Le Infezioni in Medicina, n. 2, 247-253, 2022
doi: 10.53854/liim-3002-10

The prevalence of gonococcal and non-gonococcal infections in women referred to obstetrics and gynecology clinics

Hossein Kazemian[1,2], Morteza Karami Zarandi[3], Zeinab Zargoush[1], Sobhan Ghafourian[1], Nourkhoda Sadeghifard[1], Anahita Jalilian[4], Mahnaz Shafieian[5], Iraj Pakzad[1]

[1]Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran;
[2]Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran;
[3]Department of Microbiology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran;
[4]Department of Obstetrics and Gynecology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran;
[5]Department of Midwifery, Faculty of Nursing and Midwifery, Ilam University of Medical Sciences, Ilam, Iran

Article received 08 March 2022, accepted 30 April 2022

SUMMARY

Bacterial vaginosis is a condition caused by changes in the vaginal microbial ecosystem and increases the risk of preterm delivery, premature rupture of membranes, endometritis, and weight loss of the baby. This study aimed to evaluate the frequency of gonococcal and non-gonococcal genital infections in women referred to clinics in Ilam, Iran. Two swab samples were taken from each patient using a sterile swab, one swab was placed in a THB medium for the culture of *Streptococcus agalactiae* and the other in PBS buffer for PCR. PCR method was conducted for the identification of the other bacterial agents such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and also *S. agalactiae*. Sampling was performed on 169 women with symptomatic vaginosis. The frequency of *S. agalactiae* by culture and PCR methods was 4.7% (8 samples) and 13.6% (23 samples) respectively. Also, 6.5% (11 samples), 3.5% (6 samples), 4.1% (7 samples), 1.2% (2 samples), and 0% of the samples were positive for *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, *U. urealyticum* and *C. trachomatis* by PCR method. Except for a significant association between *S. agalactiae* colonization and abortion, there was no significant correlation between the prevalence of these bacteria and the patient's age, age of marriage, number of deliveries, and number of abortions. Overall, the prevalence of gonococcal and non-gonococcal infection in women referred to clinics in Ilam is similar to the other parts of Iran.

Keywords: *Neisseria*, gonococcal infection, mycoplasma, *Streptococcus agalactiae*, PCR.

INTRODUCTION

Genital infections can be divided into gonococcal and non-gonococcal groups, the former being caused by *Neisseria gonorrhoeae*, a Gram negative diplcoccus [1]. Other important agents including *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* are causative agents for non-gonococcal infections [2, 3]. *N. gonorrhoeae* and *C. trachomatis* preferentially infect cylindrical epithelial cells on the mucosal surfaces with inflammatory responses, leading to the production of large amounts of muco-purulent secretions and bleeding [2]. Sexually Transmitted Infections (STIs) by bacterial species such as *C. trachomatis*, *N. gonorrheae*, *U. urealyticum*, and *M. genitalium*, are responsible for serious genital consequences in women [4]. Approximately 20 to 40 percent of women with *C.*

Corresponding author
Iraj Pakzad
E-mail: Pakzad_i2006@yahoo.com

trachomatis and 10 to 20 percent of women with untreated *N. gonorrhoeae* develop pelvic inflammatory disease, which potentially results in infertility, miscarriage, and ectopic pregnancy [5, 6]. Acquisition of *M. hominis* during passage through the birth canal can cause meningitis, bloodstream infection, and eye and brain abscesses in infants. *U. urealyticum* can cause prenatal and postnatal complications, neonatal pneumonia, pelvic inflammation, respiratory syndrome, chorioamnionitis, and low birth weight [3].

Additionally, *Streptococcus agalactiae* strains, as a commensal bacterium in vaginal microbiota, can potentially ascend into the uterus and cause meningitis, sepsis, and death in the neonate. Furthermore, *S. agalactiae* can cause urinary tract infections, sepsis, endometritis, and infectious abortions [7, 8]. According to the dangerous consequences of infections with these bacterial species and also the unclear prevalence of genital infections in most parts of Iran, in this study, we tried to assess the rate of gonococcal and non-gonococcal infections in women in Ilam, Iran.

