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Biofilm formation on intrauterine devices in relation to duration of use

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Intrauterine devices (IUDs) are highly effective, long-term methods of contraception; however, IUD use is limited due to concerns about an increased risk of pelvic inflammatory disease (PID) and subsequent complications. A retrospective review of clinical and microbiological data of 127 participants was carried out over a 3 year period. IUDs were removed and sent for microbiological examination. A 10 year old IUD, removed because of the symptoms of PID, was investigated via both microbial culture and scanning electron microscopy. The primary objective of this study was to examine the bacteria present on removed IUDs after different times *in situ* by using aerobic and anaerobic culture methods. A close association of the distribution of aerobic and anaerobic bacteria on the IUDs with different times *in situ* was found.

INTRODUCTION

Intrauterine devices (IUDs) are highly effective, long-term methods of contraception; however, IUD use is limited to some regions of the world due to concerns about an increased risk of pelvic inflammatory disease (PID) and subsequent complications, such as infertility and ectopic pregnancy. Some researchers have speculated that the presence of an IUD in the uterus may increase host susceptibility to infection, thus increasing the incidence of PID infections (Peterson *et al.*, 1996). In addition, some studies have shown that *Actinomyces* may proliferate significantly in the endocervix of women wearing an IUD (Bhagavan & Gupta, 1978; Gupta, 1982). It has been shown that insertion of an IUD may contaminate the endometrial cavity with bacteria (Mishell *et al.*, 1966). Indeed, IUDs are considered to cause PID by pushing vaginal and cervical bacteria into the uterus along the tail of the device. However, organisms cultured from the vagina of IUD users may or may not represent microbes present in the uterus (Ferraz do Lago *et al.*, 2003). In fact, most of the microbes recovered from such cultures originate from the vagina. Therefore, the bacteriological investigation of IUDs removed through the cervix may show contamination from the cervico-vaginal flora along with the microbial biofilm on the IUD.

The major complication associated with the use of medical implants such as IUDs, intravascular catheters and tubes is infection. Micro-organisms originating from the normal flora can colonize these devices and form biofilms consisting of layers of host cells and bacteria/fungi embedded within a matrix material. Foreign materials of this type are the most probable sites of biofilm formation (Gristina, 1994). The main component of the biofilm produced by the bacteria and/or fungi is an exopolysaccharide layer, which is the pivotal factor responsible for the behaviour of biomaterial-centred infection. The biofilm bacteria are usually resistant to attack by antimicrobial agents and host phagocytes. This is one reason why infections caused by these micro-organisms are hard to treat without removal of the devices.

The primary objective of this study was to examine the bacteria present on removed IUDs after different times *in situ* by using aerobic and anaerobic culture methods. Although the investigation of the occurrence of PID was not the goal of this study, 46 (39·3 %) of our patients had typical PID symptoms at the time of removal of the IUD. In the case of one patient with typical symptoms of PID, besides quantitative culture of the biofilm bacteria of the removed IUD, scanning electron microscopy was also used to visualize the biofilm.

Abbreviations: BV, bacterial vaginosis; IUD, intrauterine device; PID, pelvic inflammatory disease.

METHODS

Patients. A group of 127 participants was recruited for our study from women who visited the outpatients' unit of the Department of Obstetrics and Gynecology, University of Szeged, between 1 January 2001 and 31 December 2003. The mean age of the women was 41 years, ranging between 28 and 56. All participants underwent the following clinical and laboratory examinations: history and physical examination (including pelvic examination), and transvaginal ultrasound. If signs and/or symptoms of genital tract infection were present, patients were screened for vaginal and cervical infections, according to the practice of our microbiology laboratory. The IUDs were removed under antiseptic

conditions. After careful cleaning of the cervix and the vaginal wall with antiseptic solution (Braunol, B. Braun Medical AG; in the case of iodic allergy, Kodan Forte, Schülke & Mayr GmbH), the removals were performed without touching the vaginal wall or the opener instrument with the IUDs to prevent contamination by the vaginal flora.

A 41-year-old patient with two previous live births was selected with typical symptoms of PID and with a 10 year old Copper-T IUD. After removal of the IUD, it was sent for scanning electron microscopic examination simultaneously with culture in the microbiology laboratory.

