgrown by yeasts and the result was recorded as unknown. *U. urealyticum* was isolated on A7 agar from 78 specimens and detected in 82 specimens by Mycoplasma IST. A pH change was observed in 76 specimens in U10C broth which were positive for *U. urealyticum* on A7 agar, as well as in an additional 8 specimens in broth where *U. urealyticum* was not isolated on A7 agar. On subculture of six of the latter eight specimens in broth, the A7 agar was overgrown with yeasts and an unknown result was recorded.

Blind subculture (i.e., that performed routinely on all broths after overnight incubation) was found to be more sensitive for the detection of the genital mycoplasmas than the indicated subculture (i.e., that performed only after a pH change was observed in the broth). Whereas *M. hominis* was detected in 70 specimens and *U. urealyticum* was detected in 78 specimens from a total of 100 specimens by blind subculture, the two were detected in only 58 and 66 specimens, respectively, by indicated subculture. There was no occasion where *M. hominis* or *U. urealyticum* was negative by blind subculture and positive by indicated subculture. For this reason, blind subculture results are used only for comparisons with the Mycoplasma IST.

TABLE 2. Sensitivity and specificity of the broth-agar culture system and Mycoplasma IST kit for the detection of *M. hominis* and *U. urealyticum*[a]

Species	Culture against Mycoplasma IST[b]		Mycoplasma IST against culture[c]	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
M. hominis	94.2	83.9	92.9	86.7
U. urealyticum	92.7	88.9	97.4	72.7

[a] For the calculation of sensitivity and specificity, unknown results from culture (for *M. hominis*, n = 1; for *U. urealyticum*, n = 6) were regarded as negative.

[b] The positive and negative predictive values of the Mycoplasma IST are the same as the sensitivity and specificity, respectively, of culture against Mycoplasma IST.

[c] The positive and negative predictive values of culture are the same as the sensitivity and specificity, respectively, of the Mycoplasma IST against culture.

Table 2 shows that in terms of sensitivity there was good agreement between the broth-agar culture system and the Mycoplasma IST kit for the detection of *M. hominis* and *U. urealyticum*. In terms of specificity, the two systems did not compare as favorably; in particular, the specificity of the Mycoplasma IST against broth-agar culture for the detection of *U. urealyticum* was only 72.7%. However, there was no statistically significant difference in the performance of the two methods for the detection of the genital mycoplasmas ($P > 0.1$ for both *M. hominis* and *U. urealyticum*).

In addition to the detection of the genital mycoplasmas, Mycoplasma IST also provided information on the density of colonization, with a titer of $\geq 10^4$ being considered evidence of disease. Of the 69 specimens in which *M. hominis* was detected by Mycoplasma IST, *M. hominis* was present at a titer of $\geq 10^4$ in 24 specimens; 56 of the 82 specimens with *U. urealyticum* had titers of $\geq 10^4$.

Antimicrobial susceptibilities determined by Mycoplasma IST are shown in Table 3. All genital mycoplasma isolates showed full resistance to erythromycin, with two exceptions (one intermediately resistant and one susceptible strain). Of the 89 women who were colonized by genital mycoplasmas (as detected by Mycoplasma IST), 8 harbored strains resistant to three or more of the six antibiotics tested, including two strains which were resistant to all six antibiotics included in the kit.

The prevalences of *M. hominis* and *U. urealyticum* were very high in this population, as determined both by broth-agar culture and by Mycoplasma IST (Table 4), with 88% of the women being colonized by either one or both of the genital mycoplasmas as assessed by culture and 89% as assessed by Mycoplasma IST. Simultaneous colonization by both *M. hominis* and *U. urealyticum* was common, with values of 60% as

TABLE 3. Antibiotic susceptibilities of the genital mycoplasma isolates determined by using the Mycoplasma IST kit

Antibiotic	% of isolates (n = 89) with the following antibiotic susceptibility:		
	Susceptible	Intermediate	Resistant
Doxycycline	89.9	3.4	6.7
Josamycin	80.9	14.6	4.5
Ofloxacin	28.1	58.4	13.5
Erythromycin	1.1	1.1	97.8
Tetracycline	85.4	1.1	13.5
Pristinamycin	93.3	0	6.7

TABLE 4. Prevalence of vaginal colonization by *M. hominis* and *U. urealyticum*

Species	Prevalence determined by:	
	Broth-agar culture[a] (n = 100)	Mycoplasma IST kit (n = 100)
M. hominis only	10	7
U. urealyticum only	18	20
M. hominis and *U. urealyticum*	60	62
M. hominis and/or *U. urealyticum*	88	89

[a] Unknown results obtained by culture (for *M. hominis*, n = 1; for *U. urealyticum*, n = 6) were regarded as negative.

determined by broth-agar culture and 62% as determined by the Mycoplasma IST (Table 4).

