Prevalence of Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, and Trichomonas vaginalis among patients suspected of sexually transmitted infections in Evercare Hospital Dhaka during 2015 to 2022

Nazmul Hasan[1], Rummana Rahim[2], Abu Hasan[3], Rubaiya Ali[4], Q. M Mahbub Ullah[5], Mizanur Rahman[6]

1. Junior Scientific Officer, Molecular Diagnostics Lab, Evercare Hospital Dhaka
2. Associate Consultant, Molecular Diagnostics Lab, Evercare Hospital Dhaka
3. Chief Scientific Officer, Molecular Diagnostics Lab, Evercare Hospital Dhaka
4. Senior Consultant, Dermatology Department, Evercare Hospital Dhaka
5. Senior Consultant, Dermatology Department, Evercare Hospital Dhaka
6. Senior Consultant, Molecular Diagnostics Lab, Evercare Hospital Dhaka

Address for Correspondence:
Dr. Mizanur Rahman
Sr. Consultant,
Molecular Diagnostics Lab,
Evercare Hospital Dhaka.
mizanur.rahman @evercarebd.com

ABSTRACT

Background
Sexually Transmitted Infections (STIs) are often diagnosed by clinical symptoms and signs due to lack of sensitive laboratory methods. Most of the time pathogens are not identified and antibiotics are used empirically. The purpose of our study is to find out the STI pathogens detected by sensitive multiplex PCR done routinely in a tertiary care hospital.

Materials and methods
A total of 578 samples were tested from February 2015 to July 2022. Urine, prostatic secretion, urethral swab, high vaginal swab, semen, throat swabs were collected from symptomatic patients for the routine test. DNA was extracted and Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Trichomonas vaginalis were screened by using CE-IVD approved multiplex Real Time PCR kit.

Result
Out of 578 cases, 76 (13.15%) were positive for at least one pathogen and 5 were co-infections. Out of 76 positive cases, Chlamydia trachomatis was found 41 (53.9%), Neisseria gonorrhoeae was 13 (17.1%), Mycoplasma genitalium was 12 (15.8%), and Trichomonas vaginalis 5 (6.6%) and co-infections with Neisseria gonorrhoeae and Chlamydia trachomatis was 5 (6.6%). Positivity rate was remarkably higher in male (77.6%) than female (22.4%) and predominant age group was 19-40 years.

Conclusion
The predominant STIs pathogen found in our cohort is Chlamydia trachomatis followed by Neisseria gonorrhoeae. Multiplex PCR is a wide-ranging diagnostic method for the detection of multiple pathogens simultaneously which allows early and accurate diagnosis of STIs.

Keywords: *Sexually Transmitted Infections (STIs), multiplex PCR, Bangladesh*

INTRODUCTION

Sexually Transmitted Infections (STIs) are disease that are passed on during unprotected sex with an infected partner. The contact is usually through vaginal, oral, or anal sex. But sometimes they can spread through other intimate physical contact like herpes and HPV are spread by skin-to-skin contact[1]. STIs cases may be symptomatic or asymptomatic. Thus, a patient may go unnoticed until complications occur, or a partner is diagnosed. Signs and symptoms that might indicate an STIs include sores or bumps on the genitals or oral and rectal area, painful or burning urination, pain during sex, unusual discharge from urethra or vagina, lower abdominal pain, fever etc[2]. More than 30 different bacteria, viruses and parasites are known to be transmitted through sexual contact. Some STIs can also be transmitted from mother to child during pregnancy, childbirth, and breastfeeding. According to the World Health Organization (WHO), more than 1 million STIs are diagnosed every day and 374 million new cases of gonorrhoea, chlamydia, syphilis, and trichomoniasis was identified throughout the world in 2020[3]. In USA, 1.7 million cases of Chlamydia trachomatis infections had been reported in 2017 which is 22% more than reported cases of 2013. It is highly alarming that infections with Neisseria gonorrhoeae increased by 67% in 2017 over the same interval[4]. In Bangladesh very limited data is available for STIs and its causative organism. A report in 2000 showed prevalence of Neisseria gonorrhoeae and Chlamydia trachomatis was 35.5% and 25% respectively, in street-based sex workers[5] and 35.8% and 43.5% respectively, among hotel-based sex workers[6]. Later, a study published in 2009 showed prevalence of Neisseria gonorrhoeae was 39.1% and Chlamydia trachomatis was 47.8% among outpatient department in dermatology clinic in two public tertiary care hospital in Dhaka[7]. Trend in rising of non-gonococcal urethritis is also reported in another study of Chattagram[8]. However, there is no study reported so far in Bangladesh about the prevalence of

