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Isolation of *Mycoplasma pneumoniae* from the Human Urogenital Tract

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***Mycoplasma pneumoniae* is a common etiologic agent of lower respiratory tract infections in humans. However, it has been reported previously that the organism has occasionally been isolated from sites other than the oropharynx and respiratory tract. We report the isolation of 24 strains of *M. pneumoniae* from urogenital specimens obtained from 22 female patients. Most isolates were of cervical origin from patients attending several local gynecological clinics over a 2-year period. Strains were also isolated from the urethra of one of three healthy male sexual partners of female patients positive for the organism. Single serum specimens obtained from three female patients and three different male sexual partners showed antibody levels suggestive of either recent respiratory infection or genital tract colonization with *M. pneumoniae*. Although there is no apparent definitive explanation for the localized outbreak of the organism at these unusual sites, the possible transfer through sexual and/or orogenital contact remains the most likely mode of transmission. The occurrence of an organism with obvious pathogenicity for human epithelial tissue in the urogenital tract suggests such transfer could play a role in genital tract infection.**

Mycoplasma pneumoniae is one of the commonest etiologic agents of lower respiratory tract infections in humans, accounting for somewhere between 15 and 20% of all cases of pneumonia (3, 7). The organism can clearly be associated with a wide range of mild to serious extrapulmonary complications following respiratory disease in adults, children, and infants. However, the most frequent clinical manifestations observed with the organism involve tracheobronchitis and pharyngitis, with almost 20% of infections being asymptomatic (3). In an earlier study in Canada (6), we isolated *M. pneumoniae* from 10.8% of 1,660 throat and laryngeal swab specimens or sputum aspirates from patients with suspected respiratory infections.

Studies to establish a true carrier state with *M. pneumoniae* have given conflicting data. Part of the difficulty may involve the persistence of the organism in the oropharynx for as long as 4 months following respiratory tract infection (7). Carriage rates in healthy populations in nonepidemic years are generally thought to be very low, although data suggesting otherwise have been reported (7, 9). However, carriage rates as high as 10% have been recorded during epidemic *M. pneumoniae* infections in military populations (21).

Strains of *M. pneumoniae* have been isolated from a variety of extrapulmonary tissue sites, almost always following an acute respiratory infection with the organism (16). The organism has been isolated most frequently from arthritic joint infections, usually in individuals with immune deficiencies, or from cerebrospinal fluid (16). From February through December 1992 and from January through March 1994, we isolated a total of 24 strains of *M. pneumoniae* from the urogenital tracts of female patients attending several local gynecological clinics in Ottawa. During this same time, *M. pneumoniae* was also isolated from an urethral specimen of a male sexual partner of one of the female patients who harbored the organism.

Since few other reports of the isolation of *M. pneumoniae* from these anatomical locations, other than cultivation from a tubo-ovarian abscess and three vaginal isolates between 1975 and 1977 (4, 15), have been made, we considered both the numbers of isolates and the patients involved to be a significant expansion of the known host relationships of the organism. In addition, the established shared serologic relationship between *M. pneumoniae* and *Mycobacterium genitalium* (11, 14) and the occurrence of the latter organism in both the human urogenital tract and the human respiratory tract (1, 19) suggested the possibility that some of the isolates might be *M. genitalium* or that the specimens might contain mixtures of both organisms (17).

MATERIALS AND METHODS

Patients. The 22 specimens from female patients from whom *M. pneumoniae* was isolated represented approximately 2.6% of the total number (840) of urogenital tract specimens from females examined for mycoplasmas in the Ottawa Public Health Laboratory during the period from January 1992 to March 1994. All patients were visiting one of several local gynecological clinics. The ages of the patients varied from 17 to 51 years; there was no apparent history of respiratory disease during the 6 months prior to specimen collection, and most patients belonged to a low-risk, average socioeconomic group. Urethral specimens from three healthy male sexual partners of patients from whom *M. pneumoniae* was isolated were also examined during the study.

Specimen collection. Endocervical swabs were collected, inoculated into a transport medium (5), and then transferred to the laboratory within 24 h. In several instances, urine or pelvic fluid from female patients was also examined. Urethral swabs taken from male patients were also transferred to transport medium for cultivation attempts. Blood for serum was obtained from 10 of the female patients and 3 of the male sexual partners.

