Original Article

Anatomic site distribution of *Neisseria gonorrhoeae* in men who have sex with men attending a tertiary care hospital in North India

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Abstract

Background and Objectives: Anorectal and pharyngeal infections with *Neisseria gonorrhoeae* (NG) are common in men who have sex with men (MSM). However, they are often asymptomatic and found in the absence of reported risk behavior and concurrent genital infection. These serve as a hidden reservoir for ongoing transmission and may cause complications. Additionally, they drive the transmission of other sexually transmitted infections (STIs) including HIV and may contribute to the development of antimicrobial resistance. The current study was undertaken to study the anatomic site distribution of gonococcal infection in MSM as limited data are available from India. **Materials and Methods:** A total of 127 MSM patients attending the STI clinic Dermatology Outpatient Department of AIIMS were included in the study. A duplex polymerase chain reaction (PCR) targeting *opa* and *porA* psuedogene targets using in-house primers was standardized and used for testing. In addition, all samples were processed by conventional methods, i.e., microscopy and culture. **Results:** A total of 26 patients were found to be positive for NG by PCR with a prevalence rate of 20%. The prevalence rate for urethral, rectal, and pharyngeal gonorrhea was 8.7%, 9.4%, and 4.7% respectively. Out of the 26 positives, 15 patients, i.e., 57.7%, had only extragenital infections and none were positive at all three sites. On the other hand, only three patients were culture positive at the urethral site. **Discussion:** We would have missed approximately 60% of the infections if the testing was restricted to genital sites only. **Conclusion:** An expanded testing including extragenital sites for screening of gonococcal infection in MSM will have clinical and public health benefits.

Key words: Duplex polymerase chain reaction, extragenital gonorrhea, men who have sex with men, *opa*, polymerase chain reaction, *porA* pseudogene

Introduction

India has a large population of men who have sex with men (MSM). However, the prevalence data available may be misleading as a huge proportion remains hidden/unreported due to the social stigma attached, the discrimination, and the family pressure. The reported numbers may just be a tip of the iceberg. As per the World Health Organization (WHO) estimates, 82.4 million new cases of gonorrhea were acquired in 2020, of which majority were aged between 15 and 49 years.[1] The gonococcal incidence rate has been reported to be higher in men as compared to women, since 2013.[2]

In many countries, the data about MSM are very sparse, limited by the poor surveillance and lack of infrastructure. Recent reports from across the world suggest an overly high percentage of gonococcal infection in MSM attending sexual health clinics, with a positivity rate varying from 24.6% in a study from Australia to 35% in a study from Guatemala.[3,4] Further, the study from Australia reported a significant increase in incidence rate over the years from 14.1/100 person-years (PY) in 2010 to 24.6/100 PY in 2017. Reports from England, Ireland, Spain, the USA, and Canada also show a similar trend.[5-8] This has been speculated to be because of the inclusion of extragenital samples for testing, routine screening for

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sexually transmitted infections (STIs) as a part of HIV preexposure prophylaxis (PrEP) and introduction of molecular diagnostics tests in laboratories which has significantly increased the sensitivity and specificity of the diagnostic procedure worldwide. In India, a 12.1% prevalence has been reported in MSM.[9] The gonococcal case rates have been shown to vary hugely. This may be due to a variation in the incidence rate in various subgroups. Additionally, screening/testing practices and proper reporting of cases may also influence the reported rates.

As per the epidemiological data infection of the oropharynx has been found to be a major driver for the spread of gonococcal infections among MSM and may also be a contributing factor in the development and spread of antimicrobial-resistant strains. There are reports of frequent detection of gonococcus in the saliva of infected patients, emphasizing the role of the oropharynx in the spread of gonococcal infections.[10] The infections of the oropharynx are generally asymptomatic and hence may remain undiagnosed and lead to the development of resistance strains. Furthermore, the oropharynx may serve as a reservoir for the infection leading to further spread. Further, the individuals with pharyngeal/rectal infections may not have a concordant urethral infection. The gonococcal strains isolated from MSM have been shown to have a higher antimicrobial resistance as compared to the strains isolated from heterosexual individuals. Further, the transmission rate has been shown to vary with the anatomic site of infection with urethral to rectal predicted to have the highest probability of transmission (84%) followed by urethral to pharyngeal (63%).[5]

Earlier studies have demonstrated that the specificity of polymerase chain reaction (PCR) for oropharyngeal samples is extremely low, probably due to the presence of the commensal *Neisseria*.[11] In this study, we designed and performed a duplex PCR for the detection of gonorrhea in MSM patients. Further, a 2-gene targeted duplex PCR would decrease the possibility of sequence-related false negatives and simultaneously provide a confirmatory positive result. This is also in compliance of the recommendations by the Centers for Disease Control and Prevention (CDC) 2014 and Public Health England guidelines 2021 that all *Neisseria gonorrhoeae* (NG) nucleic acid amplification test-positive results for oropharyngeal samples should be confirmed by a supplementary testing.[12,13] This study therefore targets the two genes i.e., *opa* and *porA* pseudogene, to optimize and evaluate a duplex PCR assay for the detection of NG in genital and extragenital samples collected from MSM patients.