PATIENTS AND METHODS

Sample collection

In this cross-sectional study, from January to June 2019, 169 vaginal samples were collected from women referred to the Obstetrics and Gynecology clinics in Ilam. Demographic information of the patients such as age, education and marital status, and clinical history including history of abortion and pregnancy and antibiotic consumption of any sort in the last 10 days were collected. The patients with a history of consumption of antibiotics such as β-lactams, aminoglycosides, macrolides, tetracyclines, and other sorts which probably result in negative culture or PCR results were excluded from the study. Sampling was performed by a gynecologist on women with symptomatic vaginitis according to the CDC and American College of Obstetricians and Gynecologists (ACOG) guidelines [9]. Two cotton swabs were collected and one of them was inoculated in Todd-Hewitt broth (THB) (Merck & Co., Kenilworth, NJ, USA) containing 10µg/ml gentamicin and 15 µg/ml nalidixic acid. The other swab was inoculated in PBS buffer for DNA extraction and PCR assay; then the samples were immediately transported to the microbiology laboratory within 2 hours of collection.

Culture and phenotypic identification of S. agalactiae

Immediately after the samples were transported to the microbiology laboratory THB medium were incubated at 37ºC for 24h, then, each medium subculture was performed on defibrinated sheep blood agar and incubated for 24-48 h in a 5% CO2 atmosphere. Gram-positive coccui bacteria were identified by catalase test, growth in 5% NaCl, CAMP test, sensitivity to bacitracin, beta hemolysis, and pigment production under anaerobic conditions.

Molecular identification

DNA extraction for each sample was conducted according to the instructions of the manufacturer by GeneAll DNA extraction kit (Seoul, Korea) kit. Then, purified DNA was maintained at -20ºC. The Polymerase Chain Reaction was performed in a reaction mixture with a final volume of 25 µl containing 10 µl of 2 Taq Master Mix (SinaClon, Tehran, Iran), 1 µL of each primer [50 pmol/µl], 3 µL of the DNA template and 8 µl sterile purified water to complete the volume. Settings for the reaction were as follows: primary denaturation step at 94°C for 3 minutes; 40 amplification cycles each for 1 minute at 94°C, 1 minute at 59°C, and 1 minute at 72°C. This was followed by an additional extension step of 10 minutes at 72°C. The PCR products were electrophoresed on 1% agarose gel containing 1x DNA-safe stain (SinaClon, Tehran, Iran). Extracted DNA from *N. gonorrhoeae* strain PTCC1779 and *S. agalactiae* strain PTCC1884 were used as positive control samples.

Primers

Specific primers for molecular detection of *S. agalactiae*, *N. gonorrhoeae*, *U. urealyticum*, *M. genitalium*, *M. hominis*, and *C. trachomatis* (Table 1) were designed by Primer 3 software. The specificity and efficacy of designed primers were analyzed by the BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast).

Ethical consideration

The study protocol was approved by the Ethics Committee of Ilam University of Medical Sciences, Ilam, Iran (Project number 959009-93). Written informed consent was taken from all participants.

Statistical analysis

The collected data were analyzed using the Statistical Package for Social Science (SPSS Inc., Chica-

Table 1 - Target genes and their primers used in the present study.

Gene name	*Target bacteria*	*Primer sequence*	*Molecular weight*
Cfb	*S. agalactiae*	Forward-5´-TGGAACTCTAGTGGCTGGTGCAT-3´ Reverse-5´-CTGTCTCAGGGTTGGCACGCAA-3´	263 bp
16s rDNA	*N. gonorrheae*	Forward-5´-TAAAGCGGGCGCAGACGGTTAC-3´ Reverse-5´-TGTTACGGCTCCCGAAGGCACT-3´	474 bp
Urease	*U. urealyticum*	Forward-5´-ACTGAGGGGCCAACAGAAACGC-3´ Reverse-5´-ACGTGAGTCAGCAAAAGCAACGTC-3´	570 bp
16s rDNA	*M. genitalium*	Forward-5´-GCCAGCAGCCGCGGTAATACAT-3´ Reverse-5´-TTTGCTCCCCACGCTTTCGTCC-3´	170 bp
16s rDNA	*M. hominis*	Forward-5´-CGCGGTGAATACGTTCTCGGGT-3´ Reverse-5´-TGATCCACCCCCACGTTCTCGT-3´	373 bp
Omp1	*C. trachomatis*	Forward-5´-AATCCTGCTGAACCAAGCC-3´ Reverse-5´-CTGTAGGCTTGGCACCCATT-3´	100 bp

go, version 20.0). Chi-square tests (χ^2), and Fisher exact test were used for the analysis of categorical data. $P<0.05$ was considered statistically significant.

