Novel Research in Microbiology Journal (2018), 2(6): 147-155
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/NRMJ.2018.22707

Prevalence of microorganisms associated with Pelvic inflammatory disease in reproductive aged women in Onitsha North, Anambra state, Nigeria

Eze, E.M.[1*]; Unegbu, V.N.[2]; Ezebialu, C.U.[3]; Nneji, I.R.[4]

[1]Department of Microbiology, Novena University, Ogume, Delta State, Nigeria; [2]Department of Microbiology, Renaissance University Ugboawka, Enugu State, Nigeria; [3]Department of Microbiology, Godfrey Okoye University, Thinkers Corner Ugwumu-nike, Enugu State, Nigeria; [4]Department of Microbiology, Legacy University, Okija, Anambra State, Nigeria

*Corresponding author E-mail: chuksebere31@yahoo.com

Received: 26 November, 2018; Accepted: 9 December, 2018; Published online: 25 December, 2018

Abstract

The prevalence of pelvic inflammatory disease (PID) and its associated microbes among reproductive aged women in Onitsha north, Anambra state, Nigeria, were investigated. A total of 500 reproductive aged women between the ages of 10 - 50 years were examined; where 300 of them showed positive results. A total of 640 microorganisms were isolated. Nine (9) microbial genera were recovered consisting of seven bacterial genera; one yeast sp. and one protozoan isolate. Monomicrobial growth was recorded in 53 (7.17%), polymicrobial growth in 23 (7.7%) and bacterio-fungal growth in 10 cases (33%). *Staphylococcus aureus* accounted for 150 (50%) cases; followed by *Escherichia coli* 125 (41.7%), *Streptococcus pyogenes* 15 (5%), *Klebsiella pneumonia* 55 (18.3%), *Proteus mirabilis* 25 (8.3 %), *Pseudomonas aeruginosa* 64 (21.3%), *Neisseria gonorrhoeae* 62 (20.7%), *Candida albicans* 56 (18.7%), and *Trichomonas vaginalis* 88 (29.3%), respectively. Frequency of occurrence was predominant with the age groups of 21-30 and 31-40 years; conversely was least in ages of 10-20 and those age >51 years; respectively. There was significant statistical difference between microbial infection and the age-group ($p<0.05$). PID is a major public health problem, thus needs to be prevented and controlled.

Keywords: Bacteria, Yeast, Protozoa, Women, Pelvic disease, Nigeria

1. Introduction

Pelvic inflammatory disease (PID) is a polymicrobial infection and inflammatory disorder of the upper female genital tract; that primarily affects young sexually active women. These disorders may include; cervicitis, endometritis, salpingitis, parametritis, oophoritis, tubo-ovarian abscess (Banikarim, 2005; Crossman, 2006), and pelvic peritonitis (Buchan *et al*., 1993). PID may results from sexually transmitted infection; infection due to post-gynecological procedure or rarely from

hematological spread (Risch and Howe, 1995). Most cases of PID are presumed to occur in 2 stages. According to Audu, (2004), the first stage was acquisition of a vaginal or cervical infection; which was often sexually transmitted and may be asymptomatic. The second stage was direct ascent of microorganisms from the vagina or cervix to the upper genital tract; accompanied with infection and inflammation of these structures. The mechanism(s) by which microorganisms ascend from the lower genital tract involve multiple factors. Although cervical mucus provides a functional barrier against upward spread of microbes; however, the efficacy of this barrier may be decreased by vaginal inflammation and by hormonal changes that occur during ovulation and menstruation (Ehoton-Vlasak, 2000).

In addition, antibiotic treatment of sexually transmitted infections can disrupt the balance of endogenous microflora in the lower genital tract; causing nonpathogenic microorganisms to overgrow and ascend. Opening of the cervix during menstruation along with retrograde menstrual flow; may also facilitate ascent of microorganisms. Intercourse may contribute to the ascent of microbes through rhythmic uterine contractions occurring during orgasm. Bacteria may also be carried along with sperms into the uterus and fallopian tubes (Cohen and Brunham, 1999). The principal complications of PID were chronic pelvic pain, infertility and ectopic pregnancy.

