

Bacterial isolates associated with Pelvic Inflammatory Disease (PID) among Iraqi non-pregnant women and it correlated with level of interleukin-6

By

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Background : Among women of reproductive age or who are not pregnant, pelvic inflammatory disease (PID) is one of the most prevalent and dangerous illnesses. **Aim:** Among non- pregnant women patients at Al-Yarmouk teaching hospital, the current study attempts to isolate and identify pathogenic bacteria linked to pelvic inflammatory disease (PID) and it correlated with level of interleukin-6. **Materials and Methods:** In order to detect bacterial pathogens based on cultural and biochemical characterization tests, approximately 230 endocervical swabs were aseptically collected from period (January – July) 2024 from non-pregnant patients at Al-Yarmouk teaching hospital who had been diagnosed with Pelvic Inflammatory Disease (PID) based on the clinical features with estimation level of IL-6 by Elisa. **Results:** Among the 230 samples analyzed, 102(44.34%) were monomicrobial isolates, and 2 (0.86%) were polymicrobial , and 48.27 % were sterile cultures ,About 6.65% of positive cultures for both *E.coli* and *P.aeruginosa*, with *Staph.aureus* having the highest percentage , as well endocervical positive cultures ranged from 57 (47.90%) in individuals in the 25–35 age group to 20 (16.80%) in those in the 15-25 age group. so serum IL-6 levels in women with PID (32.41 ± 3.03) more than healthy control participants . **conclusion:** both *E.coli* and *P.aeruginosa* highest percentage, as well as endocervical positive cultures in patients with 25–35 age more than in those in the 15-25 age , so serum IL-6 levels in non-pregnant women with PID more than healthy control .

Keywords: pelvic inflammatory disease (PID) ; Bacterial isolates ; non pregnant women.

Introduction :

More morbidity in young women of reproductive age is caused by PID, a polymicrobial infection that primarily affects the female lower reproductive tract and spreads to the upper reproductive tract, including the uterus, fallopian tube, ovaries, and the pelvic peritoneum (Shinde *et al.*,2018). According to Vanamala *et al.*, (2018), the yearly incidence in affluent nations is predicted to be 10–13 per 1000 women, with 20 of those women falling between the 20–24 age range. PID is an ambiguous word that can relate to infections caused by viruses, fungi, parasites, and most frequently, bacteria (Loscalzo *et al.*, 2001).

Sexually transmitted infections, particularly *Neisseria gonorrhoea* and *Chlamydia trachomatis*, have been linked to numerous occurrences of PID. One study found that 10 (40%) of the 40 (60%) women with acute salpingitis who had *N. gonorrhoea* without treatment went on to develop PID. Furthermore, it is believed that *C. trachomatis* is the source of almost 60% of cases of salpingitis; of these, 20 (40%) of the infected women experienced PID (Viberga *et al.*,2006).

In the US, fallopian tube samples from 25% to 50% of women with acute PID have been found to have *C. trachomatis* in (25- 40) % of cases, *N.gonorrhoeae* in (30- 50)% of cases, and several additional anaerobes and facultative aerobes (Shafer, 1992).

PID is primarily diagnosed clinically, and female patients presenting with pelvic or lower abdominal pain together with tenderness in the genital tract should be suspected. Other possible causes of the patient's pain, such as an ectopic pregnancy, should be evaluated and ruled out. Antibiotics are used to treat PID in order to eradicate the main pathogens, which include *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Pelvic or tubo-ovarian abscesses are examples of short-term consequences. Chronic pelvic pain, infertility, and ectopic pregnancy are examples of long-term consequences, So early detection and intervention may be able to avoid problems (Woodhall *et al.*,2018; Stevens *et al.*,2018 and Basit *et al.*,2023).

Interleukin-6 (IL-6), a cytokine that is implicated in the regulation of immunological, hematopoietic, and inflammatory responses, in response to infection and damage , numerous cell types, including monocytes, lymphocytes, fibroblasts, endothelial cells, and keratinocytes, release interleukin-6 (IL-6) (Kishimoto *et al.*, 1995 and Mehsen *et al.*,2020).

