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Original Article

Neisseria Gonorrhoeae

Pak Armed Forces Med J 2021; 71 (3): 866-69

MULTIPLEX POLYMERASE CHAIN REACTION (PCR) FOR THE DETECTION OF NEISSERIA GONORRHOEAE AND THE QUINOLONE RESISTANCE GENE IN PAKISTAN

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ABSTRACT

Objective: To detect Neisseria gonorrhoeae from the urine of male patients reporting with active urethral discharge using multiplex polymerase chain reaction (PCR). And the simultaneous detection of the quinolone resistance determining region (QRDR) on the Neisseria gonorrhoeae gene using multiplex polymerase chain reaction.

Study Design: Cross sectional study.

Place and Duration of Study: Microbiology department, Army Medical College Rawalpindi Pakistan, from Mar to Dec 2018.

Methodology: Male patients with active urethral discharge with no past history of antibiotic use for urethral discharge were included in study and patients without active urethral discharge and history of antibiotic use for urethral discharge were excluded. Urine of patients of active urethral discharge was collected and multiplex polymerase chain reaction was done by using two forward primers along with common reverse primer.

Results: In this study 24 (40%) of patients who presented with active urethral discharge were positive for gonorrhea. However Quinolone Resistance Determining Region is detected in 17 (70.83%) of cases and only 7 (29.17%) were sensitive to ciprofloxacin.

Conclusion: The multiplex polymerase chain reaction is very efficient and effective method for the simultaneous detection of Neisseria gonorrhoeae and status of isolate susceptibility to ciprofloxacin. And in Pakistan ciprofloxacin cannot be used as first line drug for the treatment of gonorrhea.

Keywords: Gonorrhea, Multiplex polymerase chain reaction, Quinolone resistance determining region.

INTRODUCTION

Gonorrhea is the second most prevalent sexually transmitted bacterial disease caused by bacteria Neisseria gonorrhoeae[1]. The center for disease control (CDC) estimates that approximately 820,000 new gonococcal infections occur in United States each year, and out of these 820.000 only half are detected and reported to CDC[2].

Neisseria gonorrhoeae causes urethritis in males and cervicitis in females. More than half of gonococcus infected females and a small number of infected males are asymptomatic. But if urogenital gonorrhea remain untreated it ascends and causes serious complications like pelvic inflammatory disease, ectopic pregnancy in females and epididymitis and penile edema in males. It also encourages transmission of HIV and other sexually transmitted infections.

To control gonococcal infections the cornerstone is effective treatment with appropriate antimicrobials to treat the case and to prevent its further transmission as there is no vaccine available against gonorrhea. And the ability of Neisseria gonorrhoeae to develop resistance against antimicrobials making it complicated. It has developed resistance to each and every class of antimicrobial offered for treatment[3].

In USA and UK dual therapy regimen of ceftriaxone and azithromycin is recommended for uncomplicated gonococcal infections. But the history of Neisseria gonorrhoeae tells us that it develops antimicrobial resistance against every drug offered for treatment so this dual therapy only seems some delay rather than a permanent solution, Therefore urgent action is needed to combat this problem. Currently one of the limitations to control the antimicrobial resistance problem of Neisseria gonorrhoeae is lack of data so surveillance data need to be enhanced and implemented.

Gram stained smear is reliable, inexpensive and a fast method for detection of Neisseria gonorrhoeae but it is only reliable when patients with active urethral discharge were selected for the study. In well developed countries nucleic acid amplification test (NAAT) is commonly used for diagnosis of gonorrhea. NAAT is recommended because it has high sensitivity as compared to culture, ease of sample collection and sample transport as it can also detect gonorrhea in first void urine sample and endocervical swabs[4]. A molecular method for the simultaneous detection of Neisseria gonorrhoeae and its ciprofloxacin susceptibility status has been described. To include most conserved region

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Received: 01 Jul 2020; revised received: 22 Oct 2020; accepted: 04 Nov 2020

in gyrA sequence that is not common in non-Neisseria gonorrhoeae species the primer gyrA-W forward was designed. To include gyrA sequence harboring S91 and D95 mutations the gyrA-M forward primer was designed. These primers paired with common reverse primer.

