Original Article
https://doi.org/10.24079/CAJMS.2017.06.007 Cent Asian J Med Sci. 2017 June;3(2): 148-55.
Central Asian Journal of Medical Sciences
CAJMS

Detection of Common Bacterial Causes of STIs among STD Clinic Attendees by Gram Stain and Culture versus the Microarray Nucleic Acid Hybridization Method

Jugderjav Badrakh[1], Bulbul Aumakhan[1], Baatarkhuu Oidov[2], Unursaikhan Ganbat[3], Erdenechimeg Choijiljav[1], Naranbat Nymadawa[3], Nymadawa Pagvajav[3, 4]

[1]National Center for Communicable Diseases, Ulaanbaatar, Mongolia; [2]Department of Infectious Diseases, School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; [3]"Gyals" Medical Center, LLC, Ulaanbaatar, Mongolia; [4]Mongolian Academy of Medical Sciences, Ulaanbaatar, Mongolia

Submitted: March 15, 2017
Revised: April 15, 2017
Accepted: May 31, 2017

Corresponding Author
Jugderjav Badrakh, MSc
National Center for Communicable Diseases, Ulaanbaatar 13335, Mongolia
Tel: +976-9914-3069
Fax: +976-11-458787
E-mail: b_juugee@yahoo.com

Objectives: The purpose of the study was to determine the prevalence and etiology of predominant sexually transmitted infections (STIs) occurring among the STD and Gynecology Clinic attendees and to compare sensitivity and specificity of conventional versus molecular diagnostic methods. **Methods:** A total of 1,441 individuals were recruited from the STD clinic at the National Center for Communicable Diseases and Gynecology Clinic at the National Center for Oncology between 2014 and 2015. Urogenital tract swab samples were collected and tested for N.gonorrhoeae, C.trachomatis, and T.vaginalis using conventional (Gram stain and culture) and molecular (Microarray Nucleic Acid Hybridization - MNAHM) diagnostic methods. The final infection status of participants was determined using the "either test positive" strategy. **Results:** Of the total 1,441 participants, 19.7% were N.gonorrhoeae infected, 12.5% were C.trachomatis infected, and 7.8% were T.vaginalis infected. For T. vaginalis, conventional methods and MNAHM had sensitivities of 17.9% and 98.2%, with specificities of 96.6% and 99.6%, respectively. For N.gonorrhoeae, conventional methods had sensitivity and specificity values of 33.3% and 99.1%, and MNAHM had values of 95.1% and 98.8%, respectively. **Conclusion:** The study found a high prevalence of N.gonorrhoeae and C.trachomatis among STD and Gynecology Clinic attendees. Conventional methods for detection of N.gonorrhoeae and T.vaginalis were significantly less sensitive than molecular diagnostic methods and didn't detect other STI pathogens.

Keywords: Sexually Transmitted Diseases, Nucleic Acid Hybridization, Mongolia

Introduction

Sexually transmitted infections (STIs) are a major public health concern worldwide, especially in developing countries, due to limited resources and facilities for diagnosis and treatment [1]. According to the World Health Organization (WHO), an

estimated 106.1 million people around the world each year are newly infected with N. gonorrhoeae, 105.7 million with C. trachomatis, and 276.4 million with T. vaginalis [2].

STIs often have no or mild symptoms and therefore, can go unrecognized and subsequently, untreated [3-5]. Unrecognized and untreated STIs can lead to serious adverse effects on an individual's sexual and reproductive health. For example, most women infected with chlamydia due to C. trachomatis, an obligate intracellular pathogen, will have no symptoms and infection may clear spontaneously, but persistent infection can spread to their upper genital tract and cause long-term complications such as pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy, and infertility [6]. Gonorrhea is an infection due to N. gonorrhoeae and usually involves the mucosa of the cervix, urethra, endocervix, fallopian tubes, rectum, and throat [7,8]. Signs and symptoms of gonorrhea are generally not different from genital chlamydial infection and are clinically indistinguishable. Trichomoniasis, caused by T. vaginalis, is considered the most prevalent, curable STI, and its occurrence exceeds that of chlamydial and gonococcal infections. Many carriers of T. vaginalis are asymptomatic and, when experiencing a health problem, they often have nonspecific symptoms [9]. However, this parasite has been identified to increase risk of HIV transmission and has also been associated with prostate and cervical cancer. Adverse health consequences due to STIs result in significant costs to the public health system of most countries, although the true burden and impact of unrecognized STIs are largely unknown [10].

