Bacterial Infections and Biofilm Formation Associated with Intra Uterine Contraceptive Device among Females Attending Al- Glaa Teaching Hospital in Cairo

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ABSTRACT

Background: Intrauterine device (IUD) is a convenient, effective and one of a long term contraceptive procedures. However, it may act as a reservoir of reproductive tract infections.

Aim of the study: to isolate the microorganisms in the cervix and on the removed IUDs after different times in situ ,examine their association with microbial biofilm formation on the removed intrauterine devices, and also to detect some common sexually transmitted bacteria.

Subjects and Methods: a total of 40 women selected randomly from the Gynecological outpatient clinic of Al-Glaa Teaching Hospital in Cairo were included. Cervical swabs and the removed IUDs were bacteriologically examined. The removed IUDs were analyzed by electron microscope to identify the presence of a microbial biofilm. Real time polymerase chain reaction (PCR) was performed to detect some common sexually transmitted infections (STIs) organisms.

Results: The mean age of the studied women was 32.12±6.7 years and the mean duration of IUDs in situ was 2.55±0.87 years . Mixed organisms (*E. coli, Enterococcus faecalis, Candida , Staph aureus and others*) were detected with no significant differences between the isolated organisms from the removed IUDs (116) and that from cervical swabs (134) ; neither nor to the duration of IUDs in situ (p>0.05). *Chlamydiae trachomatis(55%,70%),Neisseria gonorrheae(30% ,30%) and* Mycoplasma *(7.5%,0.0%),* were detected STIs organisms by PCR from IUDs and swabs respectively with no statistical significant differences (p> 0.05). Thick biofilm of multiple microorganisms was detected on the surfaces of the removed IUDS.

Conclusion:The insignificant association between microorganisms that were isolated from the cervix, removed IUDs and biofilms may indicate the pre-existence of those organisms before and spread by IUDs insertion . IUD may act as a reservoir for resistant microorganisms. The presence of some asymptomatic sexually transmitted infections (STIs), may point to ,that women may act as STIs transmitters. Prior to and throughout IUDs use, appropriate management of reproductive tract infections is vital. IUDs related prospective studies are also recommended.

Keywords: IUDS, cervix infection, PCR , biofilm, microbiological examinations.

INTRODUCTION

Contraceptive methods are used to regulate inter pregnancy spacing, avoid unwanted pregnancy and prevent sexually transmitted infections [1]. The intrauterine devices (IUDs) are highly effective, long-term methods of contraception that have the benefit of being reversible. In developing countries IUDs are the most preferred means of contraception [2]. In 2015, fourteen per cent of women worldwide used the IUD[3]. However,
the presence of an IUD in the uterus may be associated with infertility, ectopic pregnancy and increased risk of pelvic inflammatory disease (PID) and increase host susceptibility to infections, including sexually transmitted infections (STIs)[4].   Worldwide, more than 1 million sexually transmitted infections are acquired with a great suffering of both men and women and profound impact on sexual and reproductive health [5].

On the other hand, IUDs like other implanted devices such as catheters and prosthetic cardiac valves were found to attract pathogenic bacteria and fungi and could form a biofilm on their inert surface. A biofilm is a complex surface-associated cells enclosed in a matrix and represent a common mode of microbial growth. Co-infection with other pathogens will affect its formation. Microbial biofilm is build up when micro-organisms irreversibly adhere to a surface and

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produce extracellular, self-produced organic material that facilitate adhesion and provide a structural polymeric matrix[1]. Usually, pathogenic cells in the biofilm are embedded within a complex matrix that may protect the microorganisms from host defenses, as well as decrease their susceptibility to antibiotic agents[7]. Moreover, the biofilm bacteria are usually resistant to attack by antimicrobial agents and host phagocytes. This is one explanation why infections caused by these micro-organisms are difficult to treat without removal of the devices[7]. The presence of a biofilm may also, constitute a reservoir of infection[8]. However, there are limited studies concerning organisms cultured from the vagina and cervix among IUD users and a debate either these organisms may or may not correspond to microbes present in the uterus [2, 9] and consequently affect the reproductive health.

Thus; objectives of the present study were to :
- Isolate the microorganisms in the cervix and on the removed IUDs after different duration of use .
- Examine their association with biofilm formation.
- Detect some common Sexually Transmitted Bacteria such as ; *Neisseria gonorrhoeae,*
Chlamydia trachomatis and Mycoplasma .

