

Interrelationship of *Chlamydia trachomatis* and other pathogens in the female genital tract

G. L. RIDGWAY AND J. D. ORIEL

From the Departments of Clinical Microbiology and Genito-urinary medicine, University College Hospital, London

SUMMARY The isolation of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Candida albicans* in the female genital tract was studied in 1323 patients attending a venereal disease clinic. Disruption of the cell monolayers used for the isolation of *C. trachomatis* was significantly associated with the presence of *T. vaginalis*; this effect was markedly reduced by the addition of vancomycin to gentamicin and amphotericin B in the transport and growth media. The only significant positive association was the more frequent isolation of *C. trachomatis* in the presence of *N. gonorrhoeae*. There was a negative association between *N. gonorrhoeae* and *C. albicans* and between *T. vaginalis* and *C. albicans*, the fungus being isolated significantly less frequently when these microorganisms were present.

The female genital tract provides a satisfactory environment for many pathogenic microorganisms, and multiple infections are therefore common (Wentworth *et al.*, 1973). In sexually transmitted diseases, the causative organisms of more than one infection may be acquired simultaneously, or one species of organism may join other species which have persisted since a previous infecting episode. The presence of multiple infections may make the interpretation of symptoms and signs relating to a particular pathogen difficult.

Bacterial interaction is a familiar phenomenon, as exemplified by the satellitism of *Haemophilus influenzae* around staphylococcal colonies, or the production of antibiotics and bacteriocines by microorganisms *in vitro* (Wickman, 1970). That bacterial interactions may occur in the genital tract has been suggested by the observations of Kraus *et al.* (1976) on the inhibitory effect of certain bacterial species against *N. gonorrhoeae*, and it has been suggested that *C. albicans* may also be inhibitory (Hipp *et al.*, 1974).

In the present investigation we have considered the incidence and interrelationship of four commonly isolated microorganisms, *N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, and *C. albicans*. Specimens were collected from a group of women attending a venereal disease clinic, and statistical methods were used to seek associations between pairs of organisms.

Patients and methods

Between January and April 1975, 1323 consecutive female patients were examined. Of these, 268 were sexual contacts of men with gonorrhoea, 485 of men with non-gonococcal urethritis, and seven of men with other sexually transmitted diseases. The remaining 563 women attended with a request for examination because of either symptoms or anxiety about possible infection.

After interview patients were examined in the lithotomy position; a Cusco speculum was used to expose the cervix. Specimens were collected from the urethra and cervix for Gram staining and culture for *N. gonorrhoeae*, from the cervix for cell culture for *C. trachomatis*, and from the vagina as a wet mount for the identification of trichomonads and yeast cells and hyphae and for culture for *T. vaginalis* and *Candida* spp.

BACTERIOLOGY

Chlamydia trachomatis

An endocervical swab was broken into 2 ml transport medium and held at 4°C for not more than two hours. After agitation, 1 ml of this material was used to inoculate a monolayer of McCoy cells pretreated with 5 iodo 2' deoxyuridine. All media contained amphotericin B, 2.5 µg/ml and gentamicin, 10 µg/ml. The preparation was centrifuged and incubated at 37°C for 48 hours; the cells were then fixed, stained

Received for publication 28 February 1977

with iodine, and examined microscopically for inclusions (Reeve *et al.*, 1975).

Neisseria gonorrhoeae

Endocervical and urethral swabs were plated directly on to warm modified Thayer-Martin medium (Phillips *et al.*, 1972) and incubated at 37°C in 10% carbon dioxide for 36 hours. Suspect colonies were identified by Gram stain, oxidase reaction, and specific immunofluorescence.

Trichomonas vaginalis

Vaginal specimens suspended in normal saline were examined immediately under a 1/6 inch high dry objective for motile trophozoites. Specimens for culture were incubated at 37°C for 48 hours in Feinberg-Whittington medium and then examined microscopically as above.

Candida albicans

Swabs from the vagina were inoculated directly on to Sabouraud's medium and incubated at 37°C for 48 hours. The presence of *C. albicans* was confirmed by subculture into serum for germ tube identification (Mackenzie, 1962).

The isolation data were subjected to computer analysis, and the level of statistical significance was assessed by calculation of the Z score between the basal isolation rate of an organism alone and in combination with other organisms. The level of significance was taken as 99% ($P < 0.01$).

Results (Table 1)

CONTAMINATION OF CHLAMYDIA CULTURES

In 187 (14.1%) of 1323 cervical specimens examined by cell culture for *C. trachomatis*, the supernatant after incubation was cloudy and the cell monolayer so disrupted that inclusion formation could not be detected; these specimens were regarded as 'contaminated' by bacterial action. It is of interest that while 40 (35.1%) of 114 specimens from patients with trichomoniasis were 'contaminated', only 147 (12.2%) of 1209 patients without trichomoniasis

yielded 'contaminated' *Chlamydia* cultures. This difference is significant ($P < 0.001$). There were no associations between 'contaminated' *Chlamydia* cultures and infection by *N. gonorrhoeae* or *C. albicans*.

