IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 24, Issue 3 Ser. 7 (March. 2025), PP 01-10
www.iosrjournals.org

Detection of Bacterial Causes of Urethritis in Men by Multiplex Polymerase Chain Reaction

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Abstract:
Background: *Urethritis is an important sexually transmitted infection in the present day. Neisseria gonorrhoeae and Chlamydia trachomatis are the most common bacterial causes of urethritis. Men with suspected urethritis should undergo evaluation to confirm the causes of urethritis for prevention of its complications and spread of this disease.*
Aim: *This cross-sectional study was conducted to detect Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium and Ureaplasma urealyticum for diagnosis of gonococcal and non-gonococcal urethritis from urethral discharge. Comparison of different microbiological techniques used for diagnosis of gonococcal urethritis was also studied here.*
Materials and methods: *A total 142 samples were collected from clinically suspected urethritis patients attending outpatient department of Skin and Venereal disease of Chattogram Medical College Hospital (CMCH) and different diagnostic centers during the period of July 2017 to June 2018. Neisseria gonorrhoeae was detected by Gram staining, culture in Chocolate agar media and Thayer Martin media and polymerase chain reaction (PCR). Urethral discharge was also tested for DNA of Chlamydia trachomatis, Mycoplasma genitalium and Ureaplasma urealyticum by multiplex PCR.*
Results: *Among the study population, the highest cases were in the age group 21-30 years. Most of the cases were day-laborer (40.84%) and were from lower income group (38.73%). Out of 142 samples, 39(27.46%) were positive in Gram stain and 35(24.65%) yielded growth in culture media and 62(43.66%) cases were positive by PCR. Of them, 45(31.69%) were Neisseria gonorrhoeae, 17(11.97%) were Chlamydia trachomatis and 3(2.1%) cases were positive for both Neisseria gonorrhoeae and Chlamydia trachomatis. In this study, no sample was found positive for DNA of Mycoplasma genitalium and Ureaplasma urealyticum. Considering culture as gold standard, the sensitivity of PCR was 100%, specificity was 90.65%, positive predictive value was 77.78%, negative predictive value was 100% and accuracy was 92.98%. Six smear negative and ten culture negative samples were positive by PCR.*
Conclusion: *The gonococcal urethritis was the common type of infection identified in this study. Mixed infections were also identified. PCR was the most sensitive and rapid method for the diagnosis of causes of urethritis. Multiplex PCR may be considered as a suitable method for early and accurate diagnosis of gonococcal and non-gonococcal urethritis in men from a single specimen and providing a more cost-effective way of screening multiple pathogens.*
Key Word: *Urethritis, Multiplex PCR, DNA*

Date of Submission: 21-03-2025 Date of Acceptance: 31-03-2025

I. Introduction

Sexually transmitted diseases are one of the most common causes of illness and are important causes of morbidity and mortality, particularly in developing countries [1]. Urethritis is one of the most common STD syndromes diagnosed in men [1]. An estimated 340 million new cases of curable sexually transmitted infections occur world-wide each year, with the largest proportion in the region of South and South East Asia, followed by Sub-Saharan Africa, Latin America and the Caribbean [2]. In United States, approximately 15 million people become infected with one or more STDs each year, often causing severe consequences and adding billions of dollars to health care cost [3]. More than 1 million people acquire a STI every day, with the largest proportion in the region of south and southeast Asia [4]. Neisseria gonorrhoeae and Chlamydia trachomatis are the two most common bacterial causes of STDs [5]. Gonorrhoea is one of the most common STDs in developing countries and is a global health problem [6]. Globally, 88 million new cases of gonorrhoea occur each year [7]. In the United States, gonorrhoea is consistently the second-most frequently notifiable infection with a rate of 100.8 cases per 100,000 population [8]. With the advent of technologies such as nucleic acid amplification

(NAAT), major improvements in the ability to detect these STIs have occurred [9-12]. PCR based tests have generally been more sensitive than traditional tests in diagnosis of C. trachomatis and N. gonorrhoeae [13-16]. Proper diagnosis and a standard treatment regimen need in community to eradicate the infection and to prevent the development of complications and also to keep important public health benefit to decreasing transmission and eliminating the reservoirs of infection.

II. Material And Methods

The study was carried out in the department of Microbiology, Chattogram Medical College, Chattogram. Collection of specimens and laboratory works were carried out during the period of July 2017 to June 2018. The study was a descriptive type cross-sectional study.

