

Bacterial Isolates Associated with Pelvic Inflammatory Diseases among Women of Reproductive Age in Babylon Province

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Abstract

Background: Pelvic inflammatory disease (PID) is a common infection and inflammation of the reproductive tract that is lining (endometritis) and fallopian tubes (salpingitis), is a recurrent condition among young women .PID and its complications, such as infertility, ectopic pregnancy, and chronic pelvic pain, are preventable by screening asymptomatic patients for sexually transmitted infections (STIs). This study aimed at identifying the microorganisms isolated, as well as the evaluation of frequency distribution for *Chlamydia trachomatis*, *Neisseria gonorrhea*, and others aerobic and anaerobic bacteria which may be associated with PID. **Materials and Methods:** Endocervical swabs totalling 43 were aseptically collected from women of reproductive age with pelvic inflammatory disease (PID), attending in two hospitals of Babylon Province: Province: Babylon Maternity and Pediatrics Hospital, and Al-Mahaweel General Hospital; as well as the samples taken from private clinics, during the period from January to October 2018. **Results:** Out of the 43 samples analysed, 30 women (69.7%) yielded pure cultures of bacterial isolates, among these 30 women, 22 (73.3%) had mixed cultures while no bacterial growths were recorded from the remaining 30.3 % samples. Organisms encountered were *Escherichia coli* the most encountered frequency (37.2%) followed by *Staphylococcus aureus* with (27.9%). *Enterococcus faecalis* (23.2%), *Gardnerella vaginalis* (16.2%), and *Candida spp* (11.6%) were less common, while *Chlamydia trachomatis*, *Klebsiella pneumonia*, and *Neisseria gonorrhoe* had the lowest occurrence frequency of (6.9%) , (6.9 %), and (3.2 %). **Conclusions:** Pelvic inflammatory disease is a major health problem in developing countries of the world; the micro-organisms frequently responsible for acute PID were genital tract *Escherichia coli*. Thus, measures should be taken regarding early diagnosis in order to provide treatment before the complications develop of PID.

Keywords: Acute pelvic inflammatory disease (PID), Female patient, Clinical presentation, Microbial isolates.

Introduction

The term “pelvic inflammatory disease” refers to the fact that numerous parts of the upper female genital tract, all of which are situated in the pelvis, may be infected separately or either at once [1]. When a woman has PID, she may have an infection that includes any or all of the ovaries, the fallopian tubes, and the uterus (endometrium).PID can severely damage the ovaries, fallopian tubes, and tissues in and around the uterus and ovaries. The damage can be permanent and may lead to several short- and long-term complications [2]. Some women with PID may also have indication of lower genital tract infection as well. The infection always begins in the lower genital

tract and ascends to the upper genital tract over time. Commonly, women with lower genital tract infections do not have any symptoms and therefore do not seek care. It is the asymptomatic nature of these infections that permits the bacteria involved to finally enter the upper genital tract and cause infection there [3].The woman may only have mild or nonspecific symptoms, when the infection has moved into the upper genital tract-in this situation, the PID is called as “silent PID”.Women with silent PID persist to have inflammation as a result of the untreated infection in their upper genital tract, this inflammation can cause scarring of the fallopian tubes.

When the fallopian tubes become scarred, they are no longer open, and eggs cannot travel to the uterus. Scarring in the fallopian tubes causes the woman to be unable to become pregnant. PID is an important cause of infertility [4]. PID is mostly common among young women and, sexually active young women who are treated in private clinic, emergency units, or physician offices [5].

The risk of acquiring sexually-transmitted pathogens with the threat of PID progress are associated with many socio-demographic and behavioral factors [6, 9] such as: previous history of sexually transmitted diseases in the infected female or their husband or partner. Gynecological equipment of the uterus / cervical barrier, as (curettage and dilatation), hysterosalpingography, insertion of intrauterine device, termination of pregnancy, *in vitro* fertilization (IVF), factors associated with sexual conduct, multiple partners, youthful age, new bedmate during 12-month follow-up, low socioeconomic condition and racial difference, bacterial vaginosis, and black ethnicity.

The diagnosis of PID is conventional primarily on clinical valuation; because of the possible for main sequel if treatment is late, so gynecologists have to treat PID patients depending on clinical decision without waiting imaging tests or laboratory results [3]. Pathogenic etiology of PID is not completely outlined, the most cases of PID are associated with one or more of microorganisms when it is demonstrated [10, 11].

Same as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, Bacterial vaginosis (BV), *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*. However, the vagina has many normal floras (e.g., *Candida albicans*, anaerobes, *Haemophilus influenzae*, *Gardnerella vaginalis*, Gram-negative enterobacteriaceae, and *Streptococcus agalactiae*) have associated with PID [12].

