Staphylococcus saprophyticus in the aetiology of nongonococcal urethritis

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SUMMARY The occurrence of *Staphylococcus saprophyticus*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* in urethral specimens of 252 men attending a venereal disease clinic was studied. When using a selective broth medium containing novobiocin and nalidixic acid, *Staph. saprophyticus* was isolated from 20.8% of 178 men with symptoms of urethritis and from 14.9% of 74 men without such symptoms. *Staph. saprophyticus* was found significantly less often in controls (7.1% of 56) than in the men with symptoms of urethritis. In the 35 men from whom *Staph. saprophyticus* was recovered more than 10 leucocytes per high power field in urethral smears occurred more often than in those from whom this organism, or either of the other two agents, were not isolated. No differences were found in the symptoms reported by the men harbouring *Staph. saprophyticus* or *C. trachomatis* or those with negative cultures. The results of the present study tend to suggest that *Staph. saprophyticus* is the aetiological agent of some cases of nongonococcal urethritis.

Introduction

In recent years 25-45% of all cases of nongonococcal urethritis (NGU) in men have been attributable to *Chlamydia trachomatis* infection (Holmes *et al.*, 1975; Oriel *et al.*, 1976; Alani *et al.*, 1977; Bowie *et al.*, 1977a; Ripa *et al.*, 1978). Herpes simplex virus, *Candida albicans*, and *Trichomonas vaginalis* are responsible for some (5-10%) cases of NGU (Catterall, 1975; Holmes *et al.*, 1975).

There is also some evidence that *Ureaplasma urealyticum* is the pathogen responsible for some cases of NGU. An association between a primary episode of NGU and *U. urealyticum* has been claimed on the basis of therapeutic studies and quantitative cultures of the organism (Prentice *et al.*, 1976; Bowie *et al.*, 1977a).

Staphylococcus saprophyticus (earlier named *Micrococcus*, subgroup III) is known to be a frequent cause of acute cystourethritis in young adult women (Maskell, 1974; Sellin *et al.*, 1975). *Staph. saprophyticus*, like *Staphylococcus cohnii* and *Staphylococcus xylosus*, differs from other

coagulase-negative staphylococcal species (for example, *Staphylococcus epidermidis*) in being less susceptible to novobiocin (minimum inhibitory concentration $>2.0 \mu\text{g/ml}$).

In the present study, we investigated the occurrence of *Staph. saprophyticus*, *C. trachomatis*, and *Neisseria gonorrhoeae* in urethral specimens of men attending a venereal disease clinic. For comparison male medical students and laboratory staff were also studied. A selective culture medium was developed for the isolation of *Staph. saprophyticus*.

CLINIC POPULATION

Groups 1 and 2

The patients consisted of 252 men between 15 and 54 years of age (median age 25 years), who attended the Department of Dermatology and Venereology, University Hospital, Lund, during two periods, namely, June to August 1977 (96 men) and August to October 1978 (156 men). Of the 252 men, 178 (70.6%) had symptoms suggesting urethritis, that is, urethral discharge, or dysuria, or both (Group 1). The remaining 74 (29.4%) men were symptomless (Group 2). The most common reason for the latter patients attending the clinic was that a sexually transmitted disease had been diagnosed in their sexual partner(s).

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CONTROLS

Group 3

Fifty-six male medical students and male laboratory staff (20 to 37 years of age, median age 25 years) were studied during the same periods as the men in groups 1 and 2. None of these persons, according to a detailed questionnaire, currently had or had previously had a history of genitourinary disease.

SAMPLING TECHNIQUE AND ISOLATION PROCEDURES

Urethral specimens from the 252 men in groups 1 and 2 were cultured for *Staph. saprophyticus*, *C. trachomatis*, and *N. gonorrhoeae*, while those from the men in group 3 were cultured only for *Staph. saprophyticus*.