In Mongolia, STIs have been consistently increasing over the past 20 years and comprise a substantial proportion of all infectious diseases reported in the country. The rising trend is expected to continue in the near future [11]. Relatively few studies on etiological cases of STIs have been performed in Mongolia. A 2008 study of pregnant women, who are generally considered the healthiest segment of the population, found surprisingly high rates of STIs, including chlamydia in 14.9%, trichomoniasis in 9.9%, and gonorrhea in 3.9% [12]. Among the 257 high-risk women participating as participants in a 2009 bio-behavioral epidemiological survey of HIV and STI distribution, chlamydia was found in 24.5%, gonorrhea in 15.6%, and trichomoniasis in 14.8% [13].

Only four STIs (syphilis, gonorrhea, trichomoniasis, and HIV/AIDS) are reported in Mongolia, which falls far behind best international practices. All reported cases of gonorrhea and trichomoniasis infections have been diagnosed using only conventional methods. STIs, such as urogenital chlamydia, genital herpes, warts, genital ureaplasma, and mycoplasma, are not systematically diagnosed due to insufficient and inconsistent inventory of laboratory detection kits and supplies. Therefore, the reported rates of these STIs significantly underestimates true rates, as shown in most data from specifically targeted studies.

New nucleic acid based molecular diagnostic methods have been shown to have high sensitivity and specificity for simultaneous detection of a broad range of STI agents [14, 15]. Public STI clinics in Mongolia mainly use conventional methods, such as Gram stain and culture, for the detection of N.gonorrhoeae,C.trachomatis, and T.vaginalis, and thus, there is a great need to introduce new molecular diagnostic methods to increase the detection of and the detection range of STIs. There have been no studies comparing the use of nucleic acid based diagnostic methods to the use of conventional diagnostic methods to diagnose the prevalence of STIs among high-risk individuals attending public sexually transmitted disease (STD) clinics in Mongolia. Therefore, the aim of this study was to determine the prevalence and etiology of common STIs occurring among the STD and Gynecology Clinic attendees and to compare the sensitivity and specificity of conventional versus molecular diagnostic methods.

Materials and Methods

The study was a collaborative effort between the National Center for Communicable Diseases (NCCD), National Center for Oncology (NCO), and the Medical Center "Gyals" LLC. The study used a hospital-based cross-sectional design to enroll an approximately equal number of men and women with and without symptoms of urogenital tract infection attending the STD clinic at the Department of HIV/AIDS and STI Surveillance and Research, NCCD, and women attending the Gynecology Clinic at the NCO between 2014 and 2015. Participants were classified into five study groups: men (n=299) and women (n=281) from STD clinic at NCCD with signs and symptoms of STIs; men (n=315) and women (n=301) from STD clinic at NCCD with no overt signs and symptoms of STIs; and women (n=245) attending Gynecology Clinic at NCO. Urethral or cervical swab specimens were collected from a total of 1,441 participants

and tested for the three most commonly occurring STIs (N. gonorrhoeae, T. vaginalis, and C. trachomatis) using conventional and molecular diagnostic methods.

1. Detection of STIs using conventional methods

For the conventional method, Gram stain and microscopy were used to identify intracellular Gram-negative diplococci. The presence of N. gonorrhoeae was confirmed using selective culture media (Thayer-Martin, Hi Media Laboratories Pvt. Ltd, India). T. vaginalis was detected via broth-based culture (Hi Media Laboratories Pvt. Ltd, India). These laboratory tests were performed by the STD Laboratory at NCCD.