The genomic regions of Neisseria gonorrhoeae gyrA and parC are associated with ciprofloxacin resistance. ParC mutations alone is not associated with ciprofloxacin resistance and gyrA mutation alone is associated with low level resistance. However gyrA mutation along with parC mutations maintains higher levels of resistance. About 145 studies from different countries including Switzerland, Canada, South Africa and Brazil support that gyrA mutation alone is sufficient to confirm ciprofloxacin susceptibility[5]. So in this study we identified ciprofloxacin resistant and sensitive isolates only by considering gyrA. However many real time polymerase chain reaction based studies were conducted on clinical samples in which only mutations at the gyrA S91 locus were detected[6,7]. But studies showed that ciprofloxacin intermediate resistance mainly due to mutations in either S91 or D95 loci while ciprofloxacin resistance arose due to mutations in both S91 and D95 loci[8]. So in this study we consider mutations both at S91 and D95 loci.

In 2013 in the United Kingdom 70% isolates of gonorrhea were susceptible to ciprofloxacin 9 and in the United States 74.1% isolates of Neisseria gonorrhoeae showed susceptibility to ciprofloxacin[10]. So prescribing ceftriaxone to all patients is a poor strategy. We should identify ciprofloxacin sensitive strains so that unnecessary ceftriaxone can be avoided and be used only for ciprofloxacin resistant isolates.

In Pakistan people are reluctant to seek medical advice due to lack of facilities, cultural constraints and taboo. This study has the potential of rapid diagnosis of Neisseria gonorrhoeae and its susceptibility to ciprofloxacin which leads to early diagnosis and timely management.

METHODOLOGY

It was a cross sectional study and the study was carried out at the department of Microbiology, Army Medical College Rawalpindi Pakistan, from March 2018 to December 2018.

The sample size was calculated by using WHO calculator. Confidence level 95 Anticipated population proportion p=0.04[11]. Absolute precision required d= 0.05 and the minimum sample size (n=60) and the sampling technique was non probability consecutive sampling technique.

All males' first time attending skin and urology department with complaint of active urethral discharge with no previous history of antibiotic use for urethral discharge were included in the study and patients without active urethral discharge and history of antibiotic use for urethral discharge were excluded from study.

After getting approval from Ethics Review Committee Army Medical College Rawalpindi samples were collected from the department of Dermatology Pak Emirates Military Hospital Rawalpindi and department of urology Benazir Bhutto Hospital Rawalpindi. Urine samples of 60 male patients with active urethral discharge were collected insterile plastic container and transported to laboratory within one hour of collection and stored at 4°C. DNA was extracted from the urine sample of each individual using commercially available kit. (PureLink Microbiome DNA purification kit Cat# A29790 M/s Invitrogen).

Forward primer gyrA-W included the most conserved region in gyrA sequence that is not common in non-Neisseria gonorrhoeae species (5″GCGATTCCGC AGTTTACGA3″) was used. The gyrA-M forward primer included the gyrA sequence harboring S91 and D95 mutations (5″TACCACCCCCACGGCGATTT3″) which was used for quinolone resistance determination. These primer was paired with common reverse primer gyrA-R (5″5CGAAATTTTGCGCCATACGGA CGAT3″). For detecting Neisseria gonorrhoeae and its ciprofloxacin status in a single amplification these primers in multiplex format has 100% sensitivity and specificity[8].

Real time polymerase chain reaction (RT-PCR) was performed using Cepheid smart cycler on a 16 well platform with the Maxima SYBR Green/ROX q PCR Master Mix (2x) (Cat #K0221 M/s Thermo Scientific). PCR reactions contained 0.5µl of each primer (10µM), 2µl of DNA template (50 ng/µl) and 5µl of 2×SYBR Green and the final reaction volume was adjusted with deionized water to 10µl. According to manufacturer guidelines PCR was conducted with the following modifications: activation and initial holding at 50°C for 2 minutes than secondary holding at 95°C for 2 minutes. PCR was performed with positive and negative controls for 25 cycles at 95°C for 15 seconds and 60°C for 30 seconds along with positive and negative controls. Post PCR melt curve was performed with 0.3°C temperature increments between 60-95°C. Data

were collected at annealing stage (amplification data) and then at melting stage. Amplification till 25 cycles were taken as positive for Neisseria gonorrhoeae and melt curve 78°C were taken as ciprofloxacin sensitive and melt curve values 80-80.3°C were taken as ciprofloxacin resistant[8].

Data was analyzed by using SPSS-22. For qualitative variables percentages and frequency were calculated. Quantitative values were expressed as mean ± standard deviation.