Several different bacteria may cause female genital tract infection, but the most common chlamydia and gonorrhea. Chlamydia and gonorrhea are the two most common bacterial sexually transmitted infections, which are caused by *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* respectively, and related to symptomatic PID infection, these

pathogens are isolated from the upper reproductive tract of more than a half of women with PID, it has furthermore been connected with a extensive spectrum of superior genital tract pathology reaching from asymptomatic endometritis with ambiguous symptoms or even asymptomatic to symptomatic. This indicates the impact of these microbial pathogens in the development of both acute PID and subclinical superior genital tract disease [13]. Symptoms in PID range from none too severe. If there are symptoms, then fever, cervical motion tenderness, lower abdominal pain, new or different discharge, painful intercourse, uterine tenderness, adnexal tenderness, or irregular menstruation may be noted [14].

Mostly, these infections are asymptomatic and, can cause severe problems, if not treated, mainly in young females. So, developments in diagnostic techniques and practices of specimen collection cause easier detection, management of these infections of universal public healthiness importance [15].

The reproductive severe consequence of PID, including tubal repeated PID, chronic pelvic pain, ectopic pregnancy and feature infertility can outcome from destruction to the cilia cover the fallopian tubes with suppurative or fibrinous inflammatory damage along the peritoneal surface and the epithelial surface, with fallopian tube obstruction or closing, or linkage formation amongst pelvic structures [16]. The purpose of this study was to identify the microbial pathogens, and evaluate frequency distribution of *Chlamydia trachomatis*, *Neisseria gonorrhoea*, and others microbes in women with PID.

Materials and Methods

Study Population and Specimen Collection

Clinical specimens were collected from patients of PID admitted to the consultant clinics of Gynecology and Obstetrics, in two hospitals of Babylon Province: Babylon Maternity and Pediatrics Hospital, and Al-Mahaweel General Hospital; as well as the samples taken from private clinics, during the period from January to October 2018. This study enrolled 43 women with PID were subjected for sampling which include endocervical swab from each female.

The age of women ranged from 18 to 50 years, according to the following criteria of national guidelines for pelvic inflammatory disease., history of frequent lower abdominal pain and presence of lower abdominal tenderness, cervical motion tenderness, adnexal motion tenderness, oral temperature more than 38°C and or leucocytosis more than 10.500/mm³ [17, 9]. In addition to the signs and symptoms, pelvic and abdominal ultrasound.

Microbiology

Direct Examination

Wet preparation was carried out in order to rule out the presence of microscopic findings (normal epithelia, polymorphous rod-shaped bacteria), suspicion of bacterial vaginosis (*Gardnerella vaginalis*) (more than 20% “clue cells” present, absence of lactobacilli, mixed bacterial flora with rods and cocci, but no increase in the number of WBCs), inflammation (more WBCs than epithelia or > 25 WBCs at a magnification pour of 400 x), which it were indicated of Candidiasis or infection with (Bacteria or Trichomonads) that were evaluated by mixing with one drop of 0.85 % a sterile normal saline solution and placed on a slide [18].

Direct Gram’s staining was done on smears made from the swab specimens for the characteristic morphological identification of organisms present, in addition to be examined for the presence of inflammatory cells (e.g., neutrophils) in gonococcal identification [19, 20].

Specimen Cultivation

Endocervical specimens were obtained after the cervix had been exposed with a speculum, swabs were directly and respectively cultivated on blood agar, McConkey agar and chocolate agar media and incubated aerobically at 37°C for 24 hrs; the chocolate agar culture was in addition incubated with increased 10% CO₂. Additional blood agar cultures of specimens were subjected to anaerobic incubation at identical temperature and time as the aerobic cultures for detecting of the presence of obligate anaerobic bacteria.

QuickVue *Chlamydia* Test

Is a qualitative detection of chlamydia directly from endocervical swab. This test was performed according to the instructions of company (Quidel-USA, cassette) by using chromatographic immunoassays technique.

Results and Desiccation

Laboratory tests isolated in 43 patients, no micro-organism in 13 women (30.3 %). At least one micro-organism was isolated in 30 women (69.7 %). Among these 30 women, 22 (73.3%) had polymicrobial infection (≥2 germs isolated). The results revealed that *Escherichia coli* were the most encountered frequency (37.2%) followed by *Staphylococcus aureus* with (27.9 %). *Enterococcus faecalis* (23.2%), *Gardnerella vaginalis* (16.2 %), and *Candida spp* (11.6 %) were less common, while *Chlamydia trachomatis*, *Klebsiella pneumoniae*, and *Neisseria gonorrhoea* had the lowest occurrence frequency of (6.9%), (6.9 %), and (3.2 %) respectively Table (1).