Staphylococcus saprophyticus

A urethral specimen was collected with the aid of a cotton-tipped swab, which was first used to inoculate a gonococcal medium, and then transferred to a selective medium (Hovelius and Mårdh, 1977a) of the following composition: tryptone broth (Oxoid), pH 7.2, with the addition of 150 µg nalidixic acid (Winthrop) and 2 µg novobiocin (Sigma) per ml. In 156 of the patients the swab was also used to inoculate blood agar plates before it was transferred to the broth medium. The broth was incubated at 37°C for 18 hours before being seeded on to fresh blood agar plates; these consisted of Blood Agar Base No. 2 (Oxoid) with 4% defibrinated horse blood. The plates were read after 24 hours' incubation at 37°C; from each plate at least six staphylococcal colonies were selected for further studies.

The staphylococci were tested for coagulase production. Coagulase-negative strains were tested by disc diffusion tests (see below) for their susceptibility to novobiocin. Strains producing an inhibitory zone of less than 15 mm in diameter when discs containing 5 µg novobiocin were used were considered to be resistant (Hovelius and Mårdh, 1977b). Such strains were further classified as to species by biochemical tests according to Kloos and Schleifer (1975) and the Subcommittee on Taxonomy of Staphylococci and Micrococci (1976). Furthermore, all strains were also tested on agar plates containing glycerol and erythromycin (Schleifer and Kloos, 1975) to establish that the strains tested were staphylococci and not micrococci.

Neisseria gonorrhoeae

Urethral specimens for the culture of gonococci were collected with a cotton-tipped swab, which, immediately after sampling, was used to inoculate a haematin agar medium (Mårdh *et al.*, 1978). After

inoculation, the plate was incubated at 37°C with regulated CO₂ atmosphere and humidity. The methods used to identify *N. gonorrhoeae* were reported earlier (Mårdh *et al.*, 1978).

Chlamydia trachomatis

Specimens for the culture of chlamydiae were collected by means of a calcium-alginate swab (Inolex Corp., Glenwood, Illinois, USA), which was gently rotated 2-3 cm into the urethra before withdrawal. The swabs were transported in a sucrose-phosphate buffer (Gordon *et al.*, 1969) supplemented with gentamicin 10 µg (Schering), amphotericin B 2.5 µg (Squibb), and vancomycin 100 µg (Upjohn) per ml. The specimens were stored at 4°C if the culture could be started within 24 hours of sampling; otherwise they were stored at -20°C until required. *C. trachomatis* was isolated on cycloheximide-treated McCoy cells as described elsewhere (Ripa and Mårdh, 1977).

MICROSCOPY

Urethral smears from all men in groups 1 and 2 were heat-fixed and stained with methylene blue for the detection of leucocytes and gonococci. The number of leucocytes per high power field (HPF) ($\times 100$ objective) in each smear was graded as follows: <10, 10-30, and >30 (mean of ten HPFs). All specimens were studied under the same microscope by four different physicians.

ANTIBIOTIC SUSCEPTIBILITY TESTS

The 48 strains of *Staph. saprophyticus* isolated from the men in groups 1 and 2 were tested for their susceptibility to penicillin V, cephalixin, nalidixic acid, oxytetracycline, sulphadimidine, and sulphamethoxazole/trimethoprim by the disc diffusion technique (Ericsson and Sherris, 1971) using Wellco susceptibility test agar. The medium and the antibiotic discs were purchased from AB Biodisk (Stockholm, Sweden).

All 48 strains were also tested for β -lactamase production by means of chromogenic cephalosporin (O'Callaghan *et al.*, 1972).

TREATMENT AND FOLLOW-UP

All patients in whom gonorrhoea was diagnosed by microscopy of urethral discharge were treated with pivampicillin 1.4 g orally (Pondocillin^R, Lövens, Denmark) and probenecid 1 g (Probecid^R, Astra, Sweden). Patients in whom microscopical examination of urethral discharge did not indicate gonorrhoea but in whom cultures were nevertheless positive for *N. gonorrhoeae*, whether or not *Staph. saprophyticus* or *C. trachomatis* had been isolated, received the same treatment when re-examined one

week after the first attendance. If *C. trachomatis* had been isolated, whether or not *N. gonorrhoeae* or *Staph. saprophyticus* had been isolated, the patients were prescribed lymecycline 0.3 g (Tetralysal^R, Carlo Erba, Italy) twice daily for one week at the first re-examination. In men who had symptoms of urethritis and from whom *Staph. saprophyticus*—but not *C. trachomatis* or *N. gonorrhoeae*—had been isolated, phenoxymethylpenicillin 0.65 g (Kåvepenin^R, Kabi, Sweden) was given three times daily for 10 days.