2. Detection of STIs using Microarray Nucleic Acid Hybridization Method (MNAHM)

For the molecular method, GenoFlowArrayTestKit [FT-PRO] was used to perform the Microarray Nucleic Acid Hybridization Method (MNAHM) (DiagCor Bioscience Inc. Ltd, Hong Kong). MNAHM relies on PCR amplification and "flow-through" hybridization technology. The genomic DNA of target pathogen is amplified using PCR. The amplicons are subsequently hybridized to pathogen-specific capturing probes via "flow-through" hybridization per the manufacturer's instruction (DiagCor Bioscience Incorporation Limited, 2013).

3. Determination of final disease status

No one test was considered the gold standard. To determine the final infection status, we used the "either test positive strategy"—positive result from at least one of two methods, conventional gram stain and culture or the MNAHM—along with the presence of clinical signs and symptoms of an urogenital tract inflammation.

4. Statistical analysis

We performed descriptive statistical analysis, including the calculation of frequencies and proportions of study groups, for categorical variables. We compared proportions between groups using chi-square tests with confidence intervals (95% CI), based on a binomial distribution. We assessed agreement between conventional and molecular methods using Cohen's Kappa coefficient. We constructed conventional two-by-two (2x2) tables with columns indicating the condition of the subjects (diseased or non-diseased) and rows indicating positive or negative test results to calculate indicators of sensitivity, specificity, and positive and negative predictive values. All analyses were performed using the STATA statistical software (STATA/IC Version 12.1, Stata Corporation, College Station, Texas, USA), with statistical significance at $p<0.05$.

5. Ethical statement

Ethical approval was obtained from the Ethical Committee of the Ministry of Health. Each patient signed a consent form to agree to participate in the study.

Results

For the presentation of results, participants were classified into five groups: asymptomatic and symptomatic males (groups I, II), asymptomatic and symptomatic females (groups III, IV), and women with suspected cervical cancer (SCC) (group V).

1. Socio-demographic characteristics

The mean age of participants differed significantly by study groups (Table 1). Among the participants, symptomatic men (Group II) were the youngest (mean age=27), while women with SCC

Table 1. Age distribution of study participants

Age/age group	I. Asymptomatic males n=315	II. Symptomatic males n=299	III. Asymptomatic females n=301	IV. Symptomatic females n=281	V. Females with SCC n=245
Mean age (range)	31 (16-58)	27 (16-67)	35 (16-59)	32 (18-60)	38 (18-58)
15-24	67(21.3%)	115(38.5%)	46(15.3%)	58(20.6%)	17(6.9%)
25-34	159(50.5%)	141(47.2%)	90(29.9%)	124(44.1%)	58(23.7%)
35-44	60(19.0%)	34(11.4%)	116(38.5%)	80(28.5%)	108(44.1%)
45-54	25(7.9%)	7(2.3%)	45(15.0%)	15(5.3%)	53(21.6%)
> 55	4(1.3%)	2(0.7%)	4(1.3%)	4(1.4%)	9(3.7%)

(Group V) were the oldest (mean age=38). Asymptomatic men and women (Group I and III) were older than their symptomatic counterparts (Group II and IV) by an average of 3 to 4 years (Table 1). Nearly half of the male participants (48.9%) and about a third of the female participants (37%) were between 25 to 34 years old. More than half of the women were older than 35 years old. Approximately 40% of asymptomatic women (Group III) and of women with SCC (Group V) were between 35 and 44 years old.

We asked the participants attending the STD clinic at the NCCD about their level of education and employment status. Over half (59.4%, 711/1196) of the participants had college and/or university degrees, 17.4% (209/1196) had completed secondary school, 22.7% (272/1196) had some secondary education, and 0.3% (4/1196) had only primary education or no education at all. With regards to employment status, 65.8% (787/1196) were employed, 27.2% (326/1196) unemployed, and 6.9% (83/1196) were students.

2. Results of testing for N. gonorrhoeae, T. vaginalis, and C. trachomatis

Overall, N.gonorrhoeae was detected in 19.7% (284/1441), C.trachomatis in 12.5% (180/1441), and T.vaginalis in 7.8% (112/1441) of all participants. Significant differences in the rates of detection were observed by sex and the presence of signs and symptoms of STIs.