STIs pathogens among patients in a private tertiary care hospital

In the absence of availability of quick, cheap, sensitive, and specific diagnostic methods, a constantly increasing number of patients are not diagnosed or rarely diagnosed. As a result, this disease is drastically spreading out in developing countries[9]. Several methods are available for detecting *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, and Mycoplasma genitalium*, including Gram staining, Giemsa stain and wet mount preparation for microscopy, bacterial culture, enzyme-linked immunosorbent assay for antigen or antibody detection[10-11]. Strand displacement amplification[12], and polymerase chain reaction (PCR) performed in monoplex or multiplex are advanced methods for STIs diagnosis[13-16].

In this study, by using multiplex Real Time PCR simultaneous detection of three bacterial pathogens named *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium* and one protozoal pathogen that is *Trichomonas vaginalis* was performed.

MATERIAL AND METHODS

Method of data collection:

The data of the patients were taken from hospital information system of Evercare Hospital Dhaka, Bangladesh. This study was carried out between February 2015 and July 2022. Age, sex, signs and symptoms, types of specimens, year of sample collection etc. were used for data analysis. To protect patients' private information except for the age and sex, all samples were de-identified and patient consent were not needed as data is extracted from the routine test result available in the hospital information system.

Clinical Samples:

The samples were sent mainly by the Dermatology, Urology and Gynecology department of Evercare Hospital Dhaka. A total of 578 samples were collected from the age group of 13-68 years old. Urine, prostatic secretion, urethral swab, semen, throat swabs were collected from male patients and urine, high vaginal swab were collected from female patients. Commonly presented symptoms among these patients were painful or burning urination, pain during sex, discharge from the penis, vaginal discharge, lower abdominal pain, ulceration on the genital area, fever etc.

All swabs were collected by trained medical personnel in sterile swab stick and were transported to laboratory. Urine and semen samples have been collected in a 50 ml sterile plastic container. In the case of swab samples, we added 2-3 ml of sterile phosphate buffer saline (PBS) to the swab stick tube and mixed it with vortex mixture. All samples were stored at 2-8°C for no longer than 24 hours until DNA extraction and then extracted DNA was stored at -80°C. All the laboratory works were performed in the molecular laboratory of Evercare Hospitals Dhaka**.**

DNA extraction & Multiplex Real time PCR:

