Simultaneous Detection of Seven Pathogens of Cervicitis Among Young Female Sex Workers by Multiplex Real Time PCR in Dhaka, Bangladesh

Sharmili Paul1, Sharmeena Ahmed2, Shaheda Anwar2, Lima Rahman3, Zubair Shams3

1Shaheed M Monsur Ali Medical College, Siraiganj, Bangladesh; 2Department of Microbiology and Immunology Bangabandhu Sheikh Mujib Medical University, Dhaka; 3Save the Children, Bangladesh.

Abstract
The prevalence of STIs related cervicitis in Bangladesh among female sex workers (FSWs) is quite high and among them young (≤ 24 years) FSWs are more sufferers. The aim of this study was to detect infectious agents of cervicitis including Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma parvum in SWs of aged 10-24 years from endocervical swabs by multiplex real time PCR. A cross sectional study was done in collaboration with department of Microbiology, BSMMU, Dhaka and Save the Children, Bangladesh between March to December 2017 among sex workers enlisted to receive HIV prevention services at different drop in centers (DICs) in Dhaka. Total 105 SWs of aged between 10-24 years and clinically suspected as cervicitis, were enrolled for the study. Endo-cervical swabs were collect-ed during examination and tested in dept of Microbiology, BSMMU by multiplex PCR and other tests for aforementioned pathogens. Data were collected by face to face interview using semi-structured questionnaire and clinical examinations were done using Casco’s vaginal speculum. Among the study population, 87 (82.9%) were between 20-24 years of age. On examination, out of 105, 67 (63.8%) patients had no cervical discharge, only 8 (7.6%) had mucopurulent discharge. Out of total, 95 (90.5%) patients were mPCR positive for at least one pathogen and only 3 (2.9%) N. gonorrhoeae isolated by culture, 8 (7.6%) cases of C. trachomatis were detected by DFA and 8 (7.6%) cases of T. vaginalis were detected by wet film. Among the mPCR positive (95) cases, 63 (66.3%) patients had mixed infections and among them, M. hominis was the highest (76.2%) followed by U. urealyticum (49.2%). In the patients having ‘no’ (67) cervical discharge, 32 (48%) had M. hominis infection followed by U. parvum (40%). Majority of FSWs had mixed infection and M. hominis was the highest. A high number of patients had no cervical discharge though it is one of the diagnostic criteria for cervicitis in current syndromic management. In comparison to other available diagnostic tests, organisms were detected efficiently by multiplex PCR and could be advised routinely in such cases of mixed infection.

Key words: Female sex workers, cervicitis, multiplex PCR

Introduction
Cervicitis, mostly caused by sexually transmitted pathogens, remains an important public health problem among female sex workers (FSWs) in developing countries like Bangladesh.1 Untreated or undiagnosed cervicitis may cause ascending infection such as endometritis2, salpingitis3,4 pelvic inflammatory disease3 and infertility.5,6 It is also related to chorioamnionitis and other pregnancy related complications.2,8,9,10,11,12,13 It enhances the risk of HIV transmission14,15 and causes cervical cancer.16,17 It serves as a reservoir that facilitates widespread transmission among multiple sexual partners.18 There are estimated 90,000 to 150,000 FSWs operating in Bangladesh and the number of FSWs between the ages of 10-24 years old (adolescent and young group) is estimated to be 31,101.19 Higher rates of STIs related to cervicitis (83.2%) in young people have been reported in Bangladesh.1

In most cases, etiologies of cervicitis are polymicrobial in nature. It is estimated that annually there are 131 million new cases of Chlamydia trachomatisand 78 million of Neisseria gonorrhoeae infections among people aged 15-49 years all over the world20. Other organisms including Mycoplasma genitalium21,22,23,24,25,26, Mycoplasma hominis, Ureaplasma urealyticum27, Trichomonas vaginalis28,29, viruses like Herpes simplex virus, Human Papilloma Virus and Ureaplasma parvum also causes cervicitisand termed as nonspecific cervicitis (NSC) or nonchlamydial-nongonococcal cervicitis. Nonspecific cervicitis (NSC) is increasing day by day.30,31,32,33,34,35,36,37

Correspondence:
Sharmili Paul
M Monsur Ali Medical College
Siraiganj, Bangladesh
WHO approaches syndromic management (SM) for the diagnosis of cervicitis and other sexually transmitted infections. But cervicitis in FSWs is mostly asymptomatic or having mild or nonspecific symptoms.\(^{1,38,39,40}\) So syndromic approach led to many cases untreated or over diagnosed.\(^{41,42}\)

Multiplex PCR are being developed to diagnose multiple organisms in a single clinical sample from endocervical specimen in both symptomatic and asymptomatic cervicitis patients which might be helpful to diagnose all the microbes in a single test and also to the clinicians to prescribe appropriate antibiotics for patients according to results. Therefore, this study was designed to detect *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum* and *Trichomonas vaginalis* from endocervical swabs among FSWs aged of 10-24 years by multiplex real time PCR and compare the results of PCR with that of other conventional methods.