SUBJECTS AND METHODS
Participants and study design

A cross sectional study was conducted from March 2014 to July 2015 at Al-Glaa Teaching Hospital for Obstetrics and Gynecology, in Cairo, where most of the attendant women were of low socioeconomic standard. A total of forty women (age ranging between 21 and 42 years) were randomly selected at twice visits per week , from the Gynecological outpatient clinic for removal of the IUDs . Informed permission for taking the cervical swab was obtained and a structured questionnaire go through data of age, parity, gynecological history ,the duration and causes of IUD removal. Pelvic and gynecological examination data were also recorded.

Inclusion and exclusion criteria
Females attending the outpatient clinic to remove the IUD (Copper-T 380 A) and approved to share were eligible for the study. While, those with heavy blood during menstrual period at the time of sample collection and antibiotic users during the previous two weeks were excluded.

Ethical consideration
- All the selected females were personally interviewed for their consent after explaining the nature , objectives and the culture swabs needed for the study.
- The study was approved by the Ethical Committee of the Faculty of Medicine for Girls, Al-Azhar University in Cairo and from Al-Glaa Teaching Hospital.
Samples: Forty cervical swabs were taken by the gynecologist, transferred in brain heart infusion (BHI) broth medium to Microbiology laboratory for culture. Careful cleaning of the cervix and the vaginal wall with antiseptic solution was done then IUD$_s$ were removed under aseptic conditions. To prevent contamination by the vaginal flora, the removal of the IUDs were performed without touching the vaginal wall. Each IUD was placed in l0 ml reduced BHI broth (Oxoid) and 20 of IUDs were sent to the Regional Center of Mycology and Biotechnology, Cairo, Egypt to be examined by electron microscope for biofilm formation.

METHODS
Microbiological examination of the samples: all samples were sent to microbiology lab within one hour and processed as follow:
-Cervical swabs were directly inoculated onto Columbia blood agar plates (Oxoid), Mac-Conkey`s, Sabouraud`s dextrose agar plates (Oxoid), incubated aerobically at 37°C for 48 hours to isolate aerobic bacteria[10].
- The removed IUD placed in reduced brain heart infusion (BHI) broth was mixed by a vortex shaker for thirty seconds. Half ml of reduced (BHI) were stored at -20°C for real time polymerase chain reaction. Culture from suspension was done on Columbia-blood agar[10].
Identification of isolated organisms was done by the conventional methods and according to [10].

Preparation of removed IUD for scanning electron microscopy examination
The removed IUD was cut into 1 cm stripes with sterile scissors, and then subjected to chemical dehydration by serial dilution of ethanol using automatic tissue processor (Lecia EM TP). Then

the samples were dried using CO_2 critical point drier (Tousimis Audosamdri-815). The samples were coated by gold sputter coater (SPI-Module). Finally the samples were exanimated by scanning electron microscopy (JEOL-JSM-5500LV) using high vacuum mode.

Real time PCR

Real time PCR was performed to detect the presence of *Neisseria gonorrhoeae , Chlamydia trachomatis and* Mycoplasma that may cause cervical infections using the following specific primers:

Micro-organisms	Primer sequence (5'to 3')	Reference
C. trachomatis	(5'-AAG ATACGTGAATTCTTAAGT T-CGGTC-3') and (5'-TAATTGATCCA-AACT CTGACTTTCCTC-3')	[10]
N. gono-rrhoeae	(5'-CCGGAACTGGTTTCATCT GATT-3') and (5'-GTTTCAGCGGCAGCATTC A-3')	[11]
Myco-plasma	(5'-CGCCTGAGTAGTACGTTC GC-3') and (5'-GCGGTGTGTACAAGACCC GA-3')	[12]

- DNA extraction done using QIAamp (Germany) DNA Mini kit.
- Normalization the primer concentration and mix gene-specific forward and reverse primer pair were done.
   Setup experiment and the PCR program was analysed on ABI Prism SDS 7000. Relative quantification calculation of the ratio between the amount of target template and a reference template in a sample according to Kit instruction (DNA–Technology , Russia).

