

Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases

K. C. NAYYAR,* J. J. O'NEILL,† M. H. HAMBLING,† AND M. A. WAUGH*

From the Department of Sexually Transmitted Diseases, General Infirmary, Leeds*, and the Virology Department, Public Health Laboratory, Leeds†

Summary

Attempts were made to isolate *Chlamydia trachomatis* from the cervix of 300 women attending a clinic for sexually transmitted diseases in Leeds. The women were divided into four groups:

- (1) 130 were consorts of men suffering from non-specific urethritis;
- (2) 66 were suffering from gonorrhoea, or were consorts of men suffering from this disease;
- (3) 56 were suffering from other sexually transmitted diseases;
- (4) 48 had no evidence of STD.

The overall isolation rate of *Chlamydia trachomatis* was 20 per cent. Positive results were obtained in 30 per cent. of Group 1, in 27.3 per cent. of Group 2, in 3.6 per cent. of Group 3, and in 2.1 per cent. of Group 4. No pathogenic sign or symptom of *Chlamydia trachomatis* infection of the cervix was detected.

Introduction

Since the isolation of *Chlamydia trachomatis* from the human genital tract in 1959 (Jones, Collier, and Smith), further studies have confirmed the isolation of chlamydial agents from patients with non-specific genital infections (NSGI). They have been isolated from 35 to 45 per cent. of male patients suffering from non-specific urethritis (NSU) by Dunlop, Vaughan-Jackson, Darougar, and Jones (1972), Oriol, Reeve, Powis, Miller, and Nicol (1972), and Richmond, Hilton, and Clark (1972).

The isolation rate of *C. trachomatis* from unselected women attending clinics for the treatment of sexually transmitted diseases (STD) has been reported as 31 per cent. by Hilton, Richmond, Milne, Hindley, and Clark (1974), 19 per cent. by Hobson, Johnson,

Rees, and Tait (1974), 18 per cent. by Oriol, Powis, Reeve, Miller, and Nicol (1974), and 12 per cent. by Burns, Darougar, Thin, Lothian, and Nicol (1975). In view of this varying incidence of chlamydial isolations reported in these publications, it was decided to carry out an investigation to determine the incidence of *C. trachomatis* among women attending the STD clinic at Leeds.

Material and Methods

The patients involved in this investigation attended the STD Clinic at Leeds General Infirmary between October 1975 and March 1976. Those investigated were either attending the clinic for the first time or were reporting with a new complaint. They were classified into the following groups:

- (1) Contacts of men suffering from NSU;
- (2) Women suffering from gonorrhoea or contacts of men suffering from gonorrhoea;
- (3) Women suffering from other sexually transmitted diseases;
- (4) Women with no evidence of any sexually transmitted disease.

A detailed history was taken with special reference to genito-urinary symptoms, sexual contacts, contraceptive practices, last menstrual period, and any recent treatment. Routine clinical examination was carried out and the appearance of the cervix carefully recorded. To attempt to achieve uniformity, all the patients were examined by one of two clinicians.

Urethral and cervical smears were stained by Gram's method and examined for the presence of Gram-negative diplococci. Specimens from the urethra and cervix were also inoculated on to the medium described by Thayer and Martin (1966) but without antibiotics and incubated at 37°C. in an atmosphere of air plus 5 per cent. CO₂. High vaginal smears were stained with Gram stain and examined for the presence of Gram-positive organisms and mycelia. High vaginal specimens were also inoculated on to Sabouraud's medium. A wet preparation of the vaginal specimen was examined for the presence of *Trichomonas vaginalis* and the specimen was also cultured in Feinberg-Whittington medium. Cervical smears for cytological examination were taken using Ayer's spatula.

Specimens for the isolation of *C. trachomatis* were collected by gently rotating a cotton-wool swab in the

cervical canal and then breaking off the tip of the swab into a small screw-capped bottle containing 2.5 ml. transport medium (see below for details). The specimens were transferred to the virology laboratory within 3 hrs, during which time they were kept at approximately 4°C. The methods used for the isolation of *C. trachomatis* in untreated McCoy cells were essentially those described by Hobson and others (1974), except that the rate of centrifugation was increased to approximately 3,000 G. McCoy cell growth medium, to which 0.5 per cent. glucose and 10 per cent. sorbitol had been added, was used as the transport medium.

Results

In this study 300 patients were investigated and classified according to the diagnosis in one of four groups (Table IV). The patients in whom the clinical and laboratory examinations revealed nothing abnormal were placed in Group 4.

C. trachomatis isolation

Specimens from 300 patients cultured for *C. trachomatis* gave sixty positive results. Of these patients 58 were Caucasian, two negroid, and none Asian, whereas the 240 negative cultures were obtained from 220 Caucasian patients, fifteen negroid, and five Asian.

The age distribution is shown in Table I. The average age of the chlamydia-positive group was 23.2 (range 16 to 42) and that of the chlamydia-negative group 21.7 (range 15 to 46). Table II shows that the majority of the patients were taking oral contraceptives, but one woman from the positive group and seven from the negative group were pregnant.

TABLE I Age distribution of 300 women

Chlamydia	Age group (yrs)							
	15-20	21-25	26-30	31-35	36-40	41-45	46-50	Totals
Positive	20	33	6	—	—	1	—	60
Negative	97	63	47	20	7	4	2	240

TABLE II Distribution of women according to their contraceptive practices

Chlamydia	Oral contraceptive	Cap/Condom	Intrauterine device	None
Positive	38	3	6	13
Negative	123	13	15	89

The stage of the menstrual cycle on the day of examination of the patients is shown in Table III. The distribution of the patients according to the diagnosis is shown in Table IV. In Group 3 *Trichomonas vaginalis* was isolated from eighteen, *Candida albicans* from 23, and *Herpesvirus hominis* type 2 from

TABLE III Stage of menstrual cycle on day of examination

Chlamydia	Stage of cycle (days)				Pregnant
	1-7	8-14	15-21	>21	
Positive	17	20	17	5	1
Negative	76	79	43	35	7

TABLE IV Distribution of patients according to diagnostic category

Chlamydia	Group 1 NSU contacts	Group 2 Gonorrhoea	Group 3 Other STD	Group 4 NAD
Positive	39	18	2	1
Negative	91	48	54	47

four patients. In two women *Pediculosis pubis* was seen, and eight patients had genital warts.