DISCUSSION

Although culture for the genital mycoplasmas is considered the gold standard for the detection of *M. hominis* and *U. urealyticum*, because of the fastidious nature of these organisms, it was decided to compare the performance of two systems, an in-house broth-agar culture system and a commercially available kit, the Mycoplasma IST kit (bioMérieux). The results for the detection of the genital mycoplasmas obtained from each system are similar, with high sensitivities, though in terms of specificity the two systems did not compare as favorably. However, statistically, there was no difference in the performance of the two tests for the detection of *M. hominis* or *U. urealyticum.*

The lower specificity value obtained from the IST kit in detecting *U. urealyticum* results from six specimens in U10C broth where the A7 agar was overgrown by yeasts following subculture. Although an unknown result for the presence of the genital mycoplasmas was recorded for these specimens, for the purposes of calculating the sensitivities and specificities of the broth-agar culture system and the Mycoplasma IST, unknown results were regarded as negative. Five of the six subcultures from U10C broth where an unknown result was recorded from broth-agar culture were positive for *U. urealyticum* by Mycoplasma IST. Whether the positive Mycoplasma IST results were actually due to the presence of *U. urealyticum* in the samples or were false positives caused by the growth of yeasts in the R2 medium resulting in a pH change in the Mycoplasma IST strip was not possible to determine. A third method for the detection of the genital mycoplasmas, perhaps PCR, would have been useful in resolving these discrepancies, but as the difficulties encountered with contamination were not anticipated, no other method was available in the laboratory at the time of specimen collection. The manufacturers of the Mycoplasma IST are aware of the possibility of obtaining false-positive results and recommend that IST strips not be read if they are contaminated by bacteria or yeasts, contamination being evident by high turbidity of the R2 broth. However, since the majority of specimens in R2 broths were turbid following rehydration with the R1 transport medium (with epithelial cells and debris from the swabs), this exclusion criterion was not practical.

It should be noted that if the positive results obtained for the yeast-contaminated cultures on Mycoplasma IST do indeed represent false positives (i.e., the samples were in fact negative for the presence of genital mycoplasmas), then the calculated sensitivity for the in-house broth-agar culture system is too low. The remaining unknown subculture from U10C broth and the unknown subculture from arginine broth were negative by Mycoplasma IST for *U. urealyticum* and *M. hominis*, respectively.

Since contamination was due primarily to the growth of yeasts, it is not surprising that contamination occurred more frequently in the U10C broth, which unlike arginine broth does not contain the antifungal agent amphotericin B. Although amphotericin B is inhibitory to some strains of *U. urealyticum* (15), it may be worth considering whether the advantage of reducing fungal contamination by incorporating amphotericin B into either the U10C broth or A7 agar would outweigh the disadvantage of inhibiting the growth of a small number of *U. urealyticum* strains.

The finding that blind subculture was more sensitive than indicated subculture for the detection of the genital mycoplasmas may be due to the fact that these organisms are very sensitive to changes in the pH of the growth medium and that exposure to an alkaline pH can in fact be lethal to these organisms (10). Although broths were checked twice daily and subcultured as soon as any pH change was observed, during incubation overnight the broths were not checked for 16 to 18 h, and therefore exposure to alkaline pH could be prolonged, perhaps resulting in reduced viability.

Although pH changes observed in the U10C and arginine broths have been reported to indicate growth of the genital mycoplasmas, the occurrence of a small number of broths from which genital mycoplasmas were isolated on A7 agar but which failed to register a pH change and of a larger number of broths where a pH change was observed (perhaps due to the growth of contaminating microorganisms) but genital mycoplasmas were not isolated on agar subculture confirmed the views of previous researchers that a pH change in the broths cannot be considered as conclusive proof for the presence of the genital mycoplasmas (6, 11, 13). Therefore, positive results for the broth-agar culture system were reported only when characteristic colonies of the genital mycoplasmas were seen on A7 agar following subculture from U10C or arginine broth.

