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Detection of Aetiological Agents of Cervicitis and Distribution of Selective Antimicrobial Resistance Genes in *Neisseria Gonorrhoeae*

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Abstract

This cross sectional study was conducted to identify Neisseria gonorrhoeae, Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma genitalium, Adenovirus, Herpes simplex virus-2, Human papilloma virus and Trichomonas vaginalis from patients having clinically suspected cervicitis attending gynecology outpatient department of Dhaka Medical College Hospital and to detect the drug resistance genes among the isolated N. gonorrhoeae. Chronic cervicitis was detected by histopathology from cervical biopsy samples of VIA positive and colposcopy positive patient. A total of 248 endocervical swab were collected. N. gonorrhoeae were isolated and identified by Gram stain, culture, biochemical test and polymerase chain reaction (PCR). Out of 248 cervical swab samples, 22 (8.87%) were positive in Gram stain, 14 (5.64%) yielded growth in culture and 26 (10.48%) were positive by PCR for N. gonorrhoeae, 16 (6.45%) for C. trachomatis and 5(2.01%) for U. urealyticum. Wet film microscopy detected T. vaginalis trophozoite in 11 (4.43%) cases. Among 14 culture positive N.

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gonorrhoeae, 100% were resistant to tetracycline and doxycycline, 92.86% were resistant to ciprofloxacin, 85.71% were resistant to penicillin and erythromycin, 14.28% were resistant to amoxicillin and clavulonic acid, 7.14% were resistant to azithromycin and all were sensitive to cefixime and ceftriaxone. 71.42% of the N. gonorrhoeae strains were identified as penicillinase producers (PPNG). Considering culture as gold standard, the sensitivity and specificity of Gram stain was 100% and 96.58% respectively and PCR sensitivity and specificity was 100% and 94.88% respectively. Out of the 26 PCR positive N. gonorrhoeae, TEM-1, gyrA and parC gene were present in 73.07%, 34.61% and 15.38% cases respectively, both gyrA and parC were present in 42.30% cases and no TEM-135 and mosaic penA genes were found in any of the isolated N. gonorrhoeae. The results of this study showed that N. gonorrhoeae isolates were sensitive to ceftriaxone, cefixime and azithromycin. PCR may be considered as a suitable method for accurate diagnosis of gonococcal cervicitis. Multiplex PCR can be introduced for detection of other causes of cervicitis like C. trachomatis and U. urealyticum. Regular surveillance of antimicrobial resistance should be done in every tertiary care hospital for detection of cephalosporin resistant strains of N. gonorrhoeae in Bangladesh.

Key words: Aetiological agents, Cervicitis, Antimicrobial resistance, *Neisseria Gonorrhoeae*

INTRODUCTION

Cervicitis is an inflammation of the uterine cervix, characteristically diagnosed by a visible, purulent or mucopurulent endocervical exudate in the endocervical canal or on an endocervical swab specimen and sustained, endocervical bleeding (CDC, 2015). Cervicitis is frequently asymptomatic, but some women complain of an abnormal vaginal discharge and intermenstrual vaginal bleeding. In developing countries women tend to suffer more because of the synergistic effects of infection, malnutrition and reproduction (Ranjan *et al.*, 2003). Most common causes of cervicitis are *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (Marrazzo *et al.*, 2006). In 2008, worldwide 498.9 million adult became infected with a curable sexually transmitted infection

such as *N. gonorrhoeae, C. trachomatis*, *T. vaginalis* and syphilis (WHO, 2008). Globally, WHO estimated 78 million new cases of gonorrhoea among adult (Newman *et al*., 2015). In different geographical area, prevalence of gonococcal infection are different, such as 16.9% in India (Desai *et al*., 2003), 2.3% among rural women in Nepal (Christin *et al*., 2005), 2.6% in Pakistan (Mahmood and Saniotis, 2011) and 15.9% in Bangladesh (Nusrat *et al*., 2014). *N .gonorrhoeae* is fastidious organism and very much susceptible to drying. The sensitivity of gonococcal cultures to be 80 to 95%, but it is not higher than approximately 50% for females with long-standing infections. Nucleic acid amplification test is being used to detect DNA of *N. gonorrhoeae* and more sensitive (≥90%) than culture (Bignell, 2009). Diagnosis is usually made by serological determination or in vitro isolation of organism by culture (Levinson, 2014) and detected mainly by PCR (Palmer *et al*., 1991). Like *Mycoplasma*, PCR also a diagnostic tool for detection of *U. urealyticum* (Povlsen *et al*., 1998). HSV can be detected by cell culture but results are obtained after several days. PCR is a well characterized method for rapid and sensitive diagnosis of HSV. Compared with culture, PCR has 93.2% and 100% sensitivity and specificity respectively (Cullen *et al*., 1997). Antimicrobial resistance in *N. gonorrhoeae* is the most significant challenge to control gonorrhoea. In Bangladesh, few studies are carried out to identify *N. gonorrhoeae, Chlamydia trachomatis, M. genitalium* and *U. urealyticum* by PCR (Jahan *et al*., 2014). Among bacterial causes of cervicitis only *N. gonorrhoeae* culture is cheap and easy but *C .trachomatis, M. genitalium* and *U. urealyticum* culture is difficult and required expert hand. Cervicitis caused by virus is also difficult to diagnose on cell line culture and these facilities are not available in all tertiary level laboratory. PCR can detect viral and bacterial agents of cervicitis which cannot be cultured. So this study is designed to detect etiological agents of cervicitis from endocervical swab and cervical biopsy specimen of suspected patients of cervicitis attending Dhaka Medical College Hospital by Gram stain, culture and histopathology. Multiplex PCR is used to detect *N. gonorrhoeae, C. trachomatis, U. urealyticum*, *M. genitalium*, HSV-2, HPV and *Adenovirus* and to detect antimicrobial resistance genes in *Neisseria gonorrhoeae*.