Study population:
A total 142 sexually active males of 20-60 years age group attending skin and venereal disease outpatient department of Chattogram Medical College Hospital for treatment of genitourinary problems were enrolled.

Inclusion criteria:
Sexually active male patients suggestive of urethtitis having urethral discharge.

Exclusion criteria:
1) Patients who have no urethral discharge.
2) Patients unwilling to give consent are excluded from the stud

Sample size:
The required sample size for the proposed study had been calculated by using the following formula:
n = Z2pq / d2
Prevalence of Urethritis in STD patients is 42.05 % [17].
Putting the value into above equation: n = 374.44.

Data collection:
Data regarding age, occupation, marital status, uro-genital symptoms, history of sexual practices, monthly income, level of education, antibiotic history were collected using predesigned data collection sheet. Consent from every individual was taken with assurance of maintenance of secrecy. Socio economic status of study population was ascertained by interviewing the patients regarding their monthly income and grouped them according to State of children of the world 2007, UNICEF. The study populations were classified into low income, lower middle income, upper middle income and higher income group. Those who had monthly income BDT<5800 were of lower income group, BDT 5801 to BDT 23000 were lower middle-income group, BDT 23001 to BDT 71500 were upper middle income group and BDT >71501 were higher income group.

Data collection tool:
A structured questionnaire was developed to collect data from the patient.

Data analysis plan:
The results of the experiments were recorded systematically and data were analyzed by computer using SPSS 23.0. Statistical analysis was done by standard statistical procedure. A p value of <0.5 is considered to be significant.

Ethical consideration:
It was approved by the Ethical Review Committee (ERC) of Chattogram Medical College. All the patients were well informed and will be explained about the nature of the study. Informed written consent was taken from all the subjects after full explanation of the nature and purpose of the procedures before sample collection.

Sample collection:
Urethral discharge was collected from the patients suffering from clinically suspected urethritis. Specimens were collected by cleaning around the urethral opening using swab moistened with sterile normal saline and gentle massage along the line of urethra from above downwards [18]. Urethral discharge was collected at least one hour after the patient had urinated. Three sterile cotton swab sticks were soaked with urethral discharge directly from each patient. If no discharge was evident, the urethra was stropped towards the orifice to evacuate exudates. If no exudates were obtained then specimen was obtained by passing the thin swab 2-3 cm deep to

urethra [19]. First swab was used for making a smear of the discharge on two separate clean glass slides, the second swab was used for culture and third swab for PCR.

Preservation of specimen for Polymerase chain reaction (PCR):
One of the urethral swabs from each patient was collected into a screw capped test tube containing 2 ml of phosphate buffered saline (PBS). The urethral secretions were released by pressing the swabs against tube wall firmly, transfer into micro centrifuge tube by pipetting and store at -20° for PCR.

Gram staining:
Smears were prepared at the collection site on properly labeled clean glass slides. The smear was prepared by gently rolling the swab on the slide or directly discharges on the slide. The prepared smear was fixed with methanol and stains by Gram´s stain and methylene blue stain and examine with the 100X objective to detect pus cells, intracellular or extracellular Gram-negative diplococci. The specimen of urethral discharge was inoculated at collection site on Chocolate agar media and Thayer Martin (TM) media with proper labeling. The inoculation was done in such a way that all area of the swab was inoculated. The inoculated plates were kept in CO2 extinction jar with soaked paper towel at the bottom and lid of the jar was closed tightly. Each time the jar was opened to keep a new inoculated plate, the candle was flamed.

Culture and isolation:
Inoculated plates in the candle extinction jar were incubated at 37˚C in a humid atmosphere. Culture plates were examined after 24 hours of incubation for growth of Neisseria gonorrhoeae. If there is no growth, the plates were examined again following additional 24 hours of incubation. Presumptive identification of the colonies of N. gonorrhoeae on Chocolate agar media and Thayer Martin media were done by colony morphology, oxidase test, superoxol test and gram staining from growth and confirmatory identification was done by carbohydrate utilization test.

Presumptive identification:
Colony morphology: Suspected colonies of N.gonorrhoeae after 24 hours of incubation were 0.5 to 1 mm in diameter, appearing gray to white, opaque, raised and glistering.

