

## Prevalence and significance of *Mycoplasma hominis* and *Ureaplasma urealyticum* in the urines of a non-venereal disease population

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### SUMMARY

*Ureaplasma urealyticum* organisms (ureaplasmas) and *Mycoplasma hominis* organisms (mycoplasmas) were sought in mid-stream urines collected from 200 men and 200 women attending hospital with conditions of a non-venereal nature. In addition, the urines from 100 male and 100 female healthy volunteers were examined. Overall, ureaplasmas were isolated four times more often than mycoplasmas. In individuals less than 50 years of age, the organisms were found in about 20% of men and about 40% of women. In individuals 50 years or older, they were found about one-third to one-half as frequently. Centrifugation of urine and examination of the resuspended deposit did not increase the isolation rates. In men, the numbers of organisms in the urine were usually small ( $\leq 10^3$  c.c.u./ml) with less than tenfold more in the urine of women. The occurrence of  $51- > 1000$  leucocytes per  $\text{mm}^3$  in some of the urines was not associated with either the presence or an increased number of ureaplasmas/mycoplasmas, whereas they were associated with the presence of  $10^5$  or more bacteria/ml. The significance of these findings in the context of defining the role of ureaplasmas/mycoplasmas in genital-tract disease is discussed.

### INTRODUCTION

There are 12 members within the family Mycoplasmataceae which are of human origin. *Ureaplasma urealyticum* and *Mycoplasma hominis* organisms, known trivially as ureaplasmas and mycoplasmas, are those found most frequently in the urogenital tract (Taylor-Robinson & McCormack, 1980). At delivery, they are transmitted from mothers who harbour them in the vagina to various anatomical sites of their infants. The organisms are found less often in the urogenital tract of the male infant than in that of the female and they usually disappear from most anatomical sites within a few months of delivery. They disappear more rapidly from the male urogenital tract than from the female, the male infant often carrying the organisms transiently but rarely becoming colonized. Around the time of puberty the organisms are found again in the urogenital tract, presumably as a consequence of sexual activity, and in increasing proportions thereafter, most frequently in patients attending Sexually Transmitted Disease (STD) clinics.

Ureaplasmas have been associated with some cases of non-gonococcal urethritis (NGU) (Taylor-Robinson, 1985) and *M. hominis* with pelvic inflammatory disease, post-partum and post-abortual fever and pyelonephritis (Taylor-Robinson & McCormack, 1980; Taylor-Robinson & Munday, 1987). One of the difficulties in incriminating these organisms as a cause of disease is their occurrence in apparently healthy subjects. While there is some information on the prevalence and numbers of ureaplasmas and *M. hominis* organisms in healthy women in different categories (Taylor-Robinson & Furr, 1973), the information for males is confined largely to young men attending STD clinics. Information on how long the organisms persist in the urogenital tract and are, therefore, likely to be found in older men is also sparse (Taylor-Robinson, 1986). In view of these limited data, we have determined the prevalence and numbers of ureaplasmas and mycoplasmas in the urines of two groups of men and women of various ages, namely those attending hospital with conditions of a non-venereal nature and apparently healthy volunteers. The relationships between the organisms and the presence of inflammatory cells found in the urines of some of the individuals was also assessed. In general, the information should provide a useful basis for determining the significance of the organisms in various clinical conditions.

#### MATERIALS AND METHODS

##### *Subjects examined*

Four hundred patients seen at Northwick Park Hospital comprised one of the study groups. They were divided according to sex (200 male, 200 female) and age (16–49 years and 50 or more years; 100 individuals in each category). The group of 100 younger female patients divided equally into pregnant and non-pregnant. Mid-stream urine samples were collected randomly, together with the clinical and antibiotic history of each patient, until the groups mentioned above were complete. Urines were excluded if there was a possibility of a current sexually transmitted disease or the patients were receiving antimycoplasmal antibiotics.

A further four groups, each of 50 apparently healthy volunteers were classified according to the above criteria of sex and age. They comprised mainly staff members of the Clinical Research Centre and Northwick Park Hospital. Details of the ages of patients and volunteers are presented in Table 1.

Urines were examined, usually within 24 h of collection, and were left at room temperature during this interval.

##### *Estimation of number of cells*

The urine was mixed thoroughly and applied to a modified Fuchs Rosenthal counting chamber. Polymorphonuclear leucocytes (PMNL) were counted and the number per mm<sup>3</sup> was recorded.

##### *Centrifugation of urine*

After the cell count, a 20 ml volume of urine was centrifuged at 2000 rpm for 10 min in a MSE Super Minor centrifuge and the deposit resuspended in 2 ml of the supernatant urine to give an approximate tenfold concentration.