RESULTS

In the present study, a total of 169 vaginal samples were collected from as many women with symptomatic vaginosis. The mean age of the participants was 26.9 (±9.2) years. Among the participants, 136 women were married and 33 women were divorced. Among the participants, 40 of them had not been pregnant, 129 women had at least one successful delivery and 40 women nonetheless had one miscarriage history (Table 2).

N. gonorrhoeae was identified in 11 (6.5%) samples by PCR assay and 10 samples belonged to non-pregnant women. *S. agalactiae* was the most commonly detected pathogen and 23 (13.6%) positive samples were detected by PCR method. Among 23 PCR positive samples, 8 (4.7%) positive samples for *S. agalactiae* were detected by culture method.

The prevalence of *M. hominis*, *M genitalium*, *U. urealyticum*, and *C. trachomatis* were 7 (4.1%), 6 (3.6%), 2 (1.2%), and 0 (0.0%), respectively. Whereas the coinfection rate of *N. gonorrhoeae* and other

Table 2 - Demographic details of patients and prevalence of infectious agents according to age, education, marriage, pregnancy and abortion.

		Age (Years old)				*Education*			*Marriage*		*Pregnancy (times)*						*Abortion (times)*				
Groups		15-25	25-35	35-40	≥45	1*	2**	3***	married	divorced	0	1	2	3	4	≥5	0	1	2	3	≥4
Distribution of 169 Patients (n)		42	53	48	26	40	85	44	136	33	40	41	30	25	12	21	129	31	5	2	2
Positive cases of infectious agent by PCR method (n)	NG (11)	5	4	2	0	4	4	3	9	3	1	3	1	1	2	3	10	1	0	0	0
	SA (23)	10	6	4	3	7	12	4	18	5	4	6	6	3	1	1	10	11	1	0	1
	MG (6)	2	3	1	0	1	4	1	5	1	3	0	1	1	1	0	4	2	0	0	0
	MH (7)	1	5	1	0	2	5	0	6	1	0	2	3	1	1	0	5	2	0	0	0
	UU (2)	2	0	0	0	0	1	1	2	0	1	1	0	0	0	0	2	0	0	0	0
	CT (0)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*illiterate, **Elementary or high school, ***University. NG: *N. gonorrheae*, SA: *S. agalactiae*, MG: *M. genitalium*, MH: *M. hominis*, UU: *U. urealyticum.*

pathogens was zero, *M. genitalium* and *S. agalactiae* in 5 samples and *S. agalactiae* and *M. hominis* in 3 samples were simultaneously detected. However, no significant correlation was found for co-infection with these bacteria. Except that a significant association between *S. agalactiae* colonization and a history of abortion, there was no significant correlation between the prevalence of these bacteria and the patient's age, age of marriage, number of deliveries, and number of abortions (Table 2).

DISCUSSION

According to previous studies in Iran, the positive culture rate of *N. gonorrhoeae* has been reported from zero to less than 2% [1, 10, 11]. According to the low sensitivity of culture and phenotypic tests for detection of *N. gonorrhoeae*, *C. trachomatis*, and Mycoplasma species, conventional microbial diagnostics were targeted in this study only to detect *S. agalactiae*.