Oxidase test: A piece of filter paper was soaked with a few drops of freshly prepared oxidase reagent. A single suspected colony was picked up with a sterile platinum wire loop, cotton swab or a wooden applicator stick and rubbing it into a filter paper freshly impregnated with oxidase reagent. In a positive reaction with young culture, a purple colour change within 10 seconds. The test was considered negative when there was no colour change or delayed colour change. N. gonorrhoeae was oxidase positive [18].

Superoxol test: The superoxol test is a modification of catalase test [20] and was performed by placing a harvest of suspected colonies on Chocolate media and Thayer martin media with a sterile toothpick into a drop of 30% w/v hydrogen peroxide contained in a small clear test tube. A positive test was defined as immediate, brisk bubbling from the surface of the bacterial colonies dipped with toothpick and a negative test was considered when there was no bubbling and/ or weak bubbling. N.gonorrhoeae was superoxol positive.

Gram staining: A suspension of the characteristics colonies giving positive result with oxidase test and superoxol test were smeared on a clean grease free slide and fixed by passing through a flame and was stained by Grams staning method and examined under microscope. Gonococci were identified as typical Gram-negative kidney shaped diplococci.

Preservation of the isolates of N. gonorrhoeae:
A thick suspension of pure culture of the isolates from an overnight was dispended growth on chocolate agar will be made in sterile 20% glycerol broth. The suspension was dispended in screw capped vial with about 0.5 ml amount and store at -70°c.

Confirmatory identification:
The isolates with presumptive identification were confirmed by Rapid Carbohydrate Utilization Test (RCUT) which differentiates N. gonoeehoeae from other Neisseria species.

Procedure:
Two full 3mm loop full of the isolates from a pure 24 hours culture were emulsified into a test tube containing 0.3 to 0.4 ml buffered salt solution. 50 µl of 10% sterile glucose, lactose, sucrose, maltose were taken to four test tubes. Finally, 50 µl bacterial suspension was added to each of the test tube and mix well. The test tubes were incubated in a water bath for 2-4 hours. A color change from red to yellow was considered as positive. On carbohydrate utilization test N.gonorrhoeae fermented only glucose.

Antimicrobial susceptibility test:
All N.gonorrhoeae isolates were tested for antimicrobial susceptibility by Kirby-Bauer modified disc-diffusion technique and following CLSI guidelines [21]. Chocolate agar media was use for disk diffusion test. The antimicrobial discs were used according to the standard panel for isolated organisms. Antibiotic discs were obtained from commercial source (Oxoid Ltd, UK). Penicillin (10 unit), tetracycline (30µg/disc), ciprofloxacin (5µg/disc), azithromycin (15µg/disc), ceftriaxone (30µg/disc), and cefixime (5µg/disc), spectinomycin (100µg/disc) were used.

Preparation of inoculums:
Using a sterile wire loop, 3-5 well isolated colonies of test organism were emulsified in 3 ml of normal saline. The turbidity of suspension was compared with McFarland turbidity standard by adding normal saline and placing a printed card behind the test and standard inoculums under proper light.

Inoculation of test organism:
Within 10 minutes after standardization of inoculums a sterile cotton swab was immersed into the bacterial suspension. The excess suspension was removed by rotating the swab with firm pressure against the inner side of the tube above the fluid level. The swab was streaked evenly on the surface of Chocolate agar plate in 3 different directions by rotating the plate approximately 60º angle each time to get uniform distribution of inoculums. Inoculated plate was allowed to dry for 3 -5 minutes. Within 30 minutes of placement of antibiotic disc, inoculated plates were incubated in a humid atmosphere with 5-10 % CO2 inside a candle jar at 37º C for 24 hours. After overnight incubation each plate was examined and the zone of inhibition was measured in mm using a ruler on the under surface of the plate and evaluated with standard chart of CLSI guideline [21]. The presence or absence of clear zone of inhibition around the disc was interpreted as sensitive and resistant respectively.

Preservation of the disc:
Discs were kept at 2º-8º C. Prior to use, the container was left at room temperature for half an hour to minimize condensation resulting from warm air reaching the cold container.