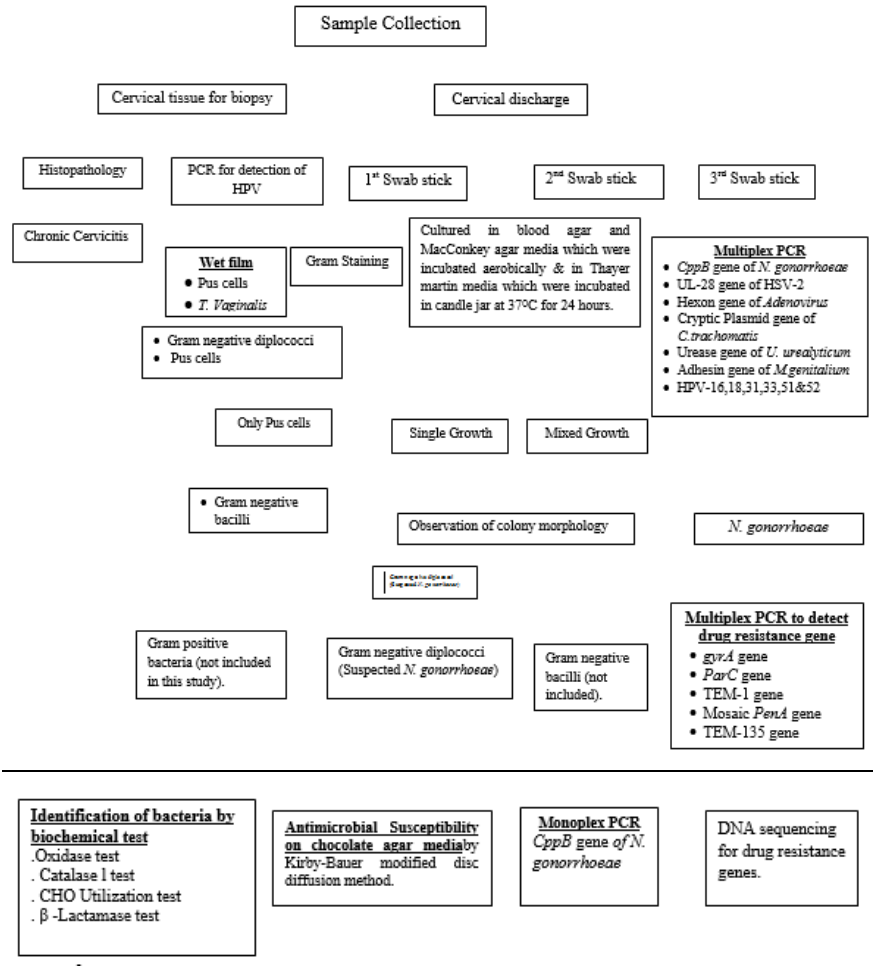
METHODS AND MATERIALS

This was a cross sectional study conducted in Department of Microbiology, Dhaka Medical College, Dhaka from January to December, 2017 with a sample of 248. Of the total 248 patients, cervical tissue biopsy samples were collected from 30 patients who were VIA and colposcopy positive. Due to time and resource constrain cervical biopsy from other patients could not be examined. Patients with clinically suspected cervicitis who presented with history of foul smelling vaginal discharge and/or painful micturition and/or abnormal per vaginal bleeding and/or itching around genital area and/or lower abdominal pain in gynaecology outpatient department in Dhaka Medical College Hospital and patients who were positive on Visual Inspection by Acetic acid and colposcopy test were included for biopsy for histopathological examination and HPV DNA detection were included in the study. Patients who were in menstrual period at the time of sample collection and who were on antimicrobial drugs or had received any antimicrobial drugs in the last 7 days were excluded. All data regarding patient were collected using pre-designed data collection sheet. Informed written consent was obtained from each patient before sample collection and professional secrecy was maintained about the diagnosis. All data were compiled and edited meticulously by through checking and rechecking. All omissions and inconsistencies were corrected and were removed methodically. The result of the study was recorded systematically. Data analysis was done by using 'Microsoft Office Excel 2013' program and SPSS and according to the objectives of the study. Results were presented in the form of table and figure. The test of significant was calculated by using Ch-square test. Endocervical swabs were collected from the patient attending outpatient department of Gynecology of DMCH suffering from clinically suspected cervicitis. After taking proper aseptic precautions, a sterile vaginal speculum was used to examined the cervix. Then cervix was cleaned by sterile normal saline and three sterile cotton swab sticks were passed one after one 20 mm into the endocervical canal. Then the sticks were rotated gently against the endocervical wall to obtain specimen. Three samples from each patient were collected. First swab was used for making smear on two separate clean glass slide by gently rolling the swab on glass slide for Gram stain

and wet film preparation. Second swab was used for culture and third swab was mixed with 2ml sterile phosphate buffer saline and kept at -20^{o} C until it was used for PCR.Women with suspected cervical lesions on screening test e.g. visual inspection with acetic acid and suspicious looking cervix were sent for colposcopy are recommended for biopsy in gynecological outpatient department in Dhaka Medical College. Two biopsy specimens were collected. One specimen was fixed in 10% formaldehyde and send to histopathology department of Dhaka Medical College. After fixation a systemic gross examination was performed and adequate tissue section was submitted and embedded in paraffin. Then histological sections with 5 micron thickness was obtained from paraffin block and stained with haematoxylin and eosine for histological assessment. Cryovial containing second specimen was preserved at -20^{o}C for PCR. Cervical discharge was placed on glass slide and a drop of sterile normal saline was placed on it, covered with a cover slip and examined under light microscope using 10x and 40x objectives with the condenser iris closed sufficiently to give good contrast. All the samples were examined microscopically for movement of motile *Trichomonas vaginalis* trophozoite. Smears were prepared from cervical swab specimen and stained with Gram stain as per standard procedure and were examined under microscope to detect pus cells, gram negative bacilli and intracellular gram negative diplococci. Gram negative intracellular kidney shaped diplococci were considered as *Neisseria gonorrhoeae*. The specimen of endocervical discharge was inoculated at collection site on blood agar, MacConkey agar and Thayer Martin media with proper labeling. The inoculation was done in such a way that all areas of the swab were inoculated. Inoculated blood agar and MacConkey agar plates were incubated aerobically and Thayer Martin media plates were incubated with 5% CO_2 inside a candle jar at 37^{o}C. Culture plates were examined after 24 hours of incubation for growth of *Neisseria gonorrhoeae*. If there was no growth, the plates were examined again following additional 24 hours of incubation. *Neisseria gonorrhoeae* colonies on Thayer Martin media were identified by colony morphology, oxidase test, catalase test, Gram staining and ß-lactamase test as per standard techniques. The isolates with presumptive identification were confirmed by rapid carbohydrate utilization test (RCUT), which differentiates *N. gonorrhoeae* from other *Neisseria* species. Finally *N. gonorrhoeae* was confirmed by detecting

DNA by PCR. Isolated *N. gonorrhoeae* were tested for antimicrobial susceptibility by Kirby-Bauer modified disc- diffusion technique. The antimicrobial discs were used according to the standard antibiotic panel for isolated organisms. Antibiotic discs were obtained from commercial source. Chocolate agar media was used for antimicrobial susceptibility test. The plates were dried in incubator at 37°C for 30 minutes before use. Zone of inhibition were interpretated according to CLSI guideline.