Statistical analysis

The Statistical Package for Social Sciences (SPSS version16.0, Inc., Chicago, IL) was used for data entry and analysis. Descriptive statistics were presented as numbers and percentages. To assess the significance in the observed differences between groups, chi-square test was used for qualitative data, while ANOVA was used for quantitative data. P. values were taken at a significance level of 0.05 and 95% confidence limit.

RESULTS

The mean age of the 40 studied females was 32.1 ± 6.7 years. According to the duration of IUDs insertion ; 10% of women had IUD insertion time for less than one year, 40% of them were IUD users from one year to less than 5 years, 35% were 5 years IUD users to less than 10 years while the remaining 15 % had IUD insertion time for more than 10 years. The mean duration for all IUDs in situ was 2.55 ± 0.87 years. The mean parity $\pm$ SD of the females was 2.37 ± 0.97 while the mean abortion was 0.62 ± 0.80 times (Table 1).

Causes of IUDs removal among the studied females:

The cause of removal of 55% of IUDs was vaginal discharge and/or irregular bleeding, 27.5% for female wish to get pregnant and 17.5% required for other IUD type (Figure 1).

Culture of cervical swabs and removed IUDs:

Table (2) demonstrated one hundred and thirty four (134) bacterial isolates from cervical swabs compared to 116   bacterial isolates from the removed IUDs . There were combined micro-organisms in the same sample. The isolated microorganisms from cervical swabs were *Enterococcus faecalis*, *E. coli,* Candida spp. and *Staph aureus ,   Coagulase negative staph Klebsiella   pneumonae and* Proteus *(*26.1 %, 23.1%, 16.4% , 15% ,12.1, 3.7, 3.7) compared to (23.2%, 24.1%, 18.9% and 16.4% ,9.5% ,5.2% ,and 2.6%   ) isolated from removed IUDs respectively   with no significant differences between them (p > 0.05).

Culture results of the removed IUDs   and cervical swabs and in relation to different times in situ among females IUDs users: Isolation of

multiple combined microorganisms were detected in the same sample and increased so as the duration of IUDs insertion were increased. Numbers of isolated species per IUDs were 9/4,21/16,53/14 and 33/6 compared to those per culture swabs(11/4,25/16,57/14 and 41/6) . The overall association between the two cultures was statistically not significant in relation to IUDs duration ($p > 0.05$) ; (Table 3).

The Scanning Electron Microscopy results:
Scanning electron microscopy (SEM) of20 removed IUDs, showed highly organized and densely packed micro-colonies of biofilm formation on the surfaces of the IUDs, involving both coccal, bacillary and Candida (*Candida albicans* was the most demonstrated SEM photo ;(Figure 2).

Real time PCR
Table (4) showed PCR similar finding for *N. gonorrhoeae* (12 cases) on both IUDs and cervical swabs ,with predominant positive finding at a duration of IUDs from 5-10 years. At the same duration of removed IUDs, *C.trachomatis* was detected among 13 removed IUDS versus 14 cervical swabs .While, only 3 cases were positive for Mycoplasma in the duration < 10 years previously inserted IUDs . No significant differences were detected between IUDs results and cervical swabs as regard to PCR finding ($p>0.05$).

Amplification curves of positive and negative real time PCR demonstrated the presence of
N. gonorrhea and C.trachomatis (Figures 3,4) respectively.

Table (1): Characteristics of the studied females

Variables	Studied Females (No. = 40)
Age - Mean ± SD / years (Range 21 - 42 years)	32.15 ± 6.73 years
Duration of IUD insertion:	No. (40) & %
< 1 year 1 - <5years 5 - <10 years >10 years	04 (10) 16 (40) 14 (35) 06 (15)
Mean duration of IUD / year	**2.55 ± 0.87**
Mean parity ± SD	**2.37 ± 0.97**
Mean abortion ± SD	**0.62 ± 0.80**
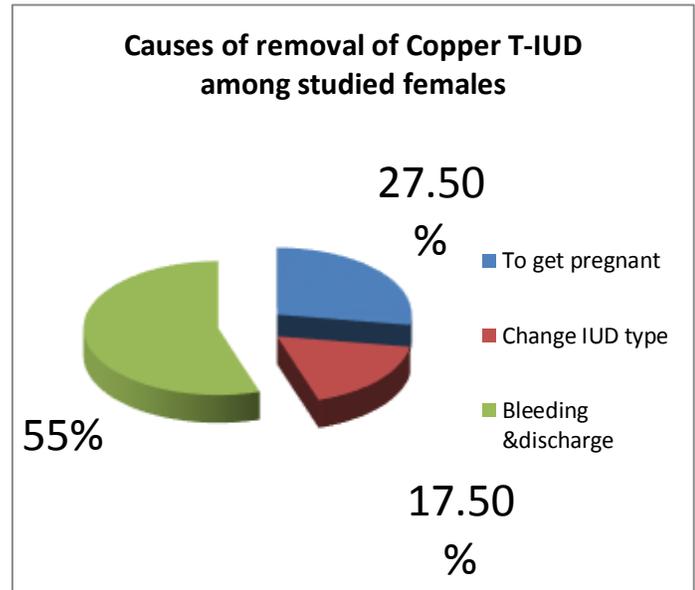
Fig. (1): Causes of IUDs removal among the studied females.

