

T-STRAIN MYCOPLASMA AND NON-GONOCOCCAL URETHRITIS

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ABSTRACT

The incidence of T-strain mycoplasma was studied in 36 men with GU, 56 men with NGU, 59 normal, symptom-free men with no leucocytes in their urethral smears and 37 symptom-free men showing leucocytes in their urethral smears. There was no significant difference in the rate of isolation of T-strain mycoplasma in our different groups. The isolation rate for *M. hominis* was significantly higher in symptom-free men than in men with GU or NGU. We could not detect serological evidence of recent infection with *M. hominis* or T-strain mycoplasmas in any of our 92 patients studied.

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INTRODUCTION

Since the isolation of T-strain mycoplasmas by Shepard (1954), followed by his subsequent finding (1956) that these organisms are highly prevalent among patients with non-gonococcal urethritis, T-strain mycoplasmas have become a hypothetical etiology of non-gonococcal urethritis.

There are several reports on the importance of T-strain mycoplasmas as a causative agent in non-gonococcal urethritis. Some of these show that there are no causal relationships between T-strain mycoplasmas and non-gonococcal urethritis (Taylor-Robinson *et al.*, 1969; Black & Rassmussen, 1968)

and others indicate the contrary (Markham *et al.*, 1972; Holmes *et al.*, 1967; Jansson *et al.*, 1971; Ford *et al.*, 1962; Ford, 1973). Finally, the third group believes that the evidence for the possible pathogenic role of these organisms is equivocal (Csonka *et al.*, 1966).

The aim of this study was to investigate the presence of T-strain mycoplasmas in groups of men referring to the Venereal Disease Section of Nejat Hospital for chlamydial study. Also, a possible antibody response against T-strain mycoplasmas (T-960), *M. hominis* (PG-21) and some isolates was studied.

MATERIAL & METHOD

a. Study Group

Ninety-two specimens of male urethral discharge were taken according to the method described by Dunlop, Vaughan-Jackson & Darougar (1972), from the patients referred to the Venereal Disease Section of Nejat Hospital for chlamydial study.

b. Control Group

Ninety-six specimens were taken from the urethras of normal men with no symptoms or visible discharge, referring to the same institution to obtain a health certificate for marriage. All of these specimens were collected on special cotton-wool swabs made by Stayne Laboratories Ltd. The swabs were put into tubes containing 2 ml PPLO transport medium and sent to the laboratory for mycoplasma isolation. These were cultured immediately upon arrival.

On the basis of microscopic examination of methylene blue stained smears of urethra, based on observation of morphologically typical intracellular diplococci and polymorphs, the patients were divided into two groups:

Group I - 36 cases of gonorrhoea positive (GU-group)

Group II- 56 cases of gonorrhoea negative (NGU-group)

However, in the absence of gram stain reaction and cultural results, this identification must, of course, be regarded as very presumptive.

Based on a microscopic observation of the presence or absence of polymorphs, the control group was also divided into two groups:

Group III- 56 cases negative for the presence of polymorphs.

Group IV- 37 cases positive for the presence of polymorphs.

The age of study group as well as the control group was between 16 and 35 years.

CULTURE

Media and Isolation Technique

All of the following media used in this study were described by Taylor-Robinson *et al.*, (1963) and Manchee & Taylor-Robinson (1968).

a. *Transport Medium*

This consisted of 7 parts Difco PPLO broth, 2 parts unheated horse serum, 1 part 25% aqueous extract of DCL (Distillers Co., Ltd., Scotland), active dried yeast and 0.002% phenol red.