In the upper tract, a number of microbial and host factors appear to influence the degree of inflammation that occurs, and thus the amount of subsequent scarring that develops. Emele *et al*., (2004) added that infection of the fallopian tubes initially affects the mucosa, but inflammation may rapidly become transmural. Inflammation may extend to uninfected parametrical structures including the bowel. In reference to Kwanwendo and Forslin, (1998), microorganisms that were implicated in PID were thought to spread in three ways: First: intra-abdominally, traveling from the cervix to the endometrium through the salpinx. Second: through the peritoneal cavity (causing endometritis, salpingitis, tubo-ovarian abscess, or pelvic peritonitis). Third: through the lymphatic systems including infections of the parametrium from an intrauterine device (IUD) (Mahon *et al*., 2005).

PID rarely occurs in pregnancy; however, chorioamnionitis can occur during the first 12 weeks of gestation, before the mucous plug solidifies and seals off the uterus from ascending bacteria. Fetal loss may thus result (Das *et al*., 2016). Concurrent pregnancy influences the choice of antibiotic therapy for PID; and demands that an alternative diagnosis of ectopic pregnancy be included (Ross *et al*., 2017). Genetically mediated variation in immune response plays an important role in susceptibility to PID (Simms and Stephenson, 2000). Variations in the genes that regulate toll-like receptors (TLRs; an important component in the innate immune system), have been associated with an increased progression of Chlamydia trachomatis infection to PID (Taylor *et al*., 2012).

PID is a polymicrobial infection which may begin as single infection due to *Neisseria gonorrhoeae* or *C. trachomatis*; which causes inflammation of the upper genital tract, thus facilitates the involvement of other pathogens (anaerobes; facultative anaerobes, and other bacteria). These pathogenic microorganisms were increasingly isolated as inflammation increased and as abscesses formed (Bevan *et al*., 1995). Microorganisms involved in PID include; *Gardnerella vaginalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, Herpes simplex virus 2 (HSV-2), *Trichomonas vaginalis*, Cytomegalovirus (CMV), *Haemophilus influenza*, *Streptococcus agalactiae*, enteric gram-negative rods, (e.g.; *E. coli*) and *Enterococcus* sp. (Ross *et al*., 2017). Women with human immunodeficiency virus (HIV) infection also have an increased risk of progression to PID (Barbosa *et al*., 1997). Younger ages have been found to be

associated with an increased risk of PID. Likely reasons include; increased cervical mucosal permeability, larger zone of cervical ectopy, lower prevalence of protective anti-chlamydial antibodies, and increased risk-taking behaviors (Taylor *et al*., 2012).

The diagnosis of PID is based primarily on clinical evaluation, because of disease potentials for significant consequences (Ness *et al*., 2005). Since these infections are polymicrobial; broad-spectrum antimicrobial agents were recommended to treat the most likely pathogens. The first option for oral treatment includes a one-time 250-mg intramuscular dose of ceftriaxone (Rocephin); and 100 mg of doxycycline used orally twice per day for 14 days. Hence, it is pertinent to investigate the microbial effects of this type of disease. Therefore, the aim of this study was to investigate the prevalence of microorganisms associated with pelvic inflammatory diseases in reproductive aged women in Onitsha North, Anambra State, Nigeria.

2. Materials and methods

2.1. Study Area

This study was carried out within Onitsha North, a major city in Anambra state, Southern Nigeria.

2.2. Collection of Specimens

High vaginal swabs were collected from 500 female patients at General hospital Onitsha, Anambra State, using sterile swab stick; and were then sent aseptically to the microbiological laboratory for bacteriological, fungal and protozoan analysis.