**Materials and methods**
This cross sectional study was done at the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh in between March, 2017 to February, 2018 and was approved by the Institutional Review Board of BSMMU. It had enrolled about 105 FSW saged between 10-24 years enlisted to receive services at different drop centers (DISCs) in Dhaka implemented by Save the Children, Bangladesh based on the inclusion criterias.

Patients who had any of the following findings per speculum examination were included in the study\(^{43,39,35}\) a) visible mucopurulent or mucoid or creamy discharge from the cervix or on endocervical swab, b) easily induced cervical bleeding by gentle passage of cotton swab, c) cervical tenderness observed on bimanual examination, d) red, edematous and hypertrophied cervix, e) signs of ectropion (The condition when lips of the cervix curl upwards and outwards to express the red looking endocervix).\(^{44}\) Menstruating women, pregnant and known case of vesico-vaginal fistula and stress incontinence were excluded from the study. After taking informed consent a pretested semi-structured data sheet gathering socio-demographic data, risk behavior and genitourinary complaints was administered.

**Sample collection procedure**
A pelvic examination was carried out by the attending gynaecologist. With all aseptic precautions, pre-moistened (warm water) sterile Casco’s self retaining bivalve vaginal speculum was introduced into the vagina. Ectocervical mucus was adequately removed with sterile cotton ball soaked with normal saline and the cervix was then inspected for the presence of cervicitis and endocervical swabs were then collected. Cotton tipped wooden sticks were introduced deeply (2-3cm) into the cervical canal and rotated gently against the endocervical wall for 15-30 seconds before removal. Total 3 cotton-tipped wooden swabs were collected and one sample was also collected using a small, nylon bristled cytobrush. First swab was used for direct inoculation into culture media at room temperature immediately after collecting the sample. Second one was immediately used for smear preparation for Gram staining and for preparing wet film. After air dry, smear for Gram staining was fixed by 99% ethanol and placed in a slide box for carrying. Saline wet mount microscopy was performed on cervical samples for detection of *T. vaginalis*. Third swab was kept in an empty screw capped test tube (5ml) for multiplex real time PCR and the cytobrush was immersed in a tube with 5ml PBS. All the tubes containing swab sticks and the slide box were transported at ambient temperature to the laboratory in a sample carrying box.

**Culture and isolation of N. gonorrhoeae**
Culture for *N. gonorrhoea* was carried out by inoculating onto modified Thayer- Martin media (HiMedia, HiMedia Laboratories Limited, Mumbai, India) blood agar, and chocolate agar media and incubated under micro-aerophilic conditions (5% O\(_2\)) at 37°C for at least 48 hours and confirmed by Gram staining, oxidase, catalase, and sugar fermentation tests. Antimicrobial susceptibility test was done for penicillin, tetracycline, ciprofloxacin, cefuroxime, ceftriaxone and cefixime following CLSI guideline.\(^{45}\) For wet film preparation, the test tubes were slightly heated and swabs were agitated vigorously in the PBS and we film was prepared. For DFA microscopy, smear was prepared as per the instruction of manufacturer (VIRCELL, Spain). Slides were stored at 2°C to 8°C for maximum 2-3 days in a tight slide box.

