

Chlamydia trachomatis in non-specific urethritis

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SUMMARY *Chlamydia trachomatis* was isolated from 58.5% of 159 patients with non-specific urethritis (NSU) using irradiated McCoy cell cultures. Patients with persistent *Chlamydia*-positive NSU remained *Chlamydia*-positive each time they were examined before treatment and patients with *Chlamydia*-negative NSU remained *Chlamydia*-negative during the course of the illness. Neither the duration of symptoms of urethritis nor a history of previous urethritis affected the chlamydial isolation rate significantly. Of 40 patients with severe discharge 30 (75%) harboured *C. trachomatis*. One-third of the *Chlamydia*-positive patients had a severe urethral discharge, while this was present in only 15% of *Chlamydia*-negative patients. Complications—such as conjunctivitis, arthritis, and epididymitis—were more severe in men with *Chlamydia*-positive NSU than in those with *Chlamydia*-negative NSU. Of 64 men matched for sexual promiscuity but without urethritis, none harboured *C. trachomatis* in his urethra. This differs significantly ($P < 0.001$) when compared with patients with NSU. *C. trachomatis* was isolated from the urogenital tract in 24 (42%) out of 57 female sexual contacts of patients with NSU. The presence of *C. trachomatis* in the women correlated significantly ($P < 0.001$) with the isolation of the agent from their male contacts. These findings give further evidence for the aetiological role of *C. trachomatis* in non-specific urethritis and its sexual transmission.

Introduction

Sexually transmitted urethritis in men is either gonococcal or nongonococcal. In Britain (where both types are reported) nongonococcal urethritis (NGU) is now more common than gonococcal urethritis. NGU accounts for more than two-thirds of all urethritis in men and is increasing more rapidly than gonococcal urethritis (Department of Health and Social Security, 1977).

In fewer than 10% of cases of NGU it is possible by the use of routine laboratory techniques to find a specific cause—such as *Trichomonas vaginalis*, *Candida albicans*, or herpesvirus hominis. The remainder of NGU is called non-specific urethritis (NSU) (Dunlop, 1975). NSU usually causes symptoms and signs that are less severe than those of gonococcal urethritis, and these may even be absent (Rodin, 1971; Oriel, 1976). NSU is diagnosed by demonstrating an inflammatory reaction in the urethra and by excluding specific causes—especially gonorrhoea (Oriel, 1976).

About 12 years ago *Chlamydia trachomatis* was first isolated from the urethra of patients who presented with NSU (Dunlop, 1965). *C. trachomatis* has since been shown to be one of the main causes of NSU (Dunlop, 1975; Oriel, 1976). It was isolated using sophisticated cell culture methods (Dunlop, 1975) from over 40% of patients with NSU and from many of their female sexual partners (Richmond and Sparling, 1976; Schachter *et al.*, 1976). In control groups of men matched for sexual activity but without urethritis the chlamydial isolation rate was 0 to 7% (Oriel, 1976).

In the present study, the isolation of *C. trachomatis* in a series of 159 men with NSU is reported, and the clinical findings of NSU are analysed in relation to the isolation of *C. trachomatis*. The isolation results in a control group of 64 men without urethritis and in 57 female sexual partners of patients with NSU are also presented.

Material and methods

STUDY POPULATION

The study population consisted of unselected Caucasian heterosexual men presenting either at the Turku Venereal Disease Clinic or the Depart-

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Received for publication 1 November 1977

ment of Dermatology and Venereology, University of Turku, between December 1973 and May 1976. Patients were included in the study provided they had not received treatment with antimicrobial agents within the previous six weeks. Each man had negative results to a urethral culture for *Neisseria gonorrhoeae*. The patients were divided into two groups.

NSU group

This group initially comprised 164 men. All had microscopical evidence of urethritis with more than 20 polymorphonuclear leucocytes in two fields or more on a Gram-stained urethral smear examined under a magnification of $\times 800$. Specimens for chlamydial isolation from five (3%) of these men were contaminated, so these patients were excluded from the study. The mean age of the remaining 159 patients was 27.5 years.