Microbiological methods for vaginal flora assessment. From those women who had signs and/or symptoms of genital tract infection, vaginal and cervical swabs were collected. The first vaginal swab was used for aerobic and anaerobic cultures; the second swab was used for detection of *Mycoplasma hominis* and *Ureaplasma urealyticum*. Vaginal swabs were directly inoculated onto: Columbia blood agar plates (Oxoid), which were incubated aerobically at 37 °C for 48 h to isolate aerobic bacteria, including lactobacilli; Columbia human blood agar plates, which were incubated anaerobically at 37 °C for 5 days to isolate *Gardnerella vaginalis* and bacterial vaginosis (BV)-associated anaerobic bacteria; Thayer-Martin agar (Oxoid), which was used to isolate *Neisseria gonorrhoeae*; Sabouraud dextrose agar plates (bioMérieux), which were incubated at 37 °C for 48 h to isolate *Candida* spp. 'Mycoplasma DUO' (Sanofi Diagnostics Pasteur) was used for quantitative assessment of the presence of *M. hominis* and *U. urealyticum* after incubation for 48 h. The bacteria and fungi isolated were identified by classical methods and/or by ATB/VITEK identification procedures (bioMérieux). The presence of *Chlamydia trachomatis* on the cervical swab was looked for by the MicroTrak II Chlamydia antigen detection kit (Trinity Biotech).

Microbiological examination of the removed IUD. All cultures were commenced within 1 h of sampling. Each IUD sample was immediately sent to the microbiology laboratory where it was placed in 10 ml reduced brain heart infusion (BHI) broth, pH 7·2 (Oxoid) and mixed on a vortex shaker for 30 s. After gentle dispersion, the suspensions were diluted (10^{-1}–10^{-6}) in reduced BHI broth and 100 µl of each dilution and 100 µl of the corresponding undiluted suspension were plated immediately on selective and non-selective media. Columbia agar base supplemented with 5 % (v/v) cattle blood, haemin and vitamin K_1 was used to enumerate the total cultivable bacterial flora; Columbia-based chocolate agar was used for calculation of the total aerobic bacterial flora. For the selective growing of *Enterobacteriaceae*, Endo agar (bioMérieux) was employed. Fungi were selectively cultured on Sabouroud dextrose agar. Black-pigmented anaerobic bacteria (*Prevotella* spp., *Porphyromonas* spp.) were isolated from kanamycin vancomycin laked blood (KVLB) agar (Oxoid). Cadmium, fluoride, acriflavine, tellurite (CFAT) agar (Oxoid) was used for isolation of anaerobic *Actinomyces* spp. For the isolation of anaerobic organisms and the determination of the total cultivable facultative aerobic and anaerobic bacterial count, cultures were performed in an anaerobic chamber with an atmosphere of 90 % N_2, 5 % H_2 and 5 % CO_2 (Bactron Sheldon) for 5 days at 37 °C. The bacteria and fungi isolated were identified by classical methods and/or ATB/VITEK identification procedures (bioMérieux).

Scanning electron microscopy. The 10-year-old IUD, removed because of the symptoms of PID, was cut into 1 cm stripes with sterile scissors, and then subjected to chemical dehydration (in 30→50→70→90→100 % ethanol for 1 h each, then in 30 : 70, 50 : 50, 70 : 30 ethanol : acetone mixtures for 20 min each). The samples were placed in the critical-point drier in 100 % acetone, and rinsed three times in liquid CO_2, then the critical point was identified, after which the samples were secured onto racks and coated in gold in a sputter coater. The examination was carried out with a Hitachi S 2400 scanning electron microscope and the pictures were digitally recorded.

RESULTS AND DISCUSSION

Out of the 127 patients involved in this study, 10 were selected as a control group with an IUD which was removed before 1 year of wearing. Due to the poor patient compliance, some extremely old IUDs were also found. Among the 117 patients investigated (study group), in 63 cases (53·8 %), the reason for the removal was the age of the device. The manufacturers' recommendation for the duration of residence of an IUD *in situ* is 4–5 years, depending on the type of the device. In our IUD material, only 22 devices (18·8 %) were younger than 5 years (1–5 years, mean time *in situ* 2·5 years) (group 1). In 44 cases (37·6 %), the patients wore their device for 5–10 years (group 2), and in 51 cases (43·5 %) we found devices which were *in situ* for more than 10 years (group 3) (Table 1). In two cases, we removed 20-year-old IUDs.