RESULTS

In our study the minimum age was 22 years and the maximum age was 38 years Mean age of patients positive for Neisseria gonorrhoeae was 26.4 ± 3.11years and mean age of patients negative for Neisseria gonorrhoeae was 27.7 ±3.98 years.

Out of the total, 21 (35%) were from patients in the age group 22-25 years, 26 (43.3%) were from patients in the age group 26-29 years, 7 (11.6%) were from patients in the age group 30-33 years and 6 (10%) were from patients in the age group with 34 years and above. A total of 60 patients were included in this study out of them 24 samples (40%) were positive for Neisseria gonorrhoeae and 36 (60%) were negative for Neisseria gonorrhoeae.

Out of total 24 positive patients for Neisseria gonorrhoeae, 9 (37.5%) were from age group 22-25 years, 11 (45.8%) were from age group 26-29 years, 3 (12.5%) were from age group 30-33 years and 1 (4.1%) were from age group 34 years and above.

Out of total 24 positive samples of Neisseria gonorrhoeae 17 (70.83%) samples were positive for Quinolone Resistance Determining Region (QRDR) and 7 (29.17%) were negative for QRDR as shown in table-II.

Table-I: Frequency of Neisseria gonorrhoeae in urethral discharge (n=60).

Samples	n (%)
Positive	24 (40%)
Negative	36 (60%)
Total	60 (100%)

Table-II: Frequency of quinolone resistance determining region (QRDR) (n=24).

Sample	n (%)
Positive	17 (70.83%)
Negative	7 (29.17%)
Total	24 (100%)

DISCUSSION

To my knowledge in Pakistan this is the first report of simultaneous detection of Neisseria gonorrhoeae and its ciprofloxacin susceptibility status in a single test that is rapid, inexpensive and 100% sensitive and specific.

In our study 40% patients of active urethral discharge were positive for Neisseria gonorrhoeae which is comparable to the studies done on this topic in Pakistan, Kuwait and Dhaka[12-14]. In these studies 27.5%, 31.5% and 30.27% patients with active urethral discharge were positive for Neisseria gonorrhoeae respectively. However rate is higher when compared with studies conducted in South Africa and India[15,16]. In these studies multiplex PCR was performed 85% and 76.8% patients with urethritis were positive for Neisseria gonorrhoeae respectively.

In current study 70.83% isolates of Neisseria gonorrhoeae were resistant to ciprofloxacin. Before that a study was conducted in Pakistan in 2012-2014 at the Aga Khan University Karachi, in which Neisseria gonorrhoeae showed 86% resistance to ciprofloxacin[17].

Study conducted from 2007 to 2011 in Pakistan, India and Bhutan on genetic characteristic and antimicrobial susceptibility of Neisseria gonorrhoeae and result showed more resistance to ciprofloxacin than our study it showed that not a single isolate of Neisseria gonorrhoeae was susceptible to ciprofloxacin, 94% were resistant to ciprofloxacin and 6% were intermediately susceptible and all isolates showed mutations in Quinolone Resistance Determining Region at gyrA and parC genes[18].

A study was conducted in India on the antimicrobial resistance of Neisseria gonorrhoeae from 2015 to 2017 which showed Neisseria gonorrhoeae was 100% resistant to quinolones and gyrA and parC were observed in all isolates of quinolone resistant Neisseria gonorrhoeae[19]. As compared to our region in the United States and the United Kingdom resistance of Neisseria gonorrhoeae to ciprofloxacin is very low.

Study in United States showed 25.9% ciprofloxacin resistance to Neisseria gonorrhoeae and according to CDC 19.2% ciprofloxacin resistance among gonococcal strains were reported[9]. In United Kingdom study reported 30% cases of gonorrhea were resistant to ciprofloxacin[20].

Studies carried out over the world on susceptibility of Neisseria gonorrhoeae to ciprofloxacin shows marked disparity of results in different regions of world so appropriate antimicrobial treatment according to susceptibility pattern in your region and according to case is essential.

Funding Source

It is funded by National Medical University (NUMS) and Department of Pathology Army Medical College Rawalpindi.

CONCLUSION

This study showed that Neisseria gonorrhoeae was positive in the urine of 40% patients of active urethral discharge and 70.83% Neisseria gonorrhoeae showed resistance to quinolone. So in Pakistan ciprofloxacin cannot be used as first line drug for the treatment of gonorrhea and we should use internationally recommended, ceftriaxone and azithromycin as first line drugs for the treatment of gonorrhea.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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