The multiplex PCR method [22-25]
This involved several key steps to detect specific genes from clinical samples. First, DNA is extracted using the Thermo Scientific GeneJET Genomic DNA Purification Kit. The DNA is then amplified in a thermal cycler using specific primers for target genes. For the amplification process, different primer sets are used for each target organism, such as N. gonorrhoeae (cppB gene), C. trachomatis (Cryptid plasmid), U. urealyticum (urease gene), and M. genitalium (adhesin gene), with the expected product sizes being 390 bp, 241 bp, 429 bp, and 865 bp, respectively. The PCR reaction mixture includes mastermix containing dNTPs, Taq polymerase, MgCl2, and PCR buffer, along with primers and DNA template. For multiplex PCR, the forward and reverse primers for all the target genes are combined, and the total reaction volume is 25 µl. The amplification process involves 36 cycles with an initial denaturation step at 94°C for 10 minutes, followed by a denaturation at 94°C, an annealing step at 55°C, and an amplification step at 72°C. The final extension occurs at 72°C for 10 minutes. After amplification, the PCR products are analyzed using gel electrophoresis, and the results are visualized under UV light to confirm the presence of the target genes. After electrophoresis, the gel was taken out carefully from the gel chamber and gel gently placed on the UV transilluminar in the dark chamber of the image documentation system. The UV light of the system was switched on and the image was viewed on the monitor by transilluminar and printed on glossy paper.

III. Result

The study population, consisting of 142 individuals, had a mean age of 30.71 ± 7.48 years, with the majority (51.40%) falling within the 21-30 age group. The age distribution showed that 9.15% were under 20 years, 32.40% were in the 31-40 age group, 4.23% were between 41-50 years, and 2.82% were between 51-60 years.

In terms of occupation, most participants were day-laborers (40.84%), followed by those in service occupations (22.54%), business (12.68%), and driving (9.86%), with fewer students (6.34%) and unemployed individuals (7.74%).

Regarding socioeconomic status, 38.73% of the population belonged to the lower income group, 29.58% were in the lower-middle income group, 21.13% were in the upper-middle income group, and 10.56% were from the high-income group. The infection rates were highest in the lower-income group, with 43.55% of individuals in this group infected, followed by 30.65% in the lower-middle income group, 20.97% in the upper-middle income group, and 4.83% in the high-income group.

Microscopic examination revealed that 27.46% of the cases were positive for Gram-negative diplococci among the 142 participants with urethral discharge.

Result of culture of *Neisseria gonorrhoeae* isolates from urethral discharge shows 35 (24.65%) cases were positive for culture. (Figure: 1)

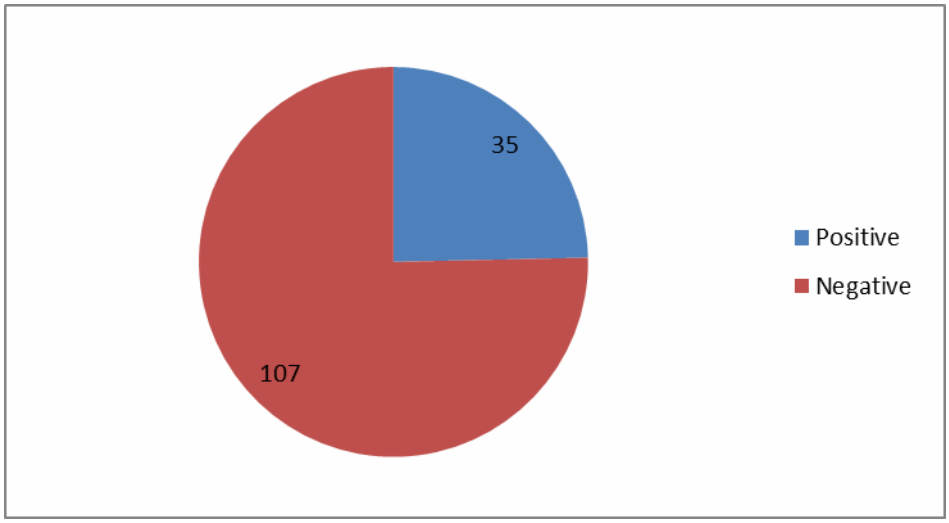

Fig: 1 Pie chart showing results of culture of *Neisseria gonorrhoeae* isolates from urethral discharge

Table: I showing total cases of *Neisseria gonorrhoeae* infection in Gram stain and culture. Total 41 cases were positive in Gram stain and culture. Among them, 33 were found in both Gram stain and culture, 06 were only in Gram stain and 02 cases were only in culture.

