

GENITAL INFECTIONS WITH CHLAMYDIA TRACHOMATIS

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INTRODUCTION

IN GENITOURINARY MEDICINE, the term "non-specific genital infection" (NSGI) refers to an inflammation of any part of the genital tract in which no established pathogen is easily detectable. The most studied form of NSGI is non-specific urethritis (NSU) in the male, which has increased in epidemic proportions in the past decade to overtake gonococcal urethritis to become the commonest notifiable sexually-transmitted disease in many countries today (Catterall, 1977). Results of extensive research into the aetiology of NSU indicate that *Chlamydia trachomatis*, an obligate intracellular parasite known to cause trachoma and a wide range of other ocular diseases, is probably the most important causative agent. From patients attending venereal disease clinics in England and USA, *C. trachomatis* was isolated from the urethra of about 40% of men with NSU (Dunlop *et al.* 1972, Oriel *et al.* 1972, Richmond *et al.* 1972, Holmes *et al.* 1975) and from about 30% of female contacts of these infected men (Oriel *et al.* 1972, Hilton *et al.* 1974). The mere isolation of *C. trachomatis* from patients does not prove that this organism is responsible for the clinical disease. However, the recovery rates from control men and women have been consistently low, around 0 to 7% (Oriel *et al.* 1972, Richmond *et al.* 1972, Schachter *et al.* 1975, Holmes *et al.* 1975). Antibodies to *C. trachomatis* are detected in patients with NSU much more often than in controls without NSU and seroconversion of specific immunoglobulin m antibody titres have been demonstrated by the

micro-immunofluorescence technique in recently acquired infections (Holmes *et al.* 1975). Clinical signs and symptoms often occur in patients from whom *C. trachomatis* is recovered. Dunlop *et al.* (1964, 1972) reported the presence of microfollicles and other changes in the marginal areas of the cervix and demonstrated papillary congestion and meatal follicles in the male urethra. Rees *et al.* (1977) studied female contacts of men with NSU and associated chlamydial infection of the cervix with hypertrophied erosion of the cervix and endocervical oedema, congestion and mucopus, all of which regressed after treatment with oxytetracycline. Experimental inoculation of *C. trachomatis* into the urethra of primates produced follicular lesions and provided further evidence that chlamydia can behave as a primary pathogen in the genital tract (Gale *et al.* 1977).

C. trachomatis has been isolated in the past with the use of embryonated hen's eggs, a rather laborious and time consuming method. Recent advances in tissue culture techniques for the culture of this agent have greatly facilitated studies on its role in infections of the genital tract. In May 1978, tissue culture techniques were set up in the Department of Medical Microbiology, University Hospital, Kuala Lumpur to isolate *C. trachomatis* from genital exudates. We report here some preliminary findings and describe the clinical histories of our first 3 chlamydia culture positive cases.

MATERIALS AND METHODS

Patients and specimens:

Outpatients from the University Hospital, Kuala Lumpur and two private clinics were examined. All male patients attended the clinics because they had symptoms of urethritis. Most female patients presented with vaginal discharge while 21 were referred for exclusion of sexually-transmitted diseases. From male patients, endo-urethral swabs were taken either with cotton-tipped wooden applicator stick swabs or with cotton-tipped wire swabs. Endocervical swabs were collected from female patients. After collection, swabs were broken into bijoux bottles containing 2 ml of Eagle's growth medium supplemented with 0.05 M glucose. These were then transported in wet ice to the laboratory for pro-

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Table 1

Isolation rate of *Chlamydia trachomatis* from genital exudates

Specimen	No. of uncontaminated specimens	No. yielding chlamydia (%)
male urethral exudates	127	2 (1.6)
cervical exudates	70	3 (4.3)
Total	197	5 (2.5)

cessing. Specimens were either inoculated directly or kept frozen at -70°C until cell cultures were available.

