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Actinomyces odontolyticus isolated from the female genital tract

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SUMMARY *Actinomyces odontolyticus* was isolated from genital tract specimens from 4·8% of 561 women fitted with intrauterine contraceptive devices and from 4% of 101 women with pelvic inflammatory disease and 1·8% of 525 women without pelvic inflammatory disease who were not known to be intrauterine contraceptive device wearers. The strains were isolated by prolonged anaerobic incubation of blood agar, with or without added 5% metronidazole or 1% neomycin. *A odontolyticus* has not been previously reported in cervico-vaginal specimens, and possible reasons for this are discussed.

Actinomyces odontolyticus was first described in 1958 by Batty,[1] who isolated strains from deep carious dentine and saliva. On blood agar, colonies initially resembled viridans streptococci, but after three or four days' incubation they developed a characteristic dark reddish brown pigment. The strains grew equally well under aerobic or anaerobic conditions. Isolation and identification of the organism were considered to be "exceedingly difficult."

A odontolyticus has only rarely been recovered from body sites other than the oral cavity, although transient bacteraemia after dental manipulation may occur.[2] Disseminated lesions due to *A odontolyticus* have occasionally been reported, including granulomatous lesions of the chest wall,[3] actinomycosis of the malar region,[4] pulmonary abscess,[5] and pleural empyema.[6,7] *A odontolyticus* was repeatedly isolated together with *Fusobacterium necrophorum* from the blood of a patient who developed a septicaemic illness with multisystem involvement attributed primarily to the fusobacterium.[8] Mouse inoculation experiments suggested that both organisms administered together showed enhanced virulence.

In all the above cases, the source of the organism, though not established, was probably the periodontal region or oropharynx.

In this laboratory we have isolated *A odontolyticus* from numerous cervico-vaginal specimens receiving prolonged anaerobic culture for the isolation of actinomyces, which is now widely implicated in genital infections associated with intrauterine contraceptive devices (IUCDs).[9] These isolates and their possible importance are reported here as we are unaware of previous accounts of this organism in the female genital tract.

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Material and methods

Initially, *A odontolyticus* was isolated sporadically from seven women wearing IUCDs but disregarded. Two years later a systematic investigation was undertaken, and over a period of 14 months specimens were obtained from 561 patients fitted with IUCDs. These included high vaginal swabs, endocervical swabs, and IUCDs themselves when removal was clinically indicated. No special transport swabs or media were used, and specimens were cultured on blood agar plates, incubated aerobically and anaerobically with additional CO_2, as well as in cooked meat medium. Anaerobic culture was also carried out on horse blood agar plates containing 1% neomycin sulphate, which were incubated for five days. For isolation of actinomyces, specimens were inoculated on to blood agar plates containing 5% metronidazole for the selective inhibition of strict anaerobes. These were incubated anaerobically and examined after five and 10 days' incubation at 37°C. Both the neomycin and metronidazole plates were examined under a low power binocular microscope for typical colonies of *A israelii* ("molar tooth") or dark brownish red colonies characteristic of *A odontolyticus*. Colonies selected for further investigation were subcultured on to blood agar plates, which were incubated anaerobically.

Cervico-vaginal specimens were obtained from a

further group of 101 patients not stated to be IUCD wearers but with suspected pelvic inflammatory disease, which warranted prolonged anaerobic culture of specimens. These were cultured by standard methods given above and inoculated on to neomycin agar plates, which were incubated anaerobically for five days.

Finally, a survey was carried out of the incidence of *A odontolyticus* in 525 vaginal swabs from patients without pelvic inflammatory disease and not stated to be IUCD wearers. Most were submitted with a diagnosis of vaginal discharge. For this purpose the routine anaerobic blood agar plate was retained and reincubated anaerobically for up to 10 days.

Colonies selected as likely *A odontolyticus* on appearances were verified as Gram positive bacteria, and submitted to the following physiological and biochemical tests:

1 Atmospheric requirements for optimal growth on blood agar.
2 Pigmentation on blood agar, Isosensitest agar containing lysed blood, and Columbia agar.
3 Twenty eight strains were identified by the Minitek Anaerobe II System (Becton Dickinson Limited), incubating anaerobically for 48 h.
4 The same strains were tested for α-fucosidase activity, using the chromogenic enzyme substrate 4-nitrophenyl-α-L-fucopyranoside (Koch-Light).[10] The organisms were first grown on Columbia agar, with anaerobic incubation with additional CO_2 for five days. A heavy suspension of each test organism was then made in 0·2 ml of a 0·1% wt/vol concentration of the substrate in phosphate buffer, 0·06 mol/l, pH 8·0, using a microtitre tray. After 4 h incubation at 37°C the development of a yellow colour, due to release of nitrophenol, indicated α-fucosidase activity.
5 Antibiotic sensitivities were determined by the disc agar gel method on blood agar plates incubated anaerobically for 18 h. Discs containing penicillin (1 unit) and metronidazole (1 μg) were included.