Table 1: Frequency distribution (%) of Pathogenic Isolates* in endocervical swab from women with pelvic inflammatory disease

Micro-organisms isolated	Number	%
<i>Escherichia coli</i>	16	37.2
<i>Staphylococcus aureus</i>	12	27.9
<i>Enterococcus faecalis</i>	10	23.2
<i>Gardnerella vaginalis</i>	7	16.2
<i>Candida spp</i>	5	11.6
<i>Chlamydia trachomatis</i>	3	6.9
<i>Klebsiella pneumoniae</i>	3	6.9
<i>Neisseria gonorrhoea</i>	1	2.3

*Some patients had more than one microorganisms isolated

The diagnosis of PID is based mainly on clinical evaluation which is vague, as no single historical, physical, or laboratory test finding is both sensitive and specific enough for the diagnosis of PID. So, combinations of

both diagnostic and clinical findings can improve sensitivity and specificity [21]. Furthermore, many episodes of PID pass unrecognized, where some cases are asymptomatic, others are not diagnosed

because the patient or the health-care worker fails to distinguish the hints of mild or nonspecific symptoms or signs; therefore health-care workers should maintain a low threshold for the diagnosis of PID [22]. Pelvic inflammatory disease is a common sexually transmitted disease occurring in young women who are sexually active.

The bacteriology of acute PID is polymicrobial in nature and this is often associated with a more severe clinical presentation, poor response to treatment, increased risk for more chronic pelvic pain, infertility and ectopic pregnancy. The most common symptoms of PID patients in this study included lower abdominal pain, abdominal tenderness with abnormal cervical or vaginal mucopurulent discharge, oral temperature $>38^{\circ}\text{C}$, all of which satisfy minimum criteria for diagnosis of PID [23].

In this study, demonstrated that the most frequently encountered pathogens were genital tract *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis*. These results were relatively agreed with results in variable studies were conducted in some developing countries such as [24] results, that showing *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus faecalis* as the predominant aerobic isolates with (34%), (21%) and (17%) percentage occurrence respectively, in contrast with other series where the most frequent genital pathogen was *Chlamydia trachomatis* [25].

Genital tract *E.coli* were thought to be pathologic, in recent studies found that they can be responsible for urethritis in men, cervicitis and PID in women [26, 27]. Intrauterine devices (IUD) are among the main risk factors for ascending facultative pathogenic bacteria such as *Staphylococcus*, *Streptococcus*, *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella* sp., *Proteus* sp., etc.) vaginal flora and the risk of PID is increased especially when an IUD is inserted in abnormal vaginal flora [28]. The most common primary infecting organisms such as *Chlamydia trachomatis* and *Neisseria gonorrhoea*, as recorded by [29], were lowest occurrence frequency encountered in this study, as the organisms isolated were mainly aerobic bacteria and facultative anaerobes.

This results were disagree with the studies carried out by [30, 31], showed that the

results of *Chlamydia trachomatis* frequency were (12.4%) and (37.1%) respectively for distribution of micro-organisms isolated from patients with PID. Several serological studies were done in various Iraqi governorates, showed wide variety of *Chlamydia trachomatis* frequency; ranging from (0%) in Kerkuk [32] to (26.5% and 39.7%) in Baghdad and Mosul respectively [33, 34]. The present study was compatible with [35], that showing *Chlamydia trachomatis* frequency, as he had demonstrated that different level of *C. trachomatis*; United Arab Emirates (2.6 %), Jordan (3.9 %), and Qatar (5.3 %). Also, [36]; in Iraq, Baghdad demonstrated that the frequency of *Neisseria gonorrhoea* (1.6%), and disagree with [37] who was presented that frequency of *Neisseria gonorrhoea* (5.1%).

Additionally, study in Iran reported that frequency of *C. trachomatis* was (22%) [38] and *Neisseria gonorrhoea* was (2.38%) [39], while in rural population of North India demonstrated the frequency of *Neisseria gonorrhoea* was (6.47%) [40]. This difference in the frequency could probably be credited to feminine diversity in social behaviours and exposure, sample size, sample type variation, population studied with variable socioeconomic factors, age of the participants, along with different practices used and some risk factors. In contrast to this lower rate frequency of *C. trachomatis*, that is expected to be lower than that in the Western countries, as UK (10.9%) [41], USA (15%) [42], and Brazil (16%) [43].

This restriction in the chlamydial positivity among study sample could be due to the Arabic and Islamic habits including the Iraqi populations are ethnically, culturally, and socially conservative in their attitudes towards free social life. Moreover, the occurrence of chlamydial genital infection and other STDs in Iraq and most Arab countries is not accurately identified, reflecting the limited specific diagnosis and treatment programs.

Additionally, the burden of this disease is nearly undervalued because most sexual infections are asymptomatic and are neither diagnosed nor reported [44]. Also that, it should be known, that there are no STI clinic in Iraq; some women go to the Obstetrics and Gynecology clinic, others might gone to another thing for consultation as private clinics or hospitals.

So, this could be biased the results. Finally, The fact that no microorganism was isolated in (30.3%) of PID patients might show that either the disease resulted from genital tract contamination during IUD procedures or the microorganisms were no more present on the

cervix, however the abnormal vaginal discharge present in all females, as well as the molecular diagnostics for the detection of microbial pathogens were not use in this study.

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