Control group

This group initially comprised 68 men who were attending the same clinic but who did not have urethritis. Specimens from four (6%) of them were invalid either because of contamination with bacteria or because of toxicity for cells. Consequently the control group comprised 64 men, whose mean age was 29.9 years. Their marital state was comparable with that of the patients with NSU.

Female sexual partners of NSU patients

Sixty female sexual partners of patients with NSU were examined. Three (5%) of them (all consorts of patients with *Chlamydia*-positive NSU) had contaminated specimens, so 57 women were included in the study.

CLINICAL EVALUATION

A standardised history was taken, special attention being paid to previous venereal diseases, recent sexual partners, possible incubation time, and duration of symptoms. General medical and genital examinations were carried out in all cases. Eyes, joints, mucous membranes, and genitalia were carefully examined to see if there were complications. Most of the patients were studied about four hours after passing urine, but in some the overnight urethral secretion was examined to reveal low-grade urethritis. Some patients had at least two examinations before medical treatment was begun. Patients were asked to return for examinations within one week of completing treatment, and then at one-monthly and two-monthly intervals. At each visit the clinical state was recorded, and relevant specimens were taken.

SPECIMENS

Urethral secretions produced spontaneously or by massaging the urethra towards the meatus were smeared with a sterile platinum loop on to a slide and stained by Gram's method. For gonococcal culture, secretions from the urethra were smeared directly with the loop on Müller-Hinton agar and/or on Columbia agar plates, which were immediately incubated in a candle-jar at 37°C. Gonococci were identified by conventional bacteriological techniques with sugar fermentation tests. All gonococcal cultures were processed at the Department of Medical Microbiology, University of Turku.

Specimens for isolating *C. trachomatis* were obtained with endourethral swabs inserted 4 cm from the meatus. The swabs were placed in 2-sucrose phosphate (2 SP) and subsequently inoculated on to irradiated McCoy cells. The technique used for isolating *C. trachomatis* is described in detail elsewhere (Terho, 1978). Briefly, irradiated McCoy cells grown on a coverglass in flat-bottomed tubes were infected by centrifugation (15 000 *g* for 1 hour at 35°C). After incubation for 72 hours in a CO₂ atmosphere at 35°C, cells were stained with iodine, and chlamydial inclusions were identified by light microscopy.

Urethral secretions from some patients were cultured for herpesvirus hominis by inoculation on to Vero cells. No attempt to cultivate mycoplasmas or ureaplasmas was made. Specimens for chlamydial isolation were taken from the urethra and from the cervical canal in women.

Statistics

Statistical analysis was made by the *t* test or χ^2 test (with Yates's correction if appropriate). Probabilities were classified as almost significant ($P < 0.05$), significant ($P < 0.01$), or highly significant ($P < 0.001$).

Results

ISOLATION OF *C. TRACHOMATIS*

C. trachomatis was isolated from 93 (58.5%) of 159 men with NSU. In no man without urethritis was *C. trachomatis* isolated. This difference is highly significant (χ_1^2 61.8; $P < 0.001$).

REPEATED ISOLATION OF *C. TRACHOMATIS*

Before treatment at least two specimens were taken for culture for *C. trachomatis* from 23 unselected men; of these 13 gave *Chlamydia*-positive results and 10 *Chlamydia*-negative. All except four men showed persistent NSU on re-examination; two of them were *Chlamydia*-positive at the initial visit but free of urethritis and *Chlamydia*-negative six

days later in the case of one and 28 days later in the other. Thus all the 11 men with persistent urethritis remained *Chlamydia*-positive after repeated examinations (one less than one week later, five between one and two weeks later, two men two weeks later, and one man three weeks later). Two patients with persistent *Chlamydia* urethritis remained positive on second re-examination, one man at one-day and at eight-week intervals, and the other at four-day and at six-week intervals; both denied consorting with a new partner. All eight patients with persistent NSU who were initially *Chlamydia*-negative were still negative on re-examination.

