

Frequency of *N. gonorrhoeae*, *T. vaginalis*, and *C. albicans* in female venereological patients

A one-year study

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Many women who attend V.D. clinics do not prove to have gonorrhoea despite the fact that they have symptoms, particularly vaginal discharge. These symptoms are often caused by other pathogens such as *Trichomonas vaginalis* and *Candida albicans*, a possibility that has hitherto received comparatively little attention in Swedish V.D. clinics. Studies in England (e.g. Catterall, 1970) have shown that *Trichomonas vaginalis* and *Candida albicans* may be found even more often than *N. gonorrhoeae* in patients with vaginal discharge visiting such clinics. In 1971 catchment areas with fixed populations were determined for hospitals in Stockholm, thus increasing the interest in an epidemiological one-year study of these three infections.

An earlier investigation of named male contacts has shown that, in those found to have gonorrhoea, this was nearly always diagnosed at the first examination (Enfors, Eriksson, Kaaman, and von Krogh, 1973). Does the same apply to corresponding female groups?

The number of cases of asymptomatic gonorrhoea in females is considered to be higher now than before (Pariser, 1972). Is there any difference in this respect between 'named contacts' and others?

In addition, the frequency, as well as type, of complications of gonorrhoea seen at first visits is a question of epidemiological interest. Moreover, we think it important to see whether complications appear during the follow-up period, particularly since the standard treatment of gonorrhoea differs from the antibiotic therapy of other infections.

Patients and methods

From November, 1971, to October, 1972, specimens were collected from all female patients at all visits for the detection of *N. gonorrhoeae*, *Trichomonas vaginalis*, and *Candida albicans*. The patients were questioned about their symptoms. Those who had abdominal pain were examined by palpation for evidence of salpingitis, and other complications were noted.

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DIAGNOSIS

N. gonorrhoeae Specimens were taken from the urethra, cervix, and rectum. As a rule, throat swabs were not taken unless requested by the patient. Direct smears were stained with methylene blue. Specimens for culture were taken with a charcoal-coated swab, which was placed in Stuart's transport medium and sent to the hospital laboratory, where it was cultured on Thayer-Martin medium within 1 to 12 hours.

Trichomonas vaginalis Specimens for direct smears were taken from the posterior fornix with a cotton-wool swab which was placed in a tube containing 1 ml. saline. The tube was gently shaken and then 1 or 2 drops were placed on a slide and examined immediately under the phase contrast microscope. The criterion for a positive smear was motile flagella. Direct microscopy was performed together with cultures during the first half of the study period, after which time only cultures were done. Direct microscopy was always performed by the same person.

Specimens for culture were taken with a charcoal-coated swab which was placed in Stuart's transport medium and sent to the laboratory, where the swab was placed in a prewarmed tube containing 8 ml. Diamond medium (Diamond, 1957). The tubes were incubated at 37°C. in a normal atmosphere. During the first 6 months the cultures were read on the second, fifth, and seventh day, and during the second 6-month period they were read on the second and fifth day. A small amount of medium was drawn up in a pipette from the bottom of the tube, placed on a slide, and examined under the phase contrast microscope. If no motile forms were seen after the seventh (fifth) day, the culture was considered negative.

Candida albicans Specimens for culture were taken from the posterior fornix with a cotton-wool swab, which was placed in Stuart's transport medium and cultured on Sabouraud's agar plates, which were kept for 48 hrs. Yeast colonies were inoculated into human serum and incubated at 37°C. for 2 to 3 hrs. The formation of germ-tubes indicated *Candida albicans*.

Treatment

Alternate patients with uncomplicated gonorrhoea were treated with either 2 g. ampicillin orally in a divided dose with a 5-hr interval or with doxycycline 0.3 g. in a single oral dose. An account of the treatment and results has been given by Enfors and Eriksson (1975). Patients with complications were treated with oral ampicillin 1 g. thrice daily for at least 7 days.

