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Bacterial Flora of the Sigmoid Neovagina

TOON A. M. TOOLENAAR,[1] INGRID FREUNDT,[1] JOHAN H. T. WAGENVOORT,[2]†
FRANS J. M. HUIKESHOVEN,[1]* MARIUS VOGEL,[2] HANS JEEKEL,[3] AND AAT C. DROGENDIJK[1]

Departments of Gynecology and Obstetrics,[1] Surgery,[3] and Microbiology,[2] University Hospital, Rotterdam Dijkzigt, Dr. Molenwaterplein 40, 3015 GD, Rotterdam, The Netherlands

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The bacterial microbiota of 15 sigmoid neovaginas, created in patients with congenital vaginal aplasia or male transsexualism, was studied. No specimen was sterile, and only normal inhabitants of the colon were cultured. The total counts of bacteria were lower than those reported for healthy sigmoid colons.

A healthy human adult colon contains a microbial flora, of which most of the species are strict anaerobes (2). The average pH is alkaline. The microbial flora of the vagina consists mainly of lactobacilli, which are responsible for the acidity of vaginal secretions. In patients with a sigmoid neovagina, an isolated segment of the colorectum is used as a neovagina. Only scarce information exists about the bacterial flora of the excluded colon, and to our knowledge, none is known about the flora of the sigmoid colon used as a vagina. The aim of the present study is to determine the microflora of the sigmoid neovagina.

The operative procedure to create a sigmoid neovagina has been described earlier (3). Briefly, preoperative mechanical bowel decontamination was performed. At the beginning of the operation a single-dose prophylactic antibiotic was given. After dissecting a plane between the bladder and rectum and isolating a sigmoid segment, the oral side of the sigmoid loop is connected with the perineum or the vulva; the aboral side is closed. Fifteen patients were studied. Ten were male-to-female transsexuals, and five patients had aplasia vaginae as a part of the Mayer-Rokitansky-Küster syndrome. None of the patients had any preexisting bowel disease. The time between surgery and the bacteriological study ranged from 23 to 87 months (mean, 52 months). After the patients provided informed consent, they filled out a questionnaire concerning the operation, complaints, and sexual habits. Eleven patients complained of sticky discharge. Thirteen patients experienced slight blood loss, eight of them spontaneously and five only after sexual intercourse. Twelve patients had regular sexual intercourse; all of them used methylcellulose as a lubricant. Condom use was not reported. No patient was using antibiotics at the time of the study. Speculum examination was done, and in most patients, the top of the neovagina contained some cellular debris with mucous strands. To measure the pH, a Neutralit pH 5 to 10 paper (E. Merck, Darmstadt, Germany) was inserted in the neovagina. A direct smear was taken from the discharge, and the smear was examined microscopically to detect the presence of *Trichomonas* species and clue cells. Also, an amine test was done for the detection of bacterial vaginosis. A sterile loop was used to collect discharge from the neovagina for quantitative bacterial analysis. This sample was placed in 2 ml of prereduced peptone yeast medium (5) with a known weight, and the mixture was immediately transported to the laboratory. Specimens were also taken to detect infections with *Candida albicans*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Chlamydia trachomatis*. On arrival at the laboratory, the sample for quantitative analysis was transferred to an anaerobic cabinet for processing. After weighing and thorough mixing, four 100-fold dilutions were made. Samples of 0.05 ml of each dilution were then plated onto several media. Blood agar medium (Oxoid Ltd., Basingstoke, England) was used for aerobic culture, and brucella blood agar medium (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Md.) was used for anaerobic culture. Several other media were used for culture of gram-positive cocci and gram-negative rods (MacConkey; Oxoid), *Haemophilus* (Levinthal; Oxoid), *Candida* species (Sabouraud; Oxoid), *Mobiluncus* species (brain heart infusion agar plus 5% horse blood, vitamin K, hemin, and 10 mg of vancomycin per liter), *Gardnerella* species (human bilayer Tween agar plate), lactobacilli and bifidobacteria (Rogosa; Difco Laboratories, Detroit, Mich.), *Trichomonas* species (Trichosel broth, modified [BBL], plus 5% horse serum), *Chlamydia trachomatis* (monolayer of HeLa 229 cells in Eagle's modified minimum essential medium; Flow Laboratories, Irvine, Scotland, as described by Thewessen et al. [11]), *Mycoplasma hominis* and *Ureaplasma urealyticum* (Trypticase soy broth agar with horse serum), and *Neisseria gonorrhoeae* (GC medium [Difco] with hemoglobin [Difco]). After an incubation period of 2 to 5 days, colony types were described, counted, and subcultured for identification. Anaerobic and facultatively anaerobic gram-negative rods were identified with the Minitek (BBL) and API 20E (BioMerieux, Marcy-l'Etoile, France) systems, respectively. Facultatively anaerobic gram-positive spheres and gram-positive rods were identified as described by others (1, 5, 10).