The numbers of inclusion-forming units (IFU) in specimens taken repeatedly before treatment were constant; in four instances there was some increase. In five there was a slight decrease, and in two the numbers were the same.

HISTORY OF PREVIOUS URETHRITIS

Ninety-two (57.9%) of patients with NSU had a history of urethritis. In this respect there was no difference between *Chlamydia*-positive and *Chlamydia*-negative men. In the control group 25 (39.1%) men had had urethritis previously. This is almost significantly less than in the NSU group (χ_1^2 6.47, $P < 0.05$). The type of previous urethritis is shown in Table 1.

There was no difference between *Chlamydia*-positive and *Chlamydia*-negative patients with NSU with regard to previous gonococcal or non-specific urethritis. Sixty-seven (42%) men were seen during their first episode of NSU. Of these 38 (57%) were *Chlamydia*-positive and 29 (43%) *Chlamydia*-negative. A history of previous NSU was given by 49 men, of whom 32 (65.3%) were *Chlamydia*-positive. This does not differ statistically from those presenting with NSU for the first time.

In the control group NSU was almost significantly less common (10 out of 64 or 15%) than in the NSU group, in which 49 out of 159 (31%) had had NSU earlier (χ_1^2 5.41, $P < 0.05$). The history of previous gonococcal urethritis was equally common in the NSU and control groups.

REASONS FOR ATTENDANCE

Most patients (134 or 84%) attended because of symptoms of urethritis—namely discharge with or without dysuria. In the NSU group 16 (10%) men were symptom-free, although they had urethritis on microscopical examination; of these nine had *Chlamydia*-positive NSU.

A casual sexual contact before admission, which (usually) meant also a recent (less than four weeks) change of sexual partner and a possible exposure to the risk of urethritis, was reported by 108 (68%) patients with NSU. There was no difference in this respect between patients with *Chlamydia*-positive and *Chlamydia*-negative NSU. In the control group a recent casual sexual contact was reported by 35 (55%) patients. If the three men in this group who denied ever having sexual intercourse are excluded the figure is 57%. Thus the control group was comparable with the NSU group in sexual promiscuity.

In the NSU group 26 men who did not admit casual sexual contact attended because of long-standing mild genital symptoms and anxiety. The possibility that the sexual partner had a sexually-transmitted disease (STD) was the reason that eight men with NSU attended the clinic; six of them had positive *Chlamydia* culture results. In the control group a possible STD in the female was the reason for attendance in seven cases. Other sexually transmitted diseases (scabies, genital herpes, or condyloma acuminatum) were more often a reason for attendance in the control group (10 out of 64 or 16%) than in NSU group (4 out of 159 or 3%). The rest of the men (12) in the control group attended requesting examination.

DURATION OF SYMPTOMS OF URETHRITIS

Patients with NSU could be divided into two groups according to whether the urethral symptoms were acute or chronic.

Acute NSU

Patients with acute NSU usually presented after a recent change of sexual partner; the mean duration of symptoms at attendance was 11.1 days in the

Table 1 Isolation of *C. trachomatis* and the history of previous urethritis

Group	Chlamydia Isolation	No.	%	No previous urethritis		Previous urethritis									
						GU		NSU		GU+NSU		Unknown		Total	
				No.	%	No.	%	No.	%	No.	%	No.	%		
NSU	Positive	93	(58.5)	38	(41)	21	(23)	20	(22)	12	(13)	2	(2)	55	(59)
	Negative	66	(41.5)	29	(44)	18	(27)	11	(17)	6	(9)	2	(3)	37	(56)
	Total	159	(100)	67	(42)	39	(25)	31	(19)	18	(11)	4	(3)	92	(58)
Control	Negative	64	(100)	39	(61)	15	(24)	6	(9)	4	(6)	—	—	25	(39)

100 men in whom it was possible to determine this. The duration of symptoms was slightly longer in the *Chlamydia*-positive group (mean 12.8 days) than in the *Chlamydia*-negative group (mean 9.8 days), but this difference does not reach statistical significance (t 1.39). Short duration of symptoms (less than seven days) was recorded in 42 (26.4%) of the 159 cases with NSU. Twenty-three (54.8%) of these 42 men had positive *Chlamydia* culture results, and 19 (45.2%) had negative results, rates similar to those of the whole (NSU) group.