Follow-up

Patients were told to return for repeat tests at weekly intervals. Those treated for uncomplicated gonorrhoea

were examined two or four times depending on the sensitivity pattern of the organism; those with complications were examined four times. According to Swedish law named women contacts must have three negative examinations before the possibility of gonococcal infection is excluded. With other patients we tried to obtain specimens on at least two occasions.

At the follow-up visits the patients were asked again about symptoms and any side-effects of the drugs. New complications were recorded.

Statistical methods

The χ^2 test with Yates' correction was used, and for small groups, Fisher's exact test.

The material was evaluated by an automatic data handling system (Eriksson, Persson, and Wessman, 1975).

Results

During the year covered by the study the number of women examined totalled 1,347. The age distribution is shown in Table I.

TABLE I Age distribution

Age group (yrs)	Gonorrhoea	No gonorrhoea	Total
-14	14	44	58
15-18	140	258	398
19-22	186	260	446
23-26	69	122	191
27-30	43	73	116
31-34	16	24	40
35-38	11	21	32
39-42	14	11	25
43-46	6	16	22
47-	7	12	19
Total	506	841	1347

A high frequency of all three pathogens under investigation was demonstrated and many patients stated that they had symptoms (Table II). The predominant symptom was vaginal discharge with or without pruritus, and in some cases the patient complained only of pronounced itching. In the further presentation of the results it is just noted whether the patient had symptoms or not.

TABLE III Frequency of different pathogens in 1,347 women at first visit

Pathogen	Total no. of patients	Concomitant pathogen											
		N. gonorrhoeae				T. vaginalis				C. albicans			
		No.	Per cent. of gonorrhoea patients	With symptoms		No.	Per cent. of Trichomonas patients	With symptoms		No.	Per cent. of Candida patients	With symptoms	
N. gonorrhoeae	506	354	70.0	223	63.0	84	30.9	55	65.5	56	24.0	41	73.2
T. vaginalis	272	84	16.6	55	65.5	152	55.9	124	81.6	24	10.3	22	91.7
C. albicans	233	56	11.1	41	73.2	24	8.8	22	91.7	141	60.5	108	76.6
T. vaginalis + C. albicans	36	12	2.4	10	83.3								

TABLE II Pathogenic findings at first visit in 1,347 women examined

Diagnosis	No. of patients			
	Total		With symptoms	
	No.	Per cent.	No.	Per cent.
N. gonorrhoeae	506	37.6	329	65.0
T. vaginalis	272	20.2	211	77.6
C. albicans	233	17.3	181	77.7
None of the above	524	38.9	362	69.3

CULTURE *v.* MICROSCOPY FOR THE DETECTION OF T. VAGINALIS

Culture gave a greater number of positive findings during the first half of the investigation, when both direct microscopy and culture were performed. During this time specimens were taken from 843 women and T. vaginalis was demonstrated in 164 cases, 150 at the first examination. Cultures were positive in all the latter cases. The direct smear was negative in 35 of the 150 women (23 per cent.). In these cases the culture often showed only a slight growth of T. vaginalis.

In no case was the direct smear positive and the culture negative. Direct smears as well as cultures were positive at the second examination in ten patients who had had negative direct smears and cultures at the first visit. Both the direct smear and the culture were positive at the third examination in four patients whose specimens had been negative earlier.

FREQUENCY OF DIFFERENT PATHOGENS

N. gonorrhoeae was detected in 506 women (38 per cent) at the first examination, and 329 of these (65 per cent) had symptoms.

Trichomonas vaginalis (positive culture) was found in a total of 272 patients (20 per cent.), 211 of whom (78 per cent.) had symptoms.

Candida albicans was found in 233 patients (17 per cent.), and 181 (78 per cent.) stated that they had symptoms (Table II).

Table III shows that 354 of the 506 women (70

per cent.) in whom *N. gonorrhoeae* was found harboured only this pathogen, and that 223 of the 354 (63 per cent.) had symptoms.

A combination of *N. gonorrhoeae* and one additional pathogen occurred in a total of 140 patients (28 per cent.).