Specifically, gonorrhea was present in 51.2% (153/299) of symptomatic men and 14.6% (41/281) of symptomatic women. More than 8% of asymptomatic men (26/315) and about 10.0% (30/301) of asymptomatic women tested positive for gonorrhea (Table 2). N.gonorrhoeae was the most prevalent pathogen among both symptomatic 153(51.2%) and asymptomatic 26(8.3%) men compared with other pathogens (p<0.001) (Table 3). Among women with SCC, N.gonorrhoeae was found in 13.9% (34/245).

C.trachomatis was most frequently found among symptomatic men (18.4%) followed by asymptomatic men (12.7%), symptomatic (12.1%), and asymptomatic women (11.0%). The lowest prevalence was observed among women with SCC (7.3%).

The high prevalence of C.trachomatis observed among asymptomatic men and women indicates the mostly vague or silent course of chlamydia infection. Additionally, N. Gonorrhoeae was detected in over half of symptomatic men confirming the predominantly symptomatic nature of gonorrheal infection among men.

No statistically significant differences were observed in gonorrhea and chlamydia rates between symptomatic and asymptomatic women except for trichomoniasis (Table 4).

3. Occurrence of multiple infections

The occurrence of co-infections among the participants was not common. Less than 10% of the participants in each group were positive for more than one of the three tested STIs

Table 2. Results of STI testing by MNAHM by study groups

	I. Asymptomatic males n=315	II. Symptomatic males n=299	III. Asymptomatic females n=301	IV. Symptomatic females n=281	V. Females with SCC n=245	p-value
T.vaginalis	15(4.8%)	22(7.4%)	24(8.0%)	37(13.2%)	14(5.7%)	0.002*
N.gonorrhoeae	26(8.3%)	153(51.2%)	30(10.0%)	41(14.6%)	34(13.9%)	<0.001*
C.trachomatis	40(12.7%)	55(18.4%)	33(11.0%)	34(12.1%)	18(7.3%)	0.003*

*p-value < 0.05 significant

Table 3. Results of STI testing by MNAHM among men with and without symptoms

	I. Asymptomatic males n=315	II. Symptomatic males n=299	p-value
T.vaginalis	15(4.8%)	22(7.4%)	0.177
N.gonorrhoeae	26(8.3%)	153(51.2%)	<0.001*
C.trachomatis	40(12.7%)	55(18.4%)	0.051

*p-value < 0.05 significant

Table 4. Results of STI testing by MNAHM among women with and without symptoms

	Symptomatic females n=281	Asymptomatic females n=301	p-value
T.vaginalis	37(13.2%)	24(8.0%)	0.041*
N.gonorrhoeae	41(14.6%)	30(10.0%)	0.089
C.trachomatis	34(12.1%)	33(11.0%)	0.668

*p-value < 0.05 significant

(Table 5). Symptomatic men (Group II) were twice as likely than symptomatic women (Group IV) to be co-infected with gonorrhea and chlamydia (7.7% vs. 3.6%). Among symptomatic women (Group IV), it was equally likely for chlamydia to occur with gonorrhea (3.6%) and trichomoniasis (3.6%). However, asymptomatic women (Group III) were more likely to be co-infected with gonorrhea and trichomoniasis. Less than 1% of participants in each group tested positive for all three infections.

4. Comparison of N.gonorrhoeae and T.vaginalis results

Among both asymptomatic men and women (Group I and III), conventional methods did not detect any intracellular diplococci typical of N.gonorrhoeae. Intracellular diplococci were found on the Gram stain in 24.1% (72/299) of symptomatic men, but 32.1% of symptomatic men (Group II) (96/299) tested positive for gonorrhea by culture. Only 1.4 % of symptomatic women (Group IV) (4/281, $p<0.05$) tested positive for gonorrhea using conventional methods (Table 6).