Chronic NSU

Duration of urethral symptoms for longer than 30 days was observed in 26 (16.4%) patients with NSU who did not have a recent change of sexual partner. Most of them (18 or 69%) harboured *Chlamydia*.

TIME FROM PREVIOUS SEXUAL INTERCOURSE

It was possible to determine the time from the previous casual sexual contact or the last sexual intercourse with a regular partner to the onset of symptoms for 86 patients with NSU. The mean time was 12.4 days. There was no difference in this between patients with positive and negative *Chlamydia* culture results.

In the control group the time from previous sexual intercourse averaged 6.3 days. This interval is significantly shorter than in the NSU group (t 3.66, $P < 0.001$).

TIME FROM LAST MICTURITION

Most patients were examined more than two hours after micturating. In the NSU groups 39 (25%) patients were examined less than two hours after passing urine, but it was possible to diagnose NSU in most of them; only five patients (three *Chlamydia*-positive and two *Chlamydia*-negative) had their overnight urethral secretion investigated so that low-grade NSU could be diagnosed. In the control group 18 (28%) men were examined within two hours of micturating. Because of practical difficulties it was not possible to investigate their overnight urethral secretion, so some cases of urethritis may have been missed (Rodin, 1971).

AMOUNT OF DISCHARGE

The amount of urethral discharge was determined as 'severe' if frank, spontaneous discharge was present. The discharge was classified as 'slight' if the urethra had to be massaged or if only a small droplet of discharge appeared spontaneously at the meatus. In patients with no discharge it was only possible to obtain the contents of the urethra by using a platinum loop. The amount of discharge is shown in Table 2.

Table 2 Isolation of *C. trachomatis* and the amount of urethral discharge

Amount of discharge	Isolation of <i>C. trachomatis</i>		
	Positive	Negative	Total
None	10	14	24
Slight	53	42	95
Severe	30	10	40
Total	93	66	159

Of the *Chlamydia*-positive patients 30 (32%) had severe discharge, while only 10 (15%) of the 66 *Chlamydia*-negative patients had. This difference is almost significant (χ_1^2 6.00, $P < 0.05$).

Thus a total of 40 patients with NSU had severe discharge, and of them 30 (75%) were *Chlamydia*-positive. When comparing this finding with patients with slight discharge, of whom 56% (53 out of 95) were *Chlamydia*-positive, it can be stated as almost significant (χ_1^2 4.39, $P < 0.05$).

COMPLICATIONS OF NSU

Chlamydia-positive NSU

Two patients had isolation-positive chlamydial conjunctivitis in addition to chlamydial urethritis. Two additional patients had arthritis of the knee concomitant with chlamydial urethritis; in one of them we attempted to isolate *C. trachomatis* from the joint fluid but the result was negative. One patient developed severe epididymitis after chlamydial NSU of long duration.

Chlamydia-negative NSU

Two patients developed a slight *Chlamydia*-negative conjunctivitis at the time of NSU. One patient had a mild epididymitis. One patient presented with Reiter's disease, which he had had twice before.

ISOLATION OF *C. TRACHOMATIS* FROM FEMALE PARTNERS

Twenty-four (42%) out of 57 female sexual partners of patients with NSU were *Chlamydia*-positive (Table 3). Of these 35 were partners of men harbouring *C. trachomatis*; the agent could be isolated from the urogenital tract of 22 (63%) (Table 3). Chlamydial isolation was possible only in two (9%) of the 22 partners of *Chlamydia*-negative patients with NSU. This difference is highly significant (χ_1^2 13.9, $P < 0.001$). The cervix gave positive results

Table 3 Isolation of *C. trachomatis* from female sexual partners

Chlamydia in women	Chlamydia in men	
	Positive	Negative
Positive	24	2
Negative	33	20

in 20 (83%) out of a total of 24 *Chlamydia*-positive women. Urethral isolation was positive in 14 cases (58%) and the urethra was the only site of recovery in four (17%) *Chlamydia*-positive cases.