2.3. Microbial isolation

Specimens were inoculated on Nutrient agar (NA), Blood agar (BA), Thayer Martin agar medium, Chocolate agar and Sabouraud Dextrose agar (SDA) plates using spread plate method (Cheesbrough, 2006). Plates were incubated at 35°C for 24 h, whereas, SDA plates were incubated for 96 h. Recovered bacterial and yeasts isolates were purified; and then subcultured on agar slants for further studies.

2.4. Characterization of bacteria

The bacterial isolates were characterized on the basis of their colony; microscopical and biochemical characteristics according methods described previously by Cheesbrough, (2006).

2.5. Characterization of yeasts

2.5.1. Microscopy of the yeast isolates

A small loopful from growing yeast slant was placed on the microscope slide containing a drop of lactophenol blue. They were teased and mixed together and then coverslip was placed on it. The yeast cells were observed with x10 and x40 objective lenses for budding; size of each yeast cell, size of daughter cells and thickness of the cell wall.

2.5.2. Germ tube test

Pasteur pipette was used to dispense 3 drops of fresh pooled human serum into test tubes. With sterile inoculating loop, yeast loopful was picked, dropped into the serum and mixed. The suspended yeast cells were incubated for 2-3 h at 37°C (Warren and Shadomy, 2011). Then a drop of this suspension was placed on a clean microscopic slide and examined with a microscope using x10 and x40 objective lenses.

2.5.3. Growth on chromogenic agar

Chromogenic agar medium (Oxoid, UK) was prepared as reported by Akter *et al*., (2014); poured in Petri dish and then allowed to solidify. A loopful from 24 h SDA slants was removed and then spread onto the surface of chromogenic agar medium with a sterile straight inoculating wire. Plates were

incubated for 24 h at 37°C and colors of colonies were recorded.

2.5.4. Growth on corn meal agar

According to the method of Warren and Shadomy, (2011); a sterile inoculating needle was used to take a loopful from 48 h yeast slant and used to streak an "X" shape in the middle on one half of a corn meal agar plate. The arms of the "X" shape were about 2 cm long. Using the same procedure, a duplicate of "X" shape was made in the middle of the other half of the agar plate. Sterile forceps was used to place a sterile cover slip over the cross of one of the "X" patterns. Plates were then incubated for 48 h at 37°C. The "X" shape without the coverslip served as a growth control. After incubation; plates were examined for development of chlamydospores, blastospores, and pseudophae using low (×10) and high power (×40) objective lenses.

2.6. Characterization of protozoa

For characterization of protozoa; two sterile swab sticks were used to collect high vaginal specimens from each woman. The first swab was placed in 2 ml of sterile saline solution (0.85 % NaCl in sterile dist. water) for direct wet mount preparation (Nourian *et al*. 2013). Briefly, a single drop of well homogenized vaginal swab content in normal saline was placed on a clean microscopic glass slide. The slide was initially scanned for motile flagellates at (x10) lens; subsequently at (x100) lens to confirm the parasite motility, flagella movement and morphological features of the organism. Moreover, other microscopical findings such as; red blood cells, pus cells and epithelial cells were also examined. The second vaginal swab content was cultivated on Trichomonas medium (Oxiod, UK); supplemented with 8 % heat inactivated horse serum following the manufacturer's instructions. To suppress bacterial and fungal growth, 0.05 mg/ ml of streptomycin and 0.05 mg /ml of chloramphenicol were supplemented to this medium; respectively. Inoculated cultures were incubated at 37°C; and followed up microscopically for the presence of motile trophozoites after 24, 48 and 72 h of incubation.

2.7. Antibiotic susceptibility testing

Susceptibility testing was carried out by disc diffusion method according to the Clinical Laboratory Standard Institute (CLSI. 2011); using the following antibiotics discs: clindamycin, doxycycline, cefotetan streptomycin, tetracycline, trimethoprim sulfamethoxazole, ampicillin, gentamycin, erythromycin, nystatin, and augmentin. Zones of inhibition of these discs were measured after incubation.