**Molecular detection of the organisms by multiplex real time PCR**
Real-time PCR amplification for seven microorganisms (*C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *M. genitalium*, *M. hominis*, *U. urealyticum*, and *U. parvum*) was performed using the FTD urethritis plus kit (Fast track Diagnostics, Luxembourg) in accordance with the manufacturer’s protocol, in a CFX96 real- time thermocycler (Bio-Rad, Hercules, CA, USA). The assay used murine Cytomegalovirus (mCMV) as an internal control which was introduced into each sample and the negative control at the lysis buffer stage of the extraction process. Extraction of nucleic acids from specimen was done according to the
Simultaneous detection of seven pathogens of cervicitis among young women in Bangladesh: A cross-sectional study. Paul et al

It contains sets of primers and Taqman probes that are specially designed from highly conserved regions of genetic sequences for the 7 pathogens. The kit has a detection limit of $10^3$ copies/ml for all pathogens except *T. vaginalis* which could be detected up to $10^2$ copies/ml. Amplification of DNA was performed on real-time PCR platform (CFX96™ Bio-Rad, USA). Two reaction mixes were prepared in two 1.5ml Eppendorf tube and labeled ‘tube 1’ and ‘tube 2’. Total reaction volume was 15 µl in each tube and prepared with 12.5 µl buffer, 1 µl enzyme and 1.5 µl of URScreen Primer-Probe mix in tube 1 and 1.5 µl UTriMyo Primer-Probe mix in tube 2. Complete reaction mix was vortexed briefly and spun down for few seconds. The amplification was performed under the following conditions: initial denaturation at 50°C for 15 minutes followed by 94°C for 1 min hold and 40 cycles (94°C for 8 sec and 60°C for 1 min).

All the data were entered into an electronic database and analysed using SPSS software (Version-20).

**Results**

Out of 105 FSWs (street, residence and hotel based) with clinical signs of cervicitis, 46 werefrom streets, 53 in residences and 6 were in the hotels. Table I shows the sociodemographic and behavioral characteristics of these FSWs.

**Table-I: Sociodemographic and behavioral characteristics in FSWs (n=105)**

| Indicators                          | Total (%) | Street based FSWs (n=46) | Residence based FSWs (n=53) | Hotel based FSWs (n=6) |
|-------------------------------------|-----------|-------------------------|-----------------------------|------------------------|
| Age (year)                          |           |                         |                             |                        |
| 14-19                               | 18 (17.1) | 11 (23.9)               | 7 (13.2)                    | 0                      |
| 20-24                               | 87 (82.9) | 35 (76.1)               | 46 (86.8)                   | 6 (100)                |
| Marital status                      |           |                         |                             |                        |
| Married*                            | 46 (43.8) | 21 (45.7)               | 23 (43.4)                   | 2 (33.3)               |
| Unmarried                           | 6 (5.7)   | 0                       | 0                           | 0                      |
| Divorced                            | 16 (15.2) | 9 (19.6)                | 0                           | 0                      |
| Separated                           | 32 (30.5) | 14 (30.4)               | 14 (26.4)                   | 4 (66.7)               |
| Widow                               | 5 (4.8)   | 2 (4.3)                 | 3 (5.7)                     | 0                      |
| Education (year)                    |           |                         |                             |                        |
| No education                        | 42 (40)   | 25 (54.3)               | 14 (26.4)                   | 3 (50)                 |
| 1-5                                 | 27 (25.7) | 16 (34.8)               | 11 (20.8)                   | 0                      |
| 6-10                                | 32 (30.5) | 5 (10.9)                | 24 (45.3)                   | 3 (50)                 |
| 11-12                               | 4 (3.8)   | 0                       | 4 (7.5)                     | 0                      |
| Income in last month (BDT)          |           |                         |                             |                        |
| 3,000-5,000                         | 32 (30.5) | 17 (37)                 | 15 (28.3)                   | 0                      |
| 5,001-10,000                        | 27 (25.7) | 11 (23.9)               | 13 (24.5)                   | 3 (50)                 |
| 10,001-20,000                       | 36 (34.3) | 16 (34.8)               | 18 (34)                     | 2 (33.3)               |
| 20,001-30,000                       | 7 (6.7)   | 2 (4.3)                 | 5 (9.4)                     | 0                      |
| More than 30000                      | 3 (2.9)   | 0                       | 2 (3.8)                     | 1 (16.7)               |

*Married means currently living with husband. Number of pregnancy includes live birth, MR (menstrual regulation) and abortion.
Table-II: Results of PCR, culture for *N. gonorrhoeae*, DFA for *C. trachomatis*, wet film for *T. vaginalis* and Gram’s stain for gram negative diplococci in endocervical swabs among the FSWs with clinically suspected cervicitis (n=105)

| Tests                      | No. of positive patients | Percentage |
|----------------------------|--------------------------|------------|
| PCR*                       | 95                       | 90.5       |
| Culture for *N. gonorrhoeae* | 3                        | 2.9        |
| DFA for *C. trachomatis*    | 8                        | 7.6        |
| Wet film for *T. vaginalis* | 8                        | 7.6        |
| Gram’s stain for gram negative diplococci | 0 | 0 |