Molecular studies in different parts of Iran have reported the prevalence of *N. gonorrhoeae* from zero to 12%. In this study, 11 (6.5%) samples were positive for *N. gonorrhoeae* by PCR test. This value is relatively higher than some of the other studies in Iran. For example, the reported incidence of *N. gonorrhoeae* was respectively 1.25% and 2.3% in Sabzevar and Kashan cities in Iran [12, 13]. In addition, in the northern provinces of Iran, the prevalence of *N. gonorrhoeae* was 0.2% [14]. Moreover, in the studies from Zanjan and Kurdistan prevalence of the *N. gonorrhoeae* was less than %1 [1, 15]. On the other hand, other studies from Iran reported more close prevalence rate for *N. gonorrhoeae* to the current study's results. For instance, the prevalence of *N. gonorrhoeae* was respectively 4.0%, 5.6%, and 4.10% in Zanjan, Fars, and Qom provinces [10, 16, 17]. It seems that the prevalence of *N. gonorrhoeae* in Ilam province is approximately similar to other provinces in Iran. Furthermore, the highest reported incidence of *N. gonorrhoeae* infection is from Isfahan (12%) between female sex workers (FSWs) [4]. None of the participants in our study declared for being FSWs and the higher prevalence of the *N. gonorrhoeae* infections in Nasirian et al study is probably related to patients' occupation [4]. The prevalence of *N. gonorrhoeae* in the neighboring countries such as UAE, Kuwait, Turkey, and Saudi Arabia has been reported 5.5%, 1.5%, 6.4%, and 7.8% [18-21] respectively. All in all the prevalence rate for *N. gonorrhoeae* in Iran and neighboring countries is similar. In the current study, while *N. gonorrhoeae* and other pathogens' coinfection rates were 0.0%, the highest co-infection rate was observed for *S. agalactiae* and *M. genitalium*. Almost all of the *M. genitalium* positive samples (5 samples out of the 6), were positive for *S. agalactiae* as well. On the other hand, *S. agalactiae* was the most prevalent pathogen among the study population. Both PCR and culture methods were applied for the detection of *S. agalactiae*. Predictably, the PCR method was more sensitive than the culture, and 23 (13.6%) and 8 (4.7%) were respectively positive by PCR and culture methods. Studies investigating *S. agalactiae* colonization in vaginal samples from Iran and other countries detected prevalence rates ranging between 11% and 19.8% [22-25]. Moreover, previous studies mentioned higher sensitivity for molecular methods in the detection of *S. agalactiae* and our results are consistent with their results [26-28]. Interestingly, the correlation between abortion history and colonization with *S. agalactiae* was significant (p value <0.05), and 13 (56.7%) women that were colonized by *S. agalactiae*, at least had one abortion. The association between maternal colonization by *S. agalactiae* and unfavorable perinatal outcomes has long been studied. Early labor and premature rupture of the membranes (PROM) are the most commonly studied complications, but the positive correlation between these complications remains controversial [29, 30]. While, some studies have found a positive relationship between preterm birth and PROM, others have not [31-33].

M. genitalium is a sexually transmitted pathogen and causes cervicitis and pelvic inflammatory disease in women [34]. In the current study, 3.6% of the patients were colonized by *M. genitalium*. This rate is reported from 0.9% in American pregnant women to more than 15% in FSWs [35]. While, the prevalence rates of *M. genitalium* vary depending on age, gender, and sexual behaviors, we did not find a significant correlation between colonization by *M. genitalium* and age, education, pregnancy, and abortion. A meta-analysis estimated the following prevalence of *M. hominis*, *M. genitalium*, and *U. urealyticum* in Iran: 17.53%, 11.33%, and 9.68%, respectively [36]. *M. hominis* colonize the mucosal surface of the cervix or vagina and are found in 21-53% of females without genitourinary infections [37]. Although several compli-

cations such as bacterial vaginosis, preterm birth, spontaneous abortion, premature birth, perinatal mortality, and infertility over time are potentially associated with *M. hominis* infections, we did not find a correlation between Mycoplasma and Ureaplasma colonization with abortion and pregnancy [36].

Although we did not find any correlation between fertility, pregnancy, and abortion with isolation of *U. urealyticum* and *M. hominis*, several studies declared a probable correlation between colonization with *U. urealyticum* and *M. hominis* and infertility. For example, in a study *U. urealyticum* and *M. hominis* were detected in 50% and 26% of endocervical swabs specimens of infertile women [38]. In addition, Christian Leli (2018) detected *U. urealyticum* in 4.7% and *M. hominis* in 3.4% of 232 cervical swab specimens of infertile women in Italy [39]. Furthermore, the prevalence of *Ureaplasma spp.* in women (14.4%) is higher than *M. hominis* in women (0.2%) with urogenital tract infection in Poland [40]. Also, Cassell estimated that the *U. urealyticum* can be found in 40 to 80% of cervicovaginal samples from sexually mature women [41]. And finally, Lee (2013) displayed *U. urealyticum* in 40% and 22.9% and *M. hominis* in 8% and 4.2% of infertile and fertile women in Korea [42]. The heterogenic prevalence of genital infections in different reports can be probably caused by differences in the geographic areas, the sexual behavior of the study group, other infection accompanied agents, the sample size, and the operator proficiency.

