

# “Microbiological Profile and Antimicrobial Susceptibility Pattern of Bacterial Isolates Associated With Intra Uterine Contraceptive Users among Females of Reproductive Age at A Tertiary Care Hospital In Kanpur”

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## Abstract:

**Introduction:** The use of contraceptives is important in preventing unplanned pregnancy and sexually transmitted diseases. However, the use of various contraceptive methods could expose women to microbial infections.

**Aim:** To study the Microbiological profile and Antimicrobial Susceptibility pattern of bacterial isolates associated with Intra uterine Contraceptive Users among females of Reproductive Age at a tertiary care hospital in Kanpur.

**Material and Methods:** In this study, high vaginal swab samples were collected from 50 women between 20 & 45 years of age. The samples were taken from the cervical canal with sterile cotton swab after cleaning the vaginal area with sterile water and inserting moistened sterile speculum into the cervix. The use of contraceptives among different age groups was determined. The microbial floras of asymptomatic individuals were assessed biochemical identification of the isolated microorganisms and their susceptibility was carried out on the samples collected and reported by CLSI guideline 2020.

**Results:** In the present study out of 50 samples the women using IUDs showed 6(12%) organisms out of which *Candida albicans* was(2%), *Candida krusei* was(2%), *Staphylococcus lugdinensis* was (2%), *Staphylococcus epidermidis* was (2%), *Klebsiella pneumoniae* was (2%) and *E.coli* was (2%). The GPC isolates were 100% sensitive to Vancomycin, Linezolid, Teicoplanine, Tetracycline, Gentamicin, Azithromycin, On the other hand the GNB isolates were 100% sensitive to Imipenem, Meropenem, Polymixin, Colistin.

**Conclusion:** This study shows that more strains of bacteria and fungi were isolated from women in age 24-40 years women sampled and indicated that the more predominant isolates in symptomatic individuals using contraceptives users.

**Key Words:** Antimicrobial activities, vaginal infection.

## Introduction

The intrauterine contraceptive device (IUCD) is one of the most widely used long acting contraceptive methods.[1-3] It offers effective protection from pregnancy and is effective for long-term use. The device can be inserted at anytime as long as pregnancy has been ruled out.[4,5] It is estimated that there are about 180 million users worldwide, with over 80% of these residing in Asia.[6] The use of contraceptives is important in preventing unplanned pregnancy and sexually transmitted diseases. However, the use of various contraceptive methods could expose women to microbial infections. [7] Effect of any contraceptive measure is always observed very keenly and intrauterine device is no exception.

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IUCD is a device, which is fitted into maternal system, evokes a more intense scrutiny. IUCD generally has shown to be safe and effective but tend to have some side effects. [8-18] While female contraceptive methods work differently and affect the quality of vaginal microbial flora both in different population and species variation, hormonal contraceptives such as estrogen and progestogen pills, depo progestin injection, vaginal ring and hormonal IUD prevent pregnancy by interfering with ovulation and suppress the release of an egg from the ovaries[19]. Reproductive tract infection is one of the major complications caused by prolonged usage of an IUCD.[8,11,13,14,18,20,21] It is recognized that use of IUCD results in quantitative and not qualitative changes in vaginal flora. IUCD has been reported to produce inflammation and changes in cervical cytopathology [8, 9, and 17] Different studies have been conducted to explore the IUCD related diseases particularly those associated with infection. Some studies linked the infection related diseases to the insertion method and technique. This is because the

post insertion pelvic infection is generally low but appears to be highest in the first 3 weeks' post placement. [13,22] With the current increase in the level of awareness on reproductive health globally, development and introduction of modern contraceptives, establishment of organized family planning and the desire of families to regulate their family sizes for a more healthy life, more women are increasingly taking to various contraceptive use [23,24]. This study was, therefore, aimed at investigating the microbiological profile and antimicrobial susceptibility of microorganisms isolated from associated with Intra uterine contraceptive users among females of reproductive age.

## Material and Methods

**Study Setting:** This study was being conducted in the Department of Microbiology Rama Medical College Hospital and Research Centre Kanpur.

Samples from outpatients and inpatients admitted to the obs & gynae department, using intra uterine contraceptive devices were collected from Rama Medical college Hospital and Research Centre as the source of the sample for the study.