Table (2): Frequency of microorganisms isolates from the removed intra uterine devices (IUDs) and cervical swabs.

Microorganisms	IUDs		Swabs	
	No.	%	No.	%
-*E.coli*	28	24.1	31	23.1
-*Enterococcus fecalis*	27	23.2	35	26.1
-**Candida**	22	18.9	22	16.4
-*Staphaureus*	19	16.4	20	15
Coagulase negative staph	11	9.5	16	11.9
Klebsiella pneumonae	6	5. 2	5	3.7
Proteus	3	2.6	5	3.7
Significant test	Chi square= 1.44 *p=0.96			
Total	116	100	134	100

*No significant difference (p> 0.05) .

Table (3): Culture results of cervical swabs and removed IUDs in relation to duration

Duration of IUD & Corresponding NO. of women		IUD (Multiple isolated microorganisms) & Mean +SD		Swab (Multiple isolated microorganisms) & Mean +SD		Test of sig. ANOVA p. value
< 1 year	4	9	2.3+0.5	11	2.8+0.5	F=1.3 *P=0.26
1 - <5 years	16	21	1.3+0.5	25	1.6+0.5	
5 - <10 years	14	53	3.8+0.4	57	4.1+0.3	
> 10 years	6	33	5.5+0.5	41	6.8+0.4	
Total	40	116	-	134	-	

*No significant difference (p> 0.05) .

Table (4): Frequency of *N.gonorrhoeae, C.trachomatis* and Mycoplasma detected on the removed IUDs and cervical swabs by the real time PCR .

Type of culture Organism	IUDs		Cervical swab		Significant test
	No.	%	No.	%	
N. gonorrhoeae:					----------
Positive	12	30	12	30	
Negative	28	70	28	70	
C. trachomatis :					Chi=1.92 * p =0.165
Positive	22	55	28	70	
Negative	18	45	12	30	
Mycoplasma :					Chi= 3.12 *p =0.077
Positive	03	7.5	00	00	
Negative	37	92.5	40	100	
Total	40	100	40	100	--------------

*No significant difference (p> 0.05) .

Table (5): Frequency of *N.gonorrhoeae ,C.trachomatis* and Mycoplasma present onthe removed IUDs and cervical Swabs by real time PCR in relation to IUDs insertion time.

IUD duration per year	N.gonorrhea				C.trachomatis				Mycoplasma			
	IUD		Swab		IUD		Swab		IUD		Swab	
	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
< 1	4	0	4	0	4	0	3	1	4	0	4	0
1- < 5	15	1	15	1	12	4	9	7	15	1	16	0
5- <10	7	7	7	7	1	13	0	14	12	2	14	0
>10	2	4	2	4	1	5	0	6	6	0	6	0
Total (40)	28	12	28	12	18	22	12	28	37	3	40	0
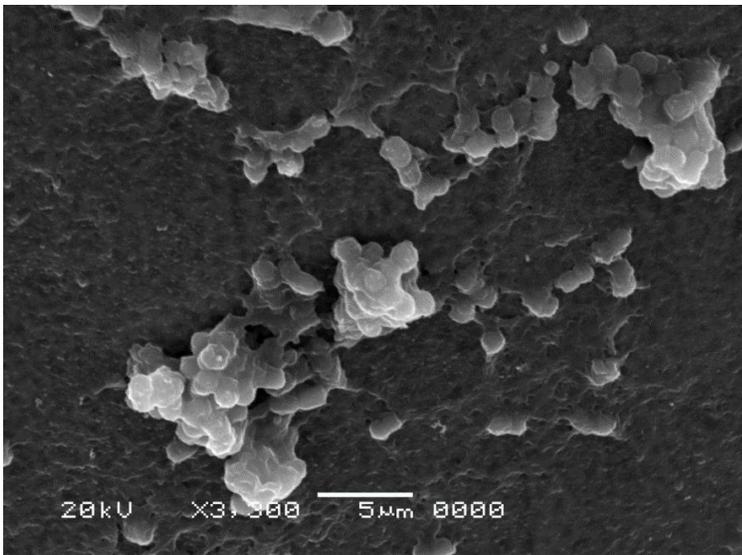
Fig. (2): Scanning electron microscope (SEM) picture of biofilm of *Candida albicans* on the surface of a removed IUD.

