The effect of a copper intra-uterine contraceptive device on the microbial ecology of the female genital tract

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Summary. Bacteria isolated from 108 intra-uterine contraceptive devices (IUCD) removed from patients with pelvic inflammatory disease (PID), haemorrhage, pregnancy and from asymptomatic women, and from the genital tracts of 66 healthy controls not wearing an IUCD, were studied. No significant differences were found in the types of micro-organisms or isolation rates from IUCDs removed from women in the various clinical groups. The isolation rate of anaerobic bacteria from IUCDs removed from asymptomatic wearers was significantly lower than that from controls, with the exception of the isolation rate of actinomyces which was significantly higher in IUCD wearers and *A. israelii* was recovered only from IUCDs. The isolation rates of the different bacterial species varied with the duration of the device *in utero*. The presence of a copper IUCD altered the bacterial flora of the female genital tract. The insertion of such a device and the ecological changes that follow play a crucial role in the development of PID.

Introduction

The requirement for contraception has led to the use of intra-uterine contraceptive devices (IUCD). This method of contraception is not without risk, and reports have shown an increased incidence of pelvic inflammatory disease (PID) amongst women wearing such devices (Nilsson *et al.*, 1981; Duguid *et al.*, 1982). *Actinomyces israelii* and related species *A. naeslundii*, *A. odontolyticus* and *Arachnia propionica* have been incriminated in these infections (Burkman *et al.*, 1982; Bonnez *et al.*, 1985; Nayar *et al.*, 1985; Peloux *et al.*, 1985). Although *Actinomyces* spp. are not part of the normal vaginal flora and are rarely recovered from this site, they have been found to colonise the genital tracts of 2.8–25% of women wearing an IUCD (Sykes and Shelley 1981; Burkman *et al.*, 1982; Keebler *et al.*, 1983; Peloux *et al.*, 1985). Some studies showed that these organisms tend to colonise mainly plastic devices and disappear when these are replaced by copper-containing ones (Duguid *et al.*, 1982; Keebler *et al.*, 1983).

Most of these findings were based on the detection of *Actinomyces* spp. on Papanicolaou-stained cervico-vaginal smears and by immunofluorescence with actinomyces antisera. Other organisms nor-

mally present in the vagina may resemble actinomyces in Papanicolaou smears (Rogosa *et al.*, 1974) and if antisera have not been tested against a wide range of anaerobes, cross-reactions with other bacterial species may occur (Jones *et al.*, 1979). Attempts were made to culture IUCDs, but the rate of isolation of *Actinomyces* spp. was low and reports on the types and frequency of other isolates were inconsistent (Hager *et al.*, 1979; Nilsson *et al.*, 1981; Sparks *et al.*, 1981; Duguid *et al.*, 1982).

In this study we used improved microbiological techniques to enable us to evaluate the effect of a copper IUCD on the bacterial flora of the female genital tract, the predisposition of wearers to colonisation by *Actinomyces* spp. and the relationship between bacterial colonisation and gynaecological symptoms.

Materials and methods

Patients

One hundred and eight patients who had IUCDs removed during the period June 1984 to May 1986 were studied. They were 21–35 years old with a mean age of 27 years. They were randomly selected from patients referred to the Maternity Hospital and Maternal Welfare Clinics, Kuwait for removal of IUCD. Reasons for removal were PID, haemorrhage, pregnancy, pelvic pain, vaginal

discharge, desire to conceive or an IUCD *in utero* for 2 years or more. Each patient was examined for evidence of PID, pregnancy and gynaecological abnormalities.

PID was diagnosed if the patient presented with fever, vaginal discharge and lower abdominal pain, and pelvic tenderness and evidence of adnexitis were found on examination. As shown in table I patients were divided into five groups according to clinical findings: group 1 (15) had PID; group 2 (21) had irregular bleeding; group 3 (10) women were pregnant; group 4 (11) was a miscellaneous group with suprapubic pain (7), vaginal discharge (3), or perforation of the uterus (1); and group 5 comprised (51) asymptomatic IUCD wearers.

Controls

Sixty-six healthy women attending the Maternity Hospital clinic for advice on contraception, with no contraception or medication for at least 6 months and no gynaecological abnormalities, served as controls. They were 20–37 years old with a mean age of 28 years.

Contraceptive devices

The devices removed were copper-containing with monofilament tails (Nova-T, Copper 7, and Cu 250). Their duration *in utero* was 2 weeks to ten years, with a mean of 20.8 months.

Collection of specimens

Specimens were collected by the two gynaecologist authors. The cervix of each patient was exposed by a sterile bivalve speculum and the IUCD was removed aseptically with sterile forceps without touching the vaginal wall or perineum.