In terms of the simplicity of specimen processing and inoculation, both the broth-agar culture system and the Mycoplasma IST kit were easy to use. However, the broth-agar culture system did require time spent in preparation, aliquoting, and storage of media, supplements, and antibiotics. Once this was done, however, large batches of U10C broth and arginine broth were prepared and stored at −20°C until required. A7 agar was prepared and stored at 4°C in sealed bags for a maximum of 2 weeks. In contrast, the Mycoplasma IST required no prior preparation of media and the reagents and strips have a shelf life of up to 12 months. Results from the Mycoplasma IST were available in 48 h, whereas those from the broth-agar culture method required an additional 6 to 18 h. In addition to determining the presence or absence of the genital mycoplasmas, the Mycoplasma IST also provided information on the density of colonization and on antimicrobial susceptibilities.

At an estimated cost of $7.50 for the Mycoplasma IST per patient recruited in this study compared to $3.00 per patient recruited for broth-agar culture, the Mycoplasma IST is more expensive. However, this estimate of cost was based on the use of laboratory consumables only (media, reagents, disposable plasticware etc) and does not include the cost of labor. It should be noted that the broth-agar culture method is considerably more labor intensive than the Mycoplasma IST. The cost was initially calculated in Papua New Guinea kina, and the resulting sum was subsequently converted into United States dollars by using the currency exchange rate at the time of the study (June and July 1995) of 1 kina equals 0.75 dollar.

The most appropriate system for use in the laboratory will

depend upon the number and frequency of specimens to be tested, the need for rapid results, and information on antimicrobial susceptibilities. In our laboratory, where the detection of genital mycoplasmas is part of a larger study to determine predisposing risk factors to the development of postpartum infection, the broth-agar culture method is the most suitable. Under these circumstances, a large number of specimens can be collected and stored at −70°C in mycoplasma transport medium, before being tested in batches. However, in laboratories where a small number of samples are received sporadically or when antibiotic susceptibilities are required (e.g., neonatal infection), then the obviated necessity to prepare media in advance and the long shelf life of the Mycoplasma IST kit may make this system more suitable for use.

Antibiotic susceptibility testing of the genital mycoplasmas is usually performed only by mycoplasma reference laboratories and so was not attempted on the individual isolates obtained here from broth-agar culture. However, the antibiotic susceptibility data provided by the Mycoplasma IST kit showed the existence of strains in the population to resistant all six antibiotics tested, though since the majority of patients were colonized by both *M. hominis* and *U. urealyticum* simultaneously, it was not possible to determine percentage resistance patterns for each organism separately. The 97.8% resistance reported for erythromycin is high and though *M. hominis* is known to be resistant to this antibiotic, *U. urealyticum* is generally thought to be susceptible (11). A high proportion of erythromycin-resistant strains of *U. urealyticum* could be a problem if this organism proves to be an important etiological agent of neonatal infection in this population, since erythromycin is the recommended treatment for neonatal infections (20). However, it is also possible that this result is aberrant, since the activity of erythromycin is known to be reduced by the acidity of the growth medium used for the genital mycoplasmas (20). The levels of resistance reported for doxycycline and tetracycline here, 6.7% and 13.5%, respectively, are similar to those that have been reported elsewhere (5, 11, 20). It has been postulated that tetracycline resistance is mediated by acquisition of the *tetM* tetracycline resistance determinant, which perhaps is transferred from streptococci also resident in the female genital tract (11).

Finally the prevalence values for colonization of the genital tract by *M. hominis* and *U. urealyticum* in this randomly selected population of women are among the highest ever reported. Both low socioeconomic status and experience with multiple sex partners are associated with high colonization rates in other settings (17). Extremely high prevalences of *Trichomonas vaginalis* and *Chlamydia trachomatis* have been reported in this same population, suggesting high levels of partner change and/or low levels of treatment (18). Further analysis of our data will determine which associations apply in this community.

Because of the ubiquitous nature of the genital mycoplasmas, the significance of these high prevalence values is uncertain, as is the pathogenic role of the genital mycoplasmas themselves. The hypothesis that colonization by the genital mycoplasmas (and other organisms) may be predisposing risk factors to the development of postpartum infection is the subject of ongoing research at the Papua New Guinea Institute of Medical Research. It is also possible that the extremely high vaginal colonization rates found in this population will increase the opportunity for these organisms to be transmitted to neonates either in utero or during passage through the birth canal. A study to determine the importance of *U. urealyticum* as an etiological agent of neonatal infection in this population is planned.

