

## Mycoplasmas in Human Pyelonephritis: Demonstration of Antibodies in Serum and Urine

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This study was carried out to elucidate by serological examination the etiological significance of mycoplasmas isolated from the upper urinary tract of patients with pyelonephritis. The occurrence of antibodies in patients with acute pyelonephritis, chronic pyelonephritis with or without exacerbation, or noninfectious urinary tract disease was compared by the indirect hemagglutination method. Antibody response was demonstrated significantly more often in patients yielding growth of *Mycoplasma hominis* from the upper urinary tract than in patients not yielding growth. Antibodies against *M. hominis* were demonstrated in ureteric and bladder urine from three patients with acute pyelonephritis and from one patient with exacerbation of chronic pyelonephritis. *M. hominis* was isolated from the upper urinary tract of all four patients. Urine antibodies could not be demonstrated in any other cases. Thus, it seems highly possible that *M. hominis* may play a role in pyelonephritis of humans. The investigations did not disclose a similar role for *Ureaplasma urealyticum*.

The reports on isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from the bladder (2, 7) and the upper urinary tract (13-15) of humans indicate a possible pathogenic role of mycoplasmas in human pyelonephritis.

The antibody response in experimental mycoplasmal pyelonephritis was studied in rats (11). All animals with pyelonephritic lesions developed antibodies in urine, whereas only rats with lesions also in the cortex and medulla had antibodies in serum.

The antibody response to bacterial inflammation of the human urinary tract has been studied intensively. Cystitis with significant bacteriuria did not result in any antibody response in serum, whereas this did happen when the inflammation involved the kidney (3, 8).

Thus, serological examinations of serum and especially of urine seem suitable for evaluating the role of mycoplasmas in pyelonephritis. The present study was undertaken to compare the occurrence of antibodies to *M. hominis* and *U. urealyticum* in serum and urine from patients with acute and chronic pyelonephritis with the antibody response in patients with noninfectious urinary tract diseases.

### MATERIALS AND METHODS

**Patients.** (i) **Group I: patients with acute pyelonephritis.** Group I included 80 patients, 13 men and 67 women, with signs of acute pyelonephritis, which consisted of acute lumbar pain, fever, and pyuria fol-

lowed by complete recovery (14). None of the patients had received antibiotics with known mycoplasma-inhibitory effects. *M. hominis* had been isolated from the upper urinary tract of seven of the patients, and *U. urealyticum* had been isolated from five (14).

(ii) **Group II: patients with chronic pyelonephritis.** Group II consisted of 40 patients, 10 men and 30 women. They all had at least three of the following signs of chronic pyelonephritis: a history of urinary tract infections, pyuria, significant bacteriuria, characteristic lesions demonstrated by histological examination or radiography, and impaired function of the kidney. The patients had not been treated within the last few months with compounds known to possess inhibitory effects on mycoplasmas.

A total of 18 patients showed signs of acute exacerbation of the chronic pyelonephritis, which consisted of lumbar pain, fever, pyuria, and progression of the kidney function impairment. *M. hominis* had been isolated from the upper urinary tract of three of these patients. A total of 22 had no signs of exacerbation, including 2 who yielded growth of *U. urealyticum* from the upper urinary tract (13).

(iii) **Group III: patients with noninfectious urinary tract diseases.** Group III was the control group and consisted of 60 patients with an age and sex distribution comparable to that of the groups with pyelonephritis. The patients suffered from different diseases of the urinary tract, but signs of infection were never observed. Two patients had received antibiotics (i.e., penicillin and nitrofurantoin). Mycoplasmas were not cultivated from the upper urinary tract (14).

**Sampling.** Two samples of sera and one of bladder urine were collected from each patient.

The first serum sample was taken within a few days of the onset of symptoms, and the second was taken 3 to 8 weeks later. Specimens of bladder urine were collected at the same time as the first serum sample, and in some cases ureteric urine was also collected on this occasion.

All sera were stored at  $-20^{\circ}\text{C}$ , and specimens of urine were stored at  $-70^{\circ}\text{C}$ .

**Serological examination.** All sera and samples of urine were tested for antibodies to mycoplasmas by the indirect hemagglutination test (IHA).

**(i) Demonstration of antibodies against *M. hominis*.** The IHA test was performed with fresh sheep erythrocytes as described by Krogsgaard-Jensen (5). All 10 strains of *M. hominis* isolated from the upper urinary tract (see above) were used as antigens.

Because of the known heterogeneity within the species of *M. hominis* (4, 9, 12), the strains were primarily separated into three groups according to a titration against hyperimmune serum to the type strain of *M. hominis* by the indirect immunofluorescence test (10; Table 1). Selection of strains to be used as separate antigen or pooled antigen was based on this titration.