At the first, as well as at subsequent, follow-up examinations (which were carried out about one week after the first visit and one week after treatment) all culture studies were repeated.

STATISTICAL METHOD

All the statistical calculations were performed using the χ^2 method with Yates's correction. The degree of freedom for each P value given was one.

Results

ISOLATION OF MICRO-ORGANISMS

Staph. saprophyticus was isolated from 37 (20.8%) of the men in group 1, who had symptoms of urethritis. The corresponding figures for the men in groups 2 and 3 without symptoms were 11 (14.9%) and four (7.1%) respectively (Table 1).

Table 1 Culture results for urethral samples from 252 men attending a venereal disease clinic

Organism isolated	No. of patients		Total
	Symptoms of urethritis (group 1)	Symptom-free (group 2)	
<i>Staph. saprophyticus</i>	25	10	35
<i>C. trachomatis</i>	31	9	40
<i>N. gonorrhoeae</i>	27	3	30
<i>Staph. saprophyticus</i> and <i>C. trachomatis</i>	9	1	10
and <i>N. gonorrhoeae</i>	3	0	3
<i>C. trachomatis</i> and <i>N. gonorrhoeae</i>	10	1	11
Total	178	74	252

Staph. saprophyticus was isolated from only six of 156 urethral specimens collected from men in groups 1 and 2 when inoculated directly on to blood agar plates, while the organism was found in 32 of the same specimens when the selective broth medium was used.

Staph. saprophyticus occurred more often in the men in group 1 than in those in group 3 ($P < 0.05$), while the difference between the isolation frequency of *Staph. saprophyticus* in groups 2 and 3 was not significant. This was also true when the isolation frequency in groups 1 and 2 was compared with that in group 3 and when that in groups 1 and 2 was compared.

N. gonorrhoeae was found in 22.5% and *C. trachomatis* in 28.1% of the men in group 1; the

corresponding figures in group 2 were 5.4% and 14.9% for *N. gonorrhoeae* and *C. trachomatis* respectively.

Staph. saprophyticus was isolated from three (6.8%) of the men with gonorrhoea and from 10 (16.4%) of the 61 men harbouring *C. trachomatis* (Table 1).

RELATION OF MICROSCOPY AND CULTURE RESULTS

The culture results of groups 1 and 2 are related to the number of leucocytes per HPF in urethral smears in Table 2. None of the men from whom more than

Table 2 Number of leucocytes per high power field (HPF) ($\times 100$ objective) in urethral smears of male patients (groups 1 and 2) in relation to micro-organisms isolated*

Organism isolated	No. of patients	No. of leucocytes/HPF		
		<10	10-30	>30
<i>Staph. saprophyticus</i>	35	15	11	9
<i>C. trachomatis</i>	40	9	9	22
<i>N. gonorrhoeae</i>	28	2	6	20
Negative results for all three organisms	123	82	21	20

*Men from whom more than one of the three organisms was isolated are not included

one of the three organisms were isolated is included in the Table. *Staph. saprophyticus* ($P < 0.05$), *N. gonorrhoeae* ($P < 0.001$), and *C. trachomatis* ($P < 0.001$) all occurred significantly more often in those men who had more than 10 leucocytes per HPF than in those with fewer. Approximately one-third of the men from whom none of these organisms was recovered also had more than 10 leucocytes per HPF. This was also true for most (20/35) of those infected with *Staph. saprophyticus*.