Discussion

C. trachomatis can be isolated significantly more often from men with NSU than from control men without urethritis (Richmond and Sparling, 1976; Schachter *et al.*, 1976). In this study the *C. trachomatis* isolation rate was 58.5% for patients with NSU. This figure is very close to the 60% suggested by Oriol *et al.* (1976) as the optimal rate for current techniques.

Repeated examinations before treatment or after treatment with placebo show that NSU may disappear spontaneously. Handsfield *et al.* (1976) recorded resolution or improvement of NSU after placebo in five out of 23 patients. In the present study NSU disappeared in four of 23 patients when repeated examinations were made.

If urethritis remained, repeated isolation of *C. trachomatis* was also possible. Thus all 11 *Chlamydia*-positive patients remained so, and all eight *Chlamydia*-negative patients remained negative on repeated examinations, which may be a proof of the sensitivity of the present isolation method. This finding is in accordance with the repeated isolations in the study of Holmes *et al.* (1975). It shows also that *C. trachomatis* does not usually disappear spontaneously and gives support for the treatment of this urethral pathogen. The present study is in agreement with previous ones (Oriol, 1976) in the absence of chlamydial isolations from men without urethritis even if their sexual promiscuity was equal to that of patients with NSU.

Patients with NSU in this study had had urethritis before (especially NSU) significantly more often than had the controls. In the NSU group the type of previous urethritis did not affect the isolation rate of *C. trachomatis*. This finding agrees with that of Oriol *et al.* (1976). Recently, however, Alani *et al.* (1977) reported significantly lower isolation rates of *C. trachomatis* from men with a third episode of NSU. They suggested some immunological phenomenon as the cause of reduced isolation rate.

Men with NSU may have no symptoms (Rodin, 1971). In the present study 10% of all patients with NSU were unaware of their urethritis. The incubation time of NSU is longer than that of gonococcal urethritis. The mean time (recorded from the previous sexual intercourse) of 12.4 days in this study correlates well with that reported by Oriol *et al.* (1976); they found that the incubation time in

Chlamydia-positive patients was 16 days and in negative ones 12 days.

Duration of symptoms before attendance had a mean of 11.1 days in those patients from whom this could be determined. This time interval did not have any influence on chlamydial isolation rates, in contrast to the report of Oriol *et al.* (1976) who found significantly higher isolation rates in patients in whom symptoms lasted more than seven days.

The amount of urethral discharge seems to correlate with chlamydial isolation rates, although the isolation was possible from men without apparent discharge. One-third of all patients with *Chlamydia* had a severe discharge. Among a selected group of patients with NSU with severe discharge 75% were *Chlamydia*-positive. This finding is similar to those of Alani *et al.* (1977), who found that chlamydial isolation was positive in 67.7% of men with severe discharge.

The clinical picture is comparable in *Chlamydia*-positive and *Chlamydia*-negative NSU (Oriol *et al.*, 1976), but in the present study *Chlamydia*-positive NSU was associated with more severe complications. *Chlamydia*-positive conjunctivitis was recorded in two patients with *Chlamydia*-positive NSU and also in one female partner of a man with *Chlamydia*-positive NSU.

Joint complications, including painful arthritis of the knee in two patients, were also more severe in the *Chlamydia*-positive NSU group. One severe case of epididymitis occurred in the *Chlamydia*-positive group and one mild case of epididymitis in the *Chlamydia*-negative group. Heap (1975) reported two cases of acute chlamydial epididymitis, one of which led to surgery. Recently, Harnisch *et al.* (1977) showed that *C. trachomatis* may be the cause of acute epididymitis in young men as often as *N. gonorrhoeae*.