In 78 cases (66·6 %), the cause of removal of the IUD was inflammation of varying degrees, including PID. In 14 cases (11·9 %), IUDs were removed because of metrorrhagia. One 41-year-old patient with two previous live births visited our outpatient unit with expressed lower abdominal pain. By bimanual examination, bilateral adnexal tenderness, cervical motion tenderness and lower abdominal tenderness was found. She had no fever at the time of the visit; however, transvaginal ultrasound showed free pelvic fluid. After PID was diagnosed, the 10-year-old Copper-T IUD was removed.

In the control group, nine of 10 (90 %) IUDs had $< 10^3$ c.f.u. bacteria per sample; however, in the study group, seven of 117 (5·9 %) IUDs had the same low level of bacteria per sample. In patient group 1, 11 patients were examined by

Table 1. Summarized culture results of 127 IUDs after different times *in situ*

Time IUD *in situ* (no.)	No. of patients with BV/ no. investigated	No. of species isolated/ no. of IUDs	No. of species isolated (range)	Mean no. of species isolated per IUD
Control (10)	0/10	12/10	0–2	1·2
< 5 years (22)	6/11	26/22	0–2	1·2
5–10 years (44)	8/15	144/44	1–4	2·6
> 10 years (51)	11/15	298/51	5–8	5·8

vaginal culture as well. In six cases, the typical BV flora was found to be dominated by black-pigmented anaerobic bacteria and by *Mobiluncus* spp. No lactobacilli were present. From the 22 IUD samples, 26 species were identified; the mean number of species per IUD was 1·2 (Table 1). In patient group 2, 15 patients had symptoms or signs of vaginal discharge. Out of these, eight had a culture result typical of BV. Altogether, 144 species were isolated, with a mean number of species per IUD of 2·6. In patient group 3, out of the 15 patients who had symptoms of vaginal discharge, 11 had BV flora in the vaginal fluid. From the 51 removed IUDs, 298 species were isolated, with a mean number of species per IUD of 5·8. Table 2 shows the prevalence of the aerobic and anaerobic bacterial species and *Candida albicans* isolated from the 51 IUDs worn for more than 10 years. Table 3 summarises the distribution of the IUDs with respect to different times *in situ*, according to the total c.f.u. per sample of bacteria and fungi found during culture. In the case of those belonging to the control group, the highest number of c.f.u. per IUD was 10^4 per sample. Low total micro-organism counts were dominant among the IUDs less than 5 years old; however, in the two other groups (IUDs removed after 5–10 years or more than 10 years *in situ*) we found significantly more IUDs with a higher number of micro-organisms per IUD. It was remarkable that not only the c.f.u. per IUD but also the number and diversity of species isolated from the IUDs increased with the time *in situ*.

Table 2. Detailed culture results for 51 IUDs removed after > 10 years

Species	No. of IUDs positive
Aerobes	
Escherichia coli	10
Enterobacteriaceae	15
Lactobacillus spp.	2
Enterococcus faecalis	4
Streptococcus agalactiae	6
Staphylococcus aureus	3
Anaerobes	
Prevotella spp.	36
Porphyromonas spp.	19
Bacteroides spp.	24
Bacteroides ureolyticus	18
Fusobacterium spp.	16
Mobiluncus spp.	17
Peptostreptococcus spp.	10
Propionibacterium spp.	8
Bifidobacterium spp.	2
Clostridium spp.	4
Actinomyces spp.	29
Others	
M. hominis	39
U. urealyticum	26
C. albicans	10

Table 3. Total c.f.u. of bacteria and yeasts isolated from the IUDs after different times *in situ*

Total viable count (c.f.u. per sample)	No. of IUDs *in situ* for:			
	< 1 year (control)	< 5 years	5–10 years	> 10 years
$\leqslant 10^3$	9	6	0	1
10^4	1	9	13	9
10^5	0	0	9	9
10^6	0	5	7	11
$\geqslant 10^7$	0	2	15	21

The distribution of aerobic and anaerobic bacteria on the IUDs with different times *in situ* is shown in Fig. 1. It is remarkable that from the biofilm on IUDs over 10 years anaerobic bacteria alone or in combination with aerobes were isolated more frequently.