Table 1. Age of patients and volunteers

Sex	Category	Age (years)		
		Group	Range	Mean
Male	Patient	< 50	16-49	34.7
	Volunteer	< 50	18-49	34.2
Female	Patient	(Non-pregnant)	< 50	16-49
		(Pregnant)	< 50	16-45
	Volunteer	< 50	18-49	34.4
		>= 50	50-94	69.3
Male	Patient	>= 50	50-94	69.3
	Volunteer	>= 50	50-81	55.8
Female	Patient	>= 50	50-95	71.9
	Volunteer	>= 50	50-84	57.3

*Bacterial medium and examination*

Cysteine-lactose-electrolyte deficient (CLED) agar was used for routine bacteriology. A standard loopful (0.002 ml) of well-mixed urine was inoculated on the agar and the plates incubated at 35 °C overnight. Colonies were identified by conventional means.

*Mycoplasmal media*

The urea- and arginine-containing broth media used to grow ureaplasmas and *M. hominis*, respectively, were as described previously (Taylor-Robinson *et al.* 1971; Manchee & Taylor-Robinson, 1968), but with two modifications. In an attempt to reduce bacterial and fungal contamination, ampicillin (1 mg/ml) was substituted for penicillin and polymyxin B (Sigma; 500 units/ml) was added.

*Estimation of numbers of organisms*

Dilutions of centrifuged and uncentrifuged urines were made by inoculating 0.2 ml of each into both urea- and arginine-containing broth media dispensed in 1.8 ml aliquots in screw-capped vials of 2.5 ml capacity. Each sample was diluted serially from  $10^{-1}$  to  $10^{-6}$  in the media and the diluted samples incubated at 37 °C. The greatest dilution at which a colour change from yellow to magenta occurred was deemed to contain one colour-changing unit (c.c.u.). Rarely, an endpoint was not obtained and then the sample was rediluted to  $10^{-8}$ .

*Identification of M. hominis*

Evidence that a change in colour of the arginine-containing medium was due to *M. hominis* was confirmed by identification with specific antiserum using the disc growth-inhibition technique (Clyde, 1964).

## RESULTS

*Isolation of Ureaplasma urealyticum and Mycoplasma hominis from urine*

As shown in Table 2, these micro-organisms, predominantly ureaplasmas, were isolated from the urines of about 20% of the younger males, whether patients or

Table 2. Isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis* from urines  
 No. of patients (P) or volunteers (V) from whom micro-organism isolated

Micro-organisms	Males < 50 yr		Males ≥ 50 yr		Females < 50 yr			Females ≥ 50 yr		
	P (n = 100)	V (n = 50)	P (n = 100)	V (n = 50)	P (n = 50 non- pregnant)	P (n = 50 pregnant)	V (n = 50)	P (n = 100)	V (n = 50)	
<i>U. urealyticum</i> only	19	7	5	5	15	14	14	9	12	
<i>M. hominis</i> only	1	0	1	0	2	1	0	2	0	
Both	3	3	0	1	3	7	5	2	1	
Total (%)	23	20	6	12	42			13	26	
GMT* for <i>U. urealyticum</i>	10 <sup>2.5</sup>	10 <sup>2.4</sup>	10 <sup>3</sup>	10 <sup>1.5</sup>	10 <sup>2.7</sup>	10 <sup>2.9</sup>	10 <sup>3.1</sup>	10 <sup>2.8</sup>	10 <sup>2.8</sup>	
GMT for <i>M. hominis</i>	10 <sup>2.25</sup>	10 <sup>2.3</sup>	10 <sup>6</sup>	10 <sup>2</sup>	10 <sup>3.4</sup>	10 <sup>3.25</sup>	10 <sup>3.8</sup>	10 <sup>4.5</sup>	10 <sup>3</sup>	

\* GMT, geometric mean titre.

Table 3. Relation between *Ureaplasma urealyticum*, *Mycoplasma hominis*, bacteria and urinary leucocytes

Micro-organisms	Sex	No. of subjects with indicated no. of leucocytes in urine (per mm <sup>3</sup> )	
		≤ 50 leucocytes	51 to > 1000 leucocytes
No ureaplasmas, mycoplasmas or bacteria	Male	126	16 (11%)
	Female	113	9 (7.4%)
Ureaplasmas/mycoplasmas only	Male	24	3 (11%)
	Female	40	8 (16.7%)
Bacteria only	Male	8	21 (72%)
	Female	11	12 (52%)

volunteers and from about 40% of the younger females. In the older age group, they were found in volunteers (mean age about 55 years; Table 1) about half as often as in the younger subjects, and in the patients (mean age about 70 years; Table 1) about one-third as frequently as in the younger subjects. Overall, the organisms were found about half as often in the urines of the older male subjects as in the older females.