Cell cultures:

McCoy cells for chlamydia isolation were kindly supplied by Dr. E.H. Sng, Department of Pathology, Singapore General Hospital. The techniques used for growing the McCoy cells and for the isolation of chlamydia were described by Reeve *et al.* (1975) but with minor modifications. The cells were grown in Eagle's minimal essential medium containing foetal calf serum 10% v/v, glutamine 1% of stock (30 mg/ml), amphotericin B 2.5 ug/ml and gentamicin 10 ug/ml. When confluent, the cells were trypsinised and then suspended in Eagle's growth medium containing 5-iodo 2' deoxyuridine (IUDR) 25 ug/ml to give a count of 10^5 cells/ml. 1 ml of this cell suspension was seeded into each of 5 ml, flat bottomed plastic tubes containing a 13 mm diameter coverslip. The tubes were used for chlamydia isolation after incubation at 37 C for 3 to 5 days when confluent growth was obtained.

Inoculation:

Each specimen was thoroughly mixed for a few seconds using a vortex mixer after which 1 ml of the specimen was inoculated into each of 2 tubes of idoxuridine-treated McCoy cells. The inoculum was centrifuged onto the cells at 3000 rpm in a MSE bench centrifuge at room temperature for 1 hour. The tubes were then incubated at 35°C for 48 — 72 hours after which the cells were fixed with methanol, stained with Giemsa and examined by darkfield illumination for characteristic intracytoplasmic inclusions of *C. trachomatis*.

Other examinations:

A separate specimen collected on a charcoal-impregnated swab was taken from each patient for the detection and isolation of other pathogens like *Trichomonas vaginalis*, *Candida albicans* and *Neisseria gonorrhoeae* by conventional methods.

RESULTS

Culture studies

A total of 243 specimens were examined out of which 46 were contaminated by bacteria. The 197 uncontaminated specimens consisted of 127 male urethral exudates and 70 cervical exudates. Out of these 197 specimens only 5 yielded chlamydia, thus giving an isolation rate of 2.5% (Table I).

Case reports

The 5 isolates of chlamydia came from 2 men and 3 women, all of whom had a history of either direct or indirect repeated exposure to sexually-transmitted diseases. *C. trachomatis* was the only pathogen isolated in 3 of the cases. One of the remaining 2 cases had concurrent *N. gonorrhoeae* infection and the other had *T. vaginalis* infection (Table II).

The lymphogranuloma venereum complement fixation test (LGVCFT) was done for 4 patients. Paired sera were obtained only from patient K.S.B. The first sample was taken about 2 weeks after onset of symptoms and the second, 6 weeks later. Both sera showed an antibody titre of 8. Low antibody titres of 4 and 8 were detected in 2 other patients. Detailed clinical histories of the first 3 cases are given below.

Table II
 Summary of clinical and laboratory data on 5 chlamydia culture positive cases

Patient	Presenting symptom	Age	Sex	Occupation	Past history of genital infection	Previous treatment for genital infection	Isolation of			Titre of LGVCFIT
							C.tr.	N.gon.	T.vag.	
M.R.	urethral discharge	35	M	Engineer	+	+	+	-	-	4
C.L.M.	low abdominal pain	23	F	Masseur	+	+	+	-	-	2
K.S.B.	vaginal discharge	NK	F	Housewife	NK	-	+	+	-	8,8
L.Y.L.	referred	15	F	Juvenile prostitute	NK	NK	+	-	+	8
T.M.C.	urethral discharge	46	M	Business man	NK	NK	+	-	-	ND

NK = not known

ND = not done

C.tr. = Chlamydia trachomatis

N.gon. = Neisseria gonorrhoeae

T.vag. = Trichomonas vaginalis

Case M.R.