2.8. Statistical analysis

Statistical analysis of results was done using SPSS (statistical package for social sciences) version 21.0. The data collected were analyzed using chi-square test. Results were tested at significance level of 0.05.

3. Results and Discussion

Findings of this study have established the existence of PID among the reproductive aged women in Onitsha north; particularly in Anambra state, eastern Nigeria, with high prevalence of 60 %. PID constitutes a major public health problem in both developed and developing countries. Although it was one of the most commonly reported infectious diseases; however, the number of infected cases increases daily. Based on the colonial; microscopical and biochemical characteristics, all isolates were identified as shown in Tables (1 and 2). Out of 500 high vaginal swabs specimens analyzed; 300 (60 %) of specimens were positive for microbial infections. Thus 7 bacterial genera, 1 yeast sp. and 1 protozoan organism were isolated and identified. A total of 640 microorganisms were isolated as clear in Table (3). Polymicrobial growth was recorded in 237 (79 %) of cases; Monomicrobial growth in 53 (17.7 %), and bacteriofungal growth in 10 (3.3 %) of cases.

Table 1: Biochemical characteristics of bacterial isolates according to Cheesbrough, (2006)

Parameter	**Characterizations of isolates**						
Isolate Code	**P**	**P_2**	**PS_2**	**PM_2**	**PC_2**	**PD_2**	**PE_2**
Colonial shape/ elevation	Circular/ raised	Circular/ raised	Circular/ flat	Circular/ raised	Circular/ raised	Circular/ raised	Irregular/ flat
Colonial color	Cream	Grey	Yellow/green	Cream	Cream	Cream	Cream
Gram reaction	Gram -	Gram -	Gram -	Gram -	Gram +	Gram +	Gram -
Oxidase	-	+	+	-	-	-	-
Catalase	+	+	+	+	+	-	-
Indole	-	-	-	+	-	-	-
Methyl red	-	+	-	+	-	-	+
VogesProskauer	+	+	-	-	-	-	-
Motiliy	+	+	+	+	-	-	+
Coagulase	-	-	-	-	+	-	-
Urease	+	-	+	-	+	+	-
Citrate utilization	-	+	-	-	+	-	+
Glucose	A	AG	A	AG	G	A	A
Maltose	AG	A	A	AG	A	A	A
Lactose	G	A	-	AG	-	-	-
Sucrose	G	A	A	A	A	A	A
Mannitol	G	AG	A	AG	AG	AG	A
Galactose	AG	A	AG	AG	A	A	-
Fructose	A	AG	A	G	A	A	A
Xylose	A	AG	A	AG	A	A	A
Sorbitol	AG	A	-	AG	A	A	A
Most Probable Organism	*Proteus mirabilis*	*Neisseria gonorrhoea*	*Pseudomonas aeruginosa*	*E. coli*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Klebsiella pneumoniae*

Key: A= acid, AG =acid and gas, G= gas

Table 2: Biochemical characteristics of yeast isolate

Parameter	**Characterizations of yeast isolate**
Isolate code	Y1
Colonial morphology	Colonies were Smooth and cream
Microscopy	Spherical budding with blastoconidia
Urease activity	Negative
Germ tube	Positive
Corn meal agar	Chlamydospores and Pseudohyphae
Chromogenic color	Green
Glucose	Acid and gas
Galactose	Acid and gas
Sucrose	Acid and gas
Maltose	Acid
Lactose	Acid and gas
Mannitol	Acid
Fructose	Acid
Xylose	Acid and gas
Sorbitol	Acid and gas

Table 3: Frequency of occurrence of isolated microorganisms

Microorganisms	Frequency of occurrence (%)
Gram-positive bacteria	
Staphylococcus aureus	150 (50%)
Streptococcus pyogyenes	15 (1.8%)
Gram-negative bacteria	
Escherichia coli	125 (41.7%)
Neisseria gonorrhea	62 (20.7%)
Klebsiella pneumonia	55 (18.3%)
Proteus mirabilis.	25 (8.3%)
Pseudomonas aeruginosa	64 (21.3%)
Yeast	
Candida sp.	56 (18.7%)
Parasites	
Trichomonas vaginalis	88 (29.3%)
Total	**640**