DNA was extracted by using QIAamp DNA Mini (Qiagen, Germany) spin column-based extraction kit. 200 µl of sample was used for DNA extraction. We added PCR kit recommended 10 µl of internal control during the DNA isolation into lysis mixture. The elution volume was 50 µl. We used CE-IVD approved Sacace *N.gonorrhoea*e / *C.trachomatis* / *M.genitalium* / *T.vaginalis* Real TM commercial multiplex real time PCR kit from Sacace Biotechnologies Srl, Italy. The total PCR volume was 25 µl where 15 µl master mix prepared for each sample, Negative control (NC) and Positive control (PC) and then 10 µl of extracted DNA, NC & PC was added respectively in 0.2 ml PCR strip tube. PCR amplification was done by Rotor-Gene Q (Qiagen, Germany) and Quant Gene 9600 (China) five plex thermocycler according to kit manufacturer's instruction which was programmed as follows: Hold-95˚C for 15 min, 5 cycles of 95˚C for 5s , 60˚C for 20s, 72˚C for 15 s , then 40 cycles of 95˚C for 5s, 60˚C for 20s (30s for Quant Gene 9600) and 72˚C for 15s. Signal was acquired at 60˚C, and analysis was performed on the linear scale. Thresholds were set manually in each run. The fluorescence was detected in FAM/ Green channel for *Neisseria gonorrhoeae*, JOE/Yellow/HEX channel for *Chlamydia trachomatis*, ROX/Orange channel for Mycoplasma genitalium, CY5.5/Crimson channel for T*richomonas vaginalis* and CY5/Red channel for amplification of internal control. The total time required for PCR amplification is less than 2 hours. The recommendations of the manufacturer were strictly followed for DNA extraction and Real time PCR.

RESULTS

Total 578 samples from February 2015 to July 2022 were tested in our laboratory. Among them 435 (75.26%) were from male patients and rest 143 (24.74%) were from female. The age range of the patients were from 13-68 years. We categorized the age group as adolescence (13-18) years, young adult (19-40) years, mid age adult (41-60) years and old adult >60 years. According to the age group classification, most patients (65.2%) were from 19-40 years old and 29.4% were from 41-60 years old. The overall positivity rate for STIs pathogen was 13.15% (76/578) among all age groups. Of them, the most detected pathogen was C. *Trachomatis* (41/53.9%), followed by N. Gonorrhoeae (13/17.1%), *M. Genitalium* (12/15.8%), *T. Vaginalis* (5/6.6%), and co-infection of *N. Gonorrhoeae* & C. Trachomatis (5/6.6%) was detected. Detection rate of STIs pathogen in male was 77.6% and female was 22.4%. Common STIs pathogen in male were C. Trachomatis (44.7%) followed by *N. Gonorrhoeae* (14.5%), *M. Genitalium* (9.2%), *T. Vaginalis* (2.6%), and *N. Gonorrhoeae* + *C. Trachomatis* co-infections was (6.6%). The reported causative STIs pathogen in female were *C. Trachomatis* (9.2%) followed by M. Genitalium (6.6%), *T. Vaginalis* (4%), and N. Gonorrhoeae (2.6%). According to pathogen distribution by age, the most infections (72.4%) were found in the age group of 19-40 years and among them predominant pathogens was *C. Trachomatis* (49.1%), while only 1.3% was observed in age group of more than 60 years. There was no pathogen found in the age group of 13-18 years. (Table: 1 and Figure: 1).

Among all positive cases burning sensation during urination/sexual intercourse and urethral / vaginal discharge were the most prevalent syndrome reported by male and female patients. About 81.4% of male and 82.3% of female patients had burning sensation. Urethral discharge in 61% of male patients and vaginal discharge in 64.3% of female patients were observed. (Figure:2)

In our study period, an average of 72 samples for STIs each year were collected. No significant difference was found in overall detection rates. The highest number (22.2%) of pathogens were detected in 2021 and followed by (18.9%) in 2020. Five co-infections were also found in these two years.

There were no co-infections found with bacteria and protozoa. (Table-2).

Different types of samples were collected in this study period. Samples were urine (321/55.5%), prostatic secretion (130/22.5%), cervico-vaginal swab (106/18.3%), urethral swab (16/2.8%), semen (4/0.7%) and throat swab (1/0.2%). Among all samples pathogens were detected 12.1%, 14.6%, 9.4%, 43.7%, 25%, 0% respectively. (Table: 3)