The isolation rate of 42% of *C. trachomatis* from female partners of patients with NSU is slightly higher than that of other studies. Hilton *et al.* (1974) found 34%, and Nayyar *et al.* (1976) 30% of partners of patients with NSU harbour *Chlamydia*.

The sexual transmissibility of *C. trachomatis* was shown by the demonstration of the agent significantly more often in the urogenital tract of partners of patients with *Chlamydia*-positive NSU. This agrees well with results of Alani *et al.* (1977).

The author thanks Ms Ulla Virtanen and Ms Anni Heikkilä for their help during clinical work and Ms Tuula Lindholm for her excellent technical assistance. Dr E. M. C. Dunlop is thanked for many valuable suggestions during the preparation of this manuscript.

References

- Alani, M. D., Darougar, S., Burns, D. C. MacD., Thin, R. N., and Dunn, H. (1977). Isolation of *Chlamydia trachomatis* from the male urethra. *British Journal of Venereal Diseases*, **53**, 88–92.
- Department of Health and Social Security (1977). Chief Medical Officer's Report on the state of the public health for the year 1975. *British Journal of Venereal Diseases*, **53**, 68–71.
- Dunlop, E. M. C. (1975). Non-specific genital infection. Laboratory aspects: The genus *Chlamydia*. In *Recent Advances in Sexually Transmitted Diseases*, pp. 275–295. Edited by R. S. Morton and J. R. W. Harris. Churchill Livingstone: Edinburgh.
- Dunlop, E. M. C., Al-Hussaini, M. K., Garland, J. A., Treharne, J. D., Harper, I. A., and Jones, B. R. (1965). Infection of urethra by TRIC agent in men presenting because of 'non-specific' urethritis. *Lancet*, **1**, 1125–1128.
- Handsfield, H. H., Alexander, E. R., Wang, S-P., Pedersen, A. H. B., and Holmes, K. K. (1976). Differences in the therapeutic response of *Chlamydia*-positive and *Chlamydia*-negative forms of nongonococcal urethritis. *Journal of the American Venereal Disease Association*, **2**, 5–9.
- Harnisch, J. P., Berger, R. E., Alexander, E. R., Monda, G., and Holmes, K. K. (1977). Aetiology of acute epididymitis. *Lancet*, **1**, 819–821.
- Heap, G. (1975). Acute epididymitis attributable to chlamydial infection—preliminary report. *Medical Journal of Australia*, **1**, 718–719.
- Hilton, A. L., Richmond, S. J., Milne, J. D., Hindley, F., and Clarke, S. K. R. (1974). *Chlamydia A* in the female genital tract. *British Journal of Venereal Diseases*, **50**, 1–10.
- Holmes, K. K., Handsfield, H. H., Wang, S-P., Wentworth, B. B., Turck, M., Anderson, J. B., and Alexander, E. R. (1975). Etiology of nongonococcal urethritis. *New England Journal of Medicine*, **292**, 1199–1205.
- Nayyar, K. C., O'Neill, J. J., Hambling, M. H., and Waugh, M. A. (1976). Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases. *British Journal of Venereal Diseases*, **52**, 396–398.
- Oriel, J. D. (1976). Nature, diagnosis, and management of non-specific urethritis. *Bulletin of the New York Academy of Medicine*, **52**, 877–885.
- Oriel, J. D., Reeve, P., Wright, J. T., and Owen, J. (1976). Chlamydial infection of the male urethra. *British Journal of Venereal Diseases*, **52**, 46–51.
- Richmond, S. J., and Sparling, P. F. (1976). Genital chlamydial infections. *American Journal of Epidemiology*, **103**, 428–435.
- Rodin, P. (1971). Asymptomatic non-specific urethritis. *British Journal of Venereal Diseases*, **47**, 452–453.
- Schachter, J., Causse, G., and Tarizzo, M. L. (1976). Chlamydiae as agents of sexually transmitted diseases. *Bulletin of the World Health Organisation*, **54**, 245–254.
- Terho, P. (1978). Isolation techniques of *Chlamydia trachomatis* from patients with nonspecific urethritis. *Dermatologische Monatschrift*, in press.