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REFERENCES

1. **Caliando, J. J., and W. M. McCormack.** 1983. Recovery of mycoplasmas from blood and special tissues. Methods Mycoplasmol. **2:**27–35.
2. **Cassels, G. H., K. B. Waites, D. T. Crouse, P. T. Rudd, K. T. Canupp, S. Stagno, and G. R. Cutter.** 1988. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birth-weight infants. Lancet **ii:**240–245.
3. **Clyde, W. A., G. E. Kenny, and J. Schacter.** 1984. Cumitech 19, Laboratory diagnosis of chlamydial and mycoplasmal infections. Coordinating ed., W. L. Drew. American Society for Microbiology, Washington, D.C.
4. **Cummings, M. C.** 1992. Mycoplasmas, p. 8.24.1.1–8.24.3.8. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 2. American Society for Microbiology, Washington, D.C.
5. **Cummings, M. C., and W. M. McCormack.** 1990. Increase in resistance of *Mycoplasma hominis* to tetracyclines. Antimicrob. Agents Chemother. **34:** 2297–2299.
6. **Fiacco, V., M. Miller, E. Carney, and W. J. Martin.** 1984. Comparison of media for isolation of *Ureaplasma urealyticum* and genital *Mycoplasma* species. J. Clin. Microbiol. **20:**862–865.
7. **Hill, G. B.** 1993. The microbiology of bacterial vaginosis. Am. J. Obstet. Gynecol. **169:**450–454.
8. **Holst, E., A. R. Goffeng, and B. Andersch.** 1994. Bacterial vaginosis and vaginal microorganisms in idiopathic premature labor and association with pregnancy outcome. J. Clin. Microbiol. **32:**176–186.
9. **Krohn, M. A., S. L. Hillier, R. P. Nugent, M. F. Cotch, J. C. Carey, R. S. Gibbs, and D. A. Eschenbach.** 1995. The genital flora of women with intra-amniotic infection. Vaginal infection and prematurity study group. J. Infect. Dis. **171:**1475–1480.
10. **Leland, D. S., M. A. Lapworth, R. B. Jones, and M. L. V. French.** 1982. Comparative evaluation of media for the isolation of *Ureaplasma urealyticum* and genital *Mycoplasma* species. J. Clin. Microbiol. **16:**709–714.
11. **McCormack, W. M.** 1993. Susceptibility of mycoplasmas to antimicrobial agents: clinical implications. Clin. Infect. Dis. **17:**S200–S201.
12. **McDonald, H. M., J. A. Oílaughlin, P. T. Jolley, R. Vigneswaran, and P. J. McDonald.** 1994. Changes in vaginal flora during pregnancy and association with preterm birth. J. Infect. Dis. **170:**724–728.
13. **Phillips, L. E., K. H. Goodrich, R. M. Turner, and S. Faro.** 1986. Isolation of *Mycoplasma* species and *Ureaplasma urealyticum* from obstetrical and gynecological patients by using commercially available medium formulations. J. Clin. Microbiol. **24:**377–379.
14. **Plummer, D. C., S. M. Garland, and G. L. Gilbert.** 1987. Bacteremia and pelvic infection in women due to *Ureaplasma urealyticum* and *Mycoplasma hominis*. Med. J. Aust. **146:**135–137.
15. **Rottem, S., E. A. Pfendt, and L. Hayflick.** 1971. Sterol requirements of T-strain mycoplasmas. J. Bacteriol. **105:**323–330.
16. **Sheppard, M. C.** 1983. Culture medium for ureaplasmas. Methods Mycoplasmol. **1:**137–146.
17. **Taylor-Robinson, D., and M. D. McCormack.** 1980. The genital mycoplasmas. N. Engl. J. Med. **302:**1003–1010.
18. **Tiwara, S., M. Passey, A. Clegg, C. Mgone, S. Lupiwa, N. Suve, and T. Lupiwa.** High prevalence of trichomonal vaginitis and chlamydial cervicitis among a rural population in the highlands. Papua New Guinea Med. J., in press.
19. **Waites, K. B., P. T. Rudd, D. T. Crouse, K. C. Canupp, K. G. Nelson, C. Ramsey, and G. H. Cassels.** 1988. Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* infections of the central nervous system in preterm infants. Lancet **ii:**17–21.
20. **Waites, K. B., D. T. Crouse, and G. H. Cassels.** 1992. Antibiotic susceptibilities and therapeutic options for *Ureaplasma urealyticum* infections in neonates. Pediatr. Infect. Dis. J. **11:**23–29.