RELATION OF SYMPTOMS AND CULTURE RESULTS

The symptoms reported by the men in group 1 are related to the culture results in Table 3. Only those

Table 3 Symptoms of men with urethritis (group 1) in relation to micro-organism isolated from urethral specimens*

Organisms isolated		No. of men (group 1) with			
		Discharge		Painful micturition	Itching
		Watery	Purulent		
<i>Staph. saprophyticus</i>	(n) 23	5	8	15	3
<i>C. trachomatis</i>	(n) 31	12	11	22	23
<i>N. gonorrhoeae</i>	(n) 27	4	22	18	3
Negative results for all three organisms	(n) 73	19	23	46	19

*Men from whom more than one of the three organisms was isolated are not included

men who harboured one of the three organisms are presented in the Table. Among the men who had symptoms, discharge was reported more often in those with gonorrhoea than in those infected with *C. trachomatis* or *Staph. saprophyticus*. Watery discharge was reported more often by men with chlamydial infection than by those with gonorrhoea, while the converse was true for purulent discharge. There was no significant difference regarding the occurrence of purulent or watery discharge in men from whom *C. trachomatis* and *Staph. saprophyticus* were isolated and from those in whom these organisms or gonococci could not be demonstrated. Dysuria was reported with approximately the same frequency regardless of the result of the culture studies.

ANTIBIOTIC SUSCEPTIBILITY AND CHROMOGENIC CEPHALOSPORIN TESTS

The disc diffusion tests indicated that, except for four strains, *Staph. saprophyticus* was susceptible to all the antibiotics tested, although all strains were resistant to nalidixic acid (Table 4). The strains did not produce β -lactamases according to the results of the chromogenic cephalosporin test.

Table 4 Antibiotic susceptibility of 48 strains of *Staphylococcus saprophyticus* isolated from urethral specimens of men attending a venereal disease clinic (groups 1 and 2)

Antibiotic	Susceptible	Intermediate	Resistant
Penicillin V	48		
Cephalexin	48		
Nalidixic acid	0		48
Oxytetracycline	45		3
Sulphadimidine	44	2	2
Sulphamethoxazole/ trimethoprim	48		

TREATMENT

In 12 of the 16 men in group 1 harbouring *Staph. saprophyticus* and who returned for re-examination, this organism could not be isolated, irrespective of whether phenoxymethylpenicillin, lymecycline, or pivampicillin had been given. All 16 men had become symptomfree. In one of the four men with persistent growth of *Staph. saprophyticus*, a renal calculus was later diagnosed. In another four men (one from group 1 and three from group 2) harbouring *Staph. saprophyticus*, and who had not been given antibiotics, the organism could no longer be isolated from urethral specimens at re-examination two weeks after the initial examination.

None of the 44 men in groups 1 and 2 with gonorrhoea was culture-positive for *N. gonorrhoeae* after treatment and 42 were symptomfree. Thirty-two of the 61 chlamydia-positive men returned for

follow up. All of them had become culture-negative for *C. trachomatis* and 29 were symptomfree.

Discussion

Staph. saprophyticus is a common cause of acute, often haemorrhagic, cystourethritis in young adult women (Maskell 1974; Sellin *et al.*, 1975). Urinary tract infections with *Staph. saprophyticus* in women occur most frequently in those age groups in which the highest incidence of sexually transmitted genital infections occur. A seasonal variation in the incidence of such infections has been noted; the highest incidence occurs in early autumn (Telander and Wallmark, 1975; Maskell and Pead, 1977; Hovelius and Mårdh, 1978), that is during the same period of the year as the present study was undertaken. A similar variation can also be shown for most sexually transmitted infections (Wright and Judson, 1978), but this cannot be demonstrated for urinary tract infections caused by *Escherichia coli* (Maskell and Pead, 1977).

In men, cystitis with *Staph. saprophyticus* most frequently occurs in men over 60 years of age with benign hyperplasia of the prostate. However, we have observed acute cystitis caused by *Staph. saprophyticus*, in young adult men without obstructive urinary tract disease (Hovelius and Mårdh, 1978). Some of these men had attended a venereal disease clinic because they suspected that they had contracted a venereal infection.