**Study Design:** Prospective study.

**Type Of Study:** Observational study.

**Study Period:** This study was conducted from January 2020 to December 2020.

**Size of Sample:** 50 sample from patients those using intra uterine contraceptive devices.

**Inclusion Criteria:** All volunteer women who were presently following Intra uterine device methods, sexually active, ages between 20 and 45 years and were not presently on any antibiotics or having history of antibiotics use three weeks prior the sampling periods were selected.

**Excision Criteria:** Women with genital infections and contraindications to IUCD insertion such as gynecological cancers, pelvic inflammatory disease (PID) and pregnancy were excluded from the study.

**Ethical Consideration:** Ethical clearance was taken from the institutional ethical committee.

**Sample Collection:** High vaginal swab samples were collected. The samples were taken from the cervical canal with sterile cotton swab after cleaning the vaginal area with sterile water and inserting moistened sterile speculum into the cervix. The cotton swabs were gently rotated against the vaginal wall to obtain specimens which were aseptically transferred into the holder, given a numerical labeling before being transferred to the laboratory within two hours of collection for processing. [25]

## Direct Smear Gram Stain

Direct smear of the swab samples were prepared on clean glass slides, allowed to air dry and Gram stained using staining method before being examined using oil

immersion objective lens (X100) with the condenser iris diaphragm being opened sufficiently to give good contrast.[25]

## Wet Preparation Using Normal Saline

The vaginal smears were prepared on clean grease free glass slides and a drop of normal saline was added to each smear, mixed thoroughly and covered with cover glass. The prepared wet smear slides were examined using X10 and X40 objective lens for detection of abnormal cells such as pus cells, white blood cells, epithelia, yeast cells and protozoa before being quantified per high power field.

## Bacteriological Examination of Samples

The vaginal swab samples were aseptically streaked on three different culture media including MacConkey agar, Blood Agar and Sabouraud Dextrose Agar. The different agars were prepared according to the manufacturer's instructions following good standard laboratory operating procedures. The prepared agar surface were dried at 45°C for 15 minutes prior to use after which the vaginal swab samples were aseptically streaked onto the different agar plates which were labeled according to the numerical identification numbers earlier assigned to swab samples. All the MacConkey agar plates were incubated at 37°C for 24 hours to produce observable growth colonies. Also, all blood agar plates were incubated at 37°C for 24 hours. Further 24 hours incubation period was allowed for the plates without growth within 24 hours before final results were recorded. Sabouraud dextrose agar plates were incubated at 37°C for 48 hour. Observable colonies on agar plates were subjected to series of bacteriological and biochemical tests according to standard protocol. [26].

## Antimicrobial Susceptibility Testing Using Disc Diffusion Method (Kirby-Bauer)

The bacterial inoculate was prepared in 1% sterile peptone water and incubated for 2 hours at 37°C to produce a slight turbidity that was compared with 0.5 McFarland standards. The adjusted inoculate were made into lawn with sterile cotton swabs on dried Mueller-Hinton agar surface. The agar surface was allowed to dry for 15 min before a commercially-prepared Gram negative multi disc containing ciprofloxacin (5 µg), ofloxacin (5 µg), ceftazidime (30 µg), cefuroxime (30 µg), gentamycin (10 µg), amoxicillin/clavulanate (30 µg) and ampicillin (10 µg), imipenem(10 µg), meropenem(10 µg), colistin(10 µg), polymyxin B(10 µg), was aseptically placed on plates containing Gram negative bacteria while Gram positive multi-disc containing erythromycin (30 µg), cloxacillin (5 µg), gentamycin (10 µg), ceftazidime (30 µg), augmentin (10 µg), vancomycin(30 µg), linezolid (30µg),teicoplanin(30µg),ciprofloxacin (5 µg) and

tetracycline (10 µg) was aseptically placed on plates containing Gram positive bacteria. The assay was done in duplicate before incubating at 37°C for 24 hours. The

inhibition zones formed around each disc were measured and recorded according to CLSI while the average from duplicate readings was recorded. [27]

[Table/Fig-1]: Social and demographic profile of 50 study population women.