The numbers of ureaplasma and *M. hominis* organisms in the urines of the younger and older males were similar (Table 2). The numbers of organisms in the urines of the younger and older women were similar and a little greater (less than tenfold) than in the urines of the males (Table 2).

The number of organisms, mainly ureaplasmas, isolated from most urines that were centrifuged was increased about tenfold, irrespective of whether cells were present. Centrifugation, however, did not enhance the isolation rate; organisms were recovered from only one urine after centrifugation but not before and this was balanced by recovery from another urine before centrifugation but not after.

#### *Relation between micro-organisms and the presence of urinary leucocytes*

As shown in Table 3, 11% of men and 7.4% of women whose urines did not contain either ureaplasmas, mycoplasmas or bacteria had 51 to > 1000 urinary leucocytes. Men whose urines contained ureaplasmas/mycoplasmas had 51 to > 1000 urinary leucocytes no more often than men whose urines did not contain any micro-organisms. Women who had ureaplasmas/mycoplasmas in their urines more often had this number of leucocytes than did those without micro-organisms, but the difference was not significant ( $\chi^2$  2.35; 1 D.F.;  $P = 0.13$ ). In contrast, however, the most striking association was seen between the presence of bacteria and urinary leucocytes. Thus, 72% of men and 52% of women whose urines contained  $\geq 10^5$  bacteria/ml had 51 to > 1000 urinary cells.

The numbers of ureaplasmas and mycoplasmas in the urines of men with large numbers of leucocytes were no greater than those in urines with small numbers of leucocytes. Several women with 51 to > 1000 urinary leucocytes had  $10^5$  c.c.u. of *M. hominis*, but the geometric mean titre of this mycoplasma ( $10^{4.2}$ ) and ureaplasmas ( $10^{3.0}$ ) in women with 51 to > 1000 leucocytes was little more than

the geometric mean titre of *M. hominis* ( $10^{3.5}$ ) and ureaplasmas ( $10^{2.7}$ ) in women with  $\leq 50$  urinary leucocytes.

#### DISCUSSION

*U. urealyticum* and *M. hominis* were isolated by the sensitive technique of detecting a colour change in the respective liquid medium. Non-specific colour changes occurred sometimes, either immediately on inoculation due to alkaline urine, or rarely later during incubation due to the presence of urease-producing bacteria such as *Proteus* sp. However, these colour changes did not recur on subculture. A colour change occurred sometimes in the first vial of arginine-containing medium even though the urine contained ureaplasmas only. This was probably due to ureaplasmas utilizing the small quantity of urea in the urine; on subculture, a colour change developed only in urea-containing medium and not in arginine-containing medium. None of these technical difficulties jeopardized the isolation of the micro-organisms. The procedure is simple and does not require centrifugation of urine because this did not enhance the isolation rate. We were surprised that centrifugation concentrated the number of organisms tenfold irrespective of the presence of urinary cells. This does not mean, of course, that some of the organisms are not cell associated. Only a multiple washing procedure would enable this to be determined.

The frequency of ureaplasmas and *M. hominis* in the urines of the younger women was similar to their frequency in the vagina, observed previously (Ross *et al.* 1981). Indeed, the presence of these organisms in urine may reflect, at least to some extent, contamination from the vagina. They were found less frequently and in smaller numbers in male subjects. Large numbers ( $\geq 10^5$  c.c.u./ml) were found rarely in the urines of either men or women. Although previous observations (Holmes, Furr & Taylor-Robinson, 1974; MacLeod, Furr & Taylor-Robinson, 1976) showed that, without sexual contact, ureaplasmas and mycoplasmas could persist in the male urethra for a year or more, it is clear that they tend to disappear, being found least frequently in both men and women in the older age group.

Urines were collected at random and were not sought from patients with urinary tract infections. Nevertheless, leucocytes were found in some urine samples. They were associated often with the presence of bacteria but an association with ureaplasmas/mycoplasmas could not be demonstrated. Furthermore, the latter micro-organisms were almost always present in small numbers and large numbers were not specifically associated with urinary leucocytes. Our failure to demonstrate an association does not necessarily militate against a role for ureaplasmas in NGU. Indeed, the information is useful as a background to investigations on this subject. As stressed previously (Taylor-Robinson, 1985), quantitative studies, as undertaken here, rather than qualitative ones on NGU are desirable and should lead to a better understanding of the exact role of ureaplasmas in this disease.

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