S. aureus accounted for 150 (50 %) of cases; followed by *E. coli* 125 (41.7 %), *S. pyogenes* 15 (5%), *K. pneumonia* 55(18.3%), *Proteus mirabilis* 25(8.3%), *P. aeruginosa* 64 (21.3%), *N. gonorrhoeae* in 62 (20.7 %), *C. albicans* in 56 (18.7 %), and *T. vaginalis* in 88 (29.3 %). *S. aureus* and *E. coli* were the predominant bacterial isolates in this study, as these pathogens were mostly isolated from the lower genital tract; and were responsible for significant proportions of sexually transmitted diseases in Nigeria. The dominance of these bacterial pathogens and their existence in the female genital tract confirmed being predisposing factors in acquisition of PID.

As evident in this study, the lower frequency of occurrence of *N. gonorrhoeae* might be attributed to; variation in the studied population, methods of microbial investigation, variations in the severity of diseases, sampling technologies, and sites of sampling. Technically; *N. gonorrhoeae* was highly fastidious fragile microorganisms, its isolation depended on; viability of the microbe in the specimen, prompt delivery to the laboratory, and suitability of isolation medium. *T. vaginalis* with prevalence of 29.3 % posed significant public health problems; because of close association of trichomoniasis with HIV infection. *T. vaginalis* was an irritating protozoa causing sexually transmitted diseases worldwide (Swygard *et al*., 2004). Buve *et al*., (2001) reported that trichomoniasis incidence was higher in cities where there were higher numbers of HIV positive individuals. High prevalence of trichomoniasis and candidiasis recorded in this study; basically revealed close association of poor personal hygienic conditions among the low socio-economic class, and diseases transmitted sexually. This was particularly obvious in cases of multiple sex partners, with high probability of PID infection.

Table (4) showed that the frequency of occurrence of infections was predominant with the ages groups of 21-30 and 31-40 years, and was least in 10-20, and >51 years; respectively. There was significant statistical difference between microbial infection and the age-group ($p<0.05$). This finding simply confirmed previous reports of Bucham *et al*., (1993) that highest rate of infection was recorded in the age group of 16-24 years. Furthermore, PID

accounted for approximately 60% of gynecological problems in women aged less than 25 years. High prevalence of PID episodes in this sexually active age group emphasized the correlation between co-existence of etiological agents in the genital tract of the females, and acquisition of PID.

The prevalence rate of PID observed in the current study agreed with those findings of previous workers (Banikarim and Chacko, 2005). A number of reasons have been attributed by researchers for the rising prevalence of PID including; increased moral laxity among young people, lack of sexual education in schools and homes, and poor hygienic conditions (WHO. 2000; Das *et al*., 2016; Ross *et al*., 2017). The *in-vitro* antimicrobial susceptibility pattern of bacterial and yeast isolates revealed high zones of inhibition observed particularly with clindamycin, cefotetan and nystatin; conversely, low zones of inhibition were recorded with trimethoprim sulphamethoxazole and ampicillin. These antibiotic susceptibility patterns were similar to those of Kayode-Isa *et al*. (2010). The reduced susceptibility of antibiotics such as; ampicillin and trimethoprim sulphamethoxazole, might be attributed to the abuse of these antimicrobial agents through self-medication practice, which was a common phenomenon in towns/cities of most developing countries. Clindamycin, cefotetan, and nystatin showed acceptable *in-vitro* susceptibility pattern; thus could serve as drugs of choice in PID treatment/management. Antibiotics susceptibility patterns and zones of inhibition of bacterial and yeast isolates are shown in Table (5).