Materials and Methods

Sample collection and processing

All MSM population in the age group of 15–65 years visiting the sexually transmitted disease (STD) clinic and Dermatology Outpatient Department of AIIMS, New Delhi, with a history of homosexual activities were enrolled in this study. A total of 127 patients were included over the time period of 1 year, April 2022 to March 2023. Samples were collected from the different anatomic sites as per standard techniques. Urethral, rectal, and oropharyngeal swabs were collected from the MSM patients in triplicate for microscopy, culture, and PCR. The culture was performed on a modified Thayer–Martin medium as per the standard protocol.[14] For PCR, DNA was extracted from the swab samples using a QIAamp DNA mini kit (Qiagen, Sciences Inc., USA), according to the manufacturer's instructions.

Duplex polymerase chain reaction

Duplex PCR targeting the *porA* pseudogene and *opa* gene was standardized and validated. The *porA* pseudogene primers were designed using the available software ClustalX and Primer3 from the gene sequences available in the GenBank database. The selected oligonucleotide sequence was analyzed further using Basic Local Alignment Search Tool (BLAST) for nonspecific binding. The forward primer was 5' CCT GCT ACT TTC ACG CTG GA 3' and the reverse primer was 5' TAT GTT CGC GTT TCT GAC TG 3'. For *opa*, prevalidated forward primer 5'-CGG TGC TTC ATC ACC TTA G-3' and reverse primer 5'-GGA TTC ATT TTC GGC TCC TT-3' were used. A 25 µL reaction was put up with a final concentration of reaction buffer (10 mM KCl, 10 mM HCl), 1.5 mM $MgCl_2$, Taq polymerase (1.5 U), 200 mM dNTP mix, and 5 pmol each forward and reverse primers of both *opa* and *porA* pseudogene with 5 µL of sample DNA. PCR conditions were denaturation at 94°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 45 s for a total of 35 cycles yielding a 188 bp and 430 bp product from positive samples.

The sensitivity of the duplex PCR was determined using serial dilutions of DNA extracted from standard strain of NG. The cross-reactivity of duplex PCR was determined using DNA from a panel of bacterial strains including *Neisseria meningitidis*, *Commensal neisseria, Lactobacillus fermentum, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Escherichia coli, Chlamydia trachomatis* (CT), *Pseudomonas aeruginosa*, Group B *Streptococcus*, *Streptococcus pyogenes, Klebsiella pneumoniae, Staphylococcus aureus,* and *Enterococcus faecalis.*

The sensitivity of the duplex PCR was further compared with that of *opa* and *porA* pseudogene monoplex PCR. The *opa* monoplex PCR was performed as described earlier.[15] The *porA* pseudogene PCR was performed as per the following conditions: denaturation at 95°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 45 s for a total of 35 cycles. Internal control for sample inhibition was checked by the amplification of the β-globin gene as previously described before performing both duplex and monoplex PCRs.[16] The DNA extracted from the clinical samples was tested using the duplex PCR. This study was approved by the Ethics Committee, AIIMS, New Delhi.

Observations/Results

The sensitivity of the duplex PCR was found to be comparable to the monoplex PCRs in the picogram range (8 pg for duplex, 0.4 pg for *opa,* and 4 pg for *porA*). The duplex PCR was found to be highly specific for NG when tested against the commensals and other pathogens of the genital tract. For identification of positive patients by PCR, patients positive at any one or two or all the anatomic sites tested were considered positive. Of the 127 patients, 26 were found to be positive for NG by PCR [Figure 1]. The details of the distribution of positives are elaborated in Figure 2 and Table 1.

There were only three culture positives from urethral sites. Among these, two demonstrated intracellular and extracellular Gram -negative diplococci (GNDC.) None

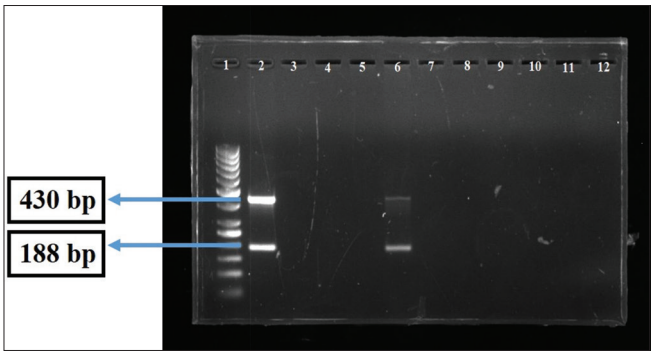

Figure 1: Gel picture for duplex polymerase chain reaction in clinical samples

of the extragenital sites were positive by culture although extracellular GNDC was observed in 28 pharyngeal and 26 rectal samples. However, no correlation was observed between the presence of extracellular GNDCs and PCR positives in extragenital samples. The details are provided in Table 2.