Among asymptomatic men and women (Group I and III), 0.9% men (3/315) and 6.3% women (19/301) tested positive for trichomoniasis by culture. Among symptomatic women

Table 5. Frequency of co-infections by study group

Co-infections	I. Asymptomatic males n=315	II. Symptomatic males n=299	III. Asymptomatic females n=301	IV. Symptomatic females n=281
NG+CT	5 (1.6%)	23 (7.7%)	3 (1%)	10 (3.6%)
NG+TV	2 (0.6%)	8 (2.7%)	10 (3.2%)	5 (1.8%)
CT+TV	5 (1.6%)	3 (1%)	5 (1.6%)	10 (3.6%)
All three	1 (0.3%)	0	2 (0.7%)	1 (0.4%)

Note: Co-infection type: NG+CT- N.gonorrhoeae and C.trachomatis; NG+TV-N.gonorrhoeae and T.vaginalis;CT+TV-C.trachomatis and T. vaginalis

Table 6. Results of N.gonorrhoeae and T.vaginalis detection using conventional (Gram stain and culture) and molecular (MNAHM) methods

Groups	N.gonorrhoeae		T.vaginalis	
	Conventional	MNAHM	Conventional	MNAHM
I. Asymptomatic men (n=315)	0	26(8.3%)	3(1.0%)	15(4.8%)
II. Symptomatic men (n=299)	96(32.1%)	153(51.2%)	21(7.0%)	21(7.4%)
III. Asymptomatic women (n=301)	0	30(10.0%)	19(6.3%)	24(8.0%)
IV. Symptomatic women (n=281)	4(1.4%)	41(14.3%)	20(7.1%)	37(13.2%)
V. Women with SCC (n=245)	1(0.4%)	34(13.9%)	1(0.4%)	14(5.7%)
Total: n=1441	101(7.0%)	284(19.7%)	64(4.4%)	112(7.8%)
Positive results agreement	90		19	
Discrepant results	205		138	
Kappa (95% CI)	0.41(0.34-0.47)		0.17(0.09-0.21)	

For N.gonorrhoeae, conventional methods had 33.3% sensitivity and 99.1% specificity, while MNAHM had 95.1% sensitivity and 98.8% specificity (Table 7). For T.vaginalis, conventional methods had low sensitivity (17.9%), while MNAHM had high sensitivity (98.2%), however, the specificities of both methods were over 95%.

Table 7. Sensitivity and specificity of conventional and MNAHM test results for N.gonorrhoeae and T.vaginalis

	N.gonorrhoeae		T.vaginalis	
	Conventional	**MNAHM**	**Conventional**	**MNAHM**
True positive (TP)	90	270	20	110
True negative (TN)	1160	1143	1284	1324
False positive (FP)	11	14	45	5
False negative (FN)	180	14	92	2
Sensitivity	33.3%	95.1%	17.9%	98.2%
95% CI	(28.0-39.2)	(91.9-97.0)	(11.9-26.0)	(93.7-99.5)
Specificity	99.1%	98.8%	96.6%	99.6%
95% CI	(98.3-99.5)	(98.0-99.3)	(95.5-97.5)	(99.1-99.8)
Positive predictive value (PPV)	89.1%	95.1%	30.8%	95.7%
95% CI	(81.5-93.8)	(91.9-97.0)	(20.9-42.8)	(90.2-98.1)
Negative predictive value (NPV)	86.6%	98.8%	93.3%	99.8%
95% CI	(84.6-88.3)	(98.0-99.3)	(91.9-94.5)	(99.5-99.9)

(Group IV), 7.1% (20/281) tested positive for trichomoniasis by culture. Conventional methods had poor agreement with MNAHM for T. vaginalis (Kappa=0.17) and moderate agreement for N. gonorrhoeae (Kappa=0.41) (Table 6).

Discussion

We have compared our study findings with the results of domestic and international investigations. A study of symptomatic women attending sexual health clinics in Scotland found 0.3% prevalence of N.gonorrhoeae, 2.8% of T.vaginalis, and 7.3% of C.trachomatis using nucleic acid based tests[16]. Our study found much higher rates among symptomatic women in Mongolia compared to symptomatic women in Scotland. Specifically, we found 14.6% of N.gonorrhoeae, 13.2% of T.vaginalis, and 12.1% of C.trachomatis. Our study findings were similar to the results reported in a study of women attending STD clinics in the USA, with the exception of N.gonorrhoeae, which was four times greater in our study [17].