Age (Years)	20-25 (n=10)	26-30 (n=10)	31-35 (n=10)	36-40 (n=10)	41-45 (n=10)
<b>Education background</b>					
a) 10th	-	2	3	5	3
b) 12th	7	3	2	3	2
c) Graduation	3	5	5	2	5
<b>Marital status</b>					
(a) Single	5	4	-	-	-
(b) Married	5	6	10	10	9
(c) Divorcee	-	-	-	-	1
<b>Occupation</b>					
(a) Govt. Job	1	2	2	3	2
(b) Pvt. Job	2	4	2	2	2
(c) Housewife	2	3	6	5	6
(d) Student	5	1	-	-	-
<b>No. of children</b>					
(a) 1-3	2	8	8	8	2
(b) 4-5	-	2	1	1	4
(c) 6 & above	-	-	1	1	4
(d) No child	8	-	-	-	-
<b>Sexual activity per week</b>					
(a) 1-2 times	2	4	7	5	7
(b) 3 & above	8	6	3	5	3

[Table/Fig-2]: Result of the microscopic examination of vaginal swab samples.

Control group	Epithelia cells	Pus cells	Yeast cells	Protozoa <i>T. vaginalis</i>
21-25	2	3	1	-
26-30	2	2	1	-
31-35	4	4	3	-
36-40	2	2	2	-
41-45	2	2	2	-
<b>Total</b>	<b>12</b>	<b>13</b>	<b>9</b>	

[Table/Fig-3]: Prevalence of microbes among the Intra uterine devices (IUD) devices.

Age group	No of population study	No of bacteria isolated	No of <i>candidat e</i> species isolated	No of <i>T. vaginali s</i>
<b>Intra uterine devices (IUD)</b>	50 (100%)	4 (8%)	2 (4%)	00 (00%)
20-25	10	-	-	-
26-30	10	-	1	-
31-35	10	1	-	-
36-40	10	2	-	-
41-45	10	1	1	-

## Results

[Table/Fig-4]: Result of susceptibility of each bacterial isolate to each of the antibiotics

	Staphylococcus lugdenensis	Staphylococcus epidermis	Escherichia coli	Klebsiella pneumoniae
CIP	S	S	R	R
OF	R	S	R	S
CAZ	R	S	S	S
CEF	R	R	R	S
GEN	S	R	R	R
AMP	R	S	S	S
AMC	R	S	S	R
E	S	S	R	S
CLOX	R	R	R	R
AG	R	R	R	R
STREP	R	R	R	R
CIP	S	S	S	S
TET	R	S	S	S
VAN	S	S	-	-
TEI	S	S	-	-
LZ	S	S	-	-
AZ	S	S	R	R
IMP	S	S	S	S
MRP	S	S	S	S
P	-	-	S	S
CL	-	-	S	S

## Discussion

The use of contraceptives is important in preventing unplanned pregnancy and sexually transmitted diseases. However, the use of various contraceptive methods could expose women to microbial infection. The socio-demographic profiles showed

S No.	Study	Year	Results
1	Viberga Vet al <sup>28</sup>	2005	The mean age of 203 study participants was 32.61±5.49 years (range: 24-42 years). Mean duration of the next visit after IUD insertion was 24.30±6.90 days (range: 12-35 days)
2	Adesumbo Idowu Odaranle et al. <sup>7</sup>	2020	The socio-demographic profiles showed ages of the 406 women in the study population were between 20-45 years (mean age 32.5 years) and 40.88% of the subjects were in 31-35 years age group. The majority of the population in the study was literates with 40% of them up to tertiary education. The majority of the populations in the study were literates with 44% of them up to Graduation education.
3	In the present study	2021	Our study showed out of 50 women's age was 20 - 45, the mean age of 50 study participants was 32 years (range: 20-45)

Microscopic examination of the vaginal specimens:  
Gram stain

S. No.	Study	Year	Results
1	Saidu, et al <sup>29</sup>	2021	Stained by Gram's technique. Using the 40 × and 100× (oil immersion) objectives, the smear was examined for pus cells and bacteria. Pus cells containing Gram-negative diplococci denote N. gonorrhoeae. Large Gram-positive yeast cells and pseudohyphae could be Candida species.
2	In the present study	2021	Our study showed stained by Gram's technique. Using 100× (oil immersion) objectives, the smear was examined for pus cells epithelial cell and bacteria like Gram-negative bacilli & Gram positive cocci in & Gram-positive yeast cells and pseudohyphae could be Candida species.