The patient was a 35 year old engine who presented with a discharge per urethra 4 days after exposure in June 1978. On each of 2 visits to his general practitioner, he was given injection kanamycin and one week's supply of Vibramycin. This was because one month prior to this, he had had a similar episode of urethral discharge appearing 3 days after exposure (culture negative for *N. gonorrhoeae*) not responding to ampicillin and probenecid but responded to tetracycline hydrochloride. However, this time, the discharge persisted despite treatment with kanamycin and vibramycin. It was a painless, scanty discharge causing staining of underpants and noticeable only in the early morning. On his third visit he was found to have no abnormal clinical features except for a small amount of whitish urethral exudate produced on penile stripping. The exudate was collected on a swab for microscopy and culture. A direct smear examination showed about 5 plus cells per high power field and no Gram-negative diplococci. The urethral exudate yielded *C. trachomatis* and no other pathogen. The patient was treated with tetracycline hydrochloride 500 milligrams 6 hourly for 3 weeks. His symptoms were aborted and a repeat culture for chlamydia after 3 weeks was negative. Subsequently he was seen again after 2 months, 3 months and 6 months. On each occasion he complained of recurrence of urethral discharge but laboratory examination of his discharge showed no pus cells in the direct smear and no growth of pathogens. He was given further courses of tetracycline and is still being followed up.

Case C.L.M.

The patient was a 23 year old masseuse who, by nature of her profession, was prone to repeated sexually-transmitted infections. She resorted to giving herself daily douches with various antiseptic solutions and swallowing 1 to 2 capsules of antibiotics now and then at her own discretion. Only when her symptoms troubled her would she visit her doctor.

She was first seen in 1976 during which she was treated on several occasions for gonococcal and non-gonococcal pelvic inflammatory disease. In 1977 she was lost from follow-up but on 19th September 1978, she turned up again complaining of a low abdominal pain. On examination, she was found to have tender fallopian tubes and cervicitis. A direct smear examination of the mucopurulent cervical exudate revealed a large number of pus cells and a typical extra-cellular Gram-negative diplococci. A presumptive diagnosis of gonococcal salpingitis was made. After taking endocervical swabs for culture, the patient was treated with spectinomycin 2 grams intramuscular-

ly on 2 consecutive days and ampicillin 500 milligrams 8 hourly for 5 days. The cultures did not grow *N. gonorrhoeae* but yielded *C. trachomatis*. After 10 days, the patient returned with no more abdominal pain but complained of post-coital bleeding. On examination, her fallopian tubes were no longer tender but there were still signs of cervicitis. A repeat cervical smear showed no Gram-negative diplococci and a repeat culture was negative for both *N. gonorrhoeae* and *C. trachomatis*. However, based on the previous chlamydia positive culture report, the patient was treated with tetracycline 500 milligrams 8 hourly for one week. She was presumably rendered asymptomatic as she did not return for further treatment.

Case K.S.B.

The patient was a middle-aged housewife who was seen on 12th January 1979 with 2 weeks history of vaginal discharge. Her husband was a travelling salesman who admitted exposure and treatment by a private practitioner for urethral discharge. On examination, patient was found to have an endocervical polyp, a normal looking cervix with some thick, white cervical exudate. Intracellular Gram-negative diplococci were seen in a direct smear and endocervical cultures grew *C. trachomatis* and a B-lactamase-producing *N. gonorrhoeae*. She was treated with injection kanamycin for her gonorrhoeae and tetracycline 500 milligrams 6 hourly for 3 weeks to clear her chlamydial infection. Six weeks later, a repeat cultural examination was negative for chlamydia.

DISCUSSION

Although asymptomatic infections occur in both men and women (Schachter *et al.* 1975) it is generally accepted that *C. trachomatis* is a pathogen in the lower genital tract. However, not much is known about the full clinical spectrum of chlamydial genital infections. The isolation of the organism from cases of acute epididymitis and prostatitis (Harnisch *et al.* 1977) *acute salpingitis* (Mardh *et al.* 1977) and Reiter's disease (Vaughan-Jackson *et al.* 1972) suggests that, like *N. gonorrhoeae*, *C. trachomatis* may cause local or generalised complications.