Of the sixty women from whom *Chlamydia trachomatis* was isolated, 46 were asymptomatic, whereas of the 240 chlamydia-negative patients 140 were asymptomatic. In the positive group seven women complained of a discharge, three of a discharge and irritation, three of a discharge, dysuria, and irritation, and one of irritation only. In the negative group forty women complained of a discharge, 23 of a discharge and irritation, sixteen of a discharge, dysuria, and irritation, and seventeen of irritation only. Clinical examination revealed that, in the positive group, the cervix appeared normal in nineteen, there was a mucopurulent or purulent cervical discharge in 36, and a cervical erosion in 29; macrofollicles were seen on the cervix of three women. In the negative group the cervix appeared normal in 150, there was a mucopurulent or purulent cervical discharge in 85, a cervical erosion in 71, and macrofollicles on the cervix in six.

Discussion

The *C. trachomatis* isolation rate of 20 per cent. found in this study may be compared with rates of 31 per cent. (Hilton and others, 1974), 19 per cent. (Hobson and others, 1974), 18 per cent. (Orlén and others, 1974), and 12 per cent. (Burns and others, 1975) reported in similar studies. The differences may be attributed to varying sensitivity of the isolation technique, difference in the prevalence of chlamydia infection in various areas at different times of the year, and the possible association of *C. trachomatis* with other sexually transmitted diseases. The largest group in this study (130 women) were consorts of men suffering from non-specific urethritis, and *C. trachomatis* was isolated from 30 per cent. of these contacts. Other workers

have reported similar isolation rates of *C. trachomatis* from female contacts of men with NSU: 34 per cent. by Hilton and others (1974), 23.5 per cent. by Hobson and others (1974), and 22 per cent. by Burns and others (1975). Oriel and others (1974) separated the contacts into two categories: women whose partners had suffered one attack of NSU and women whose partners had had recurrent attacks. They reported rates of *C. trachomatis* isolation of 33 and 14 per cent. respectively.

When comparing the Leeds rates of isolation with those of some other workers it should be noted that in the Leeds study untreated McCoy cells were used, and this lack of previous treatment, either by irradiation, Cytochalasin B, or idoxuridine, appears to have had no significant adverse effect on the isolation rate.

In the group of women suffering from gonorrhoea or in contact with men suffering from gonorrhoea, the Leeds rate of isolation (27.3 per cent) is rather lower than that described by other workers, and further investigations on this group are planned. *C. trachomatis* was isolated from one of the 48 women studied in whom there was no evidence of any sexually transmitted disease; this is similar to the isolation rate of 2 per cent. reported by Oriel and others (1974), of 3 per cent. by Hilton and others (1974), of 4 per cent. by Burns and others (1975).

The majority (76.6 per cent.) of the patients from whom *C. trachomatis* was isolated were asymptomatic. In those patients who had some symptoms no particular factor could be significantly associated with chlamydial infection. Clinically the appearance of the vagina and cervix was normal in nineteen of the sixty patients from whom *C. trachomatis* was

isolated. The macrofollicles on the cervix considered to be pathognomonic of chlamydial infection of the cervix (Dunlop, Harper, Al-Hussaini, Garland, Treharne, Wright and Jones, 1966) were seen in three of the sixty patients from whom *C. trachomatis* was isolated, but they were also seen in six of the 240 women from whom the organism was not isolated. There thus appears to be no particular sign associated with chlamydial infection of the female genital tract, and culture appears at present to be the only certain method of making a diagnosis.

References

- BURNS, D. C. MCD., DAROUGAR, S., THIN, R. N., LOTHIAN, L., and NICOL, C. S. (1975) *Brit. J. vener. Dis.*, **51**, 314
- DUNLOP, E. M. C., HARPER, I. A., KHALAF AL-HUSSAINI, M., GARLAND, J. A., TREHARNE, J. D., WRIGHT, D. J. M., and JONES, B. R. (1966) *Ibid.*, **42**, 77
- , VAUGHAN-JACKSON, J. D., DAROUGAR, S., and JONES, B. R. (1972) *Ibid.*, **48**, 425
- HILTON, A. L., RICHMOND, S. J., MILNE, J. D., HINDLEY, F., and CLARKE, S. K. R. (1974) *Ibid.*, **50**, 1
- HOBSON, D., JOHNSON, F. W. A., REES, E., and TAIT, I. A. (1974) *Lancet*, **2**, 555
- JONES, B. R., COLLIER, L. H., and SMITH, C. H. (1959) *Ibid.*, **1**, 902
- ORIEL, J. D., POWIS, P., REEVE, P., MILLER, A., and NICOL, C. S. (1974) *Brit. J. vener. Dis.*, **50**, 11
- , REEVE, P., POWIS, P., MILLER, A., and NICOL, C. S. (1972) *Ibid.*, **48**, 429
- RICHMOND, S. J., HILTON, A. L., and CLARKE, S. K. R. (1972) *Ibid.*, **48**, 437
- THAYER, J. D., and MARTIN, J. E. (1966) *Publ. Hlth Rep. (Wash.)*, **81**, 559