Staph. saprophyticus is known to occur as a pathogen solely in the urinary tract. In clinical microbiological laboratories, *Staph. saprophyticus* is seldom recovered from specimens other than urine, in which it rarely seems to occur as a contaminant. *Staph. saprophyticus* may be isolated from the skin of the arms and legs, although transiently and in low numbers (Kloos and Mammelwhite, 1975). With a selective culture medium, Pead and Maskell (1977) isolated *Micrococcus* subgroup III from rectal swabs from 10 of 156 women.

It has been argued that the capacity of a bacterium to adhere to epithelial cells is a prerequisite for its ability to cause mucosal infections (Gibbons, 1977). In *in vitro* experimental studies on bacterial adhesion to urothelial cells, we found *Staph. saprophyticus* to adhere in significantly greater numbers to such cells than to epithelial cells from other sites, for example, buccal and skin cells (Colleen *et al.*, in press). Among other genital and urinary tract pathogens only freshly isolated strains of *N. gonorrhoeae*, producing T1 and T2 colonies, adhered in greater numbers per urothelial cell than did *Staph. saprophyticus*. This pronounced capacity of *Staph. saprophyticus* to

adhere to urothelial cells may explain the organism's tropism for the urinary tract (Mårdh *et al.*, 1979).

When urethral specimens are cultured on blood agar, *Staph. saprophyticus* is seldom isolated, as shown in the present study. Bowie *et al.* (1977b) failed to isolate this organism from any of 62 patients with NGU. The poor growth of *Staph. saprophyticus* on blood agar plates inoculated with urethral specimens might be because this bacterium occurs in low numbers or because it is inhibited by microbial interaction. Thus, we have found that strains of *Staph. epidermidis* may inhibit growth of *Staph. saprophyticus* on artificial culture media (unpublished data).

When our selective culture medium was used, *Staph. saprophyticus* could be isolated from the urethral specimens of men without either a history or symptoms of urethritis or urinary tract infection. Thus, the organism was recovered from 7.1% of male students and laboratory staff.

The role of *Staph. saprophyticus* in NGU is difficult to establish, as is that of other organisms which may occur in the normal flora, namely *U. urealyticum*. *Staph. saprophyticus* was isolated significantly more often from the men with symptoms of urethritis (group 1) than from medical students and laboratory staff (group 3). The organism was found significantly more often among the men in groups 1 and 2 with an increased number of leucocytes in their urethral smears than in those men in these groups with less than 10 leucocytes per HPF. It is notable that in the patients with gonorrhoea the frequency of isolation of *Staph. saprophyticus* was as low as in the men in group 3.

Staph. saprophyticus was susceptible *in vitro* to all antibiotics tested, including a number of drugs commonly prescribed to men with genital and urinary tract infections. In this respect, however, nalidixic acid was an exception (Hovelius and Mårdh, 1977b). In a previous study this antibiotic constantly failed to cure patients with urinary infection caused by *Staph. saprophyticus* (Hovelius *et al.*, 1979). Of the 16 men studied who harboured *Staph. saprophyticus*, and who were treated with penicillin, pivampicillin or lymecycline, 12 were culture-negative and symptom-free at follow up. Three men still harboured *Staph. saprophyticus* but had no symptoms and less than 10 leucocytes per HPF in urethral smears at re-examination. In one man, in whom *Staph. saprophyticus* persisted despite antibiotic treatment, a renal calculus was diagnosed. This observation agrees with our earlier findings (Hovelius *et al.*, 1979). We have occasionally diagnosed persistent bacteriuria with *Staph. saprophyticus* which proved intractable to repeated courses of antibiotics to which the organism was

susceptible *in vitro*. These men became culture-negative after the removal of the calculus.

The finding that *Staph. saprophyticus* and symptoms of urethritis had disappeared within a fortnight in some men not given antibiotic treatment agrees with the observation of spontaneous cure in women of urinary tract infections caused by coagulase-negative staphylococci (Mabeck, 1969).

In conclusion, the result of the present study suggests that *Staph. saprophyticus* should be considered as a potential aetiological agent of some cases of NGU in men.

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