Culture procedures. Transport specimens (0.2 ml) were inoculated into the following media: U9 broth and modified A7B agar for ureaplasma isolation and identification (12), arginine broth (20), SP-4 broth (18), and mycoplasma broth and agar (5) for the isolation of conventional mycoplasmas. Agar plates were prepared with 0.8% Noble agar. All broth cultures were incubated at 37°C and were examined every third day for turbidity and a pH change over an 8-week

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period. Agar plates were incubated at 37°C in a 10% carbon dioxide environment and were examined weekly over an 8-week period. All specimens were also assessed for routine genital tract organisms, including *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus* spp., *Trichomonas* spp., *Candida albicans*, clue cells, and *Chlamydia trachomatis* by standard procedures (10).

Serological tests. Large-colony mycoplasmas cultivated from urogenital specimens, urine, or pelvic fluid were identified by either growth inhibition (2) or agar plate immunofluorescence (8) tests by using type-specific immune serum or fluorescein-conjugated antiserum. For the detection of *M. genitalium* in cultures of *M. pneumoniae* isolated from urogenital sites, agar plates containing about 200 colonies of *M. pneumoniae* were stained with a 1:64 dilution of a specific *M. genitalium* fluorescein-conjugated antiserum. The stained plates were then examined for the presence of fluorescent colonies by using incident illumination with UV light (8).

The single serum specimens obtained from each female patient positive for *M. pneumoniae* and the three male cohorts were tested for antibodies to both *M. pneumoniae* and *M. genitalium* in conventional metabolism inhibition (MI) tests (13).

RESULTS AND DISCUSSION

The patients included in the present study were not part of any designated prospective study on the occurrence of mycoplasmas in either the female urogenital tract or those of their sexual partners. Inclusion in the study was dependent on the unusual finding of at least a single isolate of *M. pneumoniae*, primarily from cervical swab specimens, submitted to the Ottawa Public Health Laboratory by various gynecological clinics in the local area.

Table 1 provides some details of the laboratory findings, including submission date, source of the specimens collected at the clinics, and the results of the culture tests for *M. pneumoniae*, *M. hominis*, and *Ureaplasma urealyticum*. Twenty-four isolates of *M. pneumoniae* were obtained from the 22 female patients. More than one isolate was obtained from the cervix of patient 1, and this occurred over a 7- to 8-week period. The male sexual partner (patient 2) of this patient was the only one of the male sexual partners examined from whom an isolate of *M. pneumoniae* was obtained from the genital tract. A majority of the female patients (14 of 20) and all 3 male cohorts had harbored *U. urealyticum* in the urogenital tract, and only 4 females harbored *M. hominis. C. trachomatis* was not detected in any specimen, and this finding was supported by the low incidence of this organism in other urogenital specimens examined over this period of time in the Ottawa Public Health Laboratory.

With the isolation of *M. pneumoniae* from urogenital specimens of patients in early 1992, attempts were made to secure serum from such individuals to determine whether a concurrent or recent respiratory infection could be detected. Although antibody measurement with a single serum specimen usually cannot establish a definitive diagnosis of *M. pneumoniae* infection, the fact that two of the male cohorts and three of the females in the group had MI titers to *M. pneumoniae* of 1:128 to 1:256 is highly suggestive of a recent infection. Since both male patients were then apparently healthy and asymptomatic, this elevated antibody response might suggest either a recent respiratory infection or a response to current urethral colonization. Likewise, the absence of overt respiratory disease in any of the three females may also suggest that the antibody response to *M. pneumoniae* could be reflective of genital tract colonization. Single MI antibody titers below 1:64 are difficult to assess and might represent early acute infection, past infection, or exposure to *M. pneumoniae.*

Several important questions were raised in the early stages of the laboratory finding of *M. pneumoniae* in urogenital specimens. The concern was raised as to whether the isolates were actually strains of *M. genitalium* that had been mistaken in identification tests, because of the known serological cross-reactions that exist between *M. pneumoniae* and *M. genitalium* (11, 14, 19). The other question related to the possibility that the *M. pneumoniae* strains might be a mixture of that organism with *M. genitalium*, an occurrence established in both the human respiratory tract and synovial fluid (1, 17). Extensive testing of 18 of the 26 urogenital isolates by an immunofluorescence technique on agar colonies with conjugate dilutions that are specific for either *Mycoplasma* species (17) confirmed that all isolates were apparently pure strains of *M. pneumoniae.*