CONCLUSIONS

Overall, the prevalence of gonococcal and non-gonococcal infection in women referred to clinics in Ilam is similar to other parts of Iran. Colonization by *S. agalactiae* is more significant in abortion than the other infectious agents and while the programs should be applied to increase the awareness about STDs in the study area, sexually active and pregnant women should be monitored for colonization of non-STD infectious agents such as *S. agalactiae*.

Author contribution

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data. They played an active role in drafting the article and gave the final approval of the version to be published.

Acknowledgements

We want to thank the 'Department of Microbiology, Ilam University of Medical Sciences, Ilam, Iran' for their kind cooperation.

Conflict interest

All of the authors declare that there are no any potentially conflicting interests related to the submitted manuscript.

Funding

None

REFERENCES

[1] Ahmadi A, Mousavi A, Salimizand H, Hedayati MA, Ramazanzadeh R, Farhadifar F, et al. Prevalence of *Neisseria gonorrhoeae* in the west of Iran. *Jpn J Infect Dis.* 2022; 75 (1), 1-4. doi: 10.7883/yoken.JJID.2021.006.

[2] Nateghi Rostami M, Hossein Rashidi B, Nazari R, Aghsaghloo F, Habibi A. A multiplex assay of *Trichomonas vaginalis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in genital specimens. J Infect Dev Ctries. 2017;11(11):833-9.

[3] Moridi K, Hemmaty M, Azimian A, Fallah MH, Khaneghahi Abyaneh H, Ghazvini K. Epidemiology of genital infections caused by *Mycoplasma hominis*, *M. genitalium* and *Ureaplasma urealyticum* in Iran; a systematic review and meta-analysis study (2000-2019). *BMC Public Health.* 2020; 20 (1), 1020.

[4.] asirian M, Kianersi S, Hoseini SG, Kassaian N, Yaran M, Shoaei P, et al. Prevalence of sexually transmitted infections and their risk factors among female sex workers in Isfahan, Iran: a cross-sectional study. *J Int Assoc Provid AIDS Care*. 2017; 16 (6), 608-14.

[5] Paavonen J. *Chlamydia trachomatis* infections of the female genital tract: state of the art. *Ann Med.* 2012; 44 (1), 18-28.

[6] Lewis D, Newton DC, Guy RJ, Ali H, Chen MY, Fairley CK, et al. The prevalence of *Chlamydia trachomatis* infection in Australia: a systematic review and meta-analysis. *BMC Infect Dis*. 2012; 12, 113.

[7] Kenyon C, Colebunders R, Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review. *Am J Obstet Gynecol.* 2013; 209 (6), 505-23.

[8] Romero P, Muñoz M, Martínez MA, Romero MI, Germain L, Maida M, et al. Ureaplasmas and mycoplasmas in vaginal samples from prepubertal girls and the reasons for gynecological consultation. *J Pediatr Adolesc Gynecol.* 2014; 27 (1), 10-3.