Separately, one strain from each group was used (strains P62, P2, and P70). The strains were cultivated, harvested by centrifugation, and suspended in phosphate-buffered saline to a volume of 50 ml before ultrasonic treatment (5). The antigen was titrated in the IHA test in twofold dilutions against hyperimmune serum to the type strain of *M. hominis* to find the optimum antigen concentration.

Pool A was a mixture of all 10 strains. To be used in this pool, the antigen from each culture was suspended to a volume of only 5 ml after harvesting. Before mixing, a sample from each of these suspensions was diluted 1:10 and titrated as above to find the optimum concentration of the antigen in the final pool.

Pool B was composed of three strains, one from each group. The strains were cultivated and harvested as above, but suspended to a volume of 17 ml before a sample from each suspension was diluted 1:3 and titrated to find the optimum antigen concentration.

After equal amounts of single antigens in optimum concentrations were mixed, the final pools were further titrated against hyperimmune serum in the IHA test. The highest dilution of the pooled antigens giving

optimum titers was used as the pooled antigen concentration in the test against specimens from patients. This final antigen titration was found to be reasonable to perform because of the quantitative and qualitative antigenic differences between the strains.

Finally, specimens from the patients were also titrated against antigen of the type strain of *M. hominis* (PG21).

**(ii) Demonstration of antibodies against *U. urealyticum*.** The IHA test was performed with Formalin-treated sheep erythrocytes by the method of Black and Krogsgaard-Jensen (1). Two strains of *U. urealyticum*, serologically identified as types I and III, were used as antigen. They were both isolated from the upper urinary tract of patients with acute pyelonephritis (14).

**(iii) Demonstration of antibodies against other human mycoplasmas.** All specimens with titers of 20 or more to *M. hominis* were examined in the IHA test, using fresh erythrocytes, for antibodies to other human-infecting species of the genus *Mycoplasma*. The type strains of *M. buccale*, *M. faucium*, *M. fermentans*, *M. orale*, *M. pneumoniae*, *M. primatum*, and *M. salivarium* were used as antigens.

## RESULTS

Specimens were recorded as positive for the occurrence of antibodies when the titer was  $\geq 20$  in one or two samples. A fourfold titer change was regarded as significant.

**Antibodies to *M. hominis*. (i) Results obtained with pooled and single antigen.** A total of 241 sera were positive to antigen pools A and B. Apart from nonsignificant differences in titers, the results were identical. When strains P62, P7, and P70 were used, antibodies were also demonstrated in all 241 sera, but when these antigens were used separately, only 219, 211, and 214 sera were positive, respectively (Table 2). With antigen of the type strain (PG21), 178 sera were positive.

In four samples of bladder urine, antibodies to *M. hominis* were demonstrated with both pool A and pool B and by the use of strains P62, P7, and P70 separately. With strains P62, P7, P70, and the type strain (PG21), antibodies were demonstrated in two, three, two, and one specimens of urine, respectively.

The following results given in the text and tables are those obtained with antigen pool B.

**(ii) Patients with acute pyelonephritis.** In serum, antibodies to *M. hominis* were demonstrated in 49 of 80 patients. A significant change in the titer occurred in 10 patients, 5 of whom yielded growth of *M. hominis* from the upper urinary tract (Table 3). The remaining two of the seven patients harboring *M. hominis* in the upper urinary tract were serologically positive, but a significant change in antibodies could not be demonstrated.

In bladder urine, antibodies were demon-

TABLE 1. Grouping of 10 strains of *M. hominis* by the indirect immunofluorescence test

Group	Strain	Titer to type strain hyper-immune serum
I	P7	640
	P62	320
	P78	640
	P113	640
II	P2	1,280
	P61	1,280
	P85	1,280
III	P53	2,560
	P70	2,560
	P71	2,560

TABLE 2. Characteristic examples of IHA titers when single and pooled *M. hominis* antigens were used

Specimen type	Titer when the following was used as antigen:					
	Type strain PG21	Strain P62	Strain P2	Strain P70	Pool A <sup>a</sup>	Pool B <sup>b</sup>
Serum	80	80	320	160	160	160
Serum	<2	80	<2	<2	80	160
Serum	160	320	<2	<2	80	160
Serum	40	<2	40	<2	40	40
Serum	<2	<2	160	<2	80	80
Serum	40	<2	<2	40	40	40
Serum	<2	<2	<2	160	320	160
Urine	<2	<2	<2	160	80	80

<sup>a</sup> Pool A was composed of 10 strains.

<sup>b</sup> Pool B was composed of 3 strains selected by the indirect immunofluorescence test as representative of the 10 strains.