T. vaginalis was found in 84 (17 per cent.), 55 of whom (66 per cent.) had symptoms.

C. albicans occurred in 56 (11 per cent.), 41 (73 per cent.) of whom reported symptoms.

A combination of *N. gonorrhoeae*, *T. vaginalis*, and *C. albicans* was encountered in twelve women (2.4 per cent. of the patients with gonorrhoea). Ten of these patients (83 per cent.) had symptoms.

Table III also shows that 152 of the 272 patients (56 per cent.) in whom *T. vaginalis* was found harboured only this pathogen, and that 124 of them (82 per cent.) had symptoms.

In those with *C. albicans* (Table III), this organism alone was present in 141 women (61 per cent.), 108 of whom (77 per cent.) had symptoms. A total of 233 patients had positive cultures for *C. albicans*.

T. vaginalis and *C. albicans* were found together in 24 of the patients and 22 of these had symptoms (Table III).

Regardless of whether pathogens were found or not, the patients were asked to return for repeat tests within a week. 1,174 patients (87 per cent.) so returned and gonorrhoea was diagnosed in an additional eleven patients. 727 patients (54 per cent.) returned for a third series of tests, and gonorrhoea was detected in two patients in whom two earlier cultures had been negative (Table IV).

TABLE IV *Diagnosis of gonorrhoea*

Examination	Cases infected			
	Total no. of patients		No. of named contacts	
	No.	Per cent.	No.	Per cent.
First	506	97.5	188	97.9
Second	11	2.1	3	1.6
Third	2	0.4	1	0.5
Total	519	100	192	100

Of the total number of patients, 292 were 'named contacts' attending according to the 'Swedish Law against Infectious Diseases'. The frequency of *N. gonorrhoeae* in these patients (66 per cent.) was found to be significantly higher ($P < 0.001$; $\chi^2 = 115.2$) than in the others (31 per cent.). Gonorrhoea was demonstrated in 188 at the first examination, in three at the second examination, and in one at the third (Table IV). 74 (38 per cent.) of the 'named contacts' in whom gonorrhoea was established had no symptoms. The frequency of *T. vaginalis* and *C. albicans* corresponded with the frequency in other patients.

The difference in the frequency of symptoms in patients infected by the different pathogens is shown

in Table V. Patients with *T. vaginalis* only had symptoms more frequently than patients infected only with *N. gonorrhoeae* ($P < 0.001$), while no difference was noted in the frequency of symptoms between groups with gonorrhoea only and those with both gonorrhoea and *T. vaginalis*. Symptoms were also significantly more frequent in patients with *T. vaginalis* only than in those with both gonorrhoea and *T. vaginalis* ($0.01 > P > 0.001$).

TABLE V *Difference in frequency of symptoms in infections with N. gonorrhoeae, T. vaginalis, and C. albicans*

Pathogen	No. of women		χ^2	P
	Total	With symptoms		
<i>N. gonorrhoeae</i>	354	233	16.19	<0.001
<i>T. vaginalis</i>	152	124		
<i>N. gonorrhoeae</i>	354	233	0.09	n.s.
<i>N. gonorrhoeae</i> + <i>T. vaginalis</i>	84	55		
<i>N. gonorrhoeae</i> + <i>T. vaginalis</i>	84	55	6.80	0.01 > P > 0.001
<i>T. vaginalis</i>	152	124		
<i>N. gonorrhoeae</i>	354	233	7.82	0.01 > P > 0.001
<i>C. albicans</i>	141	108		
<i>N. gonorrhoeae</i>	354	233	1.78	n.s.
<i>N. gonorrhoeae</i> + <i>C. albicans</i>	56	41		
<i>N. gonorrhoeae</i> + <i>C. albicans</i>	56	41	0.10	n.s.
<i>C. albicans</i>	141	108		
<i>T. vaginalis</i>	152	124	0.82	n.s.
<i>C. albicans</i>	141	108		

n.s. = not significant

Similarly, patients with *C. albicans* only had symptoms more frequently than patients with gonorrhoea only ($0.01 > P > 0.001$), while no difference was noted between the group with gonorrhoea and *C. albicans* and that with gonorrhoea only. On the other hand, there was no significant difference between patients with *Candida* only and patients with both gonorrhoea and *Candida*, whereas such a difference existed for *T. vaginalis*. Patients with *T. vaginalis* only reported symptoms as often as patients with *C. albicans* only.