*PCR for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *M. genitalium*, *M. hominis*, *U. urealyticum* and *U. parvum*

In table II, out of 105 FSWs, 90.5% patients were PCR positive for at least one pathogen and 2.9% patients were culture positive for *N. gonorrhoeae*, 7.6% patients were DFA positive for the detection of *C. trachomatis* and 7.6% patients with *T. vaginalis* were detected by wet film. None was positive by Gram’s stain for gram negative diplococci.

Table-III: Different infectious agents detected from endocervical swabs by PCR (n=95)

| Infectious agents            | No. of patients | Percentage |
|------------------------------|-----------------|------------|
| *Chlamydia trachomatis*      | 24              | 25.3       |
| *Neisseria gonorrhoeae*      | 18              | 18.9       |
| *Trichomonas vaginalis*      | 25              | 26.3       |
| *Mycoplasma genitalium*      | 16              | 16.8       |
| *Mycoplasma hominis*         | 53              | 55.8       |
| *Ureaplasma urealyticum*     | 34              | 35.8       |
| *Ureaplasma parvum*          | 43              | 45.3       |

*Sum exceed 100% because of multiple organisms were present.

Among 95 PCR positive patients, 55.8% patients were *M. hominis* positive followed by *U. parvum* (45.3%). (Table III)

Table-IV: Rate of single and mixed infections in PCR positive patients (n=95)

| No. of organisms | Organisms detected by PCR | CT (n%) | NG (n%) | TV (n%) | MG (n%) | MH (n%) | UU (n%) | UP (n%) | Total (%)
|------------------|---------------------------|---------|---------|---------|---------|---------|---------|---------|-----------|
| Single           |                           | 2(6.2)  | 1(3.1)  | 3(9.4)  | 1(3.1)  | 5(15.6)| 3(9.4)  | 17(53.1)| 32(33.7) |
| Mixed            |                           | 22(35)  | 17(27)  | 22(35)  | 15(23.8)| 48(76.2)| 31(49.2)| 26(41.3)| 63(66.3) |

**CT:** *Chlamydia trachomatis*; **NG:** *Neisseria gonorrhoeae*; **TV:** *Trichomonas vaginalis*; **MG:** *Mycoplasma genitalium*; **MH:** *Mycoplasma hominis*; **UU:** *Ureaplasma urealyticum*; **UP:** *Ureaplasma parvum*

Majority of the patients (66.3%) had mixed infections and among them, *M. hominis* was present in 76.2% of mixed infection. (Table IV)

Table-V: Distribution of per speculum findings in relation to PCR results (n=105)

| Per speculum findings | Single organism (n=32) | Mixed organisms (n=63) | No organism (n=10) |
|-----------------------|------------------------|------------------------|---------------------|
|                       | n (%)                  | n (%)                  | n (%)               |
| Cervical discharge    |                        |                        |                     |
| Mucopurulent          | 8 (7.6)                | 3 (9.4)                | 5 (7.9)             |
| Thick                 | 9 (8.6)                | 4 (12.5)               | 5 (7.9)             |
| Thin                  | 21 (20)                | 4 (12.5)               | 14 (22.2)           |
| No                    | 67 (63.8)              | 21 (65.6)              | 39 (62)             |
| Ectropion             | 27 (25.7)              | 10 (31.2)              | 16 (25.4)           |
| Easily induced cervical bleeding | 60 (57.1) | 20 (62.5) | 37 (58.7) | 3 (30) |
| Cervical tenderness on movement | 18 (17.1) | 6 (18.6) | 8 (12.7) | 4 (40) |
| Red & swollen cervix  | 33 (31.4)              | 9 (28.1)               | 21 (33.3)           | 3 (30) |

*Sum exceed 100% because of multiple signs were present.

Table V shows, most of the patients (63.8%) had no cervical discharge followed by easily induced cervical bleeding (57.1%) per speculum examination.