Table 1: Prevalence of STIs pathogens among different age groups

Age (Yrs.)	Total Case	Positive (%)	NG (%)	CT (%)	MG (%)	TV (%)	Co-infection (%) *
13-18	11 (1.9%)	0					
19-40	377 (65.2%)	55 (72.4)	10 (18.2)	27 (49.1)	10 (18.2)	4 (7.3)	4(7.3)
41-60	170 (29.4%)	20 (26.3)	3 (15)	13 (65)	2 (10)	1 (5)	1(5)
>60	20 (3.5%)	1 (1.3)		1 (100)			
Total (%)	578	76 (13.15)	13 (17.1)	41 (53.9)	12 (15.8)	5 (6.6)	5(6.6)

*Co-infection found between NG & CT (5 nos)

Abbreviations: NG- Neisseria gonorrhoeae, CT- Chlamydia trachomatis, MG- Mycoplasma genitalium, TV- Trichomonas vaginalis

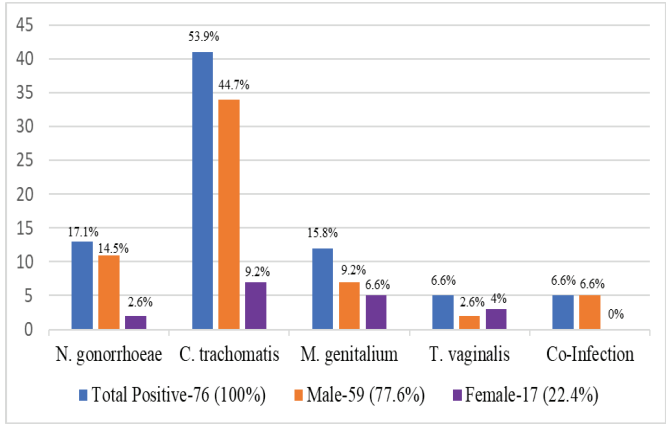

Figure.1: Distribution of STIs pathogens among different sex groups

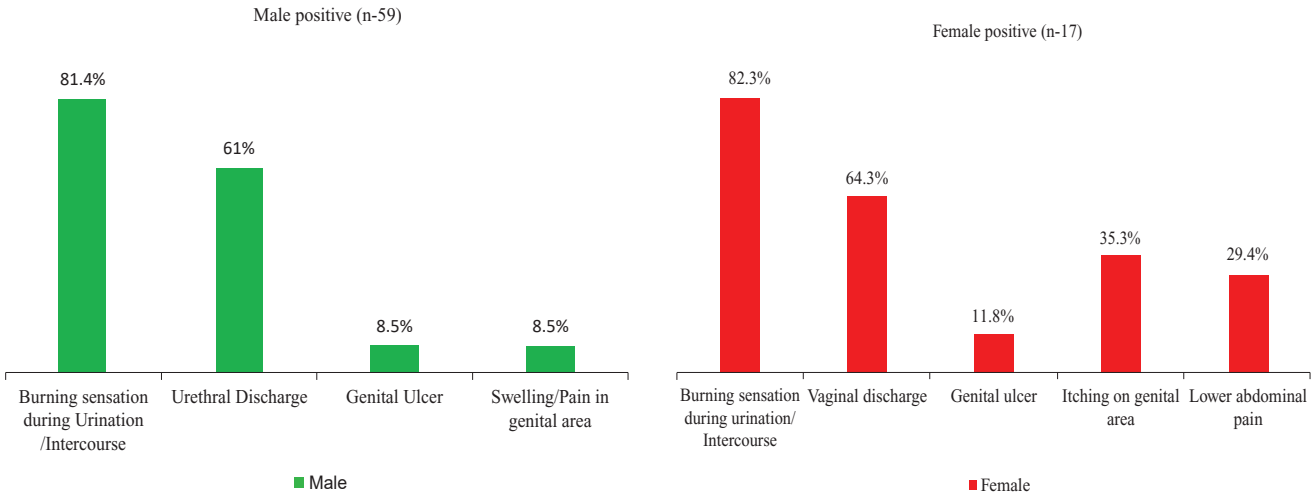

Figure.2: Signs and symptoms of STIs reported by patient.