Table 4: Distribution of recovered isolates among different age-groups of patients

	Age groups (years)					
Isolates	**10-20**	**21-30**	**31-40**	**41-50**	**>51**	**Total**
S. aureus	15	50	35	30	20	150
S. pyogenes	-	10	3	2	-	15
P. mirabilis	-	15	3	-	7	25
K. pneumonia	-	25	15	10	5	55
P. aeruginosa	10	-	-	10	44	64
E. coli	15	33	30	25	22	125
N. gonorrheae	5	42	6	9	-	62
C. albicans	7	28	16	5	-	56
T. vaginalis	12	64	8	4	-	88
Total	**64**	**267**	**116**	**96**	**98**	**640**

Table 5: Antibiotics susceptibility pattern of the bacterial and yeast isolates

	Diameter of inhibition zones (mm)										
Isolates	**CL**	**CN**	**CEF**	**DOX**	**SXT**	**AMP**	**E**	**AU**	**S**	**TET**	**NYS**
S. aureus	30	5	20	28	-	3	20	25	10	14	-
E. coli	24	23	25	20	18	16	18	15	14	14	-
K. pneumonia	20	18	12	13	5	10	15	14	12	8	-
S. pyogenes	30	22	29	20	13	5	13	12	25	25	-
P. mirabilis	25	15	18	18	10	4	14	14	15	17	-
P. aeruginosa	15	11	18	12	-	12	15	13	14	12	-
N. gonorrhea	30	23	22	24	-	4	16	14	10	14	-
C. albicans	-	-	-	-	-	-	-	-	-	-	30

Where; Clindamycin (CL), Doxycycline (DOX), Cefotetan (CEF), Strepromycin(S), Tetracycline (TET), Trimethoprim Sulfamethoxazole (SXT), Ampicillin (AMP), Gentamycin (CN), Erythromycin (E), Augmentin (AU), and Nystatin (NYS).

Conclusion

In conclusion, the prevalence (60 %) of microorganisms associated with PID recorded in this study was high and was of public health concern. It was critical that microorganisms associated with PID should be early diagnosed; and therefore appropriate chemotherapeutic treatments/ management commence, as clinical complications were always very hazardous and expensive to treat.

Conflict of interests

The authors declare no conflict of interests

Acknowledgements

We like to appreciate all patients and staff members of General Hospital Onitsha, Anambra state; who participated in the current study. And like to acknowledge all the staff members in medical laboratory department of Onitsha general hospital; for providing equipment, media and reagents used in this study. Many thanks also to sponsors of the study.

4. References

Akter, M.L.; Haque, R. and Salam, M.A. (2014). Comparative evaluation of chromogenic agar medium and conventional culture system for isolation and presumptive identification of uropathogens. Pakistan Journal of Medical Sciences. 30(5):1033-1038.

Audu, B.M. and Kudi, A.A. (2004). Microbial isolates and antibiogram from endocervial swabs of patients with pelvic inflammatory disease. Journal of Obstetrics and Gynecology. 24(6): 161-164.

Banikarim, C. and Chacko, M. (2005). Pelvic inflammatory disease in adolescents. Seminars in Pediatric Infectious Diseases. 16(3): 175-80.

Barbosa, C.; Macasaet, M.; Brockmanna, S.; Sierra, M.F.; Xia, Z. and Duerr, A. (1997). Pelvic Inflammatory Disease and Human Immunodeficiency Virus Infection. Obstetrics and Gynecology. 89(1): 65-70.

Bevan, C.D.; Johal, B.J; Mumtaz, G.; Ridgway, G.L. and Siddle, N.C. (1995). Clinical, laparoscopic and microbiologic findings in acute sapingitis: report on a United Kingdom cohort. Brazilian Journal of Obstetrics and Gynecology. 102 (66): 407-14.

Buchan, H.; Vessey, M. and Goldacre, M. (1993). Morbidity following pelvic inflammatory disease. Brazilian Journal of Obstetrics and Gynecology. 12: 558-562.