DISEASE COMPLICATIONS

At the first visit 29 patients had evidence of gonorrhoeal complications, and three developed complications during the follow-up period (altogether 6 per cent.). The types of complications are listed in

Table VI. It is noteworthy that, of the fourteen patients with confirmed or suspected salpingitis, nine were 'named contacts'. In three patients in whom uncomplicated gonorrhoea was diagnosed at the first visit and who were treated with the standard treatment regimen, salpingitis was diagnosed during the follow-up period. A closer study of the case histories of these patients indicated that the complications probably already existed at the first visit. In the group of women without gonorrhoea, bartholinitis was present at the first visit in one, salpingitis in seven, and suspected salpingitis in three. These women were referred to the gynaecological department for treatment and follow-up.

TABLE VI *Frequency and types of complications*

Complication	No. of women	
	At first visit	During follow-up
Salpingitis	9	3
Suspected salpingitis	5	
Benign gonococcal sepsis	5	
Positive throat culture	4	
Bartholinitis	3	
Fever with arthralgia	1	
Fever	1	
Arthralgia	1	
Total	29	3

Discussion

The number of cases of gonorrhoea in Sweden was 472 per 100,000 inhabitants in 1971, while the corresponding figure in 1972 was 388. In the City of Stockholm, 1,008 cases per 100,000 inhabitants were recorded in 1971 and 782 cases in 1972. Among females in the City of Stockholm, 842 cases per 100,000 were recorded in 1971 and 640 in 1972. During the period of our investigation we noted a total of 519 cases of gonorrhoea in females in our catchment area, corresponding to 364 per 100,000. This figure is lower (by about 40 per cent.) than the reported average for Stockholm. This is probably due to the fact that some of the patients are examined and treated by gynaecologists and private practitioners. 74 per cent. of the women in Uppsala reported to have gonorrhoea in 1970 were treated at the venereological clinic (Hammar and Ljungberg, 1972).

PATHOGENICITY

The pathogenicity of *T. vaginalis* has been well documented and infections caused by the organism are counted among the sexually-transmitted diseases (Chappaz, 1957; Bauer, 1957; Whittington, 1957; Catterall and Nicol, 1960).

Whether or not a finding of *C. albicans* is to be considered pathological is a subject of debate

(Hesseltine, 1959/60; Timonen, Salo, Meyer, and Haapoja, 1966). *C. albicans* exhibits the behaviour of a facultative pathogen. Oriel, Partridge, Denny, and Coleman (1972) are of the opinion that positive cultures should lead to treatment, as very little is known about what may cause some patients to develop clinical symptoms of *Candida* infection and others not. Other yeasts that occur much less frequently have also been considered to be pathogenic, e.g. *Torulopsis glabrata* (Timonen and others, 1966; Hurley, Leask, Faktor, and de Fonseka, 1973), but in our study only *C. albicans* has been recorded.

DIAGNOSTIC METHODS

Culture of *T. vaginalis* is supposed to produce more positive findings than direct microscopy (Lowe, 1965; Cox and Nicol, 1973), which accords with the results of this study. The importance of culture in chronic trichomonal infection has been stressed by Bartunek and Schultze (1972), and in evaluating therapeutic results by de Caneri, Emanuelli, and Signorelli (1968).

During the first 6 months of the investigation when wet smears were made as well as cultures, the smears were negative but the cultures positive in 23 per cent. of cases. In some cases types similar to so-called round forms (Bartunek and Schultze, 1972) were seen; in some of these cases cultures proved to be positive, while in others they were negative. The criterion for a positive direct smear was therefore distinct, motile flagella. The specimens in our investigation were taken regardless of whether the patients were menstruating or not. Rodgerson (1970) states that, in the presence of blood, *T. vaginalis* shows a greater tendency to round form transformations. Forsgren (1972) reports that there is a cytolytic factor in serum that affects *T. vaginalis*, but the clinical significance of this is not known. Such phenomena may help explain the variation in frequency of detection of cases of *Trichomonas* infection. The diagnosis of *C. albicans* was made by culture, since microscopy is known to yield fewer positive results (Timonen and others, 1966; Catterall, 1970; Oriel and others, 1972).