Discussion

In this study, about 90.5% cases, at least one pathogen was detected by PCR from the collected endocervical swabs. Similar finding was observed by Sylverken et al in 2016.38 In that, 86.5% of symptomatic women infected with at least one pathogen, were detected by PCR. Another study conducted on patients with STDs also showed higher detection (80.7%) rate of pathogens on swab and urine samples by PCR.46

Among 95 PCR positive patients, 55.8% cases were *M. hominis* positive followed by *U. parvum* (45.3%) and *U. urealyticum* (35.8%). Other organisms including *T. vaginalis*, *C. trachomatis*, *N. gonorrhoeae* and *M. genitalium* were detected in 26.3%, 25.3%, 18.9% and 16.8% patients respectively. A similar study was conducted in patients with cervicitis in Australia where the detection rate was *M. hominis* (13.7%), *U. parvum* (57%), and *U. urealyticum* (6.1%), *T. vaginalis* (3.4%), *C. trachomatis* (0.4%), *N. gonorrhoeae* (0) and *M. genitalium*(1.3%) by PCR.27

In Surat, another study conducted among FSWs showed that gonorrhoeae was 16.9%, chlamydial infection was 8.5% and trichomoniasis was 14.4%.40 In Bangladesh, Nusrat had found only 16% *C. trachomatis*, 10% *N. gonorrhoeae* and 2% *M. hominis* by PCR among cervicitis patients receiving services from outdoor of Dhaka Medical
Simultaneous detection of seven pathogens of cervicitis among young ... Paul et al

College. The lower detection rate might be due to the fact that the participants were not from sex trade.

In the present study, most (55.8%) of the cases were found to be positive for *M. hominis* followed by *U. parvum* (45.3%). Similar results were found by McKechnie *et al* in 2011 where 47.7% of *U. parvum* was detected in endocervical swabs from women attending sexual health clinics. On the contrary, Sylverken *et al* found higher rates of *M. hominis* positive (67.5%) and *U. parvum* positive (62.5%) among the study group.

Recently, cervical infection with *M. genitalium* is dramatically increasing among the high risk people including female with multiple sexual partners. In the current study, out of total 105 sex workers, *M. genitalium* was found in 16.8% patients. Similar higher prevalence rates of *M. genitalium* were reported in different parts of the world in symptomatic and high risk populations ranging between 13%-25%. A study conducted on sex workers in Ghana reported the prevalence rate of *M. genitalium* to be 26.3% in endocervical swabs whereas McKechnie and co-workers found < 5% of *M. genitalium* among non-sex workers in Australia.

Detection rate of *T. vaginalis* (26.3%) by PCR in the present study as single or mixed infections among the study population was comparable with the results of the studies in Surat (14.4%) and in Uganda (18.9%). Variable rates of microscopically *T. vaginalis* positive were found in street based and hotel based sex workers in Dhaka city that were reported to be 45.5% and 4.3% respectively. The higher rate of detection of *T. vaginalis* in the present study may be due to the use of most sensitive method of detection like PCR.

In present study, *C. trachomatis* was present in 25.3% of study patients. Similar finding was reported by Desai *et al.* In another study, higher prevalence of *C. trachomatis* (43.5%) than that of present study was found among hotel based sex workers. Rate of Chlamydial infection among asymptomatic and asymptomatic FSWs was 6.3% in Bangladesh whereas the rate was higher (25.3%) in the present study. The high result may be due to the enrollment of symptomatic patients in this study.

*N. gonorrhoeae* was found only in 18.9% of study patients which was much lower than the rates (35.5%) among the sex workers reported by Nessa *et al* and Rahman *et al.* But the result of present study was much higher than that of a previous study (5.4%) in Bangladesh.

In this study, among PCR positive cases, the rate of mixed infections (66.3%) was higher than that of single infection (33.7%). In contrast, Sylverken *et al* found higher rate of single infection (57.1%) than that of the mixed infections (42.9%). But similar result of higher prevalence of infection with mixed organisms (67%) was found by Pereyre *et al* in screening of genital infection among symptomatic and asymptomatic women in France. In the present study, out of the total single infections, *U. parvum* was the commonest (53.1%) which was followed by *M. hominis* (15.6%).

On the other hand, *M. hominis* was found as the most common pathogen (76.2%) among the mixed infections followed by *U. urealyticum* (49.2%) in the present study. The infection with two organisms was the most frequent (49.2%) co-infection among mixed infections. The combination of *M. hominis* and *U. urealyticum* was found to be higher in the infections in this study. This finding was similar to the result of a recent study described by Capoccia *et al* in 2013 with the rate of 38.7%. The different patterns of co-infection were supposed to establish a chronic infection among the cervicitis patients as well as making a threat of developing antibiotic resistance which may be evaluated by extensive research.