b. *Culture Media*

These were essentially the transport medium modified by the addition of 0.1% L-arginine-hydrochloride, 1000 units/ml penicillin G and 1:2000 thalium acetate for the isolation of arginineutilizing mycoplasmas, and 0.1% urea (oxid) for the isolation of T-strain mycoplasmas. The pH of the arginine-containing broth was adjusted to 7.2 and that of the urea-containing broth to 6.8 with N/10 HCl. The solid medium consisted of 7 parts PPLO agar, 2 parts unheated horse serum, 1 part 25% yeast extract and 1000 units/ml penicillin G. For T-strain mycoplasma, pH 6 Agar-Broth was used. It consisted of Difco PPLO broth adjusted to pH 6.0 with NHCl, 1% Difco Nobel agar, 2 parts unheated horse serum, 1 part 25% yeast extract and 1000 units/ml penicillin G. Broth cultures were incubated at 35 C under aerobic conditions. The agar cultures of T-strain were incubated in an atmosphere of 20% Co₂-80% N₂ and of arginine-utilizing mycoplasmas in 5% CO₂ and 95% N₂. The T-strain usually grew after 24-48 hours and their growth in broth was detected by a change in the color of the media from yellow (pH 6.8) to pink (pH 7.8 or above), whereas arginine-utilizing mycoplasmas grew after 3-4 days. Positive samples were kept at -20 C. The isolates were purified, using the method of three-fold cloning of the colonies, and then kept at -70°C for further studies. However, due to the small colony size of T-strain mycoplasma, we are not certain that the isolated and cloned strains were homologous.

Metabolic Inhibition Test (MI)

The tests were performed in disposable plastic microtiter plate "U-shaped cups" (Cooke Engineering Co., Alexandria, Va.) according to the method described by Purcell *et al.*, (1966), using PPLO broth containing urea (0.1% final conc.) and Guinea pig serum (GPS) in a final concentration of 1%. The sera from NGU and gonorrhoea cases were inactivated (30 min. at 56°C) and tested undiluted. Serum antibody titers were recorded when the pH of of the Medium in the cups which contained organisms but no antiserum had increased approximately 0.5 pH unit (determined by comparison of color with medium containing phenol red and adjusted to known pH values).

The highest serum dilution which prevented a change of approxi-

mately 0.25 pH unit or greater was recorded as the serum titer end point. Using the MI test and mycoplasma antisera (BBL), the isolated large colony mycoplasmas were identified. Also, the isolated large size and T-strain mycoplasmas were tested with the corresponding serum of the same person from whom the mycoplasmas had been isolated. T-strain 960 and *M. hominis* (PG-21) were used separately as the antigen for detecting MI antibodies in the patient's serum.

Mycoplasma

Strain 960 of T-strain mycoplasma and *M. hominis* (PG-21) were kindly provided by Dr. Taylor-Robinson.

Statistical analysis

Statistical analysis was done by performing X²-test using one degree of freedom and more than 95% confidence level.

RESULTS

The majority of our patients (72 cases) as well as the control group (80 cases) were in the age group 16-30 years of age. All subjects were circumcised.

Only 13% of our patients had no previous antibiotic treatment whatsoever during the month prior to their referral to the Venereal Disease Section of Nejat Hospital. The 87% treated subjects had had previous treatment with tetracyclin, sulfonamide and penicillin.

All of the large colony mycoplasma strains isolated during the course of this study were identified as *M. hominis*. The other isolated mycoplasmas were T-strains.

Table I shows that the frequency of T-strain mycoplasmas, isolated from various groups of the subjects, was higher than that of *M. hominis*. However, the isolation rate for *M. hominis* in our control groups (groups III & IV, symptomfree men) was significantly higher than in men with gonorrhoea and non-gonococcal urethritis (Table I).

There was no significant difference in the rate of isolation of T-strain mycoplasma in all of our four groups.

Table II and III show mycoplasma isolation from GU and NGU cases with regard to their past history of genital diseases there was also no significant difference in the rate of isolation of either T-strain or *M. hominis*.

All of the 92 patient's sera were tested for MI antibody to *M. hominis*, (PG--21) and T-960 mycoplasma. A total of 9 sera were positive, showing low titers to T-960 mycoplasma.

One case had a titer of 1:4, 5 cases a titer of 1:2 and 3 cases had no

titers but MI-antibody could be detected in their undiluted serum. Out of 92 sera tested, 6 had MI-antibody to *M. hominis*, (one, 1:4; three, 1:2; and two undiluted).