Between 10 to 15% of men with NSU suffer frequent relapsing attacks (Catterall 1977). Patient M.R. apparently had several recurrences of NSU after a usual course of tetracycline. During these recurrences, chlamydial cultures were repeatedly negative. This is probably because, after antibiotic therapy, isolation is unlikely to be successful, yet the disease may still be active and liable to recur (Oriel *et al.* 1972). The fact that few or no pus cells were seen in the direct smears does not preclude a bacterial cause for the recurrences as polymorphonuclear cell counts are often nor-

mal in clinical cases. A more thorough investigation is necessary to exclude possible causes of relapses like structural abnormalities and residual infections in the prostate or paraurethral glands. However, some of the "recurrences" of this patient were likely to be reinfections as the patient did admit re-exposure on at least 1 occasion and his sex partner was never investigated or treated for chlamydial infection.

Patient C.L.M. had a history of repeated episodes of pelvic inflammatory disease. As no cultures for chlamydia were done on these occasions, it is not known whether *C. trachomatis* was associated with these infections. On 19th September 1978, she was culture-negative for *N. gonorrhoeae* but *gonorrhoeae* was still a possible concurrent diagnosis for her salpingitis as she improved symptomatically on spectinomycin and ampicillin. The negative culture and atypical extracellular Gram-negative diplococci in her direct smear could have been the result of taking sub-optimal antibiotics bought under the counter. In women, *C. trachomatis* very often occurs along with other sexually-transmitted pathogens, particularly with the gonococcus. Hilton *et al.* (1974), isolated chlamydia from 63% of women with gonorrhoea. They suggested that chlamydia gives rise to persistent but frequent quiescent infections in the genital tract in promiscuous females and that *N. gonorrhoeae* may reactivate these infections. This may well be the case in patient C.L.M. A repeat pre-tetracycline therapy culture from this patient failed to yield chlamydia but this may be just due to variability in specimen collection and processing.

The low complement fixation titres in our cases of chlamydial infection were not unexpected. The LGVCFT measures antibodies to a group antigen and has been found by several workers to be very insensitive for the diagnosis of chlamydial genital infections. Dunlop *et al.* (1972), examined the sera of 44 chlamydial culture positive men and obtained only 29.5% LGVCFT positives with the highest titre being 8. Out of 34 female contacts of these men, 11 were positive in the LGVCFT with a maximum titre of only 4. Oriel *et al.* (1972), also used the LGVCFT to examine sera from chlamydia culture positive NSU cases and found 38% with antibody titres of 16 or above.

Our 2.5 % isolation rate of chlamydia is probably an under estimation due to technical reasons or it may be partly due to lower rate of chlamydia infection in this region. In Singapore, a 5.5% isolation rate of chlamydia has been reported (Ministry of Health, Singapore, 1978). Our low isolation rate must not be compared with the 30 — 40% isolation rates reported in other studies because these higher percentages were mostly obtained from a high risk population

of men with NSU and their female contacts attending venereal disease clinics. Our study population is a mixed group of patients with genital discharges attending ordinary medical and gynaecological clinics and one venereal disease clinic. In many instances, the clinical diagnosis was not NSU or NSGI but just urethritis or vaginitis for investigation and which later turned out to be gonococcal, candidal or trichomonal infections as well as infections by other microbial agents. The detailed analysis of infective causes of lower genital tract pathology forms the substance of another report under preparation.

SUMMARY

Chlamydia trachomatis is isolated for the first time in Malaysia from patients with genital tract infections. Idoxuridine treated McCoy cells were used for culturing the organisms which were demonstrated by Giemsa staining. The first 3 of 5 culture positive cases are described to illustrate some clinical features associated with chlamydial genital infections.

ACKNOWLEDGEMENTS

We are very grateful to Dr. E.H. Sng, Department of Pathology, Singapore General Hospital, for his generous gift of McCoy cells and his helpful advice.

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