Table-I: Total cases of *Neisseria gonorrhoeae* infection in Gram stain and culture (n=41)

Findings	Number	Percent (%)
Both Gram stain and culture	33	80.49
Only Gram stain	06	14.63
Only culture	02	04.88
Total	**41**	**100.00**

Table: II shows comparison between the results of Gram stain and culture for detection of *Neisseria gonorrhoeae*. Out of 142 samples, 33 cases were positive by both Gram stain and culture, whereas 101 cases were negative by both methods.

Table-II: Comparison of results of gram stain and culture for detection of *Neisseria gonorrhoea* (n=142)

Gram stain	Culture		No of cases
	Positive	Negative	
Positive	33	06	39
Negative	02	101	103
Total	35	107	142

Table: III shows molecular detection of different bacteria from urethral swab by PCR. A total 62(43.66%) cases were detected, in which 45(31.69%) cases were *N.gonorrhoeae* and 17(11.97%) cases were *C.trachomatis*. Among the isolated cases 3(2.1%) cases positive for both *N.gonorrhoeae* and *C.trachomatis.*

Table-III: Molecular detection (DNA) of different bacteria from urethral swab by PCR (n=142)

DNA of organisms	Total samples n (%)
Neisseria gonorrhoeae	45 (31.69%)
Chlamydia trachomatis	17 (11.97%)
Ureaplasma urealyticum	00
Mycoplasma genitalium	00
Total	**62 (43.66%)**

Three (2.1%) cases co-infected with both *Neisseria gonorrhoeae* and *Chlamydia trachomatis.*
Table: IV shows the comparison between the results of Gram stain with PCR. Of the 142 samples, 39 were positive by both Gram stain and PCR, whereas 97 were negative by both methods. Remaining 6 samples were positive by PCR but negative by Gram stain. The sensitivity of PCR was 100% and specificity was 94.17%.

Table-IV: Comparison of results of Gram stain with PCR for detection of *Neisseria gonorrhoeae* (n=142)

PCR	Gram stain		No of cases
	Positive	Negative	
Positive	39	06	45
Negative	00	97	97
Total	**39**	**103**	**142**

In case of PCR
Sensitivity : 100%
Specificity : 94.17%
Positive predictive value : 86.66%
Negative predictive value : 100%

Table: V shows the comparison between the results of culture with PCR. Out of the 142 samples, 35 were positive by both PCR and culture and 97 cases were negative by both methods. Remaining 10 samples were positive by PCR but negative by culture. Considering culture as gold standard, the sensitivity of PCR was 100%, specificity was 90.65%, positive predictive value was 77.78%, negative predictive value was 100% and accuracy was 92.98%.

Table-V: Comparison of results of culture with PCR for detection of *Neisseria gonorrhoeae* (n=142)

PCR	Culture		No of cases
	Positive	Negative	
Positive	35	10	45
Negative	00	97	97
Total	**35**	**107**	**142**

In case of PCR
Sensitivity : 100%
Specificity : 90.65%
Positive predictive value : 77.78%
Negative predictive value : 100%
Accuracy : 92.98%

Figure: 2 shows antibiotic sensitivity pattern of isolated *N.gonorrhoeae.* Among the 35 isolates, most susceptible antibiotic is Spectinomycin (100%) followed by Azithromycin (91.43%) and Ceftriaxone (88.57%). The most resistant antibiotic is Tetracycline (97.14%) followed by Ciprofloxacin (94.29%) and Penicillin (82.86%).

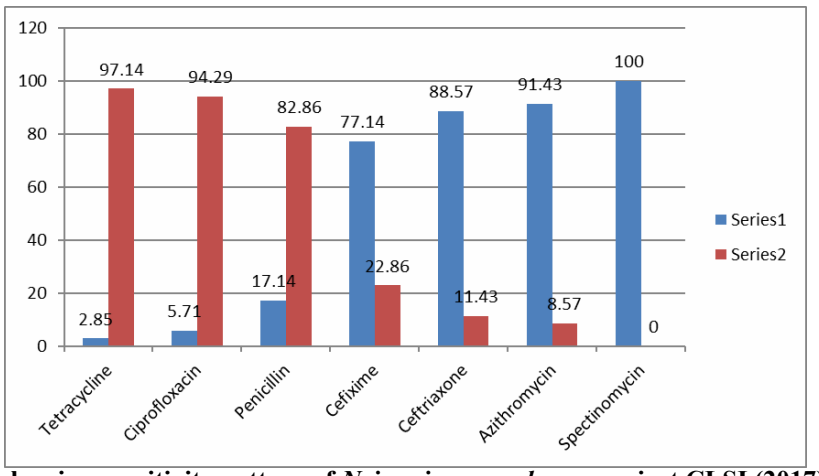

Fig: 2 Bar chart showing sensitivity pattern of *Neisseria gonorrhoeae* against CLSI (2017) recommended antibiotics.

