# BACTERIOLOGY OF PELVIC INFLAMMATORY DISEASE IN INDIAN PATIENTS.

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## SUMMARY

**The study was carried out to elucidate the spectrum of microbial flora as obtained from endocervix of 50 consecutive patients with pelvic inflammatory disease (PID) and 50 controls. Most patients belonged to the age group of 21-30 years. A total of 104 isolates were cultured from patients with PID (2.08 per patient) of which 68.9% were aerobes and 31% anaerobes. From the controls only 13 isolates were obtained (0.26 per control). Pathogens most often identified from patients with PID were E. coli and Staph. aureus each in 16%, Streptococcus faecalis and Staph. epidermidis each in 10%. Anaerobic cocci were isolated from 24% patients and Bacteroids were isolated from (10%) patients with PID. Anaerobes were not isolated from any of the controls. Ureaplasma urealyticum was isolated from 40% of the patients and 8% of controls. Chlamydia trachomatis antigen was detected from 8% of the patients and 2% of the controls. It is difficult to assign any specific role other than facultative to the isolation of coliforms, Streptococcus faecalis and Staph. epidermidis. Isolation of these facultative and anaerobic bacterial species alone or together with other STD pathogens point towards the polymicrobial etiology of PID.**


## *INTRODUCTION*

Pelvic inflammatory disease (PID) produces significant morbidity in the most productive years of human life. The 2% peak annual incidence of PID is seen among women aged less than 20 years (Westrom and Mardh, 1990). PID and PID related

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sequlae had risen to alarming proportions and are a major public health problem. This is of great importance as 30-40% of patients suffer from ectopic pregnancy and nearly 11-35% of them become infertile (Bhujwala et al, 1980, Westrom and Mardh, 1990). Apart from these major morbidities 15-20% of them suffer from other disorders like pelvic pain, dyspareunia, low backache and recurrence of infections (Muir and Belsey, 1980). Adequate and appropriate treatment of PID might prevent these sequelae and for this one has to know the aetiological microbes. Various organisms have been implicated in the causation of PID and their importance have varied over years. Bacterial species isolated from the fallopian tubes in case of salpingitis fall into two main categories: sexually transmitted disease organisms and species indigenous to lower genital tract. Neisseria gonorrhoeae was described as the classical and causative agent of PID for nearly 100 years. However, in the combined European studies, its significance has gone down and in upto 60% of cases Chlamydia trachomatis is the causative organism. (Paavonen and Hanssen, 1989). Coinfection with N. gonorrhoeae and Chlamydia trachomatis may also occur in women with PID. Facultative and anerobic organisms have been detected in a large proportion of patients with PID, alone or together with STD organisms.

Etiological role of mycoplasma has been controversial, however, recent studies have not confirmed their role (Westrom and Mardh, 1990). The causative organisms may differ among different countries. The spectrum of causative organisms of PID among Indian patients is not fully known and to elucidate it the present study was undertaken.

## *MATERIALS & METHODS*

Microbial flora in 50 consecutive patients of PID, attending the Obstetric and Gynaecology department of Postgraduate Institute of Medical Education & Research, Chandigarh, were studied by appropriate culture and side lab procedures and the isolates were compared with the samples obtained from equal number of age matched controls. Controls were patients who were attending the OPD with vague complaints of headache, backache, epigastric distress etc. and in whom pelvic examination did not reveal any abnormality.

Diagnosis of PID was made using the criteria described by Jacobson and Westrom (1969). Patients who were treated with antibiotics in the previous 14 days were excluded, likewise patients in whom PID was related to surgical procedure were excluded. Patients with a history suggestive of a recent STD and/or PID were also excluded. Three endocervical swab were taken from each patient. One swab for mycoplasma was placed in PPLO broth, shaken and squeezed to the side of tube and removed. The other two were transported to the laboratory immediately for gonococci, aerobes and anaerobic culture. One swab was used for making grams smear to look for pus cells, gonococci, aerobic and anaerobic organisms. The second swab was inoculated on two plates of blood agar, MacKonkey's agar and New York city media for the isolation of aerobic, anaerobic organisms and gonococci respectively. One blood agar and Mackonkey's agar plate were incubated at 37° C. Another blood agar plate was incubated at 37° C anaero-