Microscopic examination of the vaginal specimens: Wet mount

S. No.	Study	Year	Results
1	Abdulhadi Diyo Saidu et al. <sup>29</sup>	2017	This was carried out to detect <i>T. vaginalis</i> trophozoites, motile bacteria, Candida species, epithelial cells, and pus cells. The high vaginal swab was utilized to make the preparation. The preparation was examined using a microscope with the 10 ×and40× magnification objectives. Trophozoites of <i>T. vaginalis</i> may measure about 10–20 μm, and they are round or oval and move by an aid of undulating membrane and four anterior flagella while the fifth flagellum forms an undulating membrane.
2	Adesumbo Idowu Odaranle1et al <sup>7</sup> .	2020	Microscopic examination of the vaginal specimens in saline and potassium hydroxide solutions showed the presence of epithelial cells ranging from 3-8 cells per High Power Field (HPF) in 88.2% of the women, pus cells ranging from 2-5 cells/HPF in 37.9% of the women, yeast cells in 25.6% of the women and trophozoite stage of <i>Trichomonas vaginalis</i> in 1.5% women of the sampled population.
3	In the present study	2021	Our study, Microscopic examination of the vaginal specimens in saline solutions showed the presence of epithelial cells ranging from 2-4 cells per High Power Field (HPF) , pus cells ranging from 2-4 cells/HPF in , yeast cells in 1-3 cell/HPF and trophozoite stage of <i>Trichomonas vaginalis</i> was not seen of the sampled population.

From the bacteriological analysis of the swab samples collected:-

S. No.	Study	Year	Results
1	András Ádám30	2018	Out of the culture positive samples, 30 proved to be positive only for aerobic bacteria with one or two different species. The most frequent aerobic bacteria were <i>Staphylococcus aureus</i> (10 isolates) <i>Enter co-occurs faecalis</i> (9 isolates) <i>Streptococcus</i> spp. excluding <i>Streptococcus agalactiae</i> (8 isolates) and <i>Escherichia coli</i> (6 isolates).
2	Adesumbo Idowu Odaranle1et al <sup>7</sup> .	2020	Bacteria isolated included <i>Escherichia coli</i> (11), <i>Klebsiella oxytoca</i> (8), <i>Proteus mirabilis</i> (6), <i>Staphylococcus aureus</i> (11) and Group B beta-haemolytic streptococci (12) and the <i>Candida</i> species included <i>Candida albicans</i> (74), <i>Candida tropicalis</i> (25) and <i>Candida stellatoidea</i> (17).
3	Abdulhadi Diyo Saidu et al. <sup>29</sup>	2017	<i>Staphylococcus aureus</i> produces yellow-cream colored colonies that are 1–2 mm in diameter and they are beta hemolytic on 5% Sheep blood agar. On MacConkey agar, the <i>Staphylococcus</i> colonies appear pinkish signifying lactose fermentation. <i>Streptococcus agalactiae</i> produce grey mucoid colonies about 2 mm in diameter surrounded by a beta hemolytic zone on Blood agar. <i>Escherichia coli</i> produce 1–4 mm in diameter colonies that appear pinkish signifying lactose fermentation. They are slightly mucoid and beta hemolytic on 5% Sheep blood agar. <i>Candida albicans</i> produces pale, creamy colored, pasty colonies on blood agar with a distinctive yeasty smell. <i>Actinomyces israeli</i> produces small creamy white colonies with a rough nodular surface that glistens on 5% sheep blood agar following anaerobic incubation
4	In the present study	2021	In the present study out of 50 samples the women using IUDs showed 6(12%) organisms out of which <i>Candida albicans</i> was (2%), <i>Candida krusei</i> was (2%), <i>Staphylococcus lugdunensis</i> was (2%), <i>Staphylococcus epidermidis</i> was (2%), <i>Klebsiella pneumoniae</i> was (2%) and <i>E.coli</i> was (2%).

The antibacterial assay showed that all the bacterial species

S. No.	Study	Year	Results
1	Adesumbo Idowu Odaranle1et al <sup>7</sup> .	2020	The antibacterial assay showed that all the bacterial species were susceptible to ciprofloxacin, ofloxacin and nitrofurantoin while they were resistant to augmentin, ampicillin and cloxacillin.
2	In the present study	2021	The GPC isolates were 100% sensitive to Vancomycin, Linezolid, Teicoplanine, Tetracycline, Gentamicin, Azithromicin,. On the other hand the GNB isolates were 100% sensitive to Imipenem, Meropenem, Polymixin, Colistin,.

## Conclusion

This study shows that more strains of bacteria and fungi were isolated from women in age 24-40 years women sampled and indicated that the more predominant isolates in symptomatic individuals using contraceptives users

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