IV. Discussion

Male urethritis is an inflammation of the urethra and the periurethral glands which is widely classified as gonococcal or non-gonococcal. The most frequent microorganisms are *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* [26].

In the last three decades, the diagnosis of sexually transmitted diseases depended almost exclusively on traditional methods, such as culture, enzyme immunoassay, fluorescent antibody staining and hybridization, until the appearance of molecular techniques. Recent studies showed, multiplex PCR is the fastest and most sensitive technique for the diagnosis of gonococcal and non-gonococcal urethritis [26].

In the present study, 142 male patients having urethral discharge were enrolled for the detection of bacterial causes of urethritis. It was observed that 51.40% of the cases were in 21-30 years of age. Similar study from Bangladesh found the highest age group was belonged to 21-30 years [27]. The mean age was 30±7 years in this study. Amin *et al.* (2007) reported that mean age of the male patients having acute urethritis was 29±9 year which is comparable with the current study [28].

In our study, majority of the study population was day-laborers which were 40.84% followed by service 22.54% and business 12.68%. Ray *et al*. (2006) showed that male patients having urethritis were mainly laborers and factory workers (65.7% to 66.2%) [29]. This finding is consistent with the findings of the present study and this might be due to move in various places for their occupation and cannot live together with wife [30].

Out of 142 male patients, the socio-economic status showed 38.73% were from lower income group in this study. Ali *et al*. (2010) found 40.14% were poor which correlate with this study [31]. Low socioeconomic status is associated with higher frequency of these infections due to poverty, lack of sex education and reduced access to health care among low socioeconomic group [32].

Among 142 male patients having urethral discharge*, N. gonorrhoeae* was detected by Gram stain in 27.46% cases and confirmed by culture in 24.65% cases. In Bangladesh, Jahan *et al.* (2014) also found 27.57% cases *N. gonorrhoeae* positive by Gram stain and 26.49% by culture [33]. The rate of *N.gonorrhoeae* isolation by culture was low in this study for the reason that prior antimicrobial therapy, loss of viability of the organisms during transport, low concentrations of the organisms [34]. Moreover, a study from Pakistan by Saleem and Azim (2016) found 87% cases by Gram stained smear and 62% cases by culture which is higher than the present study which might be due to difference in selection criteria [35].

In the present study, a total of 41 cases of gonococcal infection were identified in the urethral discharge. Among them, 33(80.49%) cases were identified by both Gram stain and culture, 06(14.63%) cases were by Gram stain and 02(4.88%) were by culture. Similar study from Bangladesh was observed by Alam (2010) where 81.9% infections were identified by both Gram stain and culture, 15.2% were by Gram stain and 2.9% were by culture respectively [36]. Considering culture as gold standard, the sensitivity of gram stain was 94.28% and specificity was 94.39% in this study. Another study carried out in Egypt by Amin *et al.* (2007) reported that Gram stain had sensitivity and specificity was 75% and 100% respectively [28].

In this study, 31.69% gonococcal infections were detected by PCR among 142 sexually active male patients. Similarly, Jahan *et al*. (2014) and Nessa *et al.* (2004) found 30.27% & 35.8% gonococcal infections respectively [30, 37]. In addition among 669 patient specimens 11% were gonococci positive [38]. The difference in gonococcal infection rates reflect that the infection varies in different population, different regions and different in social and sexual behavior of patients.

Among the male urethritis patients 11.97% of *C.trachomatis* infection was identified in this study. This result is consistent with Al-Sweih *et al*. (2011) and Jahan *et al*. (2014) who reported 12.4% & 14.6% were positive for *C.trachomatis* infections [39, 33]. In contrast, in ICDDRB, Nessa *et al.* (2004) found 43.5% *C.trachomatis* infection which is higher than this study [37]. Additionally, 2.1% cases found for both gonococcal and non-gonococcal infections in this study. Similarly 2.31% cases were found as having co-infection with *N.gonorrhoeae* and *C. trachomatis* by El-Gamal *et al*. (2008) [40].