Initially the tails and bodies of 20 IUCDs were processed separately. When no difference was found in the organism isolated, the remaining 88 devices were processed *in toto*. Specimens from the controls were similarly collected from the endocervix and the posterior

fornix with albumin coated swabs. These sites were sampled because they represent areas where an IUCD lies—partly in the endocervix with the filament extending down into the upper vagina. The devices and swabs were placed in anaerobic transport tubes (Portagerm; bio-Merieux, Charbonniers-Les-Bains, France) and sent to the microbiology laboratory within 24 h.

Microbiological methods

Direct plating was not possible because of the irregular structure of the IUCD. The device was soaked in 15 ml of Brain Heart Infusion (BHI) broth (CM225, Oxoid) for 30 min. The broth was shaken for 15 s and 0.1 ml was inoculated on to each of the following pre-reduced culture media: Blood Agar (CM55, Oxoid), Schaedler Blood Agar (CM437, Oxoid) with kanamycin-vancomycin mixture (55681, bioMerieux) and Schaedler Agar with laked horse blood 5% v/v and gentamicin 80 mg/L. The plates were incubated in an anaerobic system (Model 1028, Forma Scientific, Marietta, OH, USA) with an atmosphere of N₂ 90%, H₂ 5%, CO₂ 5% and examined after 48 and 72 h.

Blood agar and MaConkey Agar (CM7, Oxoid) were similarly inoculated and incubated aerobically for 24 h. The broth containing the IUCD was then incubated anaerobically for 18 h and subcultured on to pre-reduced blood agar and Schaedler agar with laked horse blood 5% v/v and gentamicin 80 mg/L. The enriched cultures were examined after anaerobic incubation for 72 h. Swabs collected from controls were similarly processed. They were inoculated on to the above solid media and then incubated in BHI broth for 18 h anaerobically and subcultured as above.

Identification of micro-organisms

The microbial growth from the specimens and controls was evaluated qualitatively; 5–10 colonies were selected from each plate and identified. Aerobic bacteria were identified by standard microbiological techniques (Isenberg *et al.*, 1980); anaerobic bacteria were identified on

Table I. Duration of IUCDs *in utero* and reasons for removal

Reasons for IUCD removal	Number of devices <i>in utero</i> for				
	0–12 m (28)	13–24 m (49)	> 24 m (22)	Unknown (9)	Total (108)
Pelvic inflammatory disease	4	7	4	0	15
Haemorrhage	8	8	5	0	21
Pregnancy	4	3	0	3	10
Pelvic pain	1	3	2	1	7
Vaginal discharge	0	1	2	0	3
Perforation of the uterus	0	0	0	1	1
Retracted thread	6	6	2	2	16
Desire to conceive	2	18	7	0	27
Other causes	3	3	0	2	8

the basis of their colonial appearance, cell morphology, biochemically (by the API 20 A system; La Balme Le Grottes, France), and by end products of glucose metabolism as determined by gas-liquid chromatography (Shimatzu Gas Chromatograph, GC-9A, Shimatzu Corp., Kyoto, Japan). Anaerobes were definitively identified according to the scheme of Holdeman *et al.* (1977).

Statistical analysis

Analysis of the data was done with the χ^2 test.

Results

Fifteen (13.9%) patients with IUCDs were diagnosed clinically as having PID. There was no correlation between the duration of the device *in utero* and the occurrence of infection (table I). The micro-organisms isolated from IUCDs from the various clinical groups and controls are shown in table II. *Actinomyces* spp. and *Arachnia propionica*

were isolated from 16 (14.8%) devices, *A. israelii* most commonly (9.3%), whereas *A. viscosus* and *A. odontolyticus* were isolated from only two controls (3%). *Lactobacillus* spp. were isolated from 10 (9.3%) devices and from 25 (37.9%) controls, and anaerobic cocci from 13 (12.8%) devices and 24 (36.4%) controls. *Bacteroides* spp. were isolated from 15 (13.8%) devices; three (2.8%) isolates were *B. fragilis*. The *Bacteroides* strains isolated from the 12 (18.2%) controls were *B. disiens*, *B. bivius* and *B. melaninogenicus*; *B. fragilis* was not isolated from this group. *Staphylococcus* spp. and *Streptococcus* spp. were the most common aerobic isolates from devices and controls, followed by enterobacteria and *Candida* spp. *Gardnerella vaginalis* was isolated from controls only. There was no significant difference in either the types or the isolation rates of micro-organisms from devices removed from women in the different clinical groups.

