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Demonstration of *Actinomyces* and *Arachnia* Species in Cervicovaginal Smears by Direct Staining with Species-Specific Fluorescent-Antibody Conjugate

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For direct observation of microaerophilic actinomycetes by fluorescent antibody, a procedure was developed in which pepsin treatment and rhodamine conjugate of normal serum were used to reduce nonspecific staining in cervicovaginal smears. *Actinomyces israelii*, *Actinomyces naeslundii*, and *Arachnia propionica* were observed in cervicovaginal smears from women who did use and who did not use an intrauterine contraceptive device. *A. israelii* was found more commonly in women with an intrauterine contraceptive device, but no evidence was obtained that the use of an intrauterine contraceptive device influenced the presence of either *A. propionica* or *A. naeslundii.*

Although the vaginal microflora has been extensively evaluated, *Actinomyces* species or related microaerophilic actinomycetes have not been reported as part of the normal flora of the female genital tract (2, 20, 34). In some 300 cases of female genital tract actinomycoses reported in the world before 1972 (29), the original point of invasion was undetermined, as was the source of the infecting organism.

However, since 1972, a large number of cases of actinomycosis associated with the use of an intrauterine contraceptive device (IUCD) have been described (1, 5, 16, 28, 30). Gupta et al. (13) first emphasized the presence of actinomycete-like granules in cervicovaginal smears from patients who used an IUCD and who had vaginal discharge or pelvic inflammatory disease but who did not have classical actinomycosis. These workers reported the preliminary observation of such organisms in 200 cervicovaginal smears stained with the Papanicolaou stain. Others have reported similar findings with the Papanicolaou stain (10). In later studies, Gupta and collaborators identified *Actinomyces israelii* in cervicovaginal smears by using fluorescein isothiocyanate (FITC) conjugates of species-specific globulins (3, 21, 22, 32). Ultimately, actinomycete-like organisms were observed in stained smears from 520 women; in 250 out of 266 of these patients, *A. israelii* was observed by staining with the FITC conjugate (12). All of the patients used an IUCD except one, who wore a pessary, and another, who was wearing a forgotten tampon. In no case was a smear positive for *Actinomyces naeslundii.* In only two cases was *A. israelii* isolated; in two other cases a member of the genus *Actinomyces* was isolated, but the species was not determined. Actinomycosis was seen in only two of these cases.

The above results indicate a strong association between the wearer of an IUCD and pelvic inflammatory disease, associated with the presence of *Actinomyces* species (27). However, the association of an IUCD and overt actinomycosis with abscess formation and typical sulfur granules rests primarily on additional studies (8, 9, 14, 19, 30, 33). In these, virtually no cultures of *Actinomyces* species were obtained (9). The diagnosis of actinomycosis rested upon the clinical and pathological characteristics of the case, upon the demonstration of the characteristic sulfur granules (25, 31), and upon the demonstration of bacteria morphologically resembling actinomycetes in clinical materials stained with Gram stain or other stains. In their study, Hager et al. (13a) emphasized that only those patients with an IUCD showed evidence of either infection or colonization with an *Actinomyces* species, whereas none of the 50 patients in the control group showed any evidence of this bacterium.

Because of the association of pelvic actinomycosis with the use of an IUCD, questions regarding the source of the causal organism have been raised (3, 13a). We wished to determine whether *Actinomyces* or *Arachnia* species were common in the vaginas of apparently healthy women. We report here a staining procedure in which pepsin digestion and rhodamine conjugate of normal rabbit globulin are used to reduce nonspecific staining by fluorescein-conjugated

antibody and the application of this procedure, which demonstrated *A. israelii*, *A. naeslundii*, and *Arachnia propionica* in cervicovaginal smears taken from apparently healthy women who had worn and who had not worn an IUCD.

MATERIALS AND METHODS

Strains. *A. propionica* serotype I (ATCC 14257) and *A. propionica* serotype 2 (F. Lenze) were received from M. A. Gerenscer (West Virginia University Medical Center) as WVU471 and WVU346, respectively. Strains *A. israelii*, serotype 1 X523 (ATCC 22101), *A. israelii* serotype 2, W838 (ATCC 23860), and *A. naeslundii* W826 (ATCC 12104) were obtained from the Mycology Division, Center for Disease Control. These five strains were used for developing general staining procedures or for producing antisera. The five strains were grown in *Actinomyces* Casitone medium (26) for maintenance and for preparing immunogens and standard suspensions for staining procedures or agglutination tests. Other strains obtained from the Mycology Division and used for determining heterologous reactivity are described below.