Eleven sera from patients from whom T-strain mycoplasma and/or *M. hominis* were isolated were tested for MI-antibody to the homologous mycoplasma; none of the sera contained the antibody.

DISCUSSION

Our results showed that only T-strain and *M. hominis* mycoplasmas were isolated from the urethras of patients with either gonococcal (GU) or non-gonococcal (NGU) urethritis as well as from the control group. Furthermore, in all instances the isolation rates of T-strain were higher than those of *M. hominis* mycoplasmas, and were within the range of isolation rates reported by others (Ford *et al.*, 1962; Ingham *et al.*, 1966; Black & Rasmussen, 1968; Taylor-Robinson *et al.*, 1969; Janson *et al.*, 1971).

There was no significant difference in the isolation rate of T-strains in patients with gonococcal urethritis (GU) (group I) than in those with non-gonococcal urethritis (NGU) (group II). This is not in agreement with the results obtained by others (Ford *et al.*, 1962; Csonka *et al.*, 1966; Black *et al.*, 1968), who have shown that the isolation rates for T-strain mycoplasmas were greater in NGU than in GU, but it agrees with Flower & Leeming (1969) and Hass, Dorfman & Sacks (1971) who have reported that the isolation rates for T-strain in patients with GU and those with NGU were similar. The high isolation rates of *M. hominis* in our control groups (symptom-free men), is in accordance with the finding of Markham *et al.*, (1972). Also, the overall low isolation rates for *M. hominis* in our patients, all of whom had been circumcised, are in accordance with the findings of Hare *et al.* (1969) and Haas *et al.*, (1971).

We could not detect serological evidence of recent infection with *M. hominis* or T-strain mycoplasmas in any of the 92 patients studied. Only low titers of MI antibody to the T-950 strain of T-strain or to the PG-211 strain of *M. hominis* were detected in the sera from some of the patients. In addition, no MI antibody to the homologous mycoplasma isolates could be detected in the sera of patients from whom these organisms were isolated. These findings are consistent with the results reported by Purcell *et al.*, (1966) and Hill *et al.*, (1973), suggesting that neither T-strain nor *M. hominis* mycoplasmas are an important cause of NGU. However, more studies are needed to clarify the possible difference in the virulence of different strains of T-strain mycoplasmas, as well as the role of a possible cell-mediated immune response in infections due to these organisms.

TABLE I

Mycoplasma isolation from different groups of men referred to the Venereal Disease Section of Nejat Hospital

Group	No. Exam.	Mycoplasma Isolated:									
		T-strain only		M. hominis only		T-strain and M. hominis					
		No.	Percent	No.	percent	No.	percent				
Group I	36	15	42	0	0	6	17	21	60	6	17
Group II	56	14	25	1	2	10	18	24	43	11	20
Group III	59	8	13	3	5	24	40	52	54	27	45
Group IV	37	9	24	2	5	18	50	27	73	20	54

TABLE II

Mycoplasma isolation from GU cases with regard to their past history of genital diseases .

Past History of Genital Diseases	No. Exam.	Mycoplasma Isolated:							
		T-strain only		T-strain and M. hominis		Total			
		No.	Percent	No.	Percent	No.	Percent		
Positive	23	10	43	5	21	15	65	5	21
Negative	13	5	58	1	7	6	46	1	8
Total	36	15	42	6	17	21	58	6	16

TABLE III.

Mycoplasma isolation from NGU cases with regard to their past history of genital diseases .

Past History of Genital Diseases	No. Exam.	Mycoplasma Isolated:						Total			
		T-strain only		T-strain and M. hominis		M. hominis only		T-strain		M. hominis	
		No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Positive	26	7	27	3	11	0	0	10	40	3	11
Negative	30	7	23	7	23	1	3	14	47	8	27
Total	56	14	25	10	18	1	1	24	43	11	20

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