Results of the real-time PCR
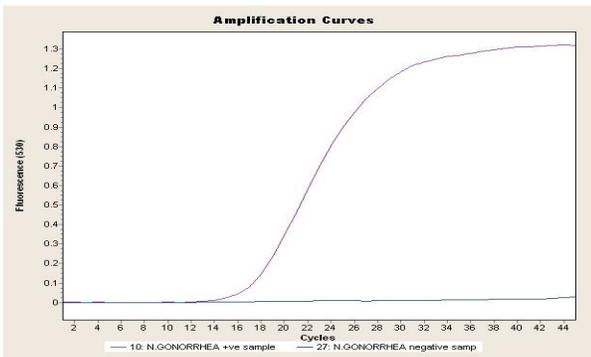
Fig. (3): Positive and negative real time PCR of *N.gonorrhoeae.*
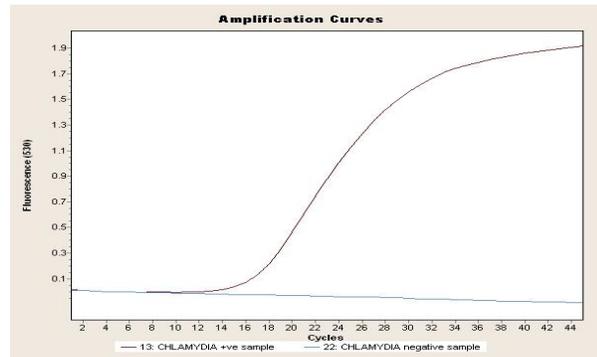
Fig. (4): Positive and negative real time PCR of *C.trachomatis.*

DISCUSSION

The present study included 40 women in child bearing period (21- 42 years old) where the risk of the pelvic inflammatory diseases (PIDs) may be higher with more negative consequences on their reproductive health [13]. They attended the outpatient clinic of Al-Glaa Teaching Hospital in Cairo, to remove their IUDs after a duration ranged from below one year to more than 10 years in situ. While, 55% of them have had non specific symptoms (e.g., abnormal bleeding and vaginal discharge), the microbiological analysis detected different and combined aerobic bacteria and fungal microorganisms in all the studied cervical swabs. Some of these organisms are opportunistic pathogens in the women genital tract and responsible for RTIs and involved with other microorganisms such as ; *E.coli, Staphylococcus aurous* and *Candida spp.* in the pathogenesis of urinary tract infections[14].