Results

Following the earlier isolation of seven strains from IUCD wearers, *A odontolyticus* was isolated from 27 of the 561 IUCD wearers (4·8%); from four of the 101 patients with pelvic inflammatory disease not known to be IUCD wearers (4%); and from seven of the 525 vaginal swabs from patients without pelvic inflammatory disease or IUCDs (1·3%). Table 1 gives the clinical details of 41 of these patients, together with accompanying bacterial isolates.

In five of the IUCD wearers colonised with *A odontolyticus*, actinomyces like organisms had been previously reported in cervical smears by Dr AI Spriggs.

The isolates generally conformed to previous descriptions of the organism.[1 11] On subculture to horse blood agar, they first appeared as small α-haemolytic colonies with surrounding browning of the medium after one to two days' incubation; they later developed a brownish red pigment with a dense centre after three to five days. Pigment production occurred earlier under anaerobic conditions. It was seen only on media containing blood and was more intense in the presence of lysed blood. The pigment was insoluble in water, alcohol, acetone, and ether. Microscopically, the organisms appeared as pleomorphic Gram positive bacteria, showing branching, beaded, or clubbed forms. On subculture, nutrient agar appeared to support growth as well as blood agar. Growth was generally improved in an atmosphere of 5% CO_2 and was maximal under anaerobic conditions with added CO_2. Since only prolonged anaerobic incubation was used for primary isolation, it was not established whether any strains were obligate anaerobes initially; but all were facultative on subculture.

Table 2 gives the results of biochemical tests carried out on 28 strains. All were identified unequivocally by the Minitek Anaerobe II System as *A odontolyticus*. Eleven of the 28 strains showed α-fucosidase activity. All strains were sensitive to penicillin and resistant to metronidazole. No strains of *A israelii* were recognised in any of the cultures included in this study.

Discussion

Several reports have indicated an association between IUCD use and colonisation of the endocervix by actinomyces like organisms.[14-17] These were first recognised microscopically in Papanicolaou stained cervical smears from IUCD wearers[9] and subsequently identified as microcolonies of *A israelii* by specific immunofluorescence staining.[14 18] In most cases the presence of these "Gupta bodies" is not associated with symptoms, but there is sometimes clinical and pathological evidence of endometritis[17 18] and, rarely, overt actinomycosis of the genital tract may develop.[19-21]

It has been surprisingly difficult to grow actinomyces from the female genital tract, and cultures may be negative even in severe established actinomycosis.[19 21] Strains isolated from cervicovaginal specimens have occasionally been identified as *A israelii*,[14 15] and *A israelii* was isolated from the brain abscess of a patient who four years previously

Table 1 *Actinomyces odontolyticus: details of isolations from genital tract specimens*

