

GENITAL OCCURRENCE OF ORAL MICROBIOTA

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Abstract. Recent studies indicate that tonsillar gonococcal infection or colonization is fairly common. Carriage rates of about 8% have been found. These studies also indicate that oro-genital contacts are common. Since very little is known about the amount of oral microbiota transmitted to the genitals, we have studied the occurrence of oral streptococci and *Neisseria* species in urethra and cervix. Among 128 patients attending an STD-clinic we found 10 carriers of oral streptococci, one *Streptococcus mitior*, four *Streptococcus sanguis*, one *Streptococcus mutans* and four *Streptococcus salivarius* and one case of urethritis due to *Neisseria meningitidis*. Seventy-three of the patients had recently had their genitals exposed to the oral flora of their partners. Despite the heavy contamination with oral microbiota that can be assumed to occur in these cases, there seems to be no colonization of the genitals with oral microbiota.

Key words: Genitals; Oral streptococci; *Neisseria* species

In an earlier study from this clinic, oro-genital contacts were recorded for 28.0% of the men and 31.1% of the women visiting the VD unit (14). Many of these patients were found to harbour *Neisseria gonorrhoeae* in the pharynx. The oro-genital route could also imply a risk of transporting microbiota of a normally oral localization to the genitals. In October 1975 a male patient with purulent urethritis and recent history of fellatio had a positive methylene blue-stained smear for intracellular diplococci. *Neisseria meningitidis* was isolated from the urethral swab.

Reports have appeared in the literature on the isolation of *Neisseria* species other than *N. gonorrhoeae* in urethral and cervical specimens from patients attending venereal disease clinics and with clinical findings mimicking those of gonorrhoea (for a review see (8)). It is generally concluded that there is a very low genital frequency of *N. meningitidis* and nonpathogenic species such as *Branhamella catarrhalis*. It also seems unlikely that the genitals are a normal habitat for these species. There is little information available on the

occurrence of viridans streptococci similar to or identical with oral streptococci in the genitals and lower urinary tract (11). Alpha- and non-haemolytic streptococci are not speciated in existing reports.

We have studied the incidence and clinical significance of the genital localization of oral streptococci.

MATERIALS AND METHODS

Clinical material

All patients between 20 and 35 years of age visiting the Venereal Disease Clinic of Uppsala University Hospital between December 1975 and March 1976 were included in the study and they were all seen by two of the authors (U. F. and J. W.). Patients with a language problem and those who had been taking antibiotics during the preceding month were excluded. It was considered highly essential for the study that the patients were well-informed and cooperative. Detailed and standardized information concerning the aims of the investigation were given by the doctor in privacy.

Only patients with a history of direct genital contact with his/her partner's mouth during the last month were included in the study group.

Those patients who denied having had oro-genital contacts during the previous 3 months were used as controls. The final material consisted of a study group of 47 men and 26 women and a control group of 32 men and 23 women.

Clinical and laboratory procedure

The majority of the patients telephoned for their appointments. They were told not to micturate for at least 3 hours before the examination. Specimens were taken with charcoal-coated swabs. In male patients two swabs were taken 1 and 2 cm into the urethra, respectively. The first was inoculated direct on GC agar and the second inserted into Stuart's medium (12). For women the same procedure was carried out but swabs were also taken from cervix. For *Neisseria* identification a specimen was also taken from the rectal mucosa of all female patients.

The GC agar used for direct inoculation had the following composition: GC agar base (BBL) 36 g, Isovitalex (BBL) 10 g, haemoglobin (BBL) 10 g, aq. dest. to 1 000 ml. In addition to a plate with this mixture, another plate using the same composition but with the addition of VCN-

Table 1. *Clinical diagnoses*

Clinical diagnosis	Men	Women
Gonorrhoea	9	7
Meningococcal urethritis	1	0
Non-gonococcal urethritis	43	—
Non-gonococcal vaginitis	—	20
Condyloma ac.	8	2
Herpes sim. gen.	5	1
Other diagnosis	5	1
Observation	12	20
Number of diagnoses	83	51
Number of patients	79	49

inhibitor (BBL) 10 ml/l was used. After the inoculation, the plates were immediately placed in a candle jar and sent to the laboratory where they were incubated for 48 h at 37°C with 5% CO₂ and 95% humidity.

Typing

All oxidase-positive *Neisseria*-suspected colonies were tested by immunofluorescence with anti-N-gonorrhoea F(ab')₂ fragments of IgG (6) and grown for 48 h on a solid sugar (dextrose, maltose and laevulose) fermentation substrate obtained from NBL, Stockholm.