Among domestic investigations, a study by B. Khandsuren et al. found the prevalence of C.trachomatis at 39.3% among individuals with signs of urogenital tract inflammation, while a study by Erdenechimeg.Ch reported a 34.1% prevalence rate [18, 19]. These rates are nearly 2 to 3-fold higher than the C. trachomatis prevalence rate of 12.5% found in our study. High rates of STIs were also found among the 257 high-risk women who participated in a 2009 bio-behavioral epidemiological survey of HIV and STI distribution, with chlamydia in 24.5%, gonorrhea in 15.6% and trichomonas in 14.8% of participants [13]. A 2008 epidemiological study among 2,000 pregnant women in antenatal care found relatively high rates of STIs as well, with chlamydia in 14.9%, trichomonas in 9.9%, gonorrhea in 3.9%, and syphilis in 3.5% of participants [12]. In our study, culture was positive for T.vaginalisin 7.1% of symptomatic women, which was nearly four times lower than the 28.4% found in a 2013 study by Giimaa N. among 109 women attending the "Red Ribbon" clinic at NCCD [20]. These domestic studies have demonstrated a high prevalence of STIs among different population groups in Mongolia.

Evangelia-Theophano Piperaki et al. studied women attending a gynecology clinic in Greece (2006) and found that trichomonas infections were more prevalent in symptomatic women (6.7%) than in asymptomatic women (2.4%). This study found that PCR was the most sensitive method (100%) while the culture had 69.6% sensitivity, and there was strong agreement among PCR, culture, and wet mount methods (κ=0.79) [21]. A 1998 study by Madico demonstrated the high sensitivity of PCR, compared to culture or wet mount methods, for the detection of T. vaginalisamong STD clinic attendees. Positive T. vaginalis results were 39% higher with PCR compared to culture [22].

Our study observed a large discrepancy in gonorrhea rates found using conventional methods between symptomatic men (32.1%) and asymptomatic women (1.4%). These results were consistent with the findings of a study from India by Bhargava A., which found greater positive results in men with urethral discharge versus in women with vaginal or cervical discharge using microscopy and culture (65.8% vs 0.5%, $p < 0.0001$). Thus,

conventional diagnostic methods may not be effective for the diagnosis of vaginal and cervical discharge syndrome [23].

Nucleic acid based diagnostic methods have been shown to have high sensitivity and specificity in multiple studies. In a study of 185 men with urethritis by Jahan F et al. N.gonorrhoeae was diagnosed in 51 men by microscopy (27.6%), 49 men by culture (26.5%), and 56 men by PCR (30.3%), and thus, they concluded that PCR has a higher sensitivity compared to conventional methods ($p<0.001$) [24]. Peuchant O et al. compared three commercial real-time PCR assays, the Abbott RealTime CT/NG, the Cobas® 4800 CT/NG, and the Cepheid Xpert® CT/NG, for the detection of C.trachomatis and N.gonorrhoeae in vaginal swabs collected prospectively from pregnant women. The sensitivity of those assays ranged from 98.9% to 99.5% with a Kappa score of 0.94 to 1.0 [25]. A study by Skulskal. E et al. also found high sensitivity of PCR, with the percent agreement between PCR and culture for gonorrhea at 96.8% for males and 85% for females [26]. Stefanski et al. reported a 75.7% sensitivity and 43.3% specificity of the smear test to detect gonorrhea and thus, concluded that the smear test should not be used solely [27]. The results of our study strongly support these findings.

In other studies, the differences in sensitivity between conventional and molecular methods were related to sex. Bartelsman M et al. reported the sensitivity of smear tests at 95.4% among symptomatic males but at 23.1% for symptomatic females, thus, the authors concluded and recommended the smear test only for high-risk males and those with visible signs and symptoms of an urogenital tract inflammation [28].