Preparation and characteristics of conjugated globulins. *A. israelii* (serotypes 1 and 2) and *A. naeslundii* FITC-labeled globulin were obtained from the Biological Products Division, Center for Disease Control. Antibody to *A. propionica* was prepared by immunizing three rabbits each with strains WVU471 and WVU346. A 20% (vol/vol) suspension of each strain was prepared in phosphate-buffered saline (0.01 M, pH 7.2), heated at 56°C for 1 h to kill the organisms, added to an equal volume of Freund incomplete adjuvant, and sonicated in an ice bath for 30 s at 130 W. Each rabbit received 0.1 ml intradermally and 0.1 ml subcutaneously between the scapulae. After 2 weeks, the animals were bled at weekly or biweekly intervals for 6 weeks. The animals were rested, given booster doses at 5 months, and bled for an additional 3 weeks. During this period, the development of antibody was monitored by performing agglutination tests in microtiter plates with 0.025-ml volumes for serum and standard suspensions of organisms (absorbance = 0.25) in phosphate-buffered saline containing 0.01% thimerosal as a preservative. Agglutination with homologous antigens (WVU471 and WVU346) and heterologous antigens (X523, W838, and W826) was monitored with dark-field optics, and the extent of clumping was rated from 0 to 5+. The highest titers were observed at 2 to 3 weeks after the initial and booster injections. One rabbit, immunized with strain WVU346 (serotype 2), had serum titers of 32 to 128 with itself and strain WVU471 (serotype 1), but showed only very minimal heterologous reactions with the other test strains.

Serum samples from this animal were pooled, the globulin was precipitated three times with 35% saturated ammonium sulfate (15), and the FITC conjugate was prepared by the method of McKinney et al. (23). The globulin was adjusted with normal rabbit serum, distributed in 1-ml volumes in tubing vials, and lyophilized. When the lyophilized serum was reconstituted to 1 ml, it gave a 4+ staining when diluted to 1:4 (endpoint). The homologous and heterologous staining characteristics of the anti-*A. israelii*, anti-*A. naeslundii*, and the anti-*Arachnia* reagents, as determined with about 80 bacterial strains, are given in Table 1.

For use as a counterstain to reduce nonspecific fluorescence, the tetramethylrhodomine isothiocyanate (TMRI) derivative of normal rabbit globulin was prepared with dimethylformamide as a solvent (23); it was distributed in 1-ml volumes in tubing vials and lyophilized.

Preparation and examination of experimental slides. To determine the effects of cervicovaginal secretions or various treatments on the staining of actinomycete cells by FITC conjugates, washed cell suspensions of the cultures were made in phosphate-buffered saline. They had an absorbance of 0.05 when read at 660 nm in a Pyrex test tube (18 by 150 mm) with a Beckman model B spectrophotometer. Samples (10 μl each) were taken, placed on a glass slide, air dried, heat fixed, and exposed to the various experimental procedures. Similarly, sets (six slides each) of cervicovaginal smears were obtained from 25 randomly chosen patients. The slides were air dried, and a known suspension of cells was added to the slides and mixed with the surface material; the slides were air dried and then heat fixed before being used. When possible, only the slides from a single patient or slides having similar amounts and appearances of surface material were used for any one experiment.

Before the slides were stained with the fluorescein conjugates, the vials of lyophilized globulin were reconstituted with 1 ml of distilled water, or if the TMRI conjugate was to be used as a counterstain to inhibit nonspecific fluorescence, only 0.5 ml of water was added. Equal volumes of the FITC and TMRI conjugates were then used. Each slide was covered with the conjugate and incubated for 20 min in a humid chamber at room temperature. The excess conjugate was tapped off; the slide was blotted with bibulous paper, immersed in phosphate-buffered saline (0.01 M, pH 7.2) for 10 min, rinsed briefly in distilled water, and air dried. Cover slips were then added with carbonate-buffered glycerol mounting medium, and the slides were examined (35).