Case no	*Date*	*Specimen*	*IUCD details where known*	*Clinical details*	*Associated organism*
1	21. 4.80	HVS	IUCD, 2 yr	Profuse white discharge	
2	4.80		IUCD	No details available	
3	21. 6.80	IUCD	IUCD, 5 mo	Abdominal pain	Group C streptococcus
4	28. 8.80	HVS	IUCD	Vaginal discharge	*Candida albicans*
5	30.10.80	Endocervical swab	Cu ML 250, 6 mo	Increasing vaginal discharge	Mixed anaerobes
6	8.12.80	HVS	IUCD in situ	Vaginal discharge	GBS, coliforms, *Cand albicans*+++, bacteroides
7	10.12.80	IUCD		Very offensive vaginal discharge	No pathogens
8	24. 9.82	HVS	IUCD	Irregular periods. Brown discharge. Painful cervix	Peptostreptococcus +
9	27. 9.82	HVS	IUCD	Vaginal discharge	Mixed anaerobes including Bacteroides
10	28. 9.82	Endocervical swab	Saf T coil, 4 yr	Tender uterus. Mid-cycle bleeds.	GBS, coliforms++, *Bacteroides fragilis*
11	29. 9.82	HVS	IUCD	Bloodstained vaginal discharge for 6 weeks	Peptostreptococcus+++
12	30. 9.82	HVS	IUCD	Vaginal discharge	Non-haemolytic streptococcus faecal streptococcus, diphtheroids
13	30. 9.82	IUCD	Lippes loop	Removal because of actinomyces like organisms in cervical smear	Peptococcus, peptostreptococcus
14	4.10.82	HVS	IUCD	Abdominal pain? pelvic inflammatory disease	*Cand albicans,* non-haemolytic streptococcus
15	11.10.82	Endocervical			Group G streptococci pepto-streptococcus ++
		IUCD	Lippes loop, 4 yr	Actinomyces organisms in cervical smear	Faecal streptococci
16	13.10.82	HVS	IUCD	Heavy vaginal discharge 6 mo Back pain	Bacteroides +++
17	19.10.82	Endocervical swab	IUCD	Lower abdominal pain 2 weeks	No pathogens
18	19.10.82	HVS		Vaginal discharge	No pathogens
19	19.10.82	HVS		Vaginal discharge	*Gardnerella vaginalis* +++
20	21.10.82	Vaginal swab		Recurrent vaginal discharge	No pathogens
21	25.10.82	HVS	IUCD	Recent abdominal pain	Peptostreptococcus, *Cand albicans,* faecal streptococcus
22	21.10.82	IUCD		Acute abdominal pain, vaginal discharge	*Streptococci milleri,* faecal streptococcus
23	24.11.82	HVS	IUCD	Vaginal discharge	Peptostreptococcus *Bact melaninogenicus*
24	1.12.82	HVS		Vaginal discharge, pregnant	No pathogens
25	20.12.82	HVS	IUCD, 3 yr	Actinomyces found in routine cervical smear	
26	31.12.82	IUCD	IUCD	Menorrhagia	Peptostreptococcus
27	20.1.83	IUCD	CuML 250	Actinomyces like organisms in smear	*Streptococcus milleri* +
28	26. 1.83	Endocervical swab	Cu 7	Pain in left ileac fossa one month. Pelvic inflammatory disease. Follicular cystitis	Faecal streptococcus, diphtheroids
29	31. 1.83	HVS	IUCD	Offensive discharge. ? actinomycosis	Faecal streptococcus
30	1. 2.83	HVS	IUCD	Discharge	No pathogens
31	4. 2.83	HVS	IUCD	Discharge	No pathogens
32	24. 2.83	HVS	IUCD	White vaginal discharge. Cervicitis	GBS
33	9. 3.83	HVS	IUCD	Vaginal discharge 3 mo	GBS, peptostreptococcus *Gardnerella vaginalis*
34	22. 3.83	HVS	IUCD	Actinomycetes seen in cervical smear. Slight irritant vaginal discharge with blood staining	GBS, Peptostreptococci
35	25. 3.83	IUCD	CuML 250		Faecal streptococcus
36	26. 5.83	Endocervical swab	IUCD	Recent onset of pain	No pathogens
37	2. 6.83	HVS	IUCD, 10 yr	Offensive vaginal discharge 4 weeks	Peptostreptococcus, faecal streptococcus
38	28. 6.83	HVS	IUCD	Vaginal discharge 3 mo	Peptostreptococcus, diphtheroids
39	11. 7.83	Endocervical swab	IUCD	Offensive vaginal loss for 2 wk	No pathogens
40	21. 9.83	IUCD	Dalkon shield, 11 yr	Pain and bleeding 3 wk	*Cand albicans,* faecal streptococcus
41	23. 9.83	HVS IUCD	IUCD	? Infection. IUCD removed because of wish to conceive	*Cand albicans,* faecal streptococcus, coliforms

GBS = Group B streptococcus.
IUCD = intrauterine contraceptive device.
HVS = high vaginal swabs.
+ = scanty; ++ = moderate; +++ = profuse growth.