In our study, conventional methods had very low sensitivity levels for T.vaginalis and N. gonorrhoeae (17.9% and 33.3%, respectively) compared with the 95%+ sensitivity levels for MNAHM, which resulted in low and moderate levels of agreement between conventional methods and MNAHM. One of the reasons for such low sensitivity could be related to the quality and/or the validity of the laboratory reagents and supplies used. Laboratory kits and supplies used at NCCD were procured through tender bids by various companies, and the quality was unknown. The vast discrepancy could also be related to the quality and validity of the MNAHM. Therefore, further quality assurance and validity studies of laboratory reagents and supplies used in laboratories in Mongolia are needed.

The main limitation of our study was the lack of local data for comparison, which could have explained with more certainty the wide discrepancy observed between conventional and molecular diagnostic methods. Our study only compared the results of the tests from the STD clinic, excluding the results from the Gynecological Clinic, and thus, further comparison studies among broader, more general population groups are needed to confirm and validate our study findings.

In summary, our study clearly demonstrated that conventional methods should not be used alone and highly sensitive nucleic acid based molecular diagnostic methods need to be urgently introduced into Mongolian public hospitals to improve the detection and management of primary pathogens of urogenital inflammation such as N.gonorrhoeae, C.trachomatis, and T.vaginalis. Our study supports the use of highly sensitive diagnostic methods to control the persistently high STI epidemic in Mongolia and to prevent complications, adverse reproductive consequences, and ensuing high cost to the health system of the country.

Conflict of Interest

The authors state no conflict of interest.

Acknowledgements

This study is based on the work supported by Mongolian Foundation for Science and Technology. Any findings, conclusions, and recommendations expressed in this study are those of the authors and do not necessarily reflect the views of Mongolian Foundation for Science and Technology of Mongolia.

References

1. Detels R, Green AM, Klausner JD, Katzenstein D, Gaydos C, Handsfield H, et al. The Incidence and correlates of symptomatic and asymptomatic Chlamydia trachomatis and Neisseria gonorrhoeae infections in selected populations in five countries. Sex Transm Dis 2011; 38: 503–509.
2. WHO. Sexually transmitted infections (STIs) [accessed on August 2016]. Available at: http://www.who.int/mediacentre/factsheets/fs110/en/.
3. CDC. Sexually Transmitted Diseases Treatment Guidelines, 2015 [accessed on June 5, 2015]. Available at: https://www.cdc.gov/mmwr/pdf/rr/rr6403.pdf.
4. Narantsetseg V, Nyamtsengel V, Battogtokh Ch. Guideline for STIs. Ulaanbaatar, Mongolia: Nom Khur Press; 2011. p 25.
5. Hamdad-Daoudi F, Petit J, Eb F. Assessment of Chlamydia

trachomatis infection in asymptomatic male partners of infertile couples. J Med Microbiol 2004; 53: 985-990.

