

## SURVEILLANCE OF MICROBIAL FLORA FOR INFERTILITY COUPLES IN AN INDIAN TERTIARY CARE TEACHING HOSPITAL

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## ABSTRACT

**Objective:** Infertility has been known to cause serious social and emotional problems in India. Microorganisms may be a primary cause for this infertility. This study was carried out to know the prevalence of microorganisms in the infertility couples of a tertiary caring teaching hospital.

**Methods:** One thousands of couples were screened for the infertility test presented at the outpatient Department of Obstetrics and Gynaecology. The suspected infertility couples were tested for microbial contamination in their reproductive sample. Simultaneously, the demographic data of all patients were documented, and all the data were analyzed with SPSS 20 software.

**Result:** From one thousand couples, 288 couples were found infertility symptoms, whereas 67.71% were primary infertility problems and rest were secondary. It is due to male factor (27.08%), female factor (50%), and both (4.86%) but 18.05 were unexplained. From vaginal swabs, 186 and semen 145 microbial colony were grown. However, Chi-square ( $\chi^2$ ) showed that there is no significant difference between the growth of microbial colony from semen or vaginal swap as  $p=0.06$ . Eight varieties of bacteria (387) and *Candida albicans* (37) were documented.

**Conclusion:** With respect to older ages of the infertile couples, it is more chances to infections. Antibiotic should be prescribed to primary infertility couples and infections can be avoided the cross transmission during intercourse.

**Keywords:** Primary infertility, *Candida*, Semen, Antibiotic, Vaginal swab.

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## INTRODUCTION

In India, childless couples are suffered both mentally and socially to live. The psychological problem is associated with the increase of infertility duration. However, the reason behind these infertile couples is varied from couple to couple. The cause may be male factor, female factor; both male and female factors or sometimes idiopathic [1]. In about 60% of married couples, 90% would achieve pregnancy in 12 months, and 95% would be able to achieve pregnancy in 18-24 months [2]. Failure to achieve pregnancy at the end of this period is not a definite criterion to arrive at a diagnosis of infertility as the age of the woman must be taken into consideration. Female fertility is at its peak between the ages of 18 and 24 years, while, it starts to decline after the age of 34 years [3].

Infertility in the female may be traced to age, tubal factor, ovarian factor, and uterine factor among others [4]. Male infertility, a common problem worldwide, is a neglected reproductive health issue in India [3]. It is estimated that the male factor accounts for 20-50% of the cause of infertility in different parts of India [4,5]. Male infertility may actually occur following the occlusion of the vas deferens [6]. Similarly, a high level of hyperprolactinemia has been associated with male infertility [7,8]. Also associated with the condition is the antisperm antibodies [9] as well as genital infections [10,11]. Studies have equally revealed a higher concentration of aflatoxin in infertile India males than those in the fertile control, indicating that aflatoxin may contribute significantly to male infertility [12]. The WHO [13] minimal criteria for normal semen include on the average a volume of 1.5 ml; minimal count of 20 millions sperms/ml; sperm motility of 60%, and no blood cells. Microbial infections have been reported to reduce sperm viability. *Chlamydia trachomatis* infections lead to blockage in the epididymis, following chronic epididymis and variably lead to azoospermia [14]. However, *Ureaplasma urealyticum* infections which

induce leukocytospermia consequently lead to sperm damage, decrease sperm numbers, and invariably impaired sperm motility [15].

Vaginal infection is a common disease of women. Causes of vaginal discharge include physiological, infective (e.g., bacterial vaginosis, candidiasis, trichomoniasis), and non-infective (foreign bodies, cervical ectopy, and genital tract malignancy) [1]. Although after 40 years, there is a fall in estrogen production. An estrogen deficient state in vagina and the immune-compromised status due to diabetes or other associated factors can lead to growth of abnormal flora which may, in turn, lead to infections. Bacterial vaginal infections are often least understood, and empirical antifungal therapy for any vaginal infection without high vaginal swab (HVS) culture is still in practice. Normal vaginal pH in women of reproductive age is acidic; pH is normally  $\leq 4.5$ . In bacterial vaginosis and infection with *trichomonas vaginalis*, vaginal pH is elevated  $>4.5$ . In vaginal candidiasis, vaginal pH is  $\leq 4.5$ . Women of reproductive age with vaginal discharge should have a HVS cultured if, postnatal or post miscarriage, vaginitis without discharge, pre or post gynecological surgery, pre or post termination of pregnancy, symptoms not characteristic of bacterial vaginosis were seen [16-19]. In this study, we have screened to the infertile couples whether the microbiota is the primary cause also documented the demographic data of the couples.