The average pH of the neovagina was 8 (range, 7 to 9). The facultatively anaerobic and anaerobic bacteria cultured are listed in Tables 1 and 2, respectively. No specimens were sterile. In one patient, we isolated only an *Escherichia coli* isolate. In all other patients we isolated more species (average, six; range, one to nine). Most often found were *Escherichia coli* (14 patients), *Bacteroides* species (13 patients), and lactobacilli (10 patients). A total of 85 different species representing 17 different genera were isolated. In one patient, we found *Ureaplasma urealyticum*, in one patient we found *Mycoplasma hominis*, and in one other patient we found both organisms. Of the 85 species isolated, 37 were anaerobic, with mean counts of 1×10^3 to 3×10^{10} CFU/g of

* Corresponding author.
† Present address: Department of Medical Microbiology, De Wever Hospital and Regional Public Health Laboratory, 6419 PC Heerlen, The Netherlands.

TABLE 1. Facultative anaerobes isolated from 15 patients with sigmoid neovaginas

Organism	No. of isolates	CFU/g of discharge
Gram-negative rods		
Escherichia coli	14	2×10^8–2×10^{11}
Morgnella morganii	2	2×10^4–4×10^7
Proteus vulgaris	1	2×10^4
Proteus mirabilis	2	2×10^5–3×10^5
Providencia sp.	1	2×10^6
Gram-positive spheres		
Staphylococcus epidermidis	2	4×10^4–2×10^4
Viridans group streptococci	3	2×10^3–8×10^7
Streptococcus spp.	5	8×10^3–2×10^7
Streptococcus haemolyticus	2	2×10^4–2×10^5
Streptococcus bovis	1	3×10^4
Streptococcus pneumoniae	1	8×10^5
Enterococcus sp.	1	8×10^7
Gram-positive rods		
Lactobacillus spp.	10	4×10^3–2×10^9
Corynebacterium spp.	3	4×10^3–4×10^7
Total	48	

TABLE 2. Anaerobes isolated from 15 patients with sigmoid neovaginas

Organism	No. of isolates	CFU/g of discharge
Gram-negative rods		
Bacteroides thetaiotaomicron	2	2×10^5–8×10^7
Bacteroides fragilis	5	8×10^3–1×10^9
Bacteroides distasonis	2	2×10^5–3×10^8
Bacteroides vulgatus	1	2×10^4
Bacteroides spp.	5	4×10^3–3×10^{10}
Prevotella bivius	1	3×10^5
Prevotella disiens	1	2×10^5
Prevotella intermedia	1	2×10^8
Porphyromonas asaccharolytica	2	3×10^5–4×10^7
Gram-negative sphere, *Veilonella parvula*	1	4×10^3
Gram-positive spheres		
Peptostreptococcus asaccharolyticus	2	3×10^5–3×10^6
Peptostreptococcus magnus	1	3×10^6
Peptostreptococcus micros	1	2×10^5
Peptostreptococcus prevotii	1	1×10^8
Peptostreptococcus sp.	1	2×10^8
Peptococcus anaerobius	2	2×10^7–1×10^9
Streptococcus parvulus	1	3×10^6
Gram-negative rods		
Bifidobacterium bifidum	1	1×10^3
Bifidobacterium spp.	3	3×10^3–4×10^8
Eubacterium lentum	3	1×10^6–4×10^8
Total	37	

discharge. Forty-eight species were facultative anaerobes, with mean counts of 2×10^3 to 2×10^{11} CFU/g. We did not detect *Trichomonas vaginalis*, *Candida albicans*, *Chlamydia trachomatis*, or *Neisseria gonorrhoeae*.

Studies performed during the past 20 years have given an appreciation of the complexity of the normal colonic microbial flora (4). The sigmoid colon and rectum, because of their stool "holding" function, have the highest bacterial counts of the intestinal tract. There are about 10^{11} bacteria per g of contents. Of the resident bacterial flora, 96 to 99% consists of anaerobes, mainly *Bacteroides* species, fusobacteria, and lactobacilli. The lower female genital tract contains a cornucopia of bacteria which are similar to the fecal flora, although with lower mean counts (6, 7). The viable counts average 10^8 to 10^{10} CFU/ml of vaginal fluid from the fornices, which is characterized by a predominance of lactobacilli (75 to 80%). In the present study, only normal inhabitants of the colon were isolated. The flora that we isolated is the remnant of the flora already present at the time of the operation or comes from contamination of bacteria from the perineal region. The average pH of 8 was similar to the pH of a healthy colorectum. The total counts of bacteria (10^3 to 10^{11}) were lower than those reported for a healthy sigmoid colon. This is probably caused by the lack of content or the lack of stasis in the sigmoid neovagina.

Little is known about the microflora of human excluded colon. Neut et al. (9) found in 16 patients with surgically excluded colorectums that the total bacterial counts were only slightly lower than those in healthy controls but that the variety of the flora was significantly reduced. This reduction was confined to strict anaerobes, mainly the genera *Eubacterium* and *Bifidobacterium* (9). Miller et al. (8) suggested that a stable microbial community can exist in parts of the colon with no connection to the upper part of the intestinal tract. We cultured mainly facultative anaerobes. The environment of the sigmoid neovagina may be more suitable for the growth of these bacteria.

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