FREQUENCY OF DIFFERENT PATHOGENS

The reported frequency of *T. vaginalis* varies according to the patient category investigated (Renkonen, Widholm, and Vartiainen, 1970; Schnell, Andrews, and Plempel, 1972; Korte, 1972).

A significantly higher frequency of *T. vaginalis* in middle-aged women was found by Schnell and others (1972). Nielsen (1973) has demonstrated that the average age of patients with gonorrhoea is considerably lower than that of those with *Trichomonas*. In our study 81 per cent. of the women were under 27 years of age, i.e. mainly in the age groups in which gonorrhoea is most prevalent. In 19 per cent. (96 out

of 506) of the patients with gonorrhoea *T. vaginalis* was also demonstrated (Table III). A similar frequency of *T. vaginalis*, 21 per cent. (176 out of 841), was observed in the patients without gonorrhoea. Tsao (1969) reported that, in a series of 1,466 patients with gonorrhoea, 42 per cent. also had trichomoniasis and that, among 1,335 patients with *Trichomonas* infection, there were 620 with gonorrhoea (46 per cent.). Ödegaard (1972) detected *T. vaginalis* in 69 (31 per cent.) of 222 patients with gonorrhoea.

This study shows that many of the patients who attend a V.D. clinic prove to be infected with pathogens other than *N. gonorrhoeae*. Thus 11 per cent. (152 out of 1,347) of the women had only a *Trichomonas* infection and 10 per cent. (141 out of 1,347) only *C. albicans*. Catterall (1970) has reported that *T. vaginalis* is the most common finding (42 per cent.) in women who present with vaginal discharge, the second commonest causative organism being *C. albicans* (34 per cent.); gonorrhoea was only the third most common finding (32 per cent.). In our investigation the most common finding (patients with and without symptoms combined) was *N. gonorrhoeae* (38 per cent.), followed by *T. vaginalis* (20 per cent.), and *C. albicans* (17 per cent.) (Table II). In the group of patients reporting symptoms *N. gonorrhoeae* was found in 20 per cent., *T. vaginalis* in 16 per cent., and *C. albicans* in 13 per cent.

RELATIONSHIP BETWEEN *C. ALBICANS* AND *T. VAGINALIS*

In the present study the ratio is 0.9:1. The frequency of vaginal *Candida* infections has increased during the last decade; according to Rohatiner (1967), *C. albicans* is a more common finding than *T. vaginalis*, and later investigations by Catterall (1971) have shown a ratio of 2:1 between the prevalence of *C. albicans* and *T. vaginalis* in patients with vaginal discharge and a ratio of 3:1 in patients with genital symptoms.

The combination of *T. vaginalis* and *C. albicans* was found in 36 patients. The frequency of *C. albicans* is said to be lower in the presence of *T. vaginalis* infections than otherwise (Oriel and others, 1972; Nielsen, 1973). According to Müller, Holtorff, and Blaschke-Hellmessen (1967), this is due to a difference in the local environmental requirements.

NUMBER OF DIAGNOSTIC EXAMINATIONS

In female patients with gonorrhoea Catterall (1970) diagnosed the condition in 66 per cent. at the first examination; he maintains that gonorrhoea cannot be excluded until at least three negative specimens have been obtained. According to the 'Swedish Law against Infectious Diseases' specimens must be taken three times in women named as contacts. In this study gonorrhoea was established at the first examination in a high percentage of cases (97.5 per cent.), and in

only 2.1 per cent. at the second examination, and 0.4 per cent. at the third (Table IV). 'Named contacts' did not differ from other patients in this respect. Similar results have been noted before with male 'named contacts' (Enfors and others, 1973). There is always a loss of patients to follow-up, but in this study a large proportion (87 per cent.) returned for at least two examinations. There is of course also the possibility of re-infection between examinations. The advantage of forcing patients to appear for repeated examinations therefore seems to be small. On the other hand, one compulsory examination for 'named contacts' is evidently of great value, since it reveals symptom-free patients, many of whom might not otherwise have reported for examination, or perhaps only when complications developed, while at the same time remaining sources of infection.