6. 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 (Yazd) 2017; 15: 75-82.
7. de Lima YA, Turchi MD, Fonseca ZC, Garcia FL, de Brito e Cardoso FA, da Guarda Reis MN, et al. Sexually transmitted bacterial infections among young women in Central Western Brazil. Int J Infect Dis 2014; 25: 16–21.
8. Johnson RE, Newhall WJ, Papp JR, Knapp JS, Black CM, Gift TL, et al. Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections-2002. MMWR Recomm Rep 2002; 51: 1–38.
9. Glehn MP, Sá LC, Silva HD, Machado ER. Prevalence of Trichomonas vaginalis in women of reproductive age at a family health clinic. J Infect Dev Ctries 2017; 11: 269-276.
10. WHO. Guideline for the treatment management of sexually transmitted infections [accessed on 2003] Available at: http://applications.emro.who.int/aiecf/web79.pdf.
11. Center for Health Development. Health Indicators 2015 [accessed on 2016]. Available at: http://www.chd.mohs.mn/images/pdf/english%20indicator-2015.pdf.
12. MOH, UNAIDS. STI Surveillance Report of Pregnant Women, Ulaanbaatar, Mongolia: 2008. p 32.
13. MOH, Global Fund Supported Project on AIDS and TB, Second Generation HIV/STI Surveillance Report, Ulaanbaatar, Mongolia: 2009. p 34.
14. Witkin SS. Immunological aspects of genital chlamydia infections. Best Pract Res Clin Obstet Gynaecol 2002; 16: 865-874.
15. Sellami H, Znazen A, Sellami A, Mnif H, Louati N, Ben Zarrouk S, et al. Molecular detection of Chlamydia trachomatis and other sexually transmitted bacteria in semen of male partners of infertile couples in Tunisia: the effect on semen parameters and spermatozoa apoptosis markers. PLoS One 2014. https://doi.org/10.1371/journal.pone.0098903.
16. Shone J, Winter A, Jones BL, Butt A, Brawley D, Cunningham C, et al. A Scottish multi-centre service evaluation examining the prevalence and diagnosis of Trichomonas vaginalis in symptomatic women attending sexual health clinics. Int J STD AIDS 2016; 27: 1066-1070.
17. Alcaide ML, Feaster DJ, Duan R, Cohen S, Diaz C, Castro JG, et al. The incidence of Trichomonas vaginalis infection in women attending nine sexually transmitted diseases clinics in the USA. Sex Transm Infect 2016; 92: 58-62.
18. Khandsuren B. Diagnosis of urogenital Chlamydial infection [dissertation]. Ulaanbaatar, Mongolia: Mongolian National University of Medical Sciences; 2001.
19. Erdenechimeg Ch, Jugderjav B. Relative result of clinical and laboratory diagnosis some indicators of chlamydial infection. Mong J Infect Dis Res 2008; 5: 10-12.
20. Giimaa N, Burnee M, Zanabazar E, Oyungerel D, Saruul E, Nyamaa G, et al. The Study of Diagnostic Methods and Sequencing for Trichomoniasis in Mongolia: Cent Asian J Med Sci 2015; 1: 41-48.
21. Piperaki ET, Theodora M, Mendris M, Barbitsa L, Pitiriga V, Antsaklis A, et al. Prevalence of Trichomonas vaginalis infection in women attending a major gynecological hospital in Greece: a cross-sectional study. J Clin Pathol 2010; 63: 249-253.
22. Madico G, Quinn TC, Rompalo A, McKee KT Jr, Gaydos CA. Diagnosis of Trichomonas vaginalis infection by PCR using vaginal swab samples. J Clin Microbiol 1998; 36: 3205–3210.
23. Bhargava A, Bala M, Singh V, Joshi NC, Kakran M, Puri P, et al. How Reliable Is Microscopy and Culture for the Diagnosis of Gonorrhea? An 11-Year Experience from INDIA. Sex Transm Dis 2017; 44: 111–113.
24. Jahan F, Shamsuzzaman SM, Akter S. Diagnosis of common bacterial causes of urethritis in men by Gram stain, culture and multiplex PCR. Malays J Pathol 2014; 36:175-180.
25. Peuchant O, de Diego S, Le Roy C, Frantz-Blancpain S, Hocké C, Bébéar C, et al. Comparison of three real-time PCR assays for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in young pregnant women. Diagn Microbiol Infect Dis 2015; 83: 335-337.
26. Skulskal E, Mlynarczyk-Bonikowska B, Walter de Walthoffen S, Mlynarczyk G, Malejczyk M, Majewski S. The Comparison of Real-Time PCR and bacterial culture in laboratory diagnostics of gonorrhea in patients of Department of Dermatology and the Venereology Medical University of Warsaw. Med Dosw Mikrobiol 2015; 67: 29-38.
27. Stepanski P, Hafner JW, Riley SL, Sunga KL, Schaefer TJ. Diagnostic utility of the genital Gram stain in ED patients. Am J Emerg Med 2010; 28:13-18.
28. Bartelsman M, van Rooijen MS, Alba S, Vaughan K, Faber WR, Straetemans M, et al. Point-of-care management of urogenital Chlamydia trachomatis via Gram-stained smear analysis in male high-risk patients. Diagnostic accuracy and cost-effectiveness before and after changing the screening indication at the STI Clinic in Amsterdam. Sex Transm Infect 2015; 91: 479-484.