Fig. 1 shows the micro-organisms isolated from IUCDs removed from asymptomatic wearers and

Table II. Micro-organisms isolated from IUCDs removed from clinical groups and from normal controls

Micro-organism	Number of isolates from groups of IUCD wearers						and controls (66)
	PID (15)	Haemorrhage (21)	Pregnancy (10)	Misc. (11)	Asymp. (51)	All (108)	
ANAEROBIC BACTERIA							
Actinomyces and Arachnia							
<i>A. israelii</i>	3	1	2	1	3	10	0
<i>A. viscosus</i>	1	0	0	0	2	3	1
<i>A. naeslundii</i>	0	0	0	0	1	1	0
<i>A. odontolyticus</i>	0	0	0	0	0	0	1
<i>Ar. propionica</i>	0	0	0	1	1	2	0
Other gram-positive bacilli							
<i>Bifidobacterium</i> spp.	0	0	0	0	1	1	0
<i>Eubacterium</i> spp.	0	2	0	1	2	5	10
<i>Lactobacillus</i> spp.	1	2	2	3	2	10	25
Gram-negative bacilli							
<i>B. fragilis</i>	1	0	1	0	1	3	0
Other species of the fragilis group	1	1	0	0	0	2	0
<i>B. melaninogenicus</i>	0	0	0	0	2	2	2
Other <i>Bacteroides</i> spp.	3	2	0	1	2	8	10
<i>Fusobacterium</i> spp.	0	2	0	0	3	5	5
Anaerobic cocci							
<i>Peptococcus</i> spp.	3	2	1	0	3	9	18
<i>Peptostreptococcus</i> spp.	1	1	0	1	1	4	6
AEROBIC BACTERIA							
<i>Staphylococcus</i> spp.	2	11	6	2	18	39	17
<i>Streptococcus</i> gp. B	4	3	0	1	9	17	12
Other streptococci	3	3	1	1	4	12	7
<i>Corynebacterium</i> spp.	0	1	2	1	3	7	3
<i>Escherichia coli</i>	1	1	1	1	5	9	6
Other enterobacteria	1	0	0	2	5	8	5
<i>Gardnerella vaginalis</i>	0	0	0	0	0	0	2
<i>Candida</i> spp.	0	3	0	4	8	15	7

Fig. 1. Micro-organisms isolated from IUCDs removed from asymptomatic women (□) and from control women (▨). 1. *Actinomyces* spp. and *Ar. propionica*; 2. *Lactobacillus* spp.; 3. Other anaerobic gram-positive bacilli; 4. *Bacteroides* spp.; 5. Anaerobic cocci; 6. *Staphylococcus* spp.; 7. *Streptococcus* spp.; 8. Enterobacteria; 9. Other aerobic bacteria; 10. *Candida* spp.

those from the genital tracts of controls. The isolation rate of anaerobes from the devices was significantly lower than that from the controls ($p < 0.01$). *Actinomyces* were isolated from seven (13.7%) devices and from only two (3%) controls ($p < 0.05$). *A. israelii*, *A. naeslundii*, and *Ar. propionica*, which were not isolated from any control, were isolated from IUCDs at a frequency of three (5.9%), one (2%) and one (2%) respectively. *Lactobacilli*, on the other hand, were isolated from only two (3.9%) devices, compared to 25 (37.9%) controls ($p < 0.01$). The other anaerobic gram positive bacilli and anaerobic cocci were also isolated with higher frequency from the controls ($p < 0.05$). No significant difference was found in the rates of isolation

of *Bacteroides* spp. or aerobic bacteria between devices and controls.

The isolation rates of micro-organisms related to duration of IUCD *in utero* and compared with controls are shown in fig. 2. The isolation rate of *actinomyces* between devices worn for less than 1 year and the controls was not significantly different, but was significantly higher from devices worn for 1–2 years, ($p < 0.01$) and from those worn for over 2 years ($p < 0.01$). *Lactobacilli* were isolated from devices worn for <1 year, 1–2 years and >2 years at a significantly lower rate than from the controls ($p < 0.01$). Anaerobic cocci were not isolated from any device *in situ* for <1 year, but their isolation rate increased steadily with the duration of wearing

Fig. 2. Micro-organisms isolated from IUCDs in relation to duration of wearing: □, controls; ▨, <1 year; ▩, 1-2 years; ■, >2 years. 1. *Actinomyces* spp. and *Ar. propionica*; 2. *Lactobacillus* spp.; 3. Other anaerobic gram-positive bacilli; 4. *Bacteroides* spp.; 5. Anaerobic cocci; 6. *Staphylococcus* spp.; 7. *Streptococcus* spp.; 8. Enterobacteria; 9. Other aerobic bacteria; 10. *Candida* spp.