Examination of cervicovaginal smears and tissue slides. Four to six cervicovaginal smears were obtained from each of 50 women who reported for routine examination and who did or did not have an IUCD. An additional set of slides was obtained from a second laboratory which submitted cervicovaginal smears of 11 asymptomatic women who wore an IUCD. These were heat fixed by two to three rapid passages through a flame. Slides prepared from patient material were then examined either by Gram stain, directly by phase-contrast microscopy, or by the fluorescent-antibody technique. Hematoxylin and eosin-stained tissue slides were used to assay actinomycetes in tissues. Cover slips of hematoxylin and eosin-stained tissue slides were removed by soaking the slides overnight in xylene. Slides were then air dried, destained with acid alcohol (1% HCl in 70% ethanol) overnight, and rehydrated by passage through a series of decreasing-alcohol, increasing-water rinses.

The final staining procedure developed and used for staining the tissue sections from 3 patients or the smears from the 61 patients was as follows. Smears or

Table 1. *Homologous and heterologous reactions of anti-actinomycete fluorescein conjugates*

Antigen species and strain no.[a]	Degree of fluorescence with conjugate to:[b]		
	A. propionica	*A. israelii*	*A. naeslundii*
Actinomyces bovis			
W827,* A9,* X521, W1755	0	0	0
Actinomyces israelii			
Serotype 1:W855,* A601,* W1691, W726, X522, X372, X523	1+[c]	4+	0
Serotype 2:W838,* X695,* W1125 W1011, W1009, W748	0	4+	0
Actinomyces naeslundii			
W826,* X600,* W569, W739, W735, W869	0	0	4+
Actinomyces odontolyticus			
W1043,* W830,* W1658, W1055, G567, D4078, W1043, W1044, G1547, WVU1596, WVU758, WVU514, W1061, W892, X362, W2312	0	0	0
Actinomyces viscosus			
W828,* X602, A755, A757	0	0	0
Arachnia propionica			
Serotype 1:W857,* W903, W973, W974, W1054, W1215	3+[d]	±	0
Serotype 2:W904,* W810, W852, W1170, W1935, W1624, WVU471, WVU346	4+	0	0
Bifidobacterium erksonii			
X407,* X573	0	0	0
Corynebacterium pyogenes			
W972*	0	±	±
Corynebacterium hemolyticum			
W967,* W1513, KC1384	0	±	±
Rothia dentocariosa			
W858,* WVU999, WVU936, W808, W876, X348, X346, X347, X599R	0	±	0

[a] All strains listed were tested with *A. propionica* conjugate. Strains indicated with an asterisk also were tested with *A. israelii* and *A. naeslundii* conjugates. In addition to the strains listed, the following cultures gave a negative reaction with the three conjugates: *Bifidobacterium bifidum* (W831), *Corynebacterium hoagii* (KC1369), *Corynebacterium xerosis* (KC1368), *Corynebacterium renale* (KC1366), *Corynebacterium pseudodiphtheriticum* (KC1364), *Propionibacterium avidum* (9064), *Propionibacterium granulosum* (9055), and *Propionibacterium acnes* (554).

[b] Fluorescence reported is the average of all cultures tested; ratings were from 0 to 4+.

[c] All cultures stained 1+, except one which did not stain.

[d] One culture stained 4+, three stained 3+, and two stained 2+.

tissue sections on slides were soaked in 0.01 N HCl containing 50 μg of pepsin A (Worthington Biochemicals Corp.; 2× crystallized; 2,800 U/mg) per ml for 4 h at 37°C. The slides were washed in phosphate-buffered saline (0.01 M, pH 7.2), rinsed in distilled water, and air dried. The surface of each slide was then demarked into three areas with a wax pencil, and 1 drop of species-specific fluorescent-antibody conjugate or 1 drop of the fluorescent-antibody conjugate and 1 drop of the TMRI conjugate were added to cover the central portion of the delineated area. The remaining steps were those given in the brochure accompanying the *Actinomyces* species reagents (35) in which the slides, after incubation for 20 min in a humid chamber, were washed, air dried, and covered with cover slips mounted with buffered-glycerol medium.

The slides were examined with a Leitz Dialux fluorescent microscope with a 100-W mercury lamp for epi-illumination, a BG-38 heat absorber, and an H cube having two KP sp. 490 excitors and a TK barrier. Stained slides of tissues or smears were made by one of us, and the interpretations were verified by another. To be recorded as positive, the fluorescence had to be of a 2+ to 4+ intensity with an absolute adherence to either diphtheroid or filamentous structure, as depicted elsewhere (4, 6, 7).