[9] Update to CDC's sexually transmitted diseases

treatment guidelines, 2006: fluoroquinolones no longer recommended for treatment of gonococcal infections. *MMWR Morb Mortal Wkly Rep*. 2007; 56 (14), 332-6.
[10] Nateghi Rostami M, Hossein Rashidi B, Habibi A, Nazari R, Dolati M. Genital infections and reproductive complications associated with *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and *Streptococcus agalactiae* in women of Qom, central Iran. *Int J Reprod Biomed.* 2017; 15 (6), 357-66.
[11] Hassanzadeh P, Mardaneh J, Motamedifar M. Conventional Agar-Based Culture Method, and Nucleic Acid Amplification Test (NAAT) of the cppB Gene for Detection of *Neisseria gonorrhea* in Pregnant Women Endocervical Swab Specimens. *Iran Red Crescent Med J.* 2013; 15 (3), 207-11.
[12] Bahador A, Mohammadzadeh M, Haghighi F. Prevalence of Chlamydia Trachomatis, *Neisseria gonorrhoeae* and *Ureaplasma urealyticum* in pregnant women of Sabzevar - Iran. *Sex Transm Infec*t. 2013; 89 (Suppl. 1), A1-A42 https://www.ijrcog.org/index.php/ijrcog/article/view/681):8.
[13] Afrasiabi S, Moniri R, Samimi M, Mousavi SG. The frequency of *Neisseria gonorrhoeae* endocervical infection among female carrier and changing trends of antimicrobial susceptibility patterns in Kashan, Iran. *Iran J Microbiol*. 2014; 6 (3), 194-7.
[14] Bakhtiari A, Firoozjahi A. *Chlamydia trachomatis* infection in women attending health centres in Babol: prevalence and risk factors. *East Mediterr Health J*. 2007; 13 (5),1124-31.
[15] Hamid B, Braham A, Mohtaram H. Prevalence of infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in women visitors of gynecology and obstetrics clinics in Zanjan Province of Iran. *African J Microbiol Res.* 2011; 5 (17), 2447-50.
[16] Molaei B, Mohmmadian F, Eftekhar M, Hatami R, Tirkan A, Kiani M. The frequency of gonorrheal and chlamydial infections in Zanjanian women in 2013-2014. *Int J Reprod Biomed.* 2017; 15 (2), 75-82.
[17] Behzadi MA, Davarpanah MA, Namayandeh M, Pourabbas B, Allahyari S, Ziyaeyan M. Molecular diagnosis of genital tract infections among HIV-positive women in Iran. *Iran J Microbiol.* 2018; 10 (4), 233-41.
[18] Mehrabani D, Behzadi MA, Azizi S, Payombarnia H, Vahdani A, Namayandeh M, et al. Cervical Infection with Herpes simplex Virus, *Chlamydia trachomatis,* and *Neisseria gonorrhoeae* among symptomatic women, Dubai, UAE: A Molecular Approach. *Interdiscip Perspect Infect Dis.* 2014; 2014, 347602.
[19] Al-Sweih NA, Khan S, Rotimi VO. Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among asymptomatic women attending the Capital Health region clinics in Kuwait. *Sex Transm Dis.* 2011; 38 (9), 793-7.
[20] Sonmez C, Usluca S, Hakki Usluca I, et al. Evaluation of Symptomatic Patients with Resistant Discharge. *Acta Dermatovenerol Croat.* 2018; 26 (1),1-7.
[21] Alzahrani AJ, Obeid OE, Hassan MI, Almulhim AA. Screening of pregnant women attending the antenatal care clinic of a tertiary hospital in eastern Saudi Arabia for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. *Indian J Sex Transm Dis AIDS.* 2010; 31 (2), 81-6.
[22] Yasini M, Safari M, Khorshidi A, Moniri R, Mousavi G, Samimi M. Frequency of group B capsular serotypes of Streptococcus using the multiplex PCR among the pregnant women in Kashan during 2011-2013. Feyz Journal of Kashan University of Medical Sciences. 2013;17(2).
[23] Shahbazian N, Rajabzadeh A, ALAVI SM. Prevalence of group-b-streptococcal colonization in vagina and rectum of 35-37 weeks pregnant women and its sensitivity to antibiotics. 2007.
[24] Bergeron MG, Ke D, Ménard C, François FJ, Gagnon M, Bernier M, et al. Rapid detection of group B streptococci in pregnant women at delivery. *New Engl J Med.* 2000; 343 (3), 175-9.
[25] Quinlan JD, Hill DA, Maxwell BD, Boone S, Hoover F, Lense JJ. The necessity of both anorectal and vaginal cultures for group B streptococcus screening during pregnancy. *J Fam Pract.* 2000; 49 (5), 447.
[26] Dmitriev A, Hu YY, Shen AD, Suvorov A, Yang YH. Chromosomal analysis of group B streptococcal clinical strains; bac gene-positive strains are genetically homogenous. *FEMS Microbiol Lett*. 2002; 208 (1), 93-8.
[27] Wollheim C, Sperhacke RD, Fontana SKR, Vanni AC, Kato SK, Araújo PR, et al. Group B Streptococcus detection in pregnant women via culture and PCR methods. *Rev Soc Bras Med Trop*. 2017; 50 (2), 179-83.
[28] Goudarzi G, Ghafarzadeh M, Shakib P, Anbari K. Culture and Real-Time PCR based maternal screening and antibiotic susceptibility for Group B Streptococcus: an iranian experience. *Glob J Health Sci.* 2015; 7 (6), 233-9.
[29] Regan JA, Klebanoff MA, Nugent RP, Eschenbach DA, Blackwelder WC, Lou Y, et al. Colonization with group B streptococci in pregnancy and adverse outcome. *Am J Obstetr Ginecol.* 1996; 174 (4), 1354-60.
[30] McDonald H, O'loughlin J, Jolley P, Vigneswaran R, McDonald P. Vaginal infection and preterm labour. *Br J Obstet Gynaecol.* 1991; 98 (5), 427-35. doi: 10.1111/j.1471-0528.1991.tb10335.x.
[31] Dechen TC, Sumit K, Ranabir P. Correlates of vaginal colonization with group B streptococci among pregnant women. Journal of global infectious diseases. 2010;2(3):236.
[32] Kubota T. Relationship between maternal group B streptococcal colonization and pregnancy outcome. *Obstet Gynecol*. 1998; 92 (6), 926-30.
[33] Valkenburg-Van Den Berg AW, Sprij AJ, Dekker FW, Dörr PJ, Kanhai HH. Association between colonization with Group B Streptococcus and preterm delivery: a systematic review. *Acta Obstet Gynecol Scand*. 2009; 88 (9), 958-67.