(12.2% and 27.3% in 1-2 and >2 years use respectively). The isolation rates of aerobic bacteria from devices removed from the various clinical groups and from the genital tracts of the controls were similar, except for enterobacteria which were isolated at a significantly higher rate ($p < 0.05$) from devices worn for >2 years.

Discussion

Fifteen (13.8%) IUCD wearers were found to have PID and a further 10 had symptoms (vaginal discharge or pain) without clinical evidence of infection. No significant difference was found in the rate of isolation of the various bacterial species between patients in the different clinical groups.

This makes incrimination of a particular organism in these infections difficult to justify, and suggests that several factors such as the micro-organisms, the state of the host, and others, are responsible for the development of PID.

Generally, anaerobic bacteria were isolated from devices at a significantly lower rate than from controls. This is in contrast to the findings of several workers (Dische *et al.*, 1974; Hager *et al.*, 1979; Nayar *et al.*, 1985) some of whom have attributed the proliferation of anaerobes in their series to the reducing effect of copper. Our findings suggest that copper has a detrimental effect on gram-positive anaerobes with the exception of *Actinomyces* spp., which were apparently favoured by its presence. The antibacterial effect of copper is known (Waler

and Röllä, 1982) and reports have shown that following insertion such devices release about 27 µg of copper daily and this amount decreases with time (Chantler *et al.*, 1984). The varying concentration of copper and the degree of susceptibility of these bacteria may explain the difference in the isolation rates of various bacterial species in relation to the duration of IUCD wearing. The absence of actinomycetes from devices worn for <1 year, which could be due to high copper concentration, led Duguid *et al.* (1982) to conclude that actinomycetes disappeared from the genital tract following the replacement of plastic devices by copper-containing ones. Perhaps, had further examination been done in subsequent years, actinomycetes could have been isolated.

Actinomyces spp. and *Ar. propionica* were recovered from 14.8% devices, a finding comparable to those detected on smears (Hager *et al.*, 1979; Burkman *et al.*, 1982; Keebler *et al.*, 1983), but greater than those reported by culture (Hager *et al.*, 1979; Sparks *et al.*, 1981). This may be attributed to improved techniques used in transporting and processing specimens. Similar to other reports (Hager *et al.*, 1979; Burkman *et al.*, 1982; Duguid *et al.*, 1982; Nayer *et al.*, 1985), *A. israelii* was the anaerobe most frequently isolated from IUCDs in our study and was not isolated from any controls. The same applied to the related species, *A. naeslundii*, and *Ar. propionica* (Bonnez *et al.*, 1985; Nayer *et al.*, 1985). Incriminating primarily *A. israelii* in IUCD-associated PID, Duguid *et al.* (1982) reported a correlation between the duration of IUCD *in utero* and the occurrence of infection. In our study no such correlation could be established, but the isolation of actinomycetes increased with the duration of IUCD *in situ*. The presence of these organisms probably represents simple colonisation and their isolation from an IUCD may not necessarily indicate infection, an observation consistent with other reports (Luff and Gupta, 1977; Jones *et al.*, 1979). Although there was no significant difference in the isolation rate of *Bacteroides* spp.

from devices and controls, they showed a tendency to proliferate in devices *in situ* for >2 years. Furthermore, the potential pathogen, *B. fragilis*, was isolated only from devices. Lomax *et al.* (1976), isolated *B. fragilis* and *Peptostreptococcus* spp. from cases of IUCD-associated PID and Elhag *et al.* (1987) demonstrated specific *B. fragilis* antibodies in sera from patients with PID. Enterobacteria and anaerobic cocci showed a similar trend to *Bacteroides* spp. The combination of bacteroides and enterobacteria in mixed infections is well documented (Gorbach and Bartlett, 1974).

Other factors may contribute to the development of PID. According to Eschenbach (1983), lactobacilli, with other vaginal bacteria, play a protective role by producing an acidic environment incompatible with the growth of many pathogens and their inhibition would promote the multiplication of various potential pathogens. Hanna *et al.* (1985) found an association between a high vaginal pH and the prevalence of pathogenic micro-organisms in vaginal secretions. Furthermore the insertion of an IUCD breaches the protective barrier of cervical mucus and the IUCD tail creates a transmission link between the sterile uterine cavity and the bacteria-rich vagina. Sparks *et al.* (1981) were able to demonstrate the migration of vaginal bacteria into the uterine cavity *via* the appendages of the device. The isolation of similar organisms from both the device and the filament in our cases gives further confirmation of those findings.

In conclusion, the present study confirms earlier findings that an IUCD promotes colonisation by *Actinomyces* spp., and further suggests that the presence of such a foreign body and the ecological changes it triggers, play a crucial role in the development of infection and that organisms other than actinomycetes could be implicated in IUCD-associated PID.

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