bically after required incubation. The plates were examined. The organisms were identified by standard methods (Cruickshank et al, 1975). For genital mycoplasmas - the PPLO broth was further subcultured into PPLO broth with urea and phenol red for U. urealyticum and PPLO broth with arginine and phenol red for M. hominis. The tubes incubated at 37° C in 10% $CO_2$ were examined daily for colour change. As soon as the colour change appeared it was subcultured on PPLO agar plates. The plates were examined after 72 hrs for colony morphology and organisms were identified by standard methods.

For C. trachomatis antigen detection STD EZE swab supplied by (Abbotts USA) were used. It was placed in the endocervix and rotated to collect epithelial cells. The ELISA test was performed for detection of C. trachomatis antigen according to the instructions given in the literature supplied alongwith the Kit (Chlamydiazyme Abbotts, U.S.A.)

*RESULTS*

All the patients and controls were married and were in the reproductive age group. Most of them belonged to the age group of 21-30 years (72% patients and controls each). Organisms were cultured from 86% patients and 20% controls. A total of 104 isolates were cultured from patients (2.08 per patient). of which 71 (68.9%) were aerobes and 33 (31%) were anaerobes. However, only 13 isolates (0.26 per control) were obtained from controls. Most of which were aerobes. Aerobes only were isolated from 42% of patients, 32% had mixed growth and from 12% patient only anaerobes were cultured. The corresponding figures for the control group were 18%, 2%, 0% respectively (Table I). The differences between the two groups are satistically significant. Types of organisms isolated are illustrated in the tables II (aerobes) and III (anaerobes).

The pathogens more often identified from patients with PID were E. coli (16%) Staph, aureus (16%), Streptococcus faecalis (10%),

**Table I**
**MICROORGANISMS ISOLATED FROM ENDOCERVIX - PATIENTS AND CONTROLS**

| Microorganism | No (%) of women from whom bacteria were isolated | | P-value |
|---|---|---|---|
| | patients n = 50 | controls n = 50 | |
| Aerobes only | 21 (42) | 9 (18) | 0.01 |
| Mixed aerobes and anaerobes | 16 (32) | 1 (2) | 0.05 |
| Anaerobes only | 6 (12) | 0 (0) | — |
| No growth | 7 (14) | 40 (80) | 0.05 |

**Table II**
**AEROBIC ORGANISMS AND FACULTATIVE SPECIES FROM ENDOCERVIX - PATIENTS AND CONTROLS**

| | Isolates No. of women from whom bacteria were isolated | |
|---|---|---|
| | patients (n=50) | controls (n=50) |
| B-haemolytic streptococci | 0 | 1 |
| Staph pyogenes | 1 (2%) | 0 |
| Staph. aureus | 8(16%) | 1 (2%) |
| Staph. epidermidis | 5(10%) | 0 |
| Streptococcus faecalis | 5 (10%) | 0 |
| E. coli | 8 (16%) | 0 |
| Klebsiella pneumoniae | 5 (10%) | 0 |
| Proteus mirabilis | 1 (2%) | 0 |
| Acintobacter spp. | 1 (2%) | 0 |
| N. gonorrhoeae | 0 | 0 |
| Diphtheroids | 2 (4%) | 0 |
| Gardnerella vaginalis | 5 (10%) | 4 (8%) |
| Mycoplasma hominis | 6 (12%) | 1 (2%) |
| Ureplasma urealyticum | 20 (40%) | 4 (8%) |
| Chalamydia trachomatis | 4 (8%) | 1 (2%) |

Staph. epidermidis (10%) and Mycoplasma hominis (12%). Anaerobic cocci were isolated from 24% and B. fragilis from 8% of the PID patients. Ureaplasma urealyticum was isolated from 40% of patients and 8% of controls. Chlamydia trachomatis antigen was detected from 8% of patients and 2% of controls. There was no difference in the isolation rates of Gardnerella vaginalis between the patients and control.