Work Flow Chart



RESULTS

Table 1 shows 248 endocervical swabs from suspected cervicitis patient were included in this study. Gram staining among 248 cervical samples showed that gram negative (intracellular) diplococci and pus cells (>10 WBC/HPF) were found in 8.87% and 26.20% cases respectively.

Table 1: Gram stain findings of endocervical swab samples (n=248)

Finding	Number	Percentage
Gram negative intracellular diplococci with pus cells >10WBC/HPF	22	8.87
Only Pus cells*	65	26.20
No gram negative diplococci and pus cells<10 WBC/HPF	161	64.93
Total	248	100.00

*Pus cells= >10 WBC/HPF (CDC, 2010).

Table 2 presents identification of *N. gonorrhoeae* by culture. Among 248suspected cases of cervicitis, *N. gonorrhoeae* was isolated in 14 (5.64%) cases. No gram negative diplococci was found in pus cells negative samples.

Table 2: *Neisseria gonorrhoeae* isolates from endocervical swab by culture (n=248)

Culture	Number	Percentage
Positive	14	5.64
Negative	234	94.36
Total	248	100.00

Detection of *N. gonorrhoeae, C. trachomatis* and *U. urealyticum* DNA in cervical swabs by PCR is shown in Table 4.3. Among 248 cervical swabs, DNA of *N. gonorrhoeae* was detected in 26 (10.48%), *C. trachomatis* in 16 (6.45%) and *U. urealyticum* in 5 (2.01%) cases.

Table 3: Distribution of DNA of different organisms detected from endocervical swab samples by PCR (n=248)

DNA of organisms	Total samples n (%)
Neisseria gonorrhoeae	26 (10.48)
Chlamydia trachomatis	16 (6.45)
Ureaplasma urealyticum	5 (2.01)
Mycoplasma genitalium	0 (0.00)
Herpes simplex-2	0 (0.00)
Adenovirus	0 (0.00)
Human Papilloma virus	0 (0.00)
Total	47 (18.94)

Table 4 demonstrates detection of *T. vaginalis* in endocervical swabs by wet film microscopy. Among 248 cases, trophozoite of *T. vaginalis* was found in 11 (4.43%) cases.

Table 4: Wet film findings of endocervical swab samples (n=248)

T. vaginalis	**Number**	**Percentage**
Positive	11	4.43
Negative	237	95.57
Total	248	100.00

Table 5 shows antimicrobial resistance pattern of *N. gonorrhoeae*. Among 14 culture positive *N. gonorrhoeae*, 100% were resistant to tetracycline and doxycycline, 92.86% were resistant to ciprofloxacin, 85.72% were resistant to penicillin and erythromycin, 14.28% were resistant to amoxiclav (amoxicillin and clavulonic acid), 7.14% were resistant to azithromycin and all the isolates were 100% sensitive to cefixime and ceftriaxone.

Table 5: Antibiotic resistance pattern of isolated *N. gonorrhoeae* (n=14)

Antimicrobial agents	**Sensitive n (%)**	**Resistance n (%)**
Tetracycline	0 (0.00)	14 (100.00)
Doxycycline	0 (0.00)	14 (100.00)
Ciprofloxacin	1 (7.14)	13 (92.86)
Penicillin	2 (14.28)	12 (85.72)
Erythromycin	2 (14.28)	12 (85.72)
Amoxiclav (Amoxicillin and clavulonic acid)	12 (85.72)	2 (14.28)
Azithromycin	13 (92.86)	1 (7.14)
Cefixime	14 (100.00)	0 (0.00)
Ceftriaxone	14 (100.00)	0 (0.00)

Table 6 demonstrates result of β-lactamase test among isolated *N. gonorrhoeae*. Among 14 *N. gonorrhoeae*, 71.42% were penicillinase producing *N. gonorrhoeae* (PPNG) and 28.58% were non-penicillinase producing *N. gonorrhoeae* (Non-PPNG).

Table 4.6: Findings of β-lactamase test among the isolated *Neisseria gonorrhoeae* (n=14)

β-Lactamase test	**Number**	**Percentage**
PPNG*	10	71.42
Non-PPNG**	4	28.58
Total	14	100.00

***** PPNG=Penicillinase producing *N. gonorrhoeae*. ****** Non-PPNG=Non penicillinase producing *N. gonorrhoeae*.

Distribution of selective antimicrobial resistance genes among the *N. gonorrhoeae* positive samples by PCR is shown in table 7. TEM-1, *gyrA* and *parC* gene were present in 73.07%, 34.61% and 15.38% samples respectively. Both *gyrA* and *parC* were present in 42.30% and no TEM-135 and mosaic *penA* genes were found.

Table 7: Detection of selective antimicrobial resistance genes in endocervical swab samples by PCR among *Neisseria gonorrhoeae* positive cases (n=26)

Genes	Number	Percentage
TEM-1 gene	19	73.07
gyrA gene	9	34.61
parC gene	4	15.38
Both *gyrA* and *parC* gene	11	42.30
TEM-135 gene	0	0.00
Mosaic *penA* gene	0	0.00

Table 8 shows comparative results of Gram stain with culture for *N. gonorrhoeae*. Out of 248 samples, 14 (100%) were positive by both Gram stain and culture and 226 (96.58%) were negative by both methods. Of the remaining samples, 8(3.42%) were positive by Gram stain but negative by culture. Considering culture as gold standard, sensitivity and specificity of Gram stain was 100% and 96.58% respectively.

Table 8: Comparison of Gram stain with culture for detection of *Neisseria gonorrhoeae* (n=248)

Gram stain	Culture		Total n (%)
	Positive n (%)	Negative n (%)	
Positive	14 (100.00)	8 (3.42)	22(8.87)
Negative	0 (0.00)	226 (96.58)	226 (91.13)
Total	14(100.00)	226 (100.00)	248 (100.00)

Note: Sensitivity of Grams stain = 100%; Specificity of Grams stain= 96.58%

Table 9 demonstrates comparative results of culture with PCR for *N. gonorrhoeae*. Among 248 suspected cases, 14 (100%) were positive by both PCR and culture and 222 (94.88%) were negative by both methods. Of the remaining samples, 12 (5.12%) were positive by PCR but negative by culture. Considering culture as gold standard, sensitivity and specificity of PCR was 100% and 94.88% respectively.