Buve, A.; Weiss, H.A. and Laga, M. (2001). The epidemiology of trichomoniasis in women in four African cities. AIDS. 4: 89-96.

Chessbrough, M. (2000). Medical Laboratory Maunal for Tropical Countries. English Language Books Society/Tropical Heath Technology, Butterworth.

CLSI. (2011). Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty-first informational supplement. Document M100-S21.

Cohen, C. and Brunham, R. (1999). Pathogenesis of Chlamydia induced pelvic inflammatory diseases. Sexually Transmitted Infections. 75 (1): 21-24.

Crossman, S. (2006). The challenge of pelvic inflammatory disease: American Family Physician. 73(5): 859-864.

Das, B.B.; Ronda, J. and Trent, M. (2016). Pelvic inflammatory disease: improving awareness, prevention, and treatment. Infection and Drug Resistance. 9: 191-197.

Ehoton-Vlasak, A. (2000). Infections and infertility. Primary Care Update for Obstetrics. Gynocology. 7 (5): 200-206.

Emele, E.; Anyiwo, E. and Fadahunsi, A. (2004). Etiological profile of vaginal infection in Sokoto, Nigeria. Journal of Biomedical Investigation. 2(2): 57-62.

Kayode-Isa, T.M.; Fasiola, K.T.; Awe, S. and Ademiju J.A. (2010). Sensitivity of bacteria isolated from jewelry to linear akylbenzesulphonate. Nigeria Journal of Microbiology. 24(1): 2193-2196.

Kwamwendo, F. and Forslin, L, (1998). Programmes to reduced pelvic inflammatory disease the Swedish experience. Lancet. 351(23): 25-28.

Mahon, B.E.; Temkit, M.; Wang, J.; Rosenman, M.B. and Katz, B.P. (2005). Pelvic inflammatory diseases during the post-partum year. Infectious Diseases in Obstetrics and Gynecology. 13(4): 191-196.

Ness, R.B.; Kip, K.E; Hillier, S.L; Soper, D.E.; Stamm, C.A.; Sweet, R.L.; Rice, P. and Richter, H.E. (2005). A cluster analysis of bacterial vaginosis associated microflora and pelvic inflammatory disease. American Journal of Epidemiology. 162(14): 585.

Nourian, A.; Shabani, N.; Fazaeli, A. and Monsavinasab, S. (2013). Prevalence of *Trichomonas vaginalis* in pregnant women in Zanjan, Northwest of Iran. Jundishapur Journal of Microbiology. 6 (17): 7258.

Risch, H. and Howe, G. (1995). Pelvic inflammatory disease and the risk of epithelial ovarian cancer. Cancer Epidemiology Biomarkers and Prevention. 4(4): 447-451.

Ross, J.; Guaschino, S.; Cusini, M. and Jensen. J. (2017). European guideline for the management of pelvic inflammatory disease. International Journal of STD and AIDS. 0(0) 1-7.

Simms, I. and Stephenson, J.M. (2000). Pelvic inflammatory disease epidemiology and I: what did we know and what do we need to know?. Sexually Transmitted Infections. 76 (13): 80-7.

Swygard, H.; Sena, A.C.; Hobbs, M.M. and Cohen, M.S. (2004). Trichomoniasis: clinical manifestations, diagnosis and Management. Sexually Transmitted Infections. 80: 91-95.

Taylor, B.D.; Darville, T.; Ferrell, R.E.; Kammerer, C.M.; Ness, R.B.; Catherine L. and Haggerty, C.L. (2012). Variants in Toll-like Receptor 1 and 4 Genes Are Associated With *Chlamydia trachomatis* Among Women With Pelvic Inflammatory Disease. The Journal of Infectious Diseases. 205: 603-609.

Warren, N. and Shadomy H.J. (2011). Yeasts of medical importance. Journal of Clinical Microbiology. 29(6): 199-206.

WHO. (2000). Women's Health, Across Age and Frontier WHO, Geneva.