## METHODS

This is a prospective study and carried out from January 2012 to December 2015 and approved from our Institutional Ethics Committee. A total of 1000 couples were screened for infertile, and 288 were enrolled for this study. For the females, HVSs are collected. While for the corresponding males (spouses), semen samples were collected using the masturbation method. All semen samples were sent to the laboratory within 15 minutes of collection and analyzed using the

WHO criteria. [13] The endocervical swabs or high vagina swabs were subjected to standard microbiological analysis. All the samples were grown in specific medium such as nutrient agar (NA), blood agar, chocolate agar, and cystine lactose electrolyte deficient agar for proper growth of bacteria and suborders dextrose agar, potato dextrose agar for fungi. Then, the colonies were identified by CLSI guideline, and with previous methods, i.e., all specimens were inoculated onto NA, MacConkey agar, and blood agar by streaking, and the plates were incubated aerobically at 37°C for 24 hrs. Discrete colonies were picked from the growth and subcultured to obtain pure colonies (Fig. 1), and biochemical tests were carried out.

In addition, the picked discrete colonies were Gram-stained, and thereafter, the consequent biochemical tests were done for the identification of microbiota with previous methods [18-30].

## RESULTS

A total number of 1000 couples studied and documented the demographic data and cause of infertility. Among the 1000 couples, 712 couples have no infertility symptoms, whereas 288 couples had infertility symptoms (Table 1). Out of 288 infertility couple, 195 primers, 93 were secondary. With questioner, it was revealed that 44.8% infertility couples were suffered since <5 years whereas 55.2% suffered >10 years. With medical tests, it was revealed that 27.08% were suffered with malefactors and 50% were revealed futures. About 4.86% were suffered both husband and wives. However, 18% of couples were not found any reasons. Among the couples, 9.02% husbands were taken medication for their infertility management, and 57.98% females were taken management for their infertility (Table 1).

All the infected couples are divided into their respective ages. It was revealed that in females, i.e., the wives are more infected as compare to the female (Tables 2 and 3).

In wives, the rate of infections increased in the older age groups as compared to elders. The two-tailed  $p=0.4160$  and by conventional criteria, this difference is considered to be not statistically significant. Hence, the organisms are found in equally in both husband and wives. In wives, at the age of the more than 40, there was more chance to infections (Table 2). In husbands, a total of 145 colonies are identifies. From the 145 colonies, 219 colonies were found in the age group on 20-39 years. Younger age group had less number of collies as compared to the older age groups (Table 3). All the samples of infertile couples were screened for microbial growth. Among the 288 couples, the

samples of 186 wives and 145 husbands were infected with microbial colony (Table 4). From the chi-square, it was revealed that the p value was more than 0.05. Hence, it is equally distributed in both husbands and wives (Table 4).

From the growth of the microbial colony, it was revealed that there is no any difference between the growth of organisms in the same couples with different couples as p was more than 0.05 from the Chi-square test (Table 5).

A total number of 414 microbial colonies are observed in this study. Among them, 37 *Candida albicans* and rest 387 bacterial colonies were revealed. Among the bacterial colonies, *Escherichia coli* was highest frequency (113), and *Staphylococcus aureus* was next to *E. coli*. Only, 3 *Streptococcus* sp. were found in this study (Table 6).

## DISCUSSION

In our study, *E. coli* arguably the dominant organism implicated in primary infertility; among males and females alike. With a prevalence rate of 27.29% isolates from the samples of infertile couple's culture, it clearly surpasses *S. aureus* which had a prevalence of 22.71% whereas 21.01%, 16.67%, 8.94%, 3.14%, 1.21%, 0.97%, and 0.72% were found for *Pseudomonas* sp., Coagulase negative *Staphylococci*, *C. albicans*, *Klebsiella* sp., *Proteus* sp., *Enterococcus faecalis*, and *Streptococcus* sp.,

**Table 1: Characteristics of infertility among the couples**

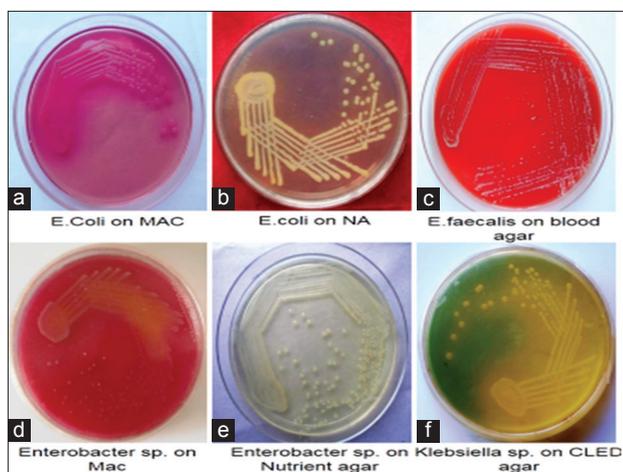
Variables/characteristics	n (%)
Presence of infertility among couples	
Absent	712 (71.2)
Present	288 (28.9)
Type of infertility among couples (n=288)	
Primary	195 (67.71)
Secondary	93 (32.30)
Duration of infertility (in years)	
<5 years	129 (44.80)
>10	159 (55.20)
Causes of infertility	
Male factors	78 (27.08)
Female factor	144 (50.00)
Both	14 (4.86)
Idiopathic	52 (18.05)
Receiving previous management for infertility	
Husbands	26 (9.02)
Wives	167 (57.98)