Table 9: Comparison of culture with PCR for detection of *Neisseria gonorrhoeae* (n=248)

PCR	Culture		Total n (%)
	Positive n (%)	Negative n (%)	
Positive	14 (100.00)	12 (5.12)	26 (10.48)
Negative	0 (0.00)	222 (94.88)	222 (89.52)
Total	14 (100.00)	234 (100.00)	248 (100.00)

Note: Sensitivity of PCR = 100%; Specificity of PCR = 94.88%

Comparative results of Gram stain with PCR for *N. gonorrhoeae* is shown in table 10. Among 248 suspected cases, 22 (100%) were positive by both PCR and Gram stain and 222 (98.23%) were negative by both methods. Of the remaining samples, 4 (1.77%) were positive by PCR but negative by Gram stain.

Table 10: Comparison of Gram stain with PCR for detection of *Neisseria gonorrhoeae* (n=248)

PCR	Gram stain		Total n (%)
	Positive n (%)	Negative n (%)	
Positive	22 (100.00)	4 (1.77)	26 (10.48)
Negative	0 (0.00)	222 (98.23)	222 (89.52)
Total	22 (100.00)	226 (100.00)	248 (100.00)

Table 11 shows MIC of ciprofloxacin among phenotypically detected 13 *N. gonorrhoeae*. Out of 13 ciprofloxacin resistant *N. gonorrhoeae*, 23.08% had MIC of 1µg/ml, 46.15% had MIC of 2µg/ml and 30.77% had MIC of 4µg/ml.

Table 11: MIC of ciprofloxacin among ciprofloxacin resistant *N. gonorrhoeae* by agar dilution method (n=13)

MIC of ciprofloxacin µg/ml	*N. gonorrhoeae* n (%)
≥ 8	0 (0.00)
4	4 (30.77)
2	6 (46.15)
1	3 (23.08)
0.5	0 (0.00)
0.25	0 (0.00)
0.12	0 (0.00)
≤0.06	0 (0.00)
Total	13 (100.00)

Note: CLSI (2016) breakpoint of MIC of ciprofloxacin for *N. gonorrhoeae*: Sensitive≤0.03µg/ml, Intermediate 0.12-0.5µg/ml, Resistant ≥1µg/ml

Table 12 demonstrates MIC of ceftriaxone among phenotypically detected 14 *N. gonorrhoeae*. All the 14 isolated *N. gonorrhoeae* were susceptible to ceftriaxone.

Table 12: MIC of ceftriaxone among ceftriaxone sensitive *N. gonorrhoeae* by agar dilution method (n=14)

MIC of ceftriaxone µg/ml	*N. gonorrhoeae* n (%)
≥ 4	0 (0.00)
2	0 (0.00)
1	0 (0.00)
0.5	0(0.00)
0.25	0(0.00)
0.12	0 (0.00)
0.06	0 (0.00)
≤0.03	4 (28.57)

Note: CLSI (2016) breakpoint of MIC of ceftriaxone for *N. gonorrhoeae*:, Sensitive≤0.25µg/ml

Table 13 shows histopathological findings of 30 cervical biopsy samples. Out of 30 samples, cervical intraepithelial neoplasia (CIN) was detected in 43.33%, squamous cell carcinoma (SCC) was in 36.67% and chronic cervicitis was in 20% cases.

Table 13: Histopathological findings of cervical tissue biopsy samples (n=30)

Histopathological findings	Number	Percentage
CIN*	13	43.33
Squamous cell carcinoma	11	36.67
Chronic cervicitis	6	20.00
Total	30	100.00

*CIN=Cervical intraepithelial neoplasia.

Table 14 demonstrates detection of human papilloma virus (HPV) genotypes among cervical tissue biopsy samples. Out of 30 samples, HPV-16 was found in 13.33% and HPV-18 was found in 3.33% cases.

Table 14: Detection of HPV genotypes among cervical tissue biopsy samples (n= 30)

HPV* genotypes	Number	Percentage
HPV-16	4	13.33
HPV-18	1	3.33
HPV-31	0	0.00
HPV-33	0	0.00
HPV-51	0	0.00
HPV-52	0	0.00
Total	5	16.66

*HPV = Human papilloma virus

Figure 1 shows DNA sequence of amplified PCR product of *cppB* gene of *Neisseria gonorrhoeae* using specific primer.

Figure 1: DNA sequence of amplified PCR product of *cppB* gene of *Neisseria gonorrhoeae* using specific primer

TTCGAAGACCTTCGAGCAGACATCACGCACCGAAGCCGCCAGCATAGAGCAACAA
ACGAAAGCAGACTTAGAGACGTTACGGAAAAATATCAACGAGGCATTGAAGCAAA
GCGAGCAGAAAATAACCGCCGATATAAACGCCCGGCAGTTACGCATGAGCAAGGC
AGTATTCAAGCCCTATCTGTGGAGCTTGCTAGGTATATCGGCGGCAGGGTTGATAG
TCATAGCAGGGCTGTTCATAGCGATATGGAGCGTCAAGAACGAGCTGGACGACTT
GAAACAGCAGAGAGCCGAAGCAGAGCGCACCCTAGACCTGTTGGAAACCAAGACC
AAAG

Figure 2 shows comparison of base sequence of the amplified PCR product of *cppB* gene which was 100% identical with the sequence from *cppB* gene of *Neisseria gonorrhoeae* MS11strain available in gene bank (Accession number CP003910.1).

Figure 2: Comparison of DNA sequence of the amplified PCR product of *cppB* gene of *Neisseria gonorrhoeae* MS11strain available in gene bank (Accession number CP003910.1)