The observations recorded here provide no obvious expla-

TABLE 1. *Mycoplasma* isolation from urogenital tracts of patients

Patient no./ age (yr)[a]	Specimen		Date (mo.day.yr)	*Mycoplasma* isolation[b]			Antibody response[c]	
	No.	Type[d]		Mp	Uu	Mh	Mp	Mg
1/27	U2	Cx	2.3.92	+	+	−		
	734/U13	Cx	3.20.92	+	+	−		
		B	4.29.92	NA[e]	NA	NA	64	64
2P/37	754/U27	U	3.30.92	+	+	−		
		B	6.29.92	NA	NA	NA	256	8
3/?	726/U29	Cx	3.5.92	+	−	−		
4/23	736/U15	Cx	3.21.92	+	+	−		
		B	7.10.92	NA	NA	NA	64	<8
5/26	737/U16	Cx	3.23.92	+	+	−		
		B	7.5.92	NA	NA	NA	32	<8
6/43	738/U17	Cx	3.23.92	+	−	−		
		B	6.22.92	NA	NA	NA	128	<8
7/31	739/U18	Cx	3.23.92	+	−	−		
8/51	740/U19	Cx	3.23.92	+	−	−		
		B	6.21.92	NA	NA	NA	128	<8
9/20	741/U20	Cx	3.23.92	+	+	−		
		B	7.7.92	NA	NA	NA	64	<8
		B	6.30.92	NA	NA	NA	128	<8
10/36	746/U22	Cx	3.27.92	+	+	−		
	794/U54	Cx biopsy	5.5.92	−	−	−		
	795/U55	Urn	5.5.92	−	−	−		
11P/34	798/U56	U	5.7.92	−	+	−		
		B	7.5.92	NA	NA	NA	256	<8
12/21	747/U23	Cx	3.27.92	+	+	−		
13/22	748/U24	Cx	3.27.92	+	+	−		
		B	9.4.92	NA	NA	NA	64	<8
	1484	Cx	12.14.92	−	−	−		
14/23	758/U30	Cx	3.31.92	+	−	+		
	827	PF	5.22.92	−	+	−		
		B	6.6.92	NA	NA	NA	16	<8
15/17	759/U31	Cx	3.31.92	+	+	−		
	858	Urn	5.28.92	−	+	−		
		B	6.19.92	NA	NA	NA	16	<8
16P/22	857	Urn	5.29.92	−	+	−		
		B	6.19.92	NA	NA	NA	64	8
17/42	760/U32	Cx	4.3.92	+	−	−		
18/31	764/U34	Cx	4.6.92	+	+	−		
19/28	765/U35	Cx	4.6.92	+	−	−		
20/37	767/U37	Cx	4.8.92	+	−	−		
21/49	1299	Cx	10.27.92	+	−	−		
22/29	1313	Cx	10.30.92	+	+	−		
23/49	16/94	Cx	1.25.94	+	−	−		
	17/94	Urn	1.25.94	−	+	−		
24/44	103/94	Cx	2.17.94	+	−	−		
25/19	219/94	Cx	3.23.94	+	−	+		

[a] P designates the male sexual partner of the preceding patient.
[b] Mp, *M. pneumoniae*; Uu, *U. urealyticum*; Mh, *M. hominis.*
[c] Antibody response measured in MI test with antigens to *M. pneumoniae* (Mp) and *M. genitalium* (Mg). The values given here are reciprocals of the MI titer, starting at a serum dilution of 1:8.
[d] Cx, endocervical swab; Urn, urine; U, male urethral swab; PF, pelvic fluid; B, blood for serum.
[e] NA, not applicable.

nation for the sudden occurrence of *M. pneumoniae* in the female urogenital tract. The number of patients involved and the number of laboratory isolations, including repeated cultivation of the organism from the same patient at different times and from multiple specimens obtained at the same time, offer strong evidence of genital tract colonization with the organism. The isolation of *M. pneumoniae* from the urethra of one male cohort suggests that the most likely mode of transmission and acquisition in the patients involved was through sexual and/or orogenital contact. Indeed, almost 50% of the individuals involved admitted to oral sexual activity. Whether normal oral carriage of the organism in asymptomatic individuals during epidemic periods of *M. pneumoniae* infection represents a focal point for transmission through later oral sexual contact can only remain speculative. However, public health laboratories should now be aware that this organism can be found at sites other than the respiratory tract. Lastly, some of the clinical responses recorded in the female patients here and the occurrence in the urogenital tract of an organism with obvious pathogenicity for human epithelial tissues warrants further exploration of the possible role of *M. pneumoniae* in genital tract infections.