So, detection of the aerobic bacterial and other microorganisms are vital, especially in relation to IUDs insertion. Coordinated seven microbial isolates were detected by culture of both removed IUDs and cervical swabs with higher percentage in the last one, but without significant difference (p>0.05) . In agreement, the microbial flora from the vaginal swabs and IUDs are matched to a large extent in a similar study[15].   However, other studies[16 ,17] found that the prevalence of cervico-vaginal infections were higher among IUDs users. In this study, *E. coli ,Enterococcus feacalis*, and *Staphylococcus aureus* were the most predominant bacteria isolated from both IUDs and cervical swabs which was in conformity with similar study (18). Moreover, different types of aerobic bacteria were isolated by endo- cervical swab culture in a comparable study[19]. These were Beta-hemolytic streptococcus, *E-coli*, *S- aurous*, Klebsiella, Coagulase -negative *Staphylococcus* and Enterobacter in the group using IUDs more than the non users. However, in the present study, the mean difference of culture results of both cervical swabs and removed IUDs in relation to different times in situ was statistically insignificant even with 20% of the studied women removed their IUDs after a long time (5 years to more than 10 years) . Although, there was an association suggested between IUDs that have been in place for a long time with more pelvic inflammatory diseases (PIDs) [20]. In contrary, another study found that IUD use is safe for all women, including women at a high risk for sexually transmitted infections [21]. However, data of a previous study[1], demonstrated a close correlation between the change in the number and type of the microbial flora with the proportion of female reproductive tract infections (RTIs) , and the longer duration of IUDs in situ . In conflicting, there was a higher risk of infection in the first weeks after IUD insertion [20]. This debate may be due to lack of continual longitudinal studies before and after IUDs insertion. Moreover, the women in the present study did not make a big effort to follow medical advice on regular follow-up visits or removal of the IUDs after 4–5 years. 20% of them had an IUD older than 5years. On the other hand , research has shown that the insertion procedure may increase the risk of PID . Moreover, the thread tail of the IUDs that protruding into the cervical region, is one of the routes of microbial migration from the vagina to the uterus[16]. Even so, another study found that there was less incidence of PIDs with IUDs that didn't have a tail[22].   However, during reproductive years , sexually transmitted infections (STIs) as well as the over growth of microorganisms present in the genital tract will increase the prevalence and negative health consequences of PIDs[13]. In the present study PCR revealed positive finding of *N. gonorrhoeae* on both IUD and swab analysis with predominant positive finding at a duration of IUD from 5- 10 years.The main causes of PID in connection with the use of IUDs were *C. trachomatis* and *N. gonorrhoeae*, which may be present in the endocervix at the time of insertion and transferred to the upper genital tract by the device. The present results recognized the presence of *C. trachomatis* in cervical swab of (70%) of the studied females compared to the removed IUDs culture (55%) determined by real-time PCR assay. Nearly detection rates of *C. trachomatis* have been reported in previous studies from developing countries[23]. While , *C. trachomatis* was detected in 20.2 % of endo cervical swabs from IUD users women in [11] by real time PCR . *Neisseria gonorrhoeae* was the most prevalent STI among the studied female (30%) . Many STI ;including *C.trachomatis* and *N.gonorrhoeae*, were the predominant sexually transmitted organism associated with *PID*[22]. Primarily, they can also be transmitted among couples and from mother to child during later pregnancy and childbirth [5]. Unfortunately, lack of

public awareness, lack of training of health workers, and long-standing, widespread stigma around STIs remain barriers to effective diagnosis and treatment of STIs [5]**.** So, Real time PCR appears very promising for detection of STIs organisms that are difficult to culture or of slow growth[12]**.** Screening for target categories of population (e.g., patients attending sexual health clinics, infertile persons or women who had infection) is necessary not only to identify symptomatic persons in order to diagnose and treat their infection but also to identify asymptomatic individuals who serve as possible infection carriers .That is help to reduce morbidity and help STDs control [5]**.** The extent of the problem of long duration of IUD insertion implied the detection of a biofilm formation . In the present study both coccal, bacillary and Candida were detected on the surfaces of the IUDs by scanning electron microscopy. A scanning and transmission electron microscopic study of the surfaces of IUDs has already been reported by a previous study[24]**.** It 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 IUDs surfaces for a long time. Microbes growing as biofilm are highly resistant to common used antimicrobial drugs [6]; consequently ,increasing burden and suffering as regards reproductive health.

CONCLUSION The insignificant association between microorganisms that were isolated from the cervix, removed IUDs and biofilms may indicate the pre-existence of those organisms before and spread by IUD insertion . IUD may act as a reservoir for resistant organisms. Additionally, the presence of some asymptomatic sexually transmitted infections, may point to that women may act as STIs transmitters. Appropriate management of reproductive tract infections prior to and throughout IUD use is vital.

Recommendations: Proper IUD insertion technique to insure sterile conditions, training of health care workers for diagnosis and appropriate management of reproductive tract infections prior to and throughout IUD use are vital. Raising the awareness of women for regular checkup after IUD insertion. Integrating STIs clinic to be available in primary health care, family planning and other routine health services. Identified target populations, and involve them in design, implementation and evaluation according to WHO eligible criteria. Further IUDs related prospective studies are also recommended.

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