TABLE 3. IHA antibodies to *M. hominis* in sera from 80 patients with acute pyelonephritis

Cultivation	No.	No. with antibodies showing:		No. with no antibodies
		Significant change	Nonsignificant change <sup>a</sup>	
<i>M. hominis</i> isolated				
Upper urinary tract	7	5 (71) <sup>b</sup>	2 (29)	0 (0)
Bladder but not upper urinary tract	4	2 (50)	2 (50)	0 (0)
Urethra only	6	0 (0)	3 (50)	3 (50)
<i>M. hominis</i> not isolated	63	3 (5)	32 (50)	28 (45)
Total	80	10 (12)	39 (49)	31 (39)

<sup>a</sup> Detected in one or both of the serum samples as a titer of  $\geq 20$ .

<sup>b</sup> Numbers within parentheses indicate percentages.

strated in three patients, all with a significant change in serum antibodies. Antibodies were also present in ureteric urine of these patients, but only from one side, and *M. hominis* was in all cases isolated from the ureter of the same side (Table 4).

(iii) **Patients with chronic pyelonephritis.** In serum, antibodies to *M. hominis* were demonstrated in 27 of 40 patients, and a significant change was demonstrated in 7 (Table 5). Of these seven, three had acute exacerbation and harbored *M. hominis* in the upper urinary tract; four had no exacerbation, and cultivation for *M. hominis* from the upper urinary tract was negative.

In bladder urine, antibodies were demonstrated in one case of acute exacerbation, and a significant serum antibody response occurred. Antibodies were also present in ureteric urine from the same side of this patient from which *M. hominis* was isolated (Table 4).

(iv) **Patients with noninfectious urinary tract diseases.** Serum antibodies were present in 21 of 60 patients. A significant change developed in two. *M. hominis* was not cultivated from the upper urinary tract.

Antibodies were not demonstrated in samples of urine.

**Antibodies to *U. urealyticum*.** (i) **Patients with acute pyelonephritis.** In serum, antibodies to *U. urealyticum* were demonstrated in 10 of 80 patients. A significant change occurred in two; one of them harbored *U. urealyticum* of the same serotype in the upper urinary tract. One patient, who harbored *U. urealyticum* in the upper urinary tract, was serologically positive to the isolated serotype, but the remaining three of the five patients yielding growth of *U. urealyticum* from the upper urinary tract were serologically negative.

In urine, antibodies to *U. urealyticum* were not demonstrated in this or the following groups.

(ii) **Patients with chronic pyelonephritis.** In serum, antibodies were present in 6 of 40 patients. A significant change developed in two. The three patients with *U. urealyticum* in the upper urinary tract had no antibodies.

(iii) **Patients with noninfectious urinary tract diseases.** In serum, antibodies to *U. urealyticum* were demonstrated in 3 of 60 patients. A significant change in antibodies did not develop in any case.

TABLE 4. Results of cultivation and serological examination with regard to *M. hominis* of four patients with acute pyelonephritis or exacerbation of chronic pyelonephritis

Patient no. and condition	Bladder urine		Ureter urine				Serum	
	Titer	Cultivation	Right side		Left side		Titer	Day after onset of symptoms
			Titer	Cultivation	Titer	Cultivation		
1. Acute pyelonephritis on right side	64	+	64	+	<2	-	512 128	1 57
2. Acute pyelonephritis on right side	32	+	32	+	NT <sup>a</sup>	NT	4 64	1 16
4. Acute pyelonephritis on right side	64	+	128	+	<2	-	64 1,024	0 7
13. Chronic pyelonephritis on left side	16	+	<2	-	32	+	32 128	0 4

<sup>a</sup> NT, Not tested.

TABLE 5. IHA antibodies to *M. hominis* in sera from 40 patients with chronic pyelonephritis<sup>a</sup>

Cultivation	No.	No. with antibodies showing:		No. with no antibodies
		Significant change	Nonsignificant change <sup>b</sup>	
<i>M. hominis</i> isolated				
Upper urinary tract	3	3 (100) <sup>c</sup>	0 (0)	0 (0)
Bladder and urethra only	4	2 (50)	2 (50)	0 (0)
<i>M. hominis</i> not isolated	33	2 (6)	18 (55)	13 (39)
Total	40	7 (17)	20 (50)	13 (33)

<sup>a</sup> In 19 of these patients, attempts to cultivate myoplasmas from the upper urinary tract and the urethra were not made, because bladder urine did not harbor myoplasmas.

<sup>b</sup> Detected in one or both of the serum samples as a titer of  $\geq 20$ .

<sup>c</sup> Numbers within parentheses indicate percentages.

**Antibodies to other human mycoplasmas.** The 241 sera positive to *M. hominis* were tested for antibodies to other human mycoplasmas, with the following numbers of sera having antibodies to the different species of *Mycoplasma*: *M. buccale*, 5; *M. faucium*, 4; *M. fermentans*, 3; *M. orale*, 8; *M. pneumoniae*, 12; *M. primatum*, 11; and *M. salivarium*, 13. Significant changes in antibodies to the strains were never demonstrated in patients with significant antibody response to *M. hominis*.