Investigation of the 'contaminated' specimens showed that, in the majority of these, *Streptococcus* spp. were isolated. The subsequent addition of vancomycin, 10 µg/ml, to the gentamicin and amphotericin B already in use has led to a marked decrease in the numbers of 'contaminated' specimens from women both with and without trichomoniasis.

ASSOCIATION OF MICROORGANISMS

C. trachomatis was recovered from cervical specimens from 151 (21.1%) of 717 patients in whom no other infecting agent was present. However, *C. trachomatis* was isolated in 75 (36.6%) of 205 cervical specimens from women infected by *N. gonorrhoeae*. This difference is significant ($P < 0.001$). *C. trachomatis* was isolated from 30 (18.8%) of 160 patients infected by *C. albicans* and from 11 (22.4%) of 49 women infected by *T. vaginalis*; there was thus no significant association between *C. trachomatis* and either *C. albicans* or *T. vaginalis*.

As sole pathogen, *C. albicans* was recovered from 184 (18.5%) of 996 patients. In the presence of *N. gonorrhoeae*, however, *C. albicans* was recovered from only 14 (6.6%) of 213 patients. This negative association is significant ($P < 0.01$). *C. albicans* was present in 8 (4.2%) of 192 patients infected by *T. vaginalis*. Again, this negative association is significant ($P < 0.01$).

PREVALENCE OF PATHOGENS

Over one half of the population studied was infected by one or more of the four selected pathogens. The commonest of these was *C. trachomatis*, which occurred in 23.7% of patients; *N. gonorrhoeae* was recovered from 17.5%, *T. vaginalis* from 7.2%, and *C. albicans* from 15.6% of women. The occurrence of multiple infections was apparent; of 1136 patients with complete pathogen information (ie, excluding 187 women with 'contaminated' cultures for *C.*

Table 1 *Interrelationship of pathogens isolated from 1323 patients*

<i>C. trachomatis</i>	<i>Pathogens isolated</i>								<i>Total</i>
	<i>None</i>	<i>N. gonorrhoeae</i>	<i>T. vaginalis</i>	<i>C. albicans</i>	<i>N. gon + T. vag</i>	<i>N. gon + C. alb</i>	<i>T. vag + C. alb</i>	<i>N. gon + T. vag + C. alb</i>	
Isolated	151	63	11	30	8	4	2	0	269
Not isolated	566	109	38	130	12	9	3	0	867
Specimen contaminated	95	27	25	24	11	1	3	1	187

N. gon = *Neisseria gonorrhoeae*; *T. vag* = *Trichomonas vaginalis*; *C. alb* = *Candida albicans*.

Table 2 Frequency of multiple isolation of pathogens from 1136 female patients

No. of pathogens	No. of patients	Percentage
0	566	49.8
1	428	37.7
2	128	11.3
3	14	1.2
Total	1136	100

trachomatis), 570 (50.2%) were infected with at least one pathogen, and 142 (12.5%) with more than one pathogen (Table 2).

Discussion

The prevalence of *C. trachomatis* in our study group (23.7%) was comparable with figures reported from other VD clinics in Britain and the USA (Wentworth *et al.*, 1973; Hilton *et al.*, 1974; Oriol *et al.*, 1974). In comparison, *N. gonorrhoeae* was less common, occurring in 18.5% of women.

The relative difficulty of obtaining valid isolation results for *C. trachomatis* in the presence of *T. vaginalis* poses a problem for the microbiologist. If 'contamination' of specimens is an effect of toxic metabolites of *T. vaginalis*, the solution might be to include an antitrichomonal drug such as metronidazole in the transport medium; this drug has little action against *C. trachomatis* (Ridgway, 1977). On the other hand, 'contamination' may be due to the release of bacteria ingested by *T. vaginalis*; in this case, additional microbials could be added to the media to eliminate possible contaminants from this source. The combination of streptomycin and vancomycin was formerly used to reduce bacterial contamination (Gordon and Quan, 1971), and we have found a marked reduction in the numbers of 'contaminated' specimens when we used gentamicin with vancomycin, and this combination seems to us to have definite advantages.