Table 2: Yearly distribution of tested and positive patients with STIs infection (Feb'15 to July' 22)

Year	No. of samples tested (n= 578)	No. of positive found (n = 76)					Total Positive (%)
		NG	CT	MG	TV	Co-infection	
2015	58	1	2	2	1	0	6(10.3)
2016	58	1	6	1	2	0	10(17.2)
2017	54	1	3	0	0	0	4(7.4)
2018	103	2	6	4	0	0	12(11.6)
2019	96	1	7	2	0	0	10(10.4)
2020	58	1	7	1	0	2	11(18.9)
2021	81	6	6	1	2	3	18(22.2)
2022	70	0	4	1	0	0	5(7.1)

*Average sample per year is 72.25

Table 3: Specimen types included in the study according to sex and pathogen distribution

Specimen types	Male	Female	Total (n=578)	Total positive (n=76)					
				NG	CT	MG	TV	Co-infection	Total Positive (%)
Urine	284	37	321(55.5)	6	24	5	2	2	39(12.1)
Prostatic secretion	130	N/A	130(22.5)	3	11	3	0	2	19(14.6)
Urethral swab	16	0	16(2.8)	3	3	0	0	1	7(43.7)
Cervicovaginal swab	N/A	106	106(18.3)	1	2	4	3	0	10(9.4)
Semen	4	N/A	4(0.7)	0	1	0	0	0	1(25)
Throat swab	1	0	1(0.2)	0	0	0	0	0	0(0)

DISCUSSION

The objective of the present study was to determine the pathogen distribution of STIs in a private tertiary care hospital by using multiplex Real Time PCR method. Multiplex Real Time PCR is a rapid, sensitive, and accurate diagnostic method of STIs which added the new dimension to screen STIs pathogens[17]. At the best of our knowledge this is the first study report in Bangladesh where multiplex PCR method was used for pathogen identification of STIs as a diagnostic tool.

In this study, Neisseria gonorrhoeae (17.1%), Chlamydia trachomatis (53.9%), Mycoplasma genitalium (15.8%), Trichomonas vaginalis (6.6%) were detected in study population. Among positive cases Chlamydia trachomatis (53.9%) was the most common pathogen, and the second most common pathogen was Neisseria gonorrhoeae (17.1%). Our data matched with other studies in Bangladesh where Chlamydia trachomatis ranked at the top and Neisseria gonorrhoeae as a second most prevalent bacterial STIs[5-7]. In 2008, a total of 78.5 million new case of Neisseria gonorrhoeae, Chlamydia trachomatis, treponema pallidum and Trichomonas vaginalis was reported by WHO for the South-East Asia region, 7.2 million were new case of Chlamydia trachomatis and 25.4 million cases were Neisseria gonorrhoeae[18].

Co-infections with Neisseria gonorrhoeae and Chlamydia trachomatis were observed in 6.6% of cases and all were male. In the United States, explorative treatment of Chlamydia trachomatis is recommended simultaneously with gonococcal treatment, if diagnostic testing for Chlamydia trachomatis was not performed[19]. We found five patients co-infected with Neisseria gonorrhoeae and Chlamydia trachomatis in our cohort. Thus, pathogen identification by multiplex PCR allows clinicians to give specific treatment of N. gonorrhoeae and C. trachomatis infection and co-infection.

In 2021, Neisseria gonorrhoeae detection rate was increased. A total of 9 cases of Neisseria gonorrhoeae were found whereas 3 were coinfected with Chlamydia trachomatis. We found 2 (2.6%) male patients with Trichomonas vaginalis infection. In other study more trichomoniasis in men were identified by PCR than by wet mount preparation and culture[20]. Overall total STIs positivity rate was increased in 2020 and 2021 in our cohort By using multiplex PCR pathogens can be detected from different types of samples. The highest number of samples was urine (55.5%) in comparison with other samples. Urine is the most preferable sample for detecting STIs in both male and female patients probably due to easy collection[21]. However, positivity rate from urine samples was 12.1% and from urethral swab was 43.7%, the highest among all kind of specimen.