**Conclusion**

Majority of FSWs had mixed infection and among the pathogens *M. hominis* was the highest. A high number of patients had no cervical discharge though it is one of the diagnostic criteria for cervicitis in current syndromic management. In comparison to other available diagnostic tests, organisms were detected efficiently by multiplex PCR and could be advised routinely in such cases of mixed infection.

**Acknowledgement**

The authors express their gratitude to Save the Children, Bangladesh, all the DIC coordinators, outreach workers and FSWs for their precious help and active assistance in getting the samples from different DICs, Dhaka.

**References**

1. Khanam R, Reza M, Ahmed D, et al. Sexually Transmitted Infections and Associated Risk Factors Among Street-Based and Residence-Based Female Sex Workers in Dhaka, Bangladesh. Sex Transm Dis 2017;44(1): 22-9.

2. Amirmozafari N, Mirnejad R, Kazemi B, et al. Comparison of polymerase chain reaction and culture for detection of genital mycoplasma in clinical samples from patients with genital infections. Saudi MedJ 2009; 30(11): 1401-5.
3. Yudin MH, Hillier SL, Wiesenfeld HC, et al. Vaginal polymorphonuclear leukocytes and bacterial vaginosis as markers for histologic endometritis among women without symptoms of pelvic inflammatory disease. Am J Obstet Gynecol 2003; 188(2):318-23.

4. Peipert JF, Ness RB, Soper DE, et al. Association of lower genital tract inflammation with objective evidence of endometritis. Infect Dis Obstet Gynecol 2000;8 (2):83-7.

5. Wiesenfeld HC, Hillier SL, Meyn LA, et al. Subclinical pelvic inflammatory disease and infertility. Obstet Gynecol 2012;120(1): 37-43

6. Brunham RC, Maclean IW, Binns B, et al. Chlamydia trachomatis: its role in tubal infertility. J Infect Dis 1985;152(6): 1275-82.

7. Cates Jr W. Sexually transmitted organisms and infertility: the proof of the pudding. Sex Transm Dis 1984;11(2):113.

8. Peerayeh SN and Sattari M. Detection of Ureaplasma urealyticum and Mycoplasma hominis in endocervical specimens from infertile women by polymerase chain reaction. Middle East Fertil Soc J 2006; 11(2):104-8.

9. Kundsin RB, Leviton A, Allred EN and Poulin SA. Ureaplasma urealyticum infection of the placenta in pregnancies that ended prematurely. Obstet Gynecol 1996;87(1):122-7.

10. Grodstein F, Goldman MB and Cramer DW. Relation of tubal infertility to a history of sexually transmitted diseases. Am J Epidemiol 1993;137:577-84.

11. Kharsany AB, Hoosen AA, Moodley J, Bagarate J and Gouws E. The association between sexually transmitted pathogens and cervical intra-epithelial neoplasia in a developing community. Genitourin Med 1993:69:357-60.

12. Hardy PH, Hardy JB, Nell EE, Grahamm DA, Spence MR and Rosenbaum RC. Prevalence of six sexually transmitted disease agents among pregnant inner-city adolescents and pregnancy outcome. Lancet ii 1984;333-7.

13. Minkoff H, Grunewald AN, Schwarz R, et al. Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. Am J Obstet Gynecol 1984;150: 965-72.

14. Saigal K, Dhawan B, Rawre J, et al. Genital Mycoplasma and Chlamydia trachomatis infections in patients with genital tract infections attending a tertiary care hospital of North India. Ind J Path Micro 2016;59(2): 194-6.

15. McClelland RS, Wang CC, Mandaliya K, et al. Treatment of cervicitis is associated with decreased cervical shedding of HIV-1. AIDS 2001;15(1): 105-10.

16. Zhang ZF and Begg CB. Is Trichomonas vaginalis a cause of cervical neoplasia? Results from a combined analysis of 24 studies. Inter J Epidemiol 1994; 23:682-90

17. Gram I, Macaluso M, Churchill J, et al. Trichomonas vaginalis (TV) and human papillomavirus (HPV) infection and the incidence of cervical intraepithelial neoplasia (CIN) grade III. Cancer Cause Control 1992;3:231-6.

18. Parks KS, Dixon PB, Richey CM, et al. spontanous clearance of Chlamydia trachomatis infection in untreated patients. Sex TransmDis 1997;24(4): 229-35.