Score	Expect	Identities	Gaps	Strand
616 bits(333)	2e-172	333/333(100%)	0/333(0%)	Plus/Minus

```
Query 3    CGAAGACCTTCGAGCAGACATCACGCACCGAAGCCGCCAGCATAGAGCAACAAACGAAAG  62
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 3118 CGAAGACCTTCGAGCAGACATCACGCACCGAAGCCGCCAGCATAGAGCAACAAACGAAAG3059

Query 63   CAGACTTAGAGACGTTACGGAAAAATATCAACGAGGCATTGAAGCAAAGCGAGCAGAAAA 122
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 3058 CAGACTTAGAGACGTTACGGAAAAATATCAACGAGGCATTGAAGCAAAGCGAGCAGAAAA2999

Query 123  TAACCGCCGATATAAACGCCCGGCAGTTACGCATGAGCAAGGCAGTATTCAAGCCCTATC 182
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2998 TAACCGCCGATATAAACGCCCGGCAGTTACGCATGAGCAAGGCAGTATTCAAGCCCTATC2939

Query 183  TGTGGAGCTTGCTAGGTATATCGGCGGCAGGGTTGATAGTCATAGCAGGGCTGTTCATAG 242
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2938 TGTGGAGCTTGCTAGGTATATCGGCGGCAGGGTTGATAGTCATAGCAGGGCTGTTCATAG2879

Query 243  CGATATGGAGCGTCAAGAACGAGCTGGACGACTTGAAACAGCAGAGAGCCGAAGCAGAGC 302
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2878 CGATATGGAGCGTCAAGAACGAGCTGGACGACTTGAAACAGCAGAGAGCCGAAGCAGAGC2819

Query 303  GCACCCTAGACCTGTTGGAAACCAAGACCAAAG 335
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        ||||||||||||||||||||||||||||||||||
Sbjct 2818 GCACCCTAGACCTGTTGGAAACCAAGACCAAAG 2786
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Figure 3 demonstrates comparison of translated nucleotide base sequence of the amplified PCR product of *cppB* gene which was 100% identical with cryptic plasmid protein B sequence of *Neisseria gonorrhoeae* available in gene bank (accession number is WP 050158798.1).

Figure 3: Comparison of translated nucleotide base sequence of the amplified PCR product of *cppB* gene cryptic plasmid protein B sequence of *Neisseria gonorrhoeae* available in gene bank (accession number is WP050158798.1)

Score	Expect	Method	Identities	Positives	Gaps	Frame
217 bits(553)	2e-70	Compositional matrix adjust.	108/108(100%)	108/108(100%)	0/108(0%)	+2

```
Query 11  FEQTSRTEAASIEQQTKADLETLRKNINEALKQSEQKITADINARQLRMSKAVFKPYLWS 190
          FEQTSRTEAASIEQQTKADLETLRKNINEALKQSEQKITADINARQLRMSKAVFKPYLWS
Sbjct 1   FEQTSRTEAASIEQQTKADLETLRKNINEALKQSEQKITADINARQLRMSKAVFKPYLWS 60

Query 191 LLGISAAGLIVIAGLFIAIWSVKNELDDLKQQRAEAERTLDLLETKTK 334
          LLGISAAGLIVIAGLFIAIWSVKNELDDLKQQRAEAERTLDLLETKTK
Sbjct 61  LLGISAAGLIVIAGLFIAIWSVKNELDDLKQQRAEAERTLDLLETKTK 108
```

Figure 4 shows DNA sequence of amplified PCR product of cryptic plasmid gene of *Chlamydia trachomatis* using specific primer.

Figure 4:DNA sequence of amplified PCR product of cryptic plasmid gene of *Chlamydia trachomatis* using specific primer

CTCTTGTAGAAGTGCATAAACTTCTGAGGATAAGTTATAATAATCCTCTTTTCTGTC
TGACGGTTCTTAAGCTGGGAGAAAGAAATGGTAGCTTGTTGGAAACAAATCTGACT
AATCTCCAAGCTTAAGACTTCAGAGGAGCGTTTACCTCCTTGGAGCATTGTCTGGG
CGATCAACCAATCCCGGGCATTGATTA

Figure 5 shows comparison of DNA sequence of the amplified PCR product of plasmid CtrE-DK-20gene which was 100% identical of *Chlamydia trachomatis* strain E-DK-20 available in gene bank (Accession number CP015305.1).

Figure 5: Comparison of DNA sequence of the amplified PCR product of plasmid CtrE-DK-20gene of *Chlamydia trachomatis* strain E-DK-20available in gene bank (Accession number CP015305.1)

Score	Expect	Identities	Gaps	Strand
355 bits(192)	2e-94	196/196(100%)	1/196(0%)	Plus/Plus

```
Query 1    CTCTTGTAGAAAGTGCATAAACTTCTGAGGATAAGTTATAATAATCCTCTTTTCTGTCTG 59
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1418 CTCTTGTAGAAAGTGCATAAACTTCTGAGGATAAGTTATAATAATCCTCTTTTCTGTCTG1477

Query 60   ACGGTTCTTAAGCTGGGAGAAAGAAATGGTAGCTTGTTGGAAACAAATCTGACTAATCTC 119
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1478 ACGGTTCTTAAGCTGGGAGAAAGAAATGGTAGCTTGTTGGAAACAAATCTGACTAATCTC1537

Query 120  CAAGCTTAAGACTTCAGAGGAGCGTTTACCTCCTTGGAGCATTGTCTGGGCGATCAACCA 179
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1538 CAAGCTTAAGACTTCAGAGGAGCGTTTACCTCCTTGGAGCATTGTCTGGGCGATCAACCA1597

Query 180  ATCCCGGGCATTGATT 195
           ||||||||||||||||
Sbjct 1598 ATCCCGGGCATTGATT 1613
```

Figure 6 demonstrates comparison of translated nucleotide base sequence of the amplified PCR product of cryptic plasmid ORF8 sequence which was 100% identical with *Chlamydia trachomatis* available in gene bank (accession number is AAZ78096.1

Figure 6: Comparison of translated nucleotide base sequence of the amplified PCR product of cryptic plasmid ORF8 sequenceof *Chlamydia trachomatis* available in gene bank (accession number is AAZ78096.1)

Score	Expect	Method	Identities	Positives	Gaps	Frame
130 bits(326)	3e-37	Compositional matrix adjust.	62/62(100%)	62/62(100%)	0/62(0%)	-3

```
Query 194  INARDWLIAQTMLQGGKRSSEVLSLEISQICFQQATISFSQLKNRQTEKRIIITYPQKFM 15
           INARDWLIAQTMLQGGKRSSEVLSLEISQICFQQATISFSQLKNRQTEKRIIITYPQKFM
Sbjct 3    INARDWLIAQTMLQGGKRSSEVLSLEISQICFQQATISFSQLKNRQTEKRIIITYPQKFM 62

Query 14   HF 9
           HF
Sbjct 63   HF 64
```

Figure 7 shows DNA sequence of amplified PCR product of TEM-1 gene of *Neisseria gonorrhoeae* using specific primer.

Figure 7: DNA sequence of **amplified PCR product of TEM-1 gene of *N. gonorrhoeae* using specific primer**

TGCTGAAGATCAGTTGGGTGCCCGGGCGGTAACATCGAACTGGATCTCAACAGCG
GTAAGATCCTTGAGAGYTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT
AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACT
CGGTCGCCGCATACACTAA

Figure 8 shows comparison of base sequence of the amplified PCR product of TEM-1 gene which was 97% identical with the sequence from *N. gonorrhoeae* bla gene for beta-lactamase available in gene bank (accession number KT391485.1). TEM-1 gene had mutation at 22, 32 and 145 position and deletion at 28 and 72 positions.

Figure 8: Comparison of DNA sequence of the amplified PCR product of beta-lactamase TEM-1 variant gene and *N. gonorrhoeae* bla gene for beta-lactamase available in gene bank (accession number is KT391485.1)

Score	Expect	Identities	Gaps	Strand
316 bits(171)	1e-82	181/186(97%)	1/186(0%)	Plus/Plus