**Table 2: Distribution of microbial isolates in relation age of wives**

Age range	Number examined	Number infected	Percent
<20	17	2	11.765
20-29	116	72	62.069
30-39	149	107	71.812
>40	6	5	83.333
Total	288	186	64.583

**Table 3: Distribution of microbial isolates in relation age of husbands**

Age of husbands	Number examined	Number infected	Percent
<30	11	2	18.182
30-39	103	56	54.369
40-49	117	61	52.137
50-59	44	17	38.636
>60	13	9	69.231
Total	288	145	50.347



**Fig. 1: Pure culture of isolated bacteria by strict plate methods.**

(a) *Escherichia coli* on MacConkey agar (MAC), (b) *E. coli* on nutrient agar (NA), (c) *Enterococcus faecalis* on blood agar, (d) *Enterobacter* sp. on MAC, (e) *Enterobacter* sp. on NA, (f) *Klebsiella* sp. on cystine lactose electrolyte deficient agar

**Table 4: Number of colony observed from respective couples**

Couple	Mixed colony	Single microbial isolate	No growth	Total
Wife	122	64	102	288
Husband	97	48	143	288
Total	219	112	145	376

**Table 5: Number of same colony observed in both husbands and wives**

Samples from couple	Same couple	Different couple	Total
Growth	182	37	219
No growth	37	108	145
Total	219	145	364

**Table 6: Frequency of microbial isolated from the couples**

Microbial isolates	Microbial isolates in frequency (n=414)	Percentage of Microbial isolated
<i>E. coli</i>	113	27.29
<i>S. aureus</i>	94	22.71
<i>Pseudomonas</i> spp.	76	21.01
Coagulase negative <i>Staphylococci</i>	69	16.67
<i>C. albicans</i>	37	8.94
<i>Klebsiella</i> spp.	13	3.14
<i>Proteus</i> spp.	5	1.21
<i>Enterobacter</i> spp.	4	0.97
<i>Streptococcus</i> spp.	3	0.72

*E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *C. albicans*: *Candida albicans*

respectively. It is pertinent to note that *E. coli*, the most prevalent Gram-negative organism implicated in urinary tract infection [11], is not the dominant cause of primary infertility in females. Its prevalence rate of 15.5% among isolates from endocervical swab, 19.4% of isolates from HVS places it behind both *Klebsiella* spp. and *Proteus* spp. as the dominant Gram-negative bacterial isolated from these cases of primary infertility. It is important to highlight the fact that ofloxacin, a second-generation fluoroquinolone is effective in the management of these infections. The study shows that basically the same group of organisms is responsible for infertility among the different couples; however, more species of bacteria were isolated from the male semen cultures. *S. aureus* and *Klebsiella* spp. were isolated more when compared to the other organisms. A statistical (Chi square) test indicate that there is a significant difference at  $p < 0.0001$  when bacteria is compared with fungi as the predominant cause of primary infertility among couples.

It is particularly difficult to define abnormal genital tract flora in infertile women. The pathogenic role of specific microorganisms in the vagina as risk factors for spontaneous preterm birth (S-PTB) varies according to the investigators because microorganisms commonly found in the lower genital tract are those that are most frequently isolated from patients with intrauterine infections. The colonization rate of microorganisms in the vagina could be affected by not only technical factors, such as detection method and sampling site but also internal and external factors of each individual [31]. Possible racial/ethnic differences in the composition of the normal vaginal microflora also exist. Usui *et al.* [32] reported that absence of vaginal lactobacilli was a better predictor of S-PTB at <33 weeks of gestation than the presence of *Mycoplasma hominis*, but its sensitivity and positive predictive value were no more than 28% and 25%, respectively. Breugelmanns *et al.* [33] reported that there was no significant correlation between the presence of abnormal vaginal flora and S-PTB, and the risk of S-PTB increased

when *Ureaplasma* species was associated with an abnormal vaginal flora.

## CONCLUSION

The study has shown the prevalent microbial cause of infertility in our region. More importantly, it highlights the fact that these organisms are common, in the different anatomical region of the female, responsible, or involved in reproduction. Although the males also have similar organisms isolated from the semen; a few other organisms were solely isolated from the males. This isolates may later be involved in cross-transmission as the couple engaged in intercourse, further compounding the problem and antibiotics will avoid these infections. We, therefore, suggest therapeutic managements strategies of couples with primary infertility to include antibiotics and couples should be managed together to avoid cross-transmission.

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