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REFERENCES

1. **Baseman, J. B., S. F. Dallo, J. G. Tully, and D. L. Rose.** 1988. Isolation and characterization of *Mycoplasma genitalium* strains from the human respiratory tract. J. Clin. Microbiol. **26:**2266–2269.
2. **Clyde, W. A., Jr.** 1983. Growth inhibition tests. Methods Mycoplasmol. **1:**405–410.
3. **Clyde, W. A., Jr.** 1993. Clinical overview of typical *Mycoplasma pneumoniae* infections. Clin. Infect. Dis. **17**(Suppl. 1)**:**S32–S36.
4. **Csütörtöki, V., and L. Stipkovits.** 1977. Nachweis von *Mycoplasma pneumoniae* Infektionen in weiblichen Geschlechtsorganen. Zentralbl. Gynäkol. **99:**877–879.
5. **Dular, R., M. Lambert, B. W. Bruce, P. H. Phipps, E. Rossier, and S. Kasatiya.** 1987. *Mycoplasma pneumoniae* infections in a rural setting in Canada. Can. Med. Assoc. J. **136:**1271–1273.
6. **Fleming, C., D. Hodge, S. Toma, R. Dular, and S. Kasatiya.** 1987. Isolation of *Mycoplasma pneumoniae* from respiratory tract specimens in Ontario. Can. Med. Assoc. J. **138:**48–50.
7. **Foy, H. M.** 1993. Infections caused by *Mycoplasma pneumoniae* and possible carrier state in different populations of patients. Clin. Infect. Dis. **17**(Suppl. 1)**:**S37–S46.
8. **Gardella, R. S., R. A. Del Giudice, and J. G. Tully.** 1983. Immunofluorescence. Methods Mycoplasmol. **1:**431–439.
9. **Gnarpe, J., A. Lundbäck, B. Sundelöf, and H. Gnarpe.** 1992. Prevalence of *Mycoplasma pneumoniae* in subjectively healthy individuals. Scand. J. Infect. Dis. **24:**161–164.
10. **Lennette, E. M., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
11. **Lind, K., B. O. Lindhardt, H. J. Schütten, J. Blom, and C. Christiansen.** 1984. Serological cross-reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. J. Clin. Microbiol. **20:**1036–1043.
12. **Shepard, M. C.** 1983. Culture media for ureaplasmas. Methods Mycoplasmol. **1:**137–146.
13. **Taylor-Robinson, D.** 1983. Metabolism inhibition tests. Methods Mycoplasmol. **1:**411–417.
14. **Taylor-Robinson, D., P. M. Furr, and J. G. Tully.** 1983. Serological cross-reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. Lancet **i:**527.
15. **Thomas, M., M. Jones, S. Ray, and B. Andrews.** 1975. *Mycoplasma pneumoniae* in a tubo-ovarian abscess. Lancet **2:**774–775.
16. **Tully, J. G.** 1993. Current status of the mollicute flora of humans. Clin. Infect. Dis. **17**(Suppl. 1)**:**S2–S9.
17. **Tully, J. G., D. L. Rose, J. B. Baseman, S. F. Dallo, A. L. Lazzell, and C. P. Davis.** 1995. *Mycoplasma pneumoniae* and *Mycoplasma genitalium* mixture in synovial fluid isolate. J. Clin. Microbiol. **33:**1851–1855.
18. **Tully, J. G., D. L. Rose, R. F. Whitcomb, and R. P. Wenzel.** 1979. Enhanced isolation of *Mycoplasma pneumoniae* from throat washings with a newly modified culture medium. J. Infect. Dis. **139:**478–482.
19. **Tully, J. G., D. Taylor-Robinson, D. L. Rose, R. M. Cole, and J. M. Bové.** 1983. *Mycoplasma genitalium*, a new species from the human urogenital tract. Int. J. Syst. Bacteriol. **33:**387–396.
20. **Velleca, W. M., B. R. Bird, and F. T. Forrester.** 1980. Laboratory diagnosis of *Mycoplasma* infections. Centers for Disease Control, Atlanta.
21. **Wenzel, R. P., R. B. Craven, J. A. Davies, J. O. Hendley, B. H. Hamory, and J. M. Gwaltney, Jr.** 1977. Protective efficacy of an inactivated *Mycoplasma pneumoniae* vaccine. J. Infect. Dis. **136**(Suppl.)**:**S204–S207.