```
Query 1   TGCTGAAGATCAGTTGGGTGCCCGGGC-GGTAACATCGAACTGGATCTCAACAGCGGTAA 59
          |||||||||||||||||||| ||||| ||| ||||||||||||||||||||||||||||||
Sbjct 99  TGCTGAAGATCAGTTGGGTGCACGGGCGGGGTTACATCGAACTGGATCTCAACAGCGGTAA 158

Query 60  GATCCTTGAGAG-TTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCT 119
          |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 159 GATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCT 218

Query 120 GCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCAT 179
          |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct 219 GCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCAT 278

Query 180 ACACTA 185
          ||||||
Sbjct 279 ACACTA 284
```

Figure 9 demonstrates comparison of translated nucleotide base sequence of the amplified PCR product of TEM-1 gene which was 91% identical with class A beta-lactamase protein sequence which is available in gene bank (accession number is WP000385876.1).TEM-1 gene had mutation at amino acid position 13,15,16,17 and 19.

Figure 9: Comparison of translated nucleotide base sequence of the amplified PCR product of TEM-1 gene and *N. gonorrhoeae* TEM family class A beta lactamase protein sequence available in gene bank (accession number is WP000385876.1)

Score	Expect	Method	Identities	Positives	Gaps	Frame
109 bits(273)	1e-28	Composition-based stats.	52/57(91%)	53/57(92%)	0/57(0%)	+1

Query 13 **V**G**CPG**G**N**IELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIH 183
+G G IELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIH
Sbjct 26 **LGARVGY**IELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIH 82

DISCUSSION

Cervicitis is an inflammation of the uterine cervix which is frequently asymptomatic, and is generally considered to be associated with sexually transmissible pathogens (Lusk and Konecny, 2008; CDC, 2015). Cervicitis is more common in the women of child bearing age (Mahmood and Saniotis, 2011). In developing countries women tend to suffer more because of the synergistic effects of infection, malnutrition and reproduction (Ranjan *et al.*, 2003). *Neisseria gonorrhoeae* and *Chlamydia trachomatis* account for about half of the causes of cervicitis. Other possible pathogens include *Trichomonas vaginalis, Ureaplasma urealyticum, Mycoplasma genitalium, Herpes simplex virus,* and *Cytomegalovirus* (Nyirjesy. 2001). *N. gonorrhoeae* is the second most prevalent bacterial sexually transmitted infection globally (Nakayama *et al.*, 2012). About more than 66.7% women with gonococcal infection (Detels *et al.*, 2011) and upto 80% women of Chlamydial infections are asymptomatic which make diagnosis and detection difficult (Watson *et al.*, 2002). *Mycoplasma* and *Ureaplasma* have been associated with cervicitis (Larsen and Hwang, 2010). *Trichomonas vaginalis* is another causative agent for cervicitis (Riley *et al*, 1992) and it is associated with adverse pregnancy outcomes such as premature rupture of membranes, preterm delivery and low birth weight (Madhivanan *et al.*, 2009). Gonococcal strains are resistant to sulphonamides, penicillins, tetracyclines, fluroquinolones and recently resistant to oral 3rd generation of cephalosporins have emerged (Patel *et al.*, 2011). In this current study, Gram stain, culture and PCR identified 8.87%, 5.64% and 10.48% *N. gonorrhoeae* respectively. Kazi (2005) showed that Gram staining identified 7.5% *N. gonorrhoeae* and

Akhter *et al*. (2016) reported culture identified 7.82% isolates. A study conducted by Nusrat (2013) reported that 9.79%, 12.65% and 15.91% *N. gonorrhoeae* were identified by Gram stain, culture and PCR respectively. In this study, among 26 *N. gonorrhoeae* positive cases, 30.77% were identified by only Gram stain (yielded no growth in culture), 15.39% were identified by PCR alone (both Gram stain and culture negative) and remaining 53.84% were identified by Gram stain, culture and PCR. Jahan *et al*. (2014) reported that only Gram stain positive samples were 27.57% and only PCR positive samples were 20.51% (Nusrat, 2013). The rate of *N. gonorrhoeae* isolation by culture was low and that might be due to frequent use of antibiotic by the patient caused reduction of bacterial loads such that it was undetectable in culture and the fastidious nature of the organisms. Gram stain also identified 26.2% pus cells (>10WBC/HPF) from cervical swab in this study. Taylor *et al*. (2013) found 23% polymorph (≥30WBC/HPF) by Gram stain in cases of mucopurulent cervicitis. In the present study, 4.43% *T. vaginalis* were diagnosed by wet film microscopy. Kaur *et al*. (2008) reported that 4.28% symptomatic and 3.66% asymptomatic patient were infected with *T. vaginalis* in North India. Andrea and Chapin (2011) showed 5.1% women were infected by *T. vaginalis*. Female infected with *T. vaginalis* was 3.1% in Bangladesh and USA (Rahman *et al*. 2012; Meites *et al*., 2013). The overall prevalence of *T. vaginalis* was 4.1% among women in Brazil (Gatti *et al*., 2017). These results are in accordance with the present study. In this study, out of the 248 suspected cases, 26 (10.48%) were positive for gonococcal infection. Divekar *et al*. (2000) reported that 9.7% women were positive for *N. gonorrhoeae*. Prevalence of *N. gonorrhoeae* was 11% in cervical specimens (Darwin *et al*., 2002). Tibedu *et al*. (2013) showed that *N. gonorrhoeae* positive cases were 8.23%. Das *et al*. (2011) reported that 14.1% female sex workers were infected by gonorrhoea in India. In Bangladesh, Nusrat *et al*. (2014) observed the rate of gonococcal infection among women was 15.9%. Ali *et al*. (2016) presented prevalence of *N. gonorrhoeae* was 11.3 %. These studies are correlated with the present study. In this study, 6.45% *C. trachomatis* were identified by PCR. Dwibedi *et al*. (2009) found the prevalence of *C. trachomatis* was 7.04% by PCR. Bikshapathi *et al.* (2012) reported 6.15% chlamydial infection occurs in India. The prevalence of *C. trachomatis* among women was 6.7% in the USA