19. NASP. Population Size Estimates for Most at Risk Populations for HIV In Bangladesh: Directorate General of Health Services, Ministry of Health and Family Welfare, Govt. of Bangladesh: Dhaka; 2009.

20. World Health Organization. Global health sector strategy on sexually transmitted infections 2016-2021: toward ending STIs; 2016.

21. Bjartling C, Osser S and Persson K. Mycoplasma genitalium in cervicitis and pelvic inflammatory disease among women at a gynecologic outpatient service. Am J Obstet Gynecol 2012;206(6): 476.1-8.

22. Ross JDC and Jensen JS. Mycoplasma genitalium as a sexually transmitted infection: implications for screening, testing, and treatment. Sex Transm Infect 2006;82(4):269-71.

23. Falk L, Fredlund H and Jensen JS. Signs and symptoms of urethritis and cervicitis among women with or without Mycoplasma genitalium or Chlamydia trachomatis infection. Sex Transm Infect 2005;81 (1):73-8.

24. Schlicht MJ, Lovrich SD, Sartin JS, Karpinsky P, Callister SM and Agger WA. High prevalence of genital mycoplasmas among sexually active young adults with urethritis or cervicitis symptoms in La Crosse, Wisconsin. J Clin Microbiol 2004;42 (10): 4636-40.

25. Manhart LE, Critchlow CW, Holmes KK, et al. Mucopurulent cervicitis and Mycoplasma genitalium. J Infect Dis 2003;187 (4): 650-7.
26. Taylor-Robinson D. Mycoplasma genitalium—an up-date. Int J STDAIDS 2002;13:145-51.

27. McIver CJ, Rismanto N, Smith C, et al. Multiplex PCR testing detection of higher-than-expected rates of cervical mycoplasma, ureaplasma, and trichomonas and viral agent infections in sexually active australian women. J Clin Microbiol 2009;47(5): 1358–63.

28. Lusk MJ and Konecny P. Cervicitis: a review. Curr Opin Infect Dis 2008;21(1): 49-55.

29. Marrazzo JM and Martin DH. Management of women with cervicitis. Clin Infect Dis 2007;44 (Supplement_3): 102-10.

30. Avolio M, Modolo ML, Stano P, De Rosa R and Camporese A. Molecular evaluation of 7 sexually transmissible microorganisms in symptomatic and asymptomatic Italian childbearing age women: is Ureaplasma parvum a real innocent bystander? Microbiologia Medica 2016;31(3): 71-75

31. World Health Organization. Global health sector strategy on sexually transmitted infections 2016-2021: toward ending STIs. 2016.

32. Taylor-Robinson D. Mycoplasma genitalium—an up-date. Int J STD AIDS 2005;20:141-5.

33. Lewis DA, Marsh K, Radeb EF, Maseko V and Hughes G. Trends and associations of Trichomonas vaginalis infection in men and women with genital discharge syndromes in Johannesburg, South Africa. Sex Transm Infect 2013;89(6): 523-7.

34. Marrazzo JM, Wiesenfeld HC, Murray PJ, et al. Risk factors for cervicitis among women with bacterial vaginosis. J Infect Dis 2006; 193(5): 617-24.

35. Pepin J, Labbé AC, Khonde N, et al. Mycoplasma genitalium: an organism commonly associated with cervicitis among west African sex workers. Sex Transm Infect 2005;81(1): 67-72.

36. Tsunoe H, Tanaka M, Nakayama H, et al. High prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in female commercial sex workers in Japan. Int J STD AIDS 2000;11(12): 790-4.

37. Nyirjesy P. Nongonococcal and nonchlamydial cervicitis. Curr Infect Dis Reports 2001;3 (6): 540-5.

38. Sylverken AA, Owusu-Dabo E, Yar DD, et al. Bacterial etiology of sexually transmitted infections at a STI clinic in Ghana; use of multiplex real time PCR. Ghana Med J 2016;50(3): 142-8.

39. Sachdeva P, Patel AL, Sachdev D, et al. Comparison of an in-house PCR assay, direct fluorescence assay and the Roche AMPLICOR Chlamydia trachomatis kit for detection of C. trachomatis. J Med Microbiol 2009;58(7): 867-73.

40. Desai VK, Kosambiya JK, Thakor HG, et al. Prevalence of sexually transmitted infections and performance of STI syndromes against aetiological diagnosis, in female sex workers of red light area in Surat, India. Sex Transm Infect 2003;79(2): 111-5.