In the case of the 41-year-old patient whose IUD was removed after 10 years because of symptoms of PID, a very complex anaerobic bacterial flora was isolated. The total c.f.u. per sample was $2{\cdot}5 \times 10^8$. Besides Gram-positive anaerobes (*Actinomyces viscosus*, *Actinomyces naeslundii*, *Bifidobacterium* sp., *Finegoldia magna*, *Anaerococcus prevotii*), Gram-negative anaerobes were dominant (*Prevotella disiens*, *Porphyromonas asaccharolyticus*, *Bacteroides ureolyticus*). One of the key pathogens in BV (*Mobiluncus* sp.) was also present. No cultures were carried out from the vaginal fluid of this patient. Fig. 2 shows the appearance of the biofilm on the removed IUD of this patient.

The use of IUDs is highly effective in preventing pregnancy and it is also very cost-effective. It is one of the most popular methods of contraception in the world today. More than 80 million women are using IUDs for contraception worldwide, and its effectiveness rivals that of tubal sterilization (Peterson *et al.*, 1996). Recent reviews suggest that the overall risk of PID with modern IUDs is lower than previously thought, at least in regions where medical advice is followed by the patients, and where there is a low prevalence of sexually transmitted infections (STIs). The risk of PID may be higher, however, in places where gonorrhoea and chlamydia are prevalent, where screening for STIs is limited and where it is difficult to ensure aseptic conditions for insertion (Steen & Shapiro, 2004). It is hard to explain the reasons behind the extreme long-term wearing of IUDs in a large number of our patients, but it seems to have a connection with a lower educational level and poor personal hygiene.

Guerreiro *et al.* (1998) found a significantly higher prevalence of infection in IUD users in comparison with users of other contraceptive methods, based on the higher prevalence of BV in this population. These data are in agreement with those published by Ferraz do Lago *et al.* (2003), who found that the prevalence of cervicovaginal infections was 29·1 % and that BV was frequently found (19·7 %) among IUD users 6 months after insertion. In the latter study, it was found that

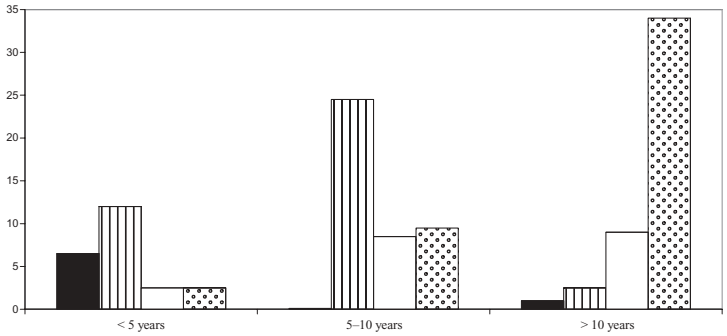

Fig. 1. Summary of culture results for IUDs after different times *in situ*. Black bars, aerobe and anaerobe negative; vertically hatched bars, aerobe positive and anaerobe negative; white bars, aerobe negative and anaerobe positive; dotted bars, aerobe and anaerobe positive.