[34] Gnanadurai R, Fifer H. *Mycoplasma genitalium:* A Review. *Microbiology (Reading).* 2020; 166 (1), 21-9.
[35] Baumann L, Cina M, Egli-Gany D, Goutaki M, Halbeisen FS, Lohrer GR, et al. Prevalence of *Mycoplasma genitalium i*n different population groups: systematic review andmeta-analysis. *Sex Transm Infect.* 2018; 94 (4), 255-62.
[36] Moridi K, Hemmaty M, Azimian A, Fallah MH, Khaneghahi Abyaneh H, Ghazvini K. Epidemiology of genital infections caused by *Mycoplasma hominis, M. genitalium* and *Ureaplasma urealyticum* in Iran; a systematic review and meta-analysis study (2000-2019). *BMC public health*. 2020; 20 (1), 1020.
[37] Jironkin A, Brown RJ, Underwood A, Chalker VJ, Spiller OB. Genomic determination of minimum multi-locus sequence typing schemas to represent the genomic phylogeny of *Mycoplasma hominis.* BMC Genomics. 2016; 17 (1),964.
[38] Moosavian M, Ghadiri A, Amirzadeh S, Rashno M, Afzali M, Ahmadi K. Investigating *Chlamydia trachomatis* and genital Mycoplasma prevalence and apoptosis markers in infertile and fertile couples. *Jundishapur J Microbiol.* 2019; 12 (1).
[39] Leli C, Mencacci A, Latino MA, Clerici P, Rassu M, Perito S, et al. Prevalence of cervical colonization by *Ureaplasma parvum, Ureaplasma urealyticum, Mycoplasma hominis and Mycoplasma genitalium i*n childbearing age women by a commercially available multiplex real-time PCR: An Italian observational multicentre study. *J Microbiol Immunol Infect.* 2018; 51 (2), 220-5.
[40] Kasprzykowska U, Sobieszczańska B, Duda-Madej A, Secewicz A, Nowicka J, Gościniak G. A twelve-year retrospective analysis of prevalence and antimicrobial susceptibility patterns of *Ureaplasma spp.* and *Mycoplasma hominis* in the province of Lower Silesia in Poland. *Eur J Obstet Gynecol Reprod Biol.* 2018; 220, 44-9.
[41] Cassell GH, Waites KB, Watson HL, Crouse DT, Harasawa R. *Ureaplasma urealyticum* intrauterine infection: role in prematurity and disease in newborns. *Clin Microbiol Rev.* 1993; 6 (1), 69-87.
[42] Lee JS, Kim KT, Lee HS, Yang KM, Seo JT, Choe JH. Concordance of *Ureaplasma urealyticum* and *Mycoplasma hominis* in infertile couples: impact on semen parameters. *Urology*. 2013; 81 (6), 1219-24.