Table 2 *Biochemical characteristics of Actinomyces odontolyticus: results of present study compared with those in previous reports*

	Batty[1]	*Bergy*[11]	*Baron et al*[5]	*Guillou et al*[6]	*Holdeman*[12]	*Holmberg Nord*[13]	*Mitchell et al*[4]	*Minitek database**	*Oxford series (28 strains)*
Aesculin hydrolysis		63	−		+	49	+	80	100
Catalase	−	0	−	−	−	0	−	0	0
Gelatin hydrolysis	−	0		−	−	0	−	0	0
H_2S production	−	0–87				17			
Indole production	−	0	−	−	−	0		0	0
Litmus milk-acid	50	80			+				
Methyl red	−	100				0			
Nitrate reduction	+	100	+	+	+	100	+	100	100
Starch hydrolysis		0		−	−	17		10	
Urease	occas	0	−		−			0	0
Voges-Proskauer	−	0				0			
Acid from:									
Adonitol		20			−	0			
Arabinose	v	50	−	−	−	17	+	50	32
Cellobiose		0			−			0	0
Fructose	−			+	+				
Galactose	v			+	−	100			
Glucose		100	+	+	+	100	+	100	100
Glycerol	−	86	−		−			50	50
Glycogen	−	0			−	0			
Lactose		77	−	−	+	100	−	90	100
Maltose	−		+	−	v		+	80	100
Mannitol	v	0	−	−	−	0	−	0	0
Mannose				−	−	0		10	0
Raffinose		50		−	−	0		30	46
Rhamnose	−	43		−	−			20	0
Ribose					−	17			
Salicin	−	70	−	−	−		−	50	29
Sorbitol		3		−	−	0		0	0
Sucrose	v		+	+	+		+	90	100
Trehalose	−	66		−	−	0		10	0
Xylose	−	53	−	−	v	83	−	50	25

*Data from BBL minitek numerical identification system percent chart.
− = negative, + = positive, v = variable. Numbers indicate percentage of positive strains.

had developed a tubovarian abscess associated with an IUCD.[20] In a recent study actinomyces were isolated from 13 of 15 women whose cervical smears showed the presence of actinomyces like bodies.[17] The cultures were identified as *A israelii* by immunofluorescence using an antiserum prepared against *A israelii* which also gave strong reactions with other actinomyces, including *A odontolyticus*. Since cultures were made on blood agar and there is no mention of the isolation of red colonies, it may be inferred that *A odontolyticus* was not isolated.

It is uncertain whether actinomyces are indigenous in the normal cervico-vaginal flora; in most studies "Gupta bodies" have been absent from the cervical smears of women not wearing IUCDs.[15 17] In a study using immunofluorescence staining of cervical smears with multiple antisera, *A israelii, A naeslundii,* and *Arachnia propionica* were commonly found in women who did not wear IUCDs.[22] These results were not confirmed in a subsequent study.[23] In an investigation of patients attending a genitourinary clinic, actinomyces were isolated from the endocervical swabs of 20 of 78 IUCD wearers and from 12 of 58 women using various forms of contraception other than IUCDs.[24] Twenty nine of the 32 isolates were identified as *A israelii* by conventional biochemical tests. The medium used for isolation in this study did not contain blood, so that red colonies of *A odontolyticus* would not have been detected. The carbohydrate fermentation reactions of *A odontolyticus* are variously reported by different authors (Table 2), the results presumably being influenced by the methods used. Identification of actinomyces ultimately depends on analysis of the chemical and antigenic composition of the cell wall,[10] but such techniques are beyond the scope of a routine laboratory. The α-fucosidase reaction appears to be specific for *A odontolyticus* and *A viscosus*,[10] which are readily distinguished by the catalase test. Only 11 of 28 strains in the present study were α-fucosidase positive, however, whereas all seven strains tested by Kilian were positive.[10] The red pigment and aerobic growth of *A odontolyticus* are important aids to identification; *A naeslundii* may develop a tan pigment on prolonged incubation but is urease positive.

During 1983 13 isolations of *A odontolyticus* from women wearing IUCDs as well as four isolations from non-genital sites were reported to the Communicable Disease Surveillance Centre, Colindale, London, by other laboratories (unpublished observations). It is surprising that previous studies have

not mentioned *A odontolyticus* in the female genital tract since it is likely that media containing blood are widely used for cervico-vaginal cultures. Equally, we cannot explain our failure to isolate *A israelii* in the present study. In five of our patients with *A odontolyticus* actinomyces like structures had been found previously in cervical smears. It seems likely that these structures may be formed from *A odontolyticus* in some patients, but this remains to be established, presumably by immunofluorescence staining of cervical smears using specific antibody to *A odontolyticus*.

The pathogenicity of this organism in the genital tract is unknown, although several of our patients had symptoms which have been attributed to endocervical infection by actinomyces.[16,17] Our findings have not shown unequivocally that IUCD usage predisposes to colonisation by *A odontolyticus* or that the organism may cause pelvic inflammatory disease in the absence of an IUCD. Further work is required to establish this beyond doubt.

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