The specimens for streptococcal screening were cultured on two Mitis-Salivarius agar plates (Difco Lab., Mich., USA), one blood agar plate and one haematin agar plate. One Mitis-Salivarius agar plate was incubated aerobically at 37°C for 48 h and the other was incubated in an anaerobic jar (BBL) for 24 h at 37°C followed by aerobic storage at room temperature before examination (9). The blood agar plate was incubated for 24 h at 37°C anaerobically. All haematin agars were incubated aerobically at 37°C before examination. The colonies were examined on the plates by using a stereoscopic microscope with 10× magnification. One representative colony of each of those colony types which did not produce catalase and in microscopic examination in Gram-stained smear showed Gram-positive chain-forming cocci were isolated from each plate.

All pure isolates were subjected to the tests, for presumptive identification of pathogenic streptococci (4, 5). Haemolysis was studied on streaked blood agar plates containing 5% defibrinated horse blood after anaerobic incubation. Susceptibility to bacitracin was tested on blood agar plates, streaked with an overnight broth culture. A bacitracin disk (Difco) was placed in the inoculum and incubated overnight. A zone of inhibition was considered a positive reaction. Tolerance to bile (10 and 40%) and sodium chloride (6.5%) was studied by growth in heart infusion broth (Difco) and the respective inhibitors. Hydrolysis of sodium hippurate was detected by the appearance of a crystalline precipitate after addition of one part of 50% sulphuric acid to two parts of culture grown in plain heart infusion (Difco) containing 1% sodium hippurate. Hydrolysis of esculin was detected by a blackening of oxoid agar containing 0.1% esculin and 0.5% ferric citrate. All tests were run at 37°C and read after 24 h

to 48 h. The tests were used as a battery and not as single entities.

In addition, a battery of physiological tests was used to identify the group D and viridans streptococci (1, 3, 5).

Production of dextran was studied in Tryptose broth (Difco) containing yeast extract, potassium acetate and 5% sucrose; dextran was detected by precipitation with one volume of ethanol. Production of acetoin was detected by the appearance of a reddish colour after shaking 2 ml of the culture in buffered Bacto-Tryptose-yeast extract containing 0.5% glucose for 4 days with 1 ml of a 1% alcohol solution of alpha-naphthol and 0.4 ml potassium hydroxide (40%).

Production of acid from 1% of lactose, raffinose, sorbitol, mannitol, salicin, arabinose, talose and 0.2% of glycerol was studied for 3 days in phenol red broth base (Difco). The group identification of streptococci groups A, B, C, D and G was made with immunoelectro-osmophoresis as described by Wadström et al. (13).

RESULTS

The final diagnoses for all patients in the study are presented in Table 1. The distribution of diagnoses does not differ markedly from that found in a long-term study at this clinic (14) and the diagnosis in patients with oro-genital contacts and the controls appear the same. The most frequent diagnosis was non-gonococcal urethritis or vaginitis. The second most frequent was gonorrhoea.

One male patient of the study group presented a heavy urethral discharge and dysuria. The methylene blue-stained smear showed intracellular diplococci indistinguishable from gonococci in morphology and the culture yielded *N. meningitidis*. The same bacterium was isolated from his partner's tonsils.

Table II lists the streptococcal species found in urethral and cervical specimens and in none was more than one streptococcal species isolated. Four group A streptococci were found without any apparent clinical significance. The groups B and D streptococci did not show any difference between study group and controls. Seven of the oral strains, *Streptococcus mitior*, *Streptococcus sanguis* and *Streptococcus salivarius* were isolated from patients with oro-genital contacts, while three were found in the female control group; two from urethral and one from cervical specimens.

Among the female patients, the cultures from urethral swabs yielded two group A strains, eleven group B strains, six group D strains and four strains of oral origin, while cultures of the cervical swabs yielded one group A strain, eight group B strains, seven group D strains and four strains of oral origin.

Table II. *Streptococci isolated from urethra and cervix in patients with and without genito-oral contacts*

Streptococcal species	Lance-field's group	Number of streptococci isolated from			
		Men with genito-oral contacts	Men without genito-oral contacts	Women with genito-oral contacts	Women without genito-oral contacts
<i>S. pyogenes</i>	A	2	0	1	1
<i>S. agalactiae</i>	B	12	6	7	7
<i>S. faecium</i>	D	0	1	0	0
<i>S. faecalis</i>	D	4	3	4	4
<i>S. mitior</i>	—	0	0	1	0
<i>S. mutans</i>	—	0	0	0	1
<i>S. sanguis</i>	—	1	0	1	2
<i>S. salivarius</i>	—	1	0	3	0
Untypable	—	0	1	1	0
No growth	—	28	21	9	9
Number of patients	—	47	32	26	23