41. Meena V and Bansal CL. Study to Evaluate Targeted Management and Syndromic Management in Women Presenting with Abnormal Vaginal Discharge. J Obstet Gynecol India 2016;66(1): 534-40.

42. Tann CJ, Mpairwe H, Morison L, et al. Lack of effectiveness of syndromic management in targeting vaginal infections in pregnancy in Entebbe, Uganda. Sex Transm Infect 2006;82(4): 285-9.

43. Beni BT, Motamedi H and Ardakani MR. Comparison of plasmid and chromosomal omp1 gene-based PCR and two DNA extraction methods for diagnosing Chlamydia trachomatis in endocervical swab samples. Asian Pac J Trop Dis 2012;2:612-6.

44. Dutta DC. Benign lesions of the cervix. In: Konar H, ed. Text book of Gynaecology, 4th edition, New Central Book agency. Kolkata, India 2003;250.

45. Clinical and Laboratory Standards Institute. Performance standard for Antimicrobial Susceptibility Testing: 24th informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

46. Lee SR, Chung JM and Kim YG. Rapid one step detection of pathogenic bacteria in urine with sexually transmitted disease (STD) and prostatitis patient by multiplex PCR assay (mPCR). J Microbiol 2007; 45(5): 453-9.

47. Nusrat F. Detection of bacterial causes of cervicitis among women of child bearing age by Gram staining, culture and multiplex PCR.[Mphil thesis] Dhaka: The University of Dhaka: 2013. 55.

48. McKechnie ML, Hillman RJ, Jones R, et al. The prevalence of urogenital micro-organisms detected by a multiplex PCR-reverse line blot assay in women attending three sexual health clinics in Sydney, Australia. J Med Microbiol 2011; 60(7): 1010-6.

49. Saigal K, Dhawan B, Rawre J, Khanna N and Chaudhry R. Genital Mycoplasma and Chlamydia trachomatis infections in patients with genital tract...
infections attending a tertiary care hospital of North India. Ind JPathMicrobiol 2016;59(2): 194-6.

50. Akya A, Aletaha M, Ghadiri K and Rezaee M. The Frequency of Mycoplasma Hominis, Mycoplasma Genitalium and Ureaplasma Urealyticum in Women with Cervicitis. J Nosocom Infect 2014;1 (2):31-7.

51. Bjartling C, Osser S and Persson K. Mycoplasma genitalium in cervicitis and pelvic inflammatory disease among women at a gynecologic outpatient service. Am J Obstet Gynecol 2012;206(6): 476.1-8.

52. Haggerty CL, Totten PA, Astete SG and Ness RB. Ureaplasma genitalium among women with nongonococcal, nonchlamydial pelvic inflammatory disease. Infect Dis Obstet Gynecol 2006;6 :40-4.

53. Pickering JM, Witworth JAG, Hughes P, et al. Aetiology of sexually transmitted infections and response to syndromic treatment in southwest Uganda. Sex Transm Infect 2005;81(6):488-93.

54. Nessa K, Waris SA, Sultan Z, et al. Epidemiology and etiology of sexually transmitted infection among hotel based sex workers in Dhaka, Bangladesh. JClinMicrobiol 2004;42(2): 618-21.

55. Rahman S, Khan AI, Razzaq R and Shams I. Operational Aspects of Syndromic Management of RTIs/STIs at a Primary Healthcare-level Clinic. In: Khan MSI, ed. ICDDR,B Working Paper No.151. Bangladesh: ICDDR,B : Centre for Health and Population Research Inc; 2001.20.

56. Pereyre S, Nadalié CL, Bébéar C, et al. Mycoplasma genitalium and Trichomonas vaginalis in France: a point prevalence study in people screened for sexually transmitted diseases. ClinMicrobiol Infect 2017; 23(2):122-5.

57. Capoccia, R, Greub G and Baud D. Ureaplasma urealyticum, Mycoplasma hominis and adverse pregnancy outcomes. Curr Opin Infect Dis 2013;26 (3): 231-40.

58. Yamazaki T, Matsuo J, Nakamura S, Oguri S and Yamaguchi H. Effect of Ureaplasma parvum co-infection on Chlamydia trachomatis maturation in human epithelial HeLa cells treated with interferon-γ. JInfectChemother 2014;20(8):460-4.