The dry weight of the material on the slide was determined as the difference between the constant

weight of a cervicovaginal smear dried at 78°C and the constant weight of the slide after it had been washed with a brush and water, thoroughly rinsed with distilled water, and dried at 78°C.

Control cervicovaginal smears from asymptomatic persons were prepared, and cells of known species were added. These slides were handled in separate dishes from the test smears to avoid any possible contamination of test specimen smears with the known cultures.

RESULTS

Effect of pepsin and rhodamine on the staining of actinomycete cells with FITC conjugates. In our initial experiments with FITC conjugates, we could not demonstrate *A. israelii* in tissue sections which we knew were positive by culture and Gram stain. In these sections and in the vaginal smears, our major difficulty was the nonspecific fluorescence of tissue or cellular elements. We attempted to remove or decrease the nonspecific fluorescence by staining with the TMRI conjugate of normal serum or by proteinase treatment. We knew that pepsin or trypsin treatment would not destroy the gross morphology or the relative antigenicity of *Actinomyces* species (18, 25). However, we recognized that proteinase treatment might adversely affect the staining of actinomycete cells with homologous FITC conjugates by (i) decreasing the intensity of the homologous reaction, (ii) increasing the heterologous reaction, (iii) increasing greater nonspecific staining of the surface material, or (iv) adversely affecting the rhodamine counterstain.

Experiments were done to determine these possible effects of pepsin on actinomycetes alone or on actinomycetes added to cervicovaginal smears. Pepsin was chosen because its reactivity at a low pH would inhibit nonspecific binding of products and bacterial growth during incubation periods; pepsin (2× recrystallized) was chosen to limit nonspecific proteolytic reactions and because it was inexpensive. The dry weight of material on the surface of cervicovaginal smears ranged from 0.6 to 4.0 mg. Given 20 mg of protein (five thickly coated slides) per Coplin dish, the solution of pepsin used could digest at least 50 times an equivalent weight of coagulated egg albumen. This amount of pepsin was taken as a satisfactory excess of the amount needed for thorough digestion of heavily coated slides or of tissue sections.

Pepsin digestion did not reduce the homologous staining reactions of the actinomycete cells when alone or when added to slides of cervicovaginal smears. Heterologous reactions among the five strains of *A. israelii*, *A. naeslundii*, and *A. propionica* were not induced or increased by the use of pepsin on the cells alone or in the presence of clinical material; but treatment with pepsin greatly reduced the amount of surface material, and microscopic examination showed much less cellular debris and fewer blood cells. Pepsin digestion also reduced the amount of nonspecific fluorescence observed in the residual material in many of the slides.

TMRI-conjugated normal globulin effectively blocked the staining of nonbacterial material in about 75% of the cervicovaginal smears but did not have any effect on the remaining slides. The use of this conjugate did not diminish the homologous fluorescence of untreated or pepsin-treated bacterial cells either stained alone or added to cervicovaginal smears. Cervicovaginal smears that had been digested with pepsin and counterstained with rhodamine had much better backgrounds for observation of fluorescein-stained actinomycete cells than did untreated material. However, in slides having only small amounts of surface material, nonspecific staining of the clinical material was minimal or nonexistent, and treatment with the TMRI conjugate after pepsin digestion did not seem to be advantageous.

Examination of tissue sections. By using the conjugates specific for each species, examination of the sections of endometrium and endocervix showed actinomycotic filaments staining only with the anti-*A. israelli* conjugate. The morphology of *A. israelii* in these slides was that of ramifying filaments to spreading mycelium identical to the morphology depicted by Blank and Georg (4) and Brock et al. (6). In no case were individual diphtheroidal cells or sulfur granules seen in these slides. Descriptions of these positive cases are published elsewhere (13a); *A. israelii* serotype 1 was isolated from the tissue sections of one case.

Examination of cervicovaginal smears. Examination of cervicovaginal smears from the first 25 patients with phase-contrast microscopy was unsuitable for observations of actinomycete elements. Staining of thè smears by the Gram method readily revealed gram-positive structures which were either filamentous or diphtheroid, and the morphology of these structures was consistent with that of the microaerophilic actinomycetes that we have seen previously. In no case did we observe granules that could be identified as either sulfur granules or mycelial colonies, although in many cases we found granules consisting of central masses of gram-positive bacilli with radiating bacillary chains. However, when slides were stained directly with the FITC conjugates without pepsin digestion, we found no cells or structures that we considered positive

for *Actinomyces* species.