Materials and methods:

The cross-sectional descriptive study design was used in the execution of the current investigation. During the period from (January- July) 2024, 230 women, ages 18 to 45, who complained of lower abdominal pain, vaginal discharge, and adnexal and cervical motion tenderness on a bimanual examination had their specimens taken. The patients had been admitted as PID patients to the consultant clinics of Gynecology and Obstetrics at Al-Yarmouk Teaching Hospital. 43 PID women who were enrolled in this study were sampled, and each woman provided an endocervical swab.

The endocervical specimens were grown on Blood agar medium and incubated aerobically for 24 hours at 37°C. In addition, the Chocolate agar culture was cultured with an enhanced concentration of CO₂ (10%). In order to potentially detect the presence of obligate anaerobes, additional Blood agar cultures of specimens are exposed to anaerobic incubation at the same temperature and time as the aerobic cultures.

Blood Sampling: about 5 milliliters of blood were drawn from both control and patient subjects in simple tubes. To separate the serum, the samples were centrifuged for five minutes at 3000 rpm. The Ray Biotics Company's ELISA IL-6 Kits were then used to complete the assay protocol (USA)

Statistical Analysis:

The data were all presented as mean \pm standard deviation ($M \pm SD$). S= Significant difference ($P < 0.005$) was the comparison of the significant (P-value) in any test. The difference is HS, or highly significant ($P < 0.001$). NS stands for Non-Significant Difference

Results :

Among the 230 samples analyzed, No.= 102 , 44.34% were monomicrobial isolates , whilst only 2 (0.86%) as polymicrobial isolates and 111 (48.27%) as non- bacterial isolate) (Figure-1).

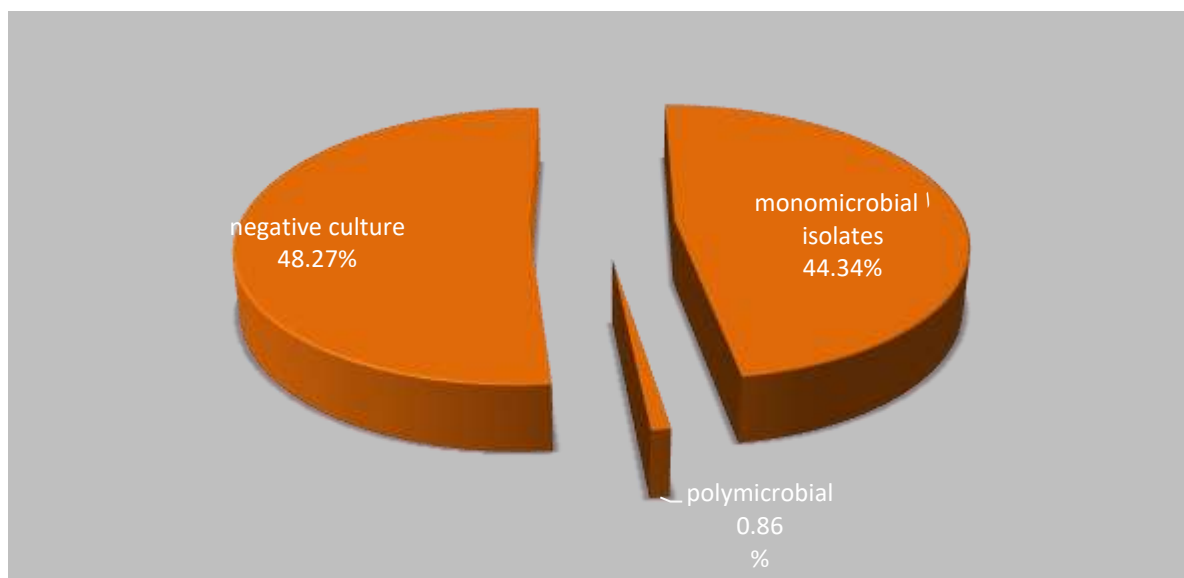


Figure (1):types of bacterial isolate

About 6.65% of positive cultures for both *Escherichia coli* and *Pseudomonas aeruginosa*, with *Staphylococcus aureus* having the highest percentage occurrence at 9.13% and *Staphylococcus epidermidis* at 21%. *N. gonorrhoea* had the lowest percentage occurrence at 0.43% table (1) .