(Ginocchio *et al*., 2012). Betha *et al*. (2016) showed the rate of *C. trachomatis* was 7.6% among symptomatic and asymptomatic women. These are in accordance with the present study. In this study, 2.01% *U. urealyticum* was identified by PCR. In Iran, *U. urealyticum* prevalence was 4.28% (Seifoleslami *et al*., 2015). Akhter (2016) found 1.68% *U. urealyticum* in her study. Cervicitis caused by HSV-2 was 5.4% and no Adenovirus have been reported (McIver *et al*., 2009; Pereira *et al*., 2012). However no such viral DNA was detected in any of the cervical swab samples in this study. In the present study, 100% of the isolated *N. gonorrhoeae* were resistant to tetracycline and doxycycline. Ahmed *et al*. (2010) reported 86% of the isolates were resistant to tetracycline and prevalence increase from 30% to 95% during 1997-2006. Chen *et al*. (2016) demonstrated that resistance to tetracycline increased from 68.3% to 82.4% in China. More than 90% isolates were found resistant to doxycycline in Bangladesh (Khanam *et al*., 2016). Between 2011-2015, the prevalence of high resistance of tetracycline increased from 73% to 91% in South Africa (Kularatne *et al*., 2017). Resistance to tetracycline and doxycycline were high due to selective pressure produced by the use of tetracycline to treat other infections and its use as adjunct therapy in the syndromic management of STDs (Bala *et al*., 2011). Ciprofloxacin resistance of *N. gonorrhoeae* was 92.86% in this study. A study conducted by Ahmed *et al*. (2010) showed ciprofloxacin resistant isolates were 87% in 2006 compared to 9% in 1997 with the highest resistance 92% in 2003 from different parts of Bangladesh. Sethi *et al*. (2013) showed that 94% isolates were resistant to ciprofloxacin in India, Pakistan and Bhutan. In China, fluoroquinolone resistant *N. gonorrhoeae* was 93.8 % (Chen *et al*., 2016). These results are similar with the present study. Khanam *et al*. (2016) reported that 95.2% isolates were resistant to ciprofloxacin. Kulkarni *et al*. (2018) found that 98% *N. gonorrhoeae* were resistant to ciprofloxacin in India during 2013-2016. This high rate of fluoroquinolone resistance might be an indicator of the overuse and misuse of this antibiotics (Sethi *et al*., 2013). In this study, Penicillin resistant *N. gonorrhoeae* was 85.71%. A study conducted by Jabeen *et al*. (2011) showed that 86.8% *Neisseria* were resistant to penicillin in Pakistan. Chen *et al*. (2016) reported that 84.2% *N. gonorrhoeae* were resistant to penicillin in China. These results are similar with this study. Akhter (2016) found penicillin resistant *N. gonorrhoeae* was 92.86% in Bangladesh. 90.6%

isolates were resistant to penicillin in Germany (Regnath *et al*., 2016). Ray *et al*. (2005) showed penicillin resistant *N. gonorrhoeae* were 20%-79% in Indian laboratories. In this study, Antimicrobial susceptibility pattern showed that *N. gonorrhoeae* was 85.72% and 7.14% resistant to erythromycin and azithromycin respectively. Ahmed *et al*. (2010) found that only 0.2% isolates were resistant to azithromycin. Sethi *et al.* (2013) observed resistant to erythromycin and azithromycin were 62% and 7.7% respectively. Muhammad *et al*. (2014) showed 6.5% isolates were resistant to azithromycin in Sweden. Akhter (2016) reported 100% isolates were resistant to erythromycin and 7.14% were resistant to azithromycin in Bangladesh. Regnath *et al*. (2016) conducted a study where 7.1% strains were resistant to azithromycin in Germany. These findings coincide with the result of this study. Whiley *et al*. (2017) showed that azithromycin resistance in *N. gonorrhoeae* was 0.2% in the northern territory of Australia which is lower than present study. In this present study, 100% isolates of *N. gonorrhoeae* were susceptible to ceftriaxone and cefixime. Khanam *et al*. (2016) presented 100% and 85.7% *N. gonorrhoeae* was susceptible to ceftriaxone and cefixime respectively. Chen *et al*. (2016) showed 0.5% isolates were resistant to ceftriaxone in China. More than 99% isolates were susceptible to extended spectrum cephalosporins and decreased cefixime susceptibility was not observed in South Africa (Kularatne *et al*., 2017). 1.4% and 1.61% isolates showed decreased susceptibility to ceftriaxone and cefixime in Australia and India respectively (Whiley *et al*., 2017; Kulkarni *et al*., 2018). These results correlated with the results of this present study. In this study, 71.42% isolates were found penicillinase producing *N. gonorrhoeae* (PPNG). WHO estimated in western pacific region that prevalence of PPNG varies from 1% to 90% (Tapsall *et al*., 2003). Ahmed *et al*. (2010) showed 44% isolates were PPNG in Bangladesh. Nakayama *et al*. (2012) found 79.33% PPNG in Japan. Bharara *et al*. (2015) reported prevalence of PPNG was 88% in India. Zhen *et al*. (2015) observed PPNG was 47.1% in China. Akhter (2016) found 92.85% PPNG in Bangladesh. Tribuddharat *et al*. (2017) reported 83.8% isolates were PPNG in Thailand. As penicillin is not been used to treat gonorrhoea now a days in Bangladesh, penicillinase production by *N. gonorrhoeae* is decreased. This might be the region of low prevalence of PPNG in this study. Out of 26 *Neisseria* positive samples, 73.07% TEM-1 gene were identified and no TEM-135 genes were

detected in this study. Nakayama *et al.* (2012) found 90.6% TEM-1 gene and 9.4% TEM-135 gene among *Neisseria* positive cases. Muhammad *et al*. (2014) showed 74.8% *N. gonorrhoeae* isolates possessed TEM-1 gene. Tribuddharat *et al*. (2016) reported prevalence of beta-lactamase positive gonococci carried 69.6% TEM-1 gene. These results are correlated with this study. Akhter (2016) showed no TEM-135 gene in *N. gonorrhoeae* in her study in Bangladesh. DNA sequences of TEM-1 gene were done to compare with the sequence from *N. gonorrhoeae* bla gene for beta-lactamase available in gene bank. In this study sequencing result of TEM-1 gene showed that the amplified PCR product of positive samples using specific primer of TEM-1 gene was 97% identical to the *Neisseria gonorrhoeae* TEM family class A beta-lactamase protein sequence available in gene bank (accession number is WP000385876). TEM-1 gene exhibited mutation at amino acid position 13,15,16,17 and 19. In this study, PCR was done to identify ciprofloxacin resistant genes *gyrA* and *parC* among 