Cervicovaginal smears from the second group, that consisting of 50 patients, were examined with the standard pepsin digestion–fluorescent-antibody-conjugate procedure described above; the TMRI conjugate of normal rabbit globulin was used as a counterstain. Of the 50 patients, 18 wore an IUCD, and actinomycetes were found in smears from 8 (44.4%) of the 18. Actinomycetes were found in smears from 10 (31.2%) of the 32 who did not wear an IUCD. Of the 18 patients positive for actinomycetes (Table 2), 11 (63%) showed *A. israelii*, 11 (63%) showed *A. propionica*, 2 (11%) showed *A. naeslundii*, and 7 (33%) showed two of these three species.

The actinomycete cells observed in most of these cervicovaginal smears were small individual diphtheroid cells or groups of diphtheroid cells. In slides from patient 11, however, a large, dense mycelial colony of *A. propionica* and several clusters of filamentous forms of *A. israelii* were observed. In slides from patients 3, 6, and 7, filamentous and mycelial forms of *A. propionica* were seen. In several of the slides, we observed large cocci, in pairs or in chains, which stained 1 to 2+ with the anti-*A. propionica* conjugate; these cells were not recorded as positive for *Arachnia* species, because they did not conform to the morphological criterion required.

Relationship of IUCD use and presence of actinomycetes. Of the smears from the 18 patients with an IUCD, 39% were positive for *A. israelii*, and 17% were positive for *A. propionica*; none was positive for *A. naeslundii* (Table 2). In contrast, only 12 and 6% of the slides from 32 patients without an IUCD stained positively for *A. israelii* and *A. naeslundii*, respectively, whereas 25% of the slides were positive for *A. propionica*. A chi-square test comparing the positive percentages in the presence and absence of an IUCD was done for each of the three species (Table 3). The statistical test for the presence of *A. israelii* versus the use of an IUCD indicated no dependence at the 5% level of probability ($P = 0.05$). However, the P value (0.07) obtained for *A. israelii* was much smaller for this comparison than that determined for *A. naeslundii* ($P = 0.74$) and for *A. propionica* ($P = 0.74$).

In addition to the 50 cases (Table 1), smears were obtained from a second laboratory. These were from 11 cases of asymptomatic women who wore an IUCD; they were examined by one of us (J.M.B.) for the presence of *A. israelii* and *A. propionica* only. Of these, all of the patients were positive for *A. israelii*, and four were positive for *A. propionica*. These overall results strongly suggest that populations of *A. israelii* are stimulated by the use of the IUCD, but do not suggest that *A. naeslundii* and *A. propionica* are similarly stimulated.

DISCUSSION

We report here observations of *A. israelii*, *A. naeslundii*, and *A. propionica* in cervicovaginal smears. These organisms were identified by direct staining with FITC-labeled rabbit globulin

TABLE 2. *Microaerophilic actinomycetes detected by fluorescent-antibody staining of cervicovaginal smears from women with and without an IUCD*[a]

Patient no.	Species observed	IUCD	Relative intensity of fluorescence with conjugate to:		
			A. israelii	*A. naeslundii*	*A. propionica*
1	*A. israelii*	–	2+, 3+	–	–
2	*A. israelii, A. naeslundii*	–	2+	3+	–
3	*A. israelli, A. propionica*	–	2+	–	4+
4	*A. israelii, A. propionica*	–	3+	–	2+
5	*A. naeslundii, A. propionica*	–	–	2+	4+
6	*A. propionica*	–	–	–	2+
7	*A. propionica*	–	–	–	3+
8	*A. propionica*	–	–	–	3+, 4+
9	*A. propionica*	–	–	–	3+
10	*A. propionica*	–	–	–	3+
11	*A. israelii*	+	2+	–	–
12	*A. israelii*	+	3+, 4+	–	–
13	*A. israelii*	+	2+, 3+	–	–
14	*A. israelii*	+	2+, 3+	–	–
15	*A. israelii*	+	2+, 3+	–	–
16	*A. israelii, A. propionica*	+	4+	–	2+
17	*A. israelli, A. propionica*	+	3+, 4+	–	3+
18	*A. propionica*	+	–	–	2+, 3+

[a] Material from 50 patients was tested. No actinomycetes were observed in 32 of the patients.