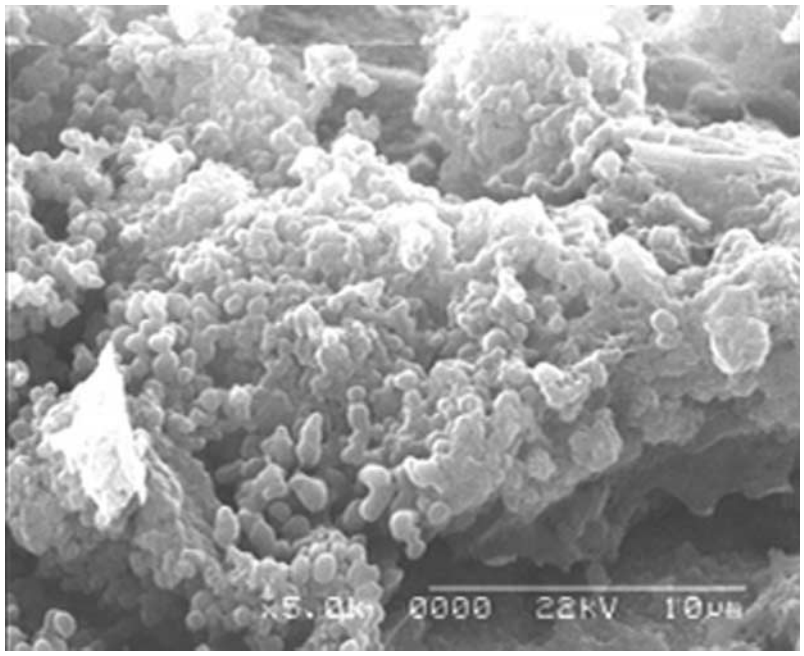

Fig. 2. SEM picture of mature bacterial biofilm on the surface of a removed IUD. Bar, 10 μm.

dysmenorrhoea and a tendency to abnormal bleeding were more recurrent in patients with BV. The incidence of BV among IUD users can be even higher (47·2 %), as noted by Joesoef *et al.* (2001). In our study, quite a few patients in all three patient groups who had symptoms or signs of genital infection had BV flora, when their vaginal flora was tested. In these cases, BV-specific anaerobic bacteria were also found more frequently and in higher numbers on the removed IUDs (not shown).

There are numerous publications in the literature showing a higher risk of PID in the first weeks after IUD insertion (Grimes, 1987; Farley *et al.*, 1992). The main agents responsible for PID in connection with the use of IUDs are *Chl. trachomatis* and *N. gonorrhoeae*, which can be present in the endocervix at the time of insertion and transferred to the upper genital tract by the device. In our study, no patient was found, among those who were tested, with *Chl. trachomatis* or *N. gonorrhoeae* positive results. But Farley *et al.* (1992) also suggest that the reason why IUDs that have been in place for a long time are associated with more PID is that aseptic practices during insertion have significantly improved over the years, so that more recently inserted devices are less likely to be infected during the procedure. Because of this, the routine administration of prophylactic antibiotics at the time of insertion is necessary only if the patient has a positive culture result (Tsanadis *et al.*, 2002). However, in a meta-analysis by Grimes & Schultz (1999), antibiotic prophylaxis for IUD insertion (either oral doxycyclin or azithromycin) significantly reduced the frequency of unscheduled return visits. The protection against PID was higher if antibiotic prophylaxis was used, but not statistically significant. In a technology-assessment study, which analysed the effectiveness of follow-up visits after IUD insertion, Neuteboom *et al.* (2003) compared a group of women with regular follow-up visits in the first year with women who had no regular follow-up visits. Patients in the regular follow-up visit group came more frequently for unscheduled visits. They concluded that regular follow-up after the insertion of an IUD is not effective. The patients in the present study did not make a big effort to follow medical advice on regular follow-up visits or removal of the IUD after 4–5 years. The majority of the patients, 95 out of 117 (81 %), had an IUD older than 5 years. Our data show a close correlation between the change in the

number and type of the microbial flora and the proportion of patients with BV, the longer the IUD was in place. This supports the recommendation for wearing the IUD for 5 years only to ensure safety. The complexity of the biofilm flora and the dominance of anaerobic bacteria on the IUDs older than 5 years was remarkable, regardless of whether the patient had BV flora in their vagina or had no symptoms or signs of genital infection.

There are few recent data in the literature about the investigation by quantitative culture methods of bacterial biofilm formation on IUDs after different times *in situ*. A scanning and transmission electron microscopic study of the surfaces of IUDs has already been reported by Marrie & Costerton (1983). Their transmission electron microscopy study showed highly organized and often densely packed micro-colonies of bacteria, a reflection of the possibility that the majority of these bacteria had been present on these surfaces for a long time. In our case, a 10-year-old IUD was examined for biofilm formation in parallel with culture. Biofilm formation, involving both coccal and bacillary forms, was detected on the surface of the IUD by scanning electron microscopy. Quantitative culture of aerobic and anaerobic bacteria showed a dominance of anaerobic bacteria in this biofilm. Bacteria living in such a biofilm are usually resistant to attack by antimicrobial agents and host phagocytes. To treat PIDs which develop in connection with IUD wearing requires the immediate removal of the IUD and treatment of the inflammation with an antibiotic that is active against the bacteria colonizing the IUDs.

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