Two combinations of pathogens were uncommon—*N. gonorrhoeae* and *C. albicans*, and *T. vaginalis* and *C. albicans*. Like Rothenberg *et al.* (1976), we were unable to show a significant association between *N. gonorrhoeae* and *T. vaginalis*, although this has been found by others (Nielsen *et al.*, 1974). However, in the present study *N. gonorrhoeae* was significantly associated with *C. trachomatis*: infection by this organism was noted in 75 (36.6%) of 205 women with gonorrhoea. In a previous study (Oriol *et al.*, 1974) we reported that 32% of such women were infected, and Hilton *et al.* (1974) have recorded a group of 40 women with gonorrhoea, of whom 25 (62%) were infected by *C. trachomatis*. This double infection is of importance. There is good evidence

that, after the treatment of gonorrhoea, *C. trachomatis* often remains in the genital tract of both sexes (Oriol *et al.*, 1976). In men, the symptoms and signs of infection persist or recur ('postgonococcal urethritis'); it is not known whether a clinical syndrome of postgonococcal chlamydial infection can be defined in women, but *C. trachomatis* has been shown to be statistically associated with clinical evidence of cervicitis (Oriol *et al.*, 1974).

The enhancement or diminution of the isolation of one microorganism in the presence of another is due to the operation of several simultaneous factors—the direct action of one organism against another, environmental conditions which selectively favour the growth of one organism, the differential operation of host factors, and epidemiological considerations. These factors are all operative within the lower genital tract in women. In planning diagnostic microbiology for women with sexually transmitted diseases, the possibility of multiple infections must be kept in mind, and the interaction of pathogenic microorganisms should also be remembered.

We are grateful to Dr E. Joan Stokes for providing laboratory facilities and to Miss Jean Owen and Miss Lam Po Tang for technical assistance.

This work was supported by a grant from the Medical Research Council.

References

- Gordon, F. B. and Quan, A. L. (1971). Isolation of *Chlamydia trachomatis* from the human genital tract by cell culture. *Trachoma and Related Disorders*, edited by R. L. Nichols, pp. 476-484. Excerpta Medica Amsterdam.
- 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.
- Hipp, S. S., Lawton, W. D., Chen, N. C., and Gaafar, H. A. (1974). Inhibition of *Neisseria gonorrhoeae* by a factor produced by *Candida albicans*. *Applied Microbiology*, **27**, 192-196.
- Kraus, S. J., Geller, R. C., Perkins, G. H., and Rhoden, D. L. (1976). Interference of *Neisseria gonorrhoeae* growth by other bacterial species. *Journal of Clinical Microbiology*, **4**, 288-295.
- Mackenzie, D. W. R. (1962). Serum tube identification of *Candida albicans*. *Journal of Clinical Pathology*, **15**, 563-565.
- Nielsen, R., Søndergaard, J., and Ullman, S. (1974). Simultaneous occurrence of *Neisseria gonorrhoeae*, *Candida albicans* and *Trichomonas vaginalis*. *Acta Dermatovenereologica*, **54**, 413-415.
- Oriol, J. D., Powis, P. A., Reeve, P., Miller, A., and Nicol, C. S. (1974). Chlamydial infections of the cervix. *British Journal of Venereal Diseases*, **50**, 11-16.
- Oriol, J. D., Ridgway, G. L., Reeve, P., Beckingham,

- D. C., and Owen, J. (1976). The lack of effect of ampicillin plus probenecid given for genital infections with *Neisseria gonorrhoeae* on associated infections with *Chlamydia trachomatis*. *Journal of Infectious Diseases*, **133**, 568-571.
- Phillips, I., Humphrey, D., Middleton, A., and Nicol, C. S. (1972). Diagnosis of gonorrhoea by culture on a selective medium containing vancomycin, colistin, nystatin and trimethoprim (VCNT). A comparison with Gram-staining and immunofluorescence. *British Journal of Venereal Diseases*, **48**, 287-292.
- Reeve, P., Owen, J., and Oriel, J. D. (1975). Laboratory procedures for the isolation of *Chlamydia trachomatis* from the human genital tract. *Journal of Clinical Pathology*, **28**, 910-914.
- Ridgway, G. L. (1977). Unpublished data.
- Rothenberg, R. B., Simon, R., Chipperfield, E., and Catterall, R. D. (1976). Efficacy of selected diagnostic tests for sexually transmitted diseases. *Journal of the American Medical Association*, **235**, 49-51.
- Wentworth, B. B., Bonin, P., Holmes, K. K., Gutman, L., Wiesner, P., and Alexander, E. R. (1973). Isolation of viruses, bacteria and other organisms from venereal disease clinic patients: methodology and problems associated with multiple isolations. *Health Laboratory Science*, **10**, 75-81.
- Wickman, K. (1970). Studies of bacterial interference in experimentally produced burns in guinea pigs. *Acta Pathologica Microbiologica Scandinavica*, **78B**, 15-28.