26 *N. gonorrhoeae* positive cases. Among these positive isolates *gyrA*, *parC* and both *gyrA* and *parC* were detected in 34.61%, 15.38% and 42.30% cases respectively. Akhter (2016) found ciprofloxacin resistant genes *gyrA*, *parC* and both *gyrA* and *parC* were 26.53%, 16.33% and 46.94% respectively. In the present study, mosaic *penA* gene were not identified by PCR which had an association with decreased susceptibility to cephalosporins. Pandori *et al*. (2009) reported that mosaic *penA* genes were found in 9.25% cases. Ochiai *et al*. (2008) found that 41.3% mosaic *penA* gene was detected in Japan. The absence of mosaic *penA* gene in *N. gonorrhoeae* in this study might be due to difference in geographical distribution of these genes. Out of 248 suspected cases of cervicitis, 14 were positive by both Gram stain and culture, 8 were positive by Gram stain but negative by culture. Considering culture as gold standard sensitivity of Gram stain was 100% and specificity was 96.58%. Akhter (2016) shows sensitivity of Gram stain was 100% and Nusrat (2013) shows specificity was 98.13% in detecting cervicitis. In the present study, *N. gonorrhoeae* was identified directly from specimen by PCR. Out of 248 samples, 12 were positive by PCR but negative by culture. Considering culture as gold standard the sensitivity of PCR was 100% and specificity was 94.88%. Diemart *et al*. (2002) presented sensitivity of PCR was 100% and specificity was 99.9% in Canada. Mayta *et al.* (2006) reported that PCR showed sensitivity and specificity was 100%

and 99.7% respectively compare with culture. Nusrat (2013) showed sensitivity and specificity of PCR was 100% and 96.26% respectively. Jahan *et al*. (2014) reported that sensitivity of PCR was 100% and specificity was 94.85% which were similar with this study. In this study, MIC of ciprofloxacin among the ciprofloxacin resistant *N. gonorrhoeae* ranged from 1µg/ml to ≥4µg/ml, out of which 23.08% had MIC≥1 µg/ml, 46.15% had MIC of ≥2µg/ml and 30.77% had MIC ≥4µg/ml. Zhao and Zhao (2013) found that 100% had MIC ≥1µg/ml and 63.55% had MIC ≥4µg/ml. Sood *et al*. (2017) showed that overall MIC ≥1µg/ml was seen in 96.4% isolates in India and 28.31% had MIC ≥4µg/ml which correlated with this study. All culture positive strain of *N. gonorrhoeae* were susceptible to ceftriaxone and 28.57% had MIC ≤0.03µg/ml. Crannante *et al*. (2012) showed that all strains of *N. gonorrhoeae* were fully susceptible to ceftriaxone with a MIC range of 0.002-0.094 µg/ml. Li *et al*. (2014) found all isolates were sensitive to ceftriaxone with MIC ≤0.25 µg/ml. Kulkarni *et al*. (2018) reported that 1.61% showed decreased susceptibility to ceftriaxone and cefixime but no resistant strains were found. In the present study base sequence of the amplified PCR product of *cppB* gene was 100% identical with the sequence from *cppB* gene of *Neisseria gonorrhoeae* MS11strain available in gene bank (Accession number CP003910.1). Also comparison of translated nucleotide base sequence of of the amplified PCR product of *cppB* gene which was 100% identical with cryptic plasmid protein B sequence of *Neisseria gonorrhoeae* available in gene bank (accession number is WP 050158798.1). *cppB* gene had no mutation or deletion in its nucleotide base sequence. In this study base sequence of the amplified PCR product of plasmid CtrE-DK-20gene was 99% identical of *Chlamydia trachomatis* strain E-DK-20 available in gene bank (Accession number CP015305.1). Plasmid CtrE-DK-20gene had deletion at 10 position. Also comparison of translated nucleotide base sequence of the amplified PCR product of cryptic plasmid ORF8 sequence which was 100% identical with *Chlamydia trachomatis* available in gene bank (accession number is AAZ78096.1). Cryptic plasmid gene had no mutation or deletion in its nucleotide base sequence. In the present study, out of 30 cervical biopsy samples, histopathology report showed that 20% were chronic cervicitis, 43.33% were cervical intraepithelial neoplasia (CIN) and 36.67% were squamous cell carcinoma (SCC). Among these 30 cervical biopsy

samples, HPV-16 and HPV-18 were in 13.33% and 3.33% cases respectively. Prevalence of human papillomavirus (HPV) infection varies from 7-14% (Sankaranarayanan *et al*., 2008).Yuan *et al*., (2011) showed that HPV prevalence was 14.2% in China. Banik *et al*. (2013) found 14.9% HPV-16 infection in cervical biopsy samples. Srivastava *et al*. (2014) reported 10% cervicitis cases were positive for HPV-16. Khandkar *et al*. (2016) found 18.2% HPV among chronic cervicitis cases. Abedin *et al*. (2018) reported that prevalence of HPV-16 and HPV-18 were 13.75% and 2.5% respectively among VIA positive cases in Bangladesh. HPV-16 is the most prevalent strain detected from cervical cancer followed by HPV-18 (Clifford *et al*., 2003). About 70% cases of cervical cancer and 86-95% cases of non cervical cancer (vulvar, vaginal, penile and anal cancers) are caused by HPV-16 and HPV-18 strains (Gillison *et al*., 2008).

CONCLUSION

Neisseria gonorrhoeae was the most common cause of cervicitis followed by *C. trachomatis, T. vaginalis* and *U. urealyticum* in endocervical swabs. HSV-2, *Adenovirus* and *Mycoplasma genitalium* also were not detected in any of cervical swab samples in this study. Antimicrobial sensitivity pattern of *N. gonorrhoeae* showed that 92.86% *N. gonorrhoeae* were resistant to ciprofloxacin and 100% were sensitive to cefixime and ceftriaxone. Penicillinase producing *N. gonorrhoeae* were identified in 71.42% in this study and no mosaic *penA* and TEM-135 gene were identified which were related to decreased susceptibility to cephalosporins. Among different procedures, PCR had the highest sensitivity to detect DNA of *N. gonorrhoeae* directly from endocervical swab samples. Multiplex PCR may be recommended specially for diagnosis of cervicitis caused by *C. trachomatis* and *U. urealyticum.* HPV-16 was the most commonly (13.33%) identified strain in cervical biopsy specimens followed by HPV-18 (3.33%). No HPV-31, HPV-33, HPV-51 and HPV-52 were detected in cervical biopsy samples in this study.

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