DISCUSSION

Group B streptococci in venereal disease clinic patients have no relation to clinical signs and symptoms of genital infection (15). In this study, streptococci of oral origin were found in the genitals without conclusive evidence of any major role of oro-genital contact as a means of transmission. *S. sanguis*, *S. mutans* and *S. salivarius* comprise about 80 to 90% of the facultative anaerobic streptococci in the oral microbiota (7). *S. mutans* and *S. sanguis* have also been isolated in faecal specimens in very low frequency (10). The seven isolates from women could be of faecal origin. However, all *S. salivarius* strains were isolated from patients with oro-genital contacts. It is also difficult to interpret the two isolates of oral streptococci in male urethra as being of faecal origin. It would thus appear that, although in oro-genital contacts the genitals will receive a massive dose of oral flora, there seems to be very little genital colonization. Factors contributing to this fact could be bacterial interactions with the resident flora and environmental factors such as pH, humidity and nutritional requirements.

Non-gonococcal *Neisseria* infections of the genitals are diagnosed as urethritis, cervicitis, vaginitis and asymptomatic carrier state in existing reports (see review in (8)). The speculations in these sparse reports on the mode of transmission are those of oro-genital contact and transfer by hand. In this study we found only one patient with highly probable oro-genital transmission of *N. meningitidis* to the urethra and the findings thus confirm the im-

pression of a very low rate of non-gonococcal *Neisseria* infections of the genitals.

The clinical importance of the findings in this study thus appears to be the presence of non-gonococcal *Neisseria* infections in rare cases with confusing clinical and laboratory findings, while oral streptococci seem to be of lesser importance.

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REFERENCES

1. Carlsson, J.: Zooglea-forming streptococci, resembling *Streptococcus sanguis*, isolated from dental plaque in man. *Odont Revy* 16: 348-358, 1965.
2. Colman, G. & Williams, R. E. O.: *In Streptococci and Streptococcal Diseases* (ed. L. W. Wannamaker & J. M. Matsen), p. 282. Academic Press, New York, 1972.
3. Facklam, R. R.: Recognition of group D streptococcal species of human origin by biochemical and physiological tests. *Appl Microbiol* 23: 1131-1139, 1972.
4. Facklam, R. R.: *In Manual of Medical Microbiology* (ed. E. H. Lennette, E. H. Spaulding & J. P. Truant). American Society of Microbiology, Washington, D.C., 1974.
5. Facklam, R. R., Padula, J. F., Thacker, L. G., Wortham, E. C. & Sconyers, B. J.: Presumptive identification of group A, B and D streptococci. *Appl Microbiol* 27: 107-113, 1974.
6. Forsum, U.: Characterization of FITC-labelled F(ab')₂ fragments of IgG and a rapid technique for the separation of optimally labelled fragments. *J Immunol Meth* 2: 183-195, 1972.

7. Gibbons, R. J.: *In Streptococci and Streptococcal Diseases* (ed. L. W. Wannamaker & J. M. Matsen), p. 371. Academic Press, New York, 1972.
8. Givan, K. F., Thomas, B. W. & Johnston, A. G.: Isolation of *Neisseria meningitidis* from the urethra, cervix, and anal canal: Further observations. *Br J Vener Dis* 53: 109-112, 1977.
9. Jordan, H. V., Krasse, B. & Möller, A.: A method of sampling human dental plaque for certain 'caries-inducing' streptococci. *Arch Oral Biol* 13: 919-927, 1968.
10. Kilian, M. E., Theilade, E. & Schiott, C. R.: Isolation of *Streptococcus mutans* from human feces. *Arch Oral Biol* 16: 553-554, 1971.
11. Rosebury, T.: *Microorganisms Indigenous to Man*. McGraw-Hill, New York, 1962.
12. Stuart, R. D.: Transport medium for specimens in public health bacteriology. *Publ Health Rep* 74: 431-438, 1959.
13. Wadström, T., Nord, C. E., Lindberg, A. A. & Möllby, R.: Rapid grouping of streptococci by immunoelectroosmophoresis. *Med Microbiol Immunol* 159: 191-200, 1974.
14. Wallin, J.: Gonorrhoea in 1972. A 1-year study of patients attending the VD unit in Uppsala. *Br J Vener Dis* 51: 41-47, 1975.
15. Wallin, J. & Forsgren, A.: Group B streptococci in venereal disease clinic patients. *Br J Vener Dis* 51: 401-404, 1975.

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