## DISCUSSION

The present serological study supports the possibility that *M. hominis* is of etiological significance in some of the cases of acute pyelonephritis and exacerbation of chronic pyelonephritis.

In acute pyelonephritis, *M. hominis* had been isolated from the upper urinary tract of seven patients, in four cases in pure culture (14). Five of these seven patients, including the four with

*M. hominis* in pure culture, developed a significant antibody response. Three of the patients, all harboring pure culture of *M. hominis* in the upper urinary tract and all with significant serum antibody response, had antibodies against *M. hominis* in urine.

In acute exacerbation of chronic pyelonephritis, *M. hominis* had been isolated from the upper urinary tract of three patients, in all cases in pure culture (13). These three patients all had a significant change in antibodies, and one of them had antibodies to *M. hominis* in urine.

In patients with chronic pyelonephritis without exacerbation and in those with noninfectious urinary tract diseases, *M. hominis* was not cultivated from the upper urinary tract, but a significant antibody response was demonstrated in four and two patients, respectively.

Thus, a significant antibody response was demonstrated in 8 of 10 patients harboring *M. hominis* in the upper urinary tract and in 11 of 170 patients not yielding growth of *M. hominis* from the upper urinary tract. This difference in

the development of antibody response is significant ( $2P < 10^{-6}$  by Fisher's exact test) and seems to indicate that the occurrence of *M. hominis* in the renal pelvis is, at least in some cases, followed by a host response.

A strong indication that *M. hominis* is pathogenic in the upper urinary tract was provided by the detection of specific antibodies in bladder urine from patients harboring *M. hominis* in the upper urinary tract. The antibodies were also present in ureteric urine, but only from the side yielding *M. hominis*. In some cases, the titers were higher than in simultaneously taken serum samples. This indicates that the antibodies originated from the affected kidney.

In experimental pyelonephritis due to *M. arthritidis*, antibodies in urine have also been demonstrated (11), thus confirming studies of bacterial pyelonephritis (6).

From the present study, it is not clear to what extent *M. hominis* is involved in the development of chronic pyelonephritis because the patients with chronic pyelonephritis without exacerbation did not differ from the control group with respect to antibodies to *M. hominis*. However, statistical evidence that *M. hominis* is a cause of chronic pyelonephritis will demand a series of experiments greater than the present one, because many healthy individuals have antibodies.

Concerning etiological diagnosis of acute pyelonephritis, it must be kept in mind that the demonstration of a significant antibody response does not implicate the occurrence of *M. hominis* in the upper urinary tract. In 11 patients, a significant antibody response was demonstrated, but cultivation for *M. hominis* from the upper urinary tract was negative. On the other hand, two patients had *M. hominis* without responding with an antibody change. Demonstration of antibodies in urine seems to indicate an *M. hominis* infection in the upper urinary tract; urine antibodies were in every case related to isolation of *M. hominis*. However, *M. hominis* may be present in the upper urinary tract without the occurrence of urine antibodies; in fact, this happened in six patients in this study.

The use of pooled *M. hominis* antigen in the IHA test for the detection of antibodies in sera of patients is recommended in view of the results of this study. When single-strain antigen only was used, 85 to 90% of all positive sera was detected. This confirms other studies; a significant antibody response in volunteers infected with strain DC63 of *M. hominis* could be demonstrated in all when the same strain was used as antigen, but only in 50% when the type strain of *M. hominis* (PG21) was used as antigen (12).

The antigenic heterogeneity within the species of *M. hominis* (9) offers an explanation for these observations. It seems that the major difference occurs in the surface antigens (4); in accordance with this, in the present study it was possible to form three groups of the 10 strains of *M. hominis* by the indirect immunofluorescence test, and, by using one strain from each of these groups, a pool of antigen was composed, giving results identical to those obtained with a pool consisting of all 10 strains.

The demonstrated IHA antibody response seems to be specific, at least in regard to mycoplasmas, because the change of titer in the cases concerned was demonstrable only against *M. hominis* and not against other human mycoplasmas.

*U. urealyticum* does not seem to be of importance as a cause of pyelonephritis, as judged from this study. The antibody response to *U. urealyticum* did not differ within the groups of patients, and only one of seven patients yielding *U. urealyticum* from the upper urinary tract developed a significant antibody response. In previous cultivation studies, *U. urealyticum* could not be related to the occurrence of any form of pyelonephritis (13, 14).

Thus, from the cultivation studies (13, 14) and the present serological study, it seems reasonable to conclude that *M. hominis* may play a role in the development of acute attacks of pyelonephritis and that *U. urealyticum* does not play a similar role.

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