In the present study, out of 142 cases, 45 were identified by PCR and 39 were identified by Gram stain. Among them six were positive by PCR but negative by Gram stain. The sensitivity of PCR was 100% and specificity was 94.17%. Amin *et al*. (2007) reported that the sensitivity of PCR was 100% and specificity was 85.71% which is consistent with this study [28].

In this study, 142 urethral discharge samples were compared for diagnosis of gonorrhoea infection using culture and PCR. 45 cases were identified by PCR and 35 were by culture. Among them, 10 cases were positive by PCR but negative by culture. Considering culture as gold standard, the sensitivity of PCR was 100% and specificity was 90.65%. Jahan *et al.* (2014) reported the sensitivity of PCR was 100% and specificity was 94.85% [33]. This finding was similar to the findings of the present study.

Among the isolated *N.gonorrhoeae*, 97.14% were found resistant to Tetracycline. A study from Indonesia by Lesmana *et al*. (2001) showed 98% resistant to Tetracycline [41]. Similarly, Akter (2016) from Bangladesh found 100% resistant to Tetracycline in her study [27]. On the other hand, Shirin *et al*. (2009) from Bangladesh found 44.4% sensitive and 55.6% were resistant to Tetracycline which is not coinciding with the finding of the present study [42]. The resistance rate of tetracycline was increasing due to antibiotic pressure.

Regarding Ciprofloxacin, 94.29% were resistant to ciprofloxacin. Similarly, 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 [43]. This emergence of ciprofloxacin resistance was probably the consequences of multiple factors, which include overuse and misuse of this antibiotic due to over-the-counter availability, self-medication and most worryingly they are still being used excessively by quacks [44]. However, Shirin *et al*. (2009) showed that 51.1% *N.gonorrhoeae* were sensitive and 48.9% were resistant to ciprofloxacin [42].

In our study, among the isolated *N.gonorrhoeae* 82.86% were resistant to Penicillin. Another study from China by Jiang *et al*. (2017) reported 73.8% and from Pakistan by Jabeen *et al.* (2011) reported 70.8% were resistant to Penicillin which is accordance with this study [45, 46]. The high-level penicillin resistance may be due to a consequence of therapy with penicillin and/or its derivatives for unrelated illnesses, e.g., syphilis and upper respiratory tract infections [47].

In this study, among the isolated *N.gonorrhoeae*, 88.57% were sensitive to Ceftriaxone. Previous studies from Bangladesh by Jahan *et al*. (2014) and from India by Sharif and Balamurugan (2017) found 83.67% and 92.5% sensitive to Ceftriaxone respectively [33, 48]. In contrast to the present study, Nusrat *et al*. (2014) observed 56.41% were sensitive to Ceftriaxone [49]. Among the isolated *N.gonorrhoeae*, 77.14% were sensitive to Cefixime was found in this study. Similar study was done by Jahan *et al.* (2014) where 75.51% were sensitive to Cefixime [33].

Among the isolated *N.gonorrhoeae* cases, 91.43% were sensitive to Azithromycin in this study. Similar findings were reported by Kulkarni *et al.* (2018) in India where they found 95.2% susceptible to Azithromycin [50]. In our study, none of the isolates were found to be resistant to Spectinomycin. Sethi *et al*. (2013) from Pakistan also found similar findings [44].

Gonorrhoeae is one of the most common venereal disease in developing countries and a global health problem. The problem is compounded by the development of resistance to antimicrobials in *N. gonorrhoeae*. So, periodic monitoring of the antimicrobial susceptibility of *N. gonorrhoeae* provides essential clues regarding the rapidly changing pattern of antimicrobial susceptibility and the sensitivity pattern of bacteria may vary in different countries.

V. Conclusion

The gonococcal urethritis was the common type of infection identified in this study. Mixed infections were also identified. PCR was the most sensitive and rapid method for the diagnosis of causes of urethritis. Multiplex PCR may be considered as a suitable method for early and accurate diagnosis of gonococcal and non-gonococcal urethritis in men from a single specimen and providing a more cost effective way of screening multiple pathogens.

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