SYMPTOMS

Higher frequencies of asymptomatic gonorrhoea in women have been reported in earlier Swedish investigations (43 per cent. by Molin, 1970; 48 per cent. by Lidén, 1969) than in the present study (35 per cent.). Patients in the 'named contact' group did not differ in this respect from other gonorrhoea patients.

Patients were asked about subjective discomfort. Of course there is a great variation between individuals with regard to what is felt to be 'discomfort', e.g. vaginal discharge. However, even with this coarse parameter, significant variations are noted. The observation that patients with *T. vaginalis* infections have symptoms more often than patients with gonorrhoea (Table V) may be due to the fact that infections with *T. vaginalis* are located mainly in the vagina, whereas in gonorrhoea the main site of infection is the cervix. But this does not explain why patients with only *T. vaginalis* infections have symptoms significantly more often than those with both gonorrhoea and *T. vaginalis*. Another aspect of the problem is dealt with by Cohen (1969), who showed that women with the most marked symptoms were also the ones who had the lowest vaginal pH. The findings did not seem to be due to the fact that the infections cause a decrease in the vaginal pH, however. Women with low pH values exhibited more pronounced desquamation from the epithelium, which would facilitate penetration by micro-organisms. Cohen further observed that a woman with a high pH may be a carrier of *Trichomonas* or yeasts and still be symptomless.

DISEASE COMPLICATIONS

Krook and Juhlin (1965) stated that 15 per cent. of all female patients with gonorrhoea have salpingitis. We noted salpingitis or suspected salpingitis at the first visit in only 3 per cent. of the patients with gonorrhoea. A tentative explanation for this low

frequency is that patients with symptoms of salpingitis usually come under the care of a gynaecologist.

During the period of investigation, five cases of gonococcal sepsis were diagnosed, corresponding to 1 per cent. of the gonorrhoea patients. Other Swedish studies (Barr and Danielsson, 1971) have shown a frequency of 3 per cent. in female patients with gonorrhoea.

The finding of gonococci in the pharynx has been regarded as a complication of the disease in our study, primarily because experience has shown that the treatment recommended for uncomplicated gonorrhoea is not sufficient for pharyngeal infections. As specimens were not taken routinely, an assessment of the frequency has not been possible.

Slightly more than a third of the women were not found to have any of the pathogens under investigation at the first visit. However, a fairly large proportion of these patients had symptoms. Gonorrhoea or *T. vaginalis* was detected in a small number of them at the second examination. In other patients it is possible that the symptoms were caused by other pathogens not investigated by us, such as *Mycoplasma* and *Chlamydia*.

By virtue of the high frequency of other pathogens encountered, the present investigation shows that, if patients are to be given proper help, the concept of venereal disease must be widened to include other pathogens besides *N. gonorrhoeae*.

Summary

The frequency of *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Candida albicans* has been studied over a period of one year in women attending a venereal diseases clinic. A total of 1,347 women were investigated, all coming from the same catchment area. Gonorrhoea was established at the first visit in 506 patients (38 per cent.), who constituted 97.5 per cent. of the total number of cases of gonorrhoea. *Trichomonas vaginalis* was found in 272 (20 per cent.) and *Candida albicans* in 233 (17 per cent.). 176 patients (13 per cent.) had more than one pathogen.

Of the patients attending, 22 per cent. (292 women) were so-called 'named contacts'. The frequency of gonorrhoea established at the first visit in these patients (64 per cent.), was significantly higher, but the frequency of symptoms did not differ from that in other gonorrhoea patients. The number of asymptomatic cases was so large that a single compulsory examination is undoubtedly very useful from the epidemiological point of view, but the value of repeated specimen collections for gonorrhoea is debatable. Complications of gonorrhoea were observed in 29 patients (6 per cent.) at the first visit.

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