Blood for VDRL was non-reactive in all patients.

## *DISCUSSION*

Isolation of bacteria in 86% of patients, as compared to the figure of 20% of control groups, indicates that in normal individuals, the endocervix is mostly sterile. Isolation of more than one organism from most of the patients suffering from PID signifies that they have multiple organisms infecting the endocervix. This is reflected by the observation that 32% of patients are infected with both aerobes and anaerobes. Rates of isolation of anaerobes (65%) are similar

**Table III**
**ANAEROBIC ISOLATES FROM ENDOCERVIX - PATIENTS AND CONTROLS.**

| Anaerobic | No. of women from whom bactria were isolated | |
|---|---|---|
| | Patients (n = 50) | Control (n = 50) |
| **Gram positive** | | |
| 1. Anaerobic cocci | 12 (24%) | 1 (2%) |
| 2. Eubacterium lentum | 5 (10%) | 0 |
| 3. Propionibacterium Spp. | 1 (2%) | 0 |
| 4. Clostridum Spp. | 1 (2%) | 0 |
| **Gram negative** | | |
| 1. Veillonella parvula | 8 (16%) | 0 |
| 2. Bacteriodes fragilis | 4 (8%) | 0 |
| 3. Melaninogenicus | 1 (2%) | 0 |
| **Total** | **32 (64%)** | **1 (2%)** |

to those reported by other workers (Escenbach et al, 1975).

From none of the patients in the present study N. gonorrhoeae was isolated. This decreasing trend of the role of N. gonorrhoeae in PID has also been observed earlier (Mardh, 1980). Isolation rate of Mycoplasmas in our patients (12%) was comparatively less compared to the reported figure of 50%-80% (Mardh and Westrom, 1970 Eschenbach et al 1975, Kinghorn et al, 1986). However, the incidence of 12% isolation of Mycoplasma honimis was more than that from the controls (2%). In studies from the 1970s, M. hominis was reported to be isolated in pure culture from the fallopian tubes of women with salpingitis (Mardh and Westrom, 1970). However, later studies have not confirmed that M. hominis can cause acute salpingitis by itself (Westrom and Mardh, 1990).

The isolation rate of 40% Ureaplasma urealyticum from our patients is similar to the earlier reported figures ranging from 18-80% (Mardh and Westrom 1970, Eschenbach et al, 1975, Kinghorn et al, 1986). Though ureaplasma urealyticum was isolated in significantly more number of patients with PID as compared to controls, its precise role is yet to be ascertained.

Chlamydia trachomatis, though an important pathogen in actiology of PID was isolated from only a small number of patients (8%) compared to the higher

detection rate of 11-47% reported from Western countries (Eschenbach et al, 1975, Gjonnaess et al, 1982) and even from India (Lal et al, 1992). The lower rate of isolation may be due to the self limiting nature of Chlamydial infection in the endocervix.

Overall the isolates which are implicated in the pathogenesis of PID were similar to the isolates reported from developed countries except for lower rate of isolation of chlamydia trachomatis. It is difficult to assign any specific role to the isolation of Coliforms, Streptococcus faecalis, Staph aureus and Staph epidermidis and at best they can be considered as facultative pathogens. Isolation of facultative and anaerobic bacterial species alone or together with other STD pathogens has earned for pelvic inflammatory disease the concept of it being microbial (Eschenbach et al, 1975; Monif et al, 1976). Notwithstanding the amount of information we have on the pathogenesis of PID, we are only at the beginning of a full understanding of its complex dynamics (Westrom and Hanssen, 1993). More knowledge on the subject will help us to prevent sequlae such as infertility and ectopic pregnancy.

## *CONCLUSION*

The mocrobial flora isolated from the endocervix of patients with PID from India is almost similar to that from developed countries except chlamydia trachomatis which is present more often in patients from western countries. The presence of more than one organism from most patients with PID confirms its polymicrobial etiology even though some organisms may be considered more consistently present than others.

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