Among all pathogen positive cases, positivity rate of STIs in male were 77.6% and female were 22.4%. In this study, the overall male patients were 75.26% and female were 24.74%. The male-female ratio shows male predominance, and it is exactly 3:1. It is comparable with another study of Bangladesh where male: female ratio was found 2.59:1[8]. An Indian study of STIs showed male: female ratio is 2:1[22]. Smaller number of female patients could be due to shame, or unwillingness to express their problems regarding STIs. In developing nations, women have limited access to information or health services than men and are normally not conscious about their health problem. Moreover, they are highly busy in maintaining household tasks and with child caring activities[23-25]

CONCLUSION

In our Study, *Chlamydia trachomatis* is the most detected sexually transmitted infection, followed by Neisseria gonorrhoeae. Our finding indicates that STIs is common in male having age group of 19-40 years. By using multiplex PCR method multiple causative organisms associated STIs can be detected simultaneously. In future, Multiplex PCR may be the standard diagnostic test for STIs instead of culture, serology, and other routine diagnostics tests. As this study is based on a single hospital of Bangladesh it may not represents actual scenario of our whole locality. Multicenter based study is required to know the exact pathogen prevalence associated with STIs

ACKNOWLEDGEMENT

We are thankful to Malay Biswas, Mr. Abu Sobhan Murad and Abdus Sobur, Molecular Lab, Evercare Hospital Dhaka for their continuous support.

REFERENCE

1. https://medlineplus.gov/sexuallytransmitteddiseases.html
2. https://www.mayoclinic.org/diseasesconditions/sexually-transmitted-diseases-stds/ symptoms-causes.
3. https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections.
4. Centers for Disease Control and Prevention (2017) Sexually transmitted disease surveillance 2017. Available:https://www.cdc.gov/std/stats17/default.htm. Accessed: 10 April 2019.