Table (1): Bacterial isolates associated with PID among Iraqi non-pregnant women

Bacterial isolate From PID	No.	%
Monomicrobial isolates		
<i>Staphylococcus aureus</i>	21	9.13
<i>Staphylococcus epidermidis</i>	14	6.08
<i>Escherichia coli</i>	13	5.65
<i>Pseudomonas aeruginosa</i>	13	5.65
<i>Streptococcus pyogenes</i>	11	4.78

<i>Klebsiella pneumoniae</i>	10	4.34
<i>Streptococcus faecalis</i>	9	3.91
<i>Streptococcus agalactiae</i>	4	1.73
<i>Enterococcus faecalis</i>	4	1.73
<i>Proteus mirabilis</i>	2	0.86
<i>N. gonorrhoea</i>	1	0.43
Total (No.= 102 , 44.34%)		
polymicrobial isolates	2 (0.86%)	
Other organisms (No.= 15, 6.52%)		
<i>T. vaginalis</i>	2	0.86
<i>C. albicans</i>	13	5.65
Total	119	51.73
Non- bacterial isolate	111	48.27
No. of swab	230	100

Age-wise, endocervical positive cultures ranged from 57 (47.90%) in individuals in the 25–35 age group to 20 (16.80%) in those in the 15-25 age group (Table 2).

Table (2): endocervical positive cultures according to age groups

Age groups (years)	No. (%)	Total
15 – 25	20	16.80
35-25	57	47.90
35≤	42	35.30
Total of endocervical positive cultures	119	100

Serum IL-6 levels were determined in the current investigation in 20 healthy control participants (5.14 ± 0.39) compare to non-pregnant women with PID (32.41 ± 3.03) (table-3).

Table (3): serum level of interleukin (IL-6) in Iraqi non-pregnant women with Pelvic Inflammatory Disease (PID)

IL-6 level (Pg/ml)	Study group	Control group	p-value
	M±SD		
	32.41 ± 3.03	5.14 ± 0.39	0.01

Discussion :

One of the most common disorders in women worldwide, pelvic inflammatory disease (PID), was once thought to be caused by organisms linked to STDs, but it has since come to be understood as a polymicrobial infection (Meštrović, 2017).

It has been demonstrated that endometritis and PID are linked to anaerobic bacteria prevalent in BV, such as anaerobic gram negative rods, diphtheroides, black pigmented gram negative rods, and anaerobic gram positive cocci (Ness *et al.*, 2005).

The present investigation's findings are entirely in line with those of Spencer *et al.*, (2014), who reported a prevalence of 16% for *Staphylococcus aureus*, followed by *E. coli* at 12% and *Streptococcus species* at 8% (Spencer *et al.*, 2014).

Staphylococcus aureus had the greatest percentage of all the aerobic pathogens that were isolated, according to a study by Goel and Kumar women should therefore take precautions to prevent contaminating their intimate areas with things because *Staphylococcus aureus* can attach to surfaces. This implies the necessity to ascertain and supply coverage of all potentially harmful PID infections with an emphasis on long-term morbidity in analyses of treatment efficacy (Goel and Kumar 2015).

According to Jennings and Krywko's 2019 research, there were over 750,000 cases of PID in the US, with women between the ages of 15 and 25 being the most commonly affected. In a 2006 study, Simms and colleagues found that 77 out of 140 (or 55%) PID patients in the United Kingdom were between the ages of 16 and 24.

So according to the findings of Spencer *et al.* (2014), there were 68 instances of PID in the age range of 26 to 35 years, compared to 27 and 5 cases in the age categories of 15 to 25 years and 36 to 45 years, respectively. The cause of the extremely high incidence of PID in the 26–35 age group is yet unclear. But it's thought that only a small percentage of them may have gone to hospitals for treatment or counseling since they were either poorly informed about the importance of routine medical exams or completely unaware of them.

Buyalos *et al.* concluded that the elevated IL-6 levels in adhesive disease was not directly related to the presence of fibrosis, but possibly to the presence of subclinical infection. In the current investigation the serum IL-6 levels in non-pregnant women with PID (32.41 ± 3.03) more than 20 healthy control participants (5.14 ± 0.39), Martinez *et al.*, 2007 who found endometriosis was associated with a higher level of IL-6 compare to control group.

Conclusion : current study that conclude :

- A. Most isolated bacteria were *Escherichia coli* and *Pseudomonas aeruginosa*.
B. Most endocervical positive cultures ranged in individuals in the 25–35 age group.
C. Serum IL-6 levels in non-pregnant women with PID more than healthy control participants

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