TABLE 3. *Relationship of the presence of an IUCD and the observation of actinomycete species*

Species	IUCD	No IUCD	χ^2	*P*
A. israelii	7/18[a]	4/32	3.26	0.07
A. naeslundii	0/18	2/32	0.11	0.74
A. propionica	3/18	8/32	0.11	0.74

[a] Values represent a number positive/number tested ratio.

specific for each organism and by observing for a typical actinomycete morphology (4, 6, 7, 17, 31). Although these criteria of specific staining and morphology are specific for the identification of these species in clinical material, it would be desirable to support our observations with direct isolations of each of the three species. Microaerophilic actinomycetes can be isolated from clinical materials under circumstances where there is relatively little contamination with other bacteria or where sulfur granules or calculus fragments can be washed free of contaminating bacteria. Direct attempts to isolate *Actinomyces* species from samples of the vaginal pool associated with the presence of an IUCD were successful in 4 out of 11 cases (3), but such attempts often have not led to positive isolations (2, 20, 34). Specific procedures for isolating these bacteria from heavily contaminated materials need to be developed. Nevertheless, our results constitute strong presumptive evidence that these three species are common inhabitants of the vagina.

Of particular interest to us during the examination of the cervicovaginal smears and tissue sections were the overall morphological aspects of the actinomycetes from different patients. In 14 of our cases in which the smears were positive for actinomycetes, we observed the organisms primarily as diphtheroidal forms, either as a few joining cells or as small groups of cells. This morphology is commonly observed in liquid cultures, but in smears from four patients, only one of whom had an IUCD, the organisms were either small, spider-like mycelial colonies within masses of the neutrophiles or ramifying filaments dispersed throughout the cellular elements. These latter smears seem to be from patients in which the vaginal environment induced the organism to grow with a morphology commonly observed in cases of actinomycosis (31). The medical significance of these observations is unknown, but they suggest that formation of mycelial elements by the actinomycete growing within the vagina represents a departure from the common diphtheroid aspect observed in most of our cases. Although none of the 18 patients having positive smears for *Actinomyces* or *Arachnia* species was described as clinically ill, many of the smears showed a heavy discharge with leukocytes or erythrocytes, some of which could have been endometrial in origin.

The boundaries between infection and colonization by microaerophilic actinomycetes are not clear; most certainly we have not established the differentiating criteria. Only *A. israelii* and *A. propionica* have been reported as causal agents of numerous cases of human actinomycosis characterized by abscesses, the progressive formation of draining sinuses, sulfur granules, and typical gram-positive, filamentous branching elements (6, 31). *A. naeslundii* has been isolated from other types of abscesses in humans (11). Thus, all of these species represent a potential for infection in the host, particularly if they gain entrance to the deeper tissues (31), but *A. israelii* and *A. propionica* may colonize tissue surfaces without penetrating deeper areas of the organs. Thus, both may grow in the lacrimal canal and form concrements that are large enough to erode the membrane or to completely block drainage, but neither species has been reported as having aused progressive actinomycosis emanating from the lacrimal canal (24, 31). imilarly, *A. israelii* present in tonsillar crypts appears to lack direct invasive ability (4). Hager et al. (13a) have described colonization of the endocervix by *A. israelii* in the absence of symptomatic pelvic infection in a patient with an IUCD; but on the basis of the description of some 29 cases of overt actinomycosis associated with an IUCD, one or more of the above species, in association with the IUCD, would appear to be able to induce invasive disease. That *A. israelii* in the presence of the IUCD may also cause pelvic inflammatory disease has not been proven (M. R. Spence and W. D. Hager, personal communication), but Bhagavan and Gupta (3) have pointed out that of the IUCD users in which *A. israelii* was found, 25% had pelvic inflammatory disease as compared with the 2 to 8% of all IUCD users who had pelvic inflammatory disease.

The results presented here show that *A. israelii*, *A. naeslundii*, and *A. propionica* are common to the human vagina. These results and those of the numerous workers cited above strongly support the conclusion that the origin of pelvic actinomycosis stems primarily from the indigenous population of *A. israelii*, if not other actinomycetes. This conclusion is in contrast to that of Gupta et al. (12), who suggested that the oropharynx is the source of the infecting organism. The numerous cases of pelvic actinomycosis reported in association with the use of an IUCD suggest an increased risk of infection with the

use of this device, but further studies are required to determine the extent of this increased risk, with a clear delineation between colonization and infection and with identification of the causal organism.

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