5. Rahman M, Alam A, Nessa K, Hossain A, Nahar S, Datta D, et al. Etiology of sexually transmitted infections among street based female sex workers in Dhaka, Bangladesh. J Clin Microbiol 2000; 38(3): 1244-1246.
6. Nessa K, Waris SA, Sultan Z, Monira S, Hossain M, Nahar S, et al. Epidemiology and etiology of sexually transmitted infection among hotel-based sex workers in Dhaka, Bangladesh. J Clin Microbiol 2000; 42(2): 618-621.
7. Shirin T, Rahman S, Rabbi FJ, Kabir MH, Mamun K. Prevalence of Sexually Transmitted Diseases and Transmission of HIV in Dhaka, Bangladesh. Bangladesh J Med Microbiol. 2009;3(1):27-33. doi:10.3329/bjmm.v3i1.2968
8. Rafiqul Mowla M, Ara S, Rahman M. Current trends in sexually transmitted infections-Bangladesh experience Forum Dermatologicum. 2016;4(2):144-149.
9. Sachdev D, Patel AL, Sonkar SC, Kumari I, Saluja D (2015) Diagnosis of Neisseria gonorrhoeae using molecular beacon. Biomed Res Int 2015: 597432.
10. Clad A, Freidank HM, Kunze M, Schnoeckel U, Hofmeier S, Flecken U, Petersen EE (2000) Detection of seroconversion and persistence of Chlamydia trachomatis antibodies in five different serological tests. Eur J Clin Microbiol Infect Dis 19:932–937.
11. Otero-Guerra L, Fernández-Blázquez A, Vazquez F. Rapid diagnosis of sexually transmitted infections. Enfermedades Infecc y Microbiol Clin (English ed). 2017;35(7):444-450. doi:10.1016/j.eimce.2017.01.035
12. Cosentino LA, Landers DV, Hillier SL (2003) Detection of Chlamydia trachomatis and Neisseria gonorrhoeae by strand displacement amplification and relevance of the amplification control for use with vaginal swab specimens. J Clin Microbiol 41:3592–3596.
13. Chan EL, Brandt K, Stoneham H, Antonishyn N, Horsman GB (2000) Comparison of the effectiveness of polymerase chain reaction and enzyme immunoassay in detecting Chlamydia trachomatis in different female genitourinary specimens. Arch Pathol Lab Med 124:840–843.
14. Ryu JS, Chung HL, Min DY, Cho YH, Ro YS, Kim SR (1999) Diagnosis of trichomoniasis by polymerase chain reaction. Yonsei Med J 40:56–60.
15. Geraats-Peters CW, Brouwers M, Schneeberger PM, van der Zanden AG, Bruisten SM, Weers-Pothoff G, Boel CH, van den Brule AJ, Harmsen HG, Hermans MH (2005) Specific and sensitive detection of Neisseria gonorrhoeae in clinical specimens by real-time PCR. J Clin Microbiol 43:5653–5659.
16. Diaz N, Dessi D, Dessole S, Fiori PL, Rappelli P (2010) Rapid detection of coinfections by Trichomonas vaginalis, Mycoplasma hominis, and Ureaplasma urealyticum by a new multiplex polymerase chain reaction. Diagn Microbiol Infect Dis 67:30–36.
17. Markoulatos P, Siafakas N, Moncany M (2002) Multiplex polymerase chain reaction: a practical approach. J Clin Lab Anal 16:47–51.
18. Global incidence and prevalence of selected curable sexually transmitted infections – 2008 [Internet]. Geneva: World Health Organization; 2012 [cited 2014 Nov 21]. Available from: http://apps.who.int/iris/bitstream/10665/75181/1/9789241503839_eng. pdf?ua=1
19. Cyr SS, Barbee L, Workowski KA, Bachmann LH, Pham C, Schlanger K. CDC STI Gonorrhea Treatment Guidelines. Morb Mortal Wkly Rep Updat. 2020;69(50):1911-1916.
20. Hobbs MM, Lapple DM, Lawing LF, Schwebke JR, Cohen MS, Swygard H, Atashili J, Leone PA, Miller WC, Seña AC. Methods for detection of Trichomonas vaginalis in the male partners of infected women: implications for control of trichomoniasis. J Clin Microbiol. 2006 Nov;44(11):3994-9. doi: 10.1128/JCM.00952-06. Epub 2006 Sep 13. PMID: 16971646; PMCID: PMC1698299.
21. Grad AI, Vica ML, Matei HV, Grad DL, Coman I, Tataru DA. Polymerase Chain Reaction as a Diagnostic Tool for Six Sexually Transmitted Infections - Preliminary Results. Clujul Med. 2015;88(1):33-7. doi: 10.15386/cjmed-373. Epub 2015 Jan 28. PMID: 26528045; PMCID: PMC4508611.
22. Thappa D.M., Devi S.A., Vetrichevvel T.P., Pise G.A. Pattern of Sexually Trans mitted Infections in a tertiary Care Centre at Puducherry. Indian J. Dermatol. 2009; 54 (4): 347–349.
23. Sirirassame B., Sethaput C., Yoddumnern-Attig B. Gender perspectives in Reproductive health Practices in Thailand: A synthesis of research findings. Nakhonpathom, Thailand: Institute for population and social research, Mahidul University, 2003.
24. Dixon-Muller R. Gender inequalities and reproductive health: Changing priorities in an era of social transformation and globalization Policy. Popul. Res. Pa. 1999; 16: 1–29.
25. Voeten H., O'Hara H.B., Kusimba J. et al. Gender differences in health care seeking behavior for sexually transmitted diseases. Sex Transm. Dis. 2004; 31: 265–272.