Discussion

The prevalence rate of gonorrhea in MSM in our study was 20.5%. The prevalence of urethral gonorrhea was 8.7%, rectal gonorrhea was 9.4%, and pharyngeal gonorrhea was 4.7%. Further, 3 (2.4%) patients had simultaneous infections at two different sites, whereas none of the patients had infection at all the three anatomic sites. A region-wise difference in gonococcal prevalence rate has been reported worldwide, 35% from a study in Guatemala, 22% from China, and 11.6% from Vietnam.[4,17,18] The reported rate varies depending on the laboratory facilities available, provision for extragenital testing and reporting, and the societal constraints. The prevalence of extragenital infections among MSM has been reported to vary from 0.2%-24.0% for rectal and 0.5%-16.5% for pharyngeal gonorrhea, globally which is in concordance with our study.[19] Further, approximately 60% of the positives in our study were extragenital infections and would have been missed if testing was limited to genital sites. This is in accordance with the CDC report stating a miss of approximately 70% if only genital testing was done.[20]

In an earlier study, for the period February 2017–November 2018, we had reported a prevalence rate of 31.5%, with 24% pharyngeal gonorrhea, 7% rectal gonorrhea, and 3.7% urethral gonorrhea.[21] A major decrease in the reported cases of pharyngeal gonorrhea has been observed. Furthermore, in the earlier study, 35% of the patients were HIV positive. In comparison, in the current study, only 11% of the patients enrolled were positive for HIV. Gonorrhea has been reported to be closely linked to HIV, hence a higher proportion of participants with HIV may be a reason for disproportionately high number of oropharyngeal positives. Further, as reported earlier, the extragenital infections may or may not have a corresponding genital infection, hence the cases may have been missed out/already treated, hence affecting the reported numbers. It may also indicate a change in the sexual practices, and one of the drivers for the change is the introduction of PrEP. Possibly, an indiscriminate use of over-the-counter antibiotics such as azithromycin during and post-COVID-19 may have contributed to a decline. Another study from India has reported a 1.6% prevalence of oropharyngeal gonorrhea for a period from April 2021 to November 2022.[22]

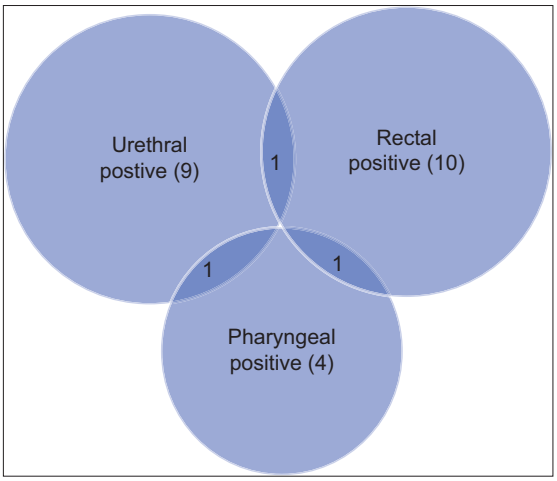

Figure 2: Anatomic site distribution of *Neisseria gonorrhoeae* infection in men who have sex with men

Table 1: Anatomic site distribution of infection for positive samples

Urethral	Rectal	Pharyngeal	Urethral + rectal	Urethral + pharyngeal	Rectal + pharyngeal
9	10	4	1	1	1
Total positives			26		

Table 2: Extracellular GNDC status in extragenital samples

GNDC extracellular	PCR			
	Pharyngeal samples		Rectal samples	
	Positive	Negative	Positive	Negative
Positive	2	26	7	19
Negative	4	95	5	96
Subtotal	6	121	12	115
Total number of patients	127		127	

PCR=Polymerase chain reaction; GNDC=Gram-negative Diplococci

The CDC recommends rectal and pharyngeal testing for all MSM at least once a year. Further, frequent screening for STIs (for gonorrhea, chlamydia, and syphilis) at 3–6-month interval has been recommended for those at higher risk. Although a high prevalence of extragenital NG/CT infections has been noted in MSM from several countries, there are limited data available from India. Routine universal screening of extragenital sites of all MSM, irrespective of behavioral exposure and symptoms, is imperative if control of these infections is to be achieved. Further, the low sensitivity and specificity of Gram stain in extragenital sites are known, therefore not recommended. Culture also has a very low sensitivity for these sites due to low pathogen load, hence advocating the use of molecular testing for these samples.

Most of the patients attending the STD clinic were in the age group of 21–30 years with a median age of 34 years. Furthermore, the highest percentage of positives were also from the same age group, i.e., 21–30 years [Table 3]. Worldwide, the highest prevalence rate of STIs has been reported from young adults (<25 years) accounting for about half the new STD infections.[20,23] In accordance, in the present study also, 31% of the infections were reported in the youth (15–24 years as defined by the WHO). The reported infection rate may not be the true representation of the actual

Table 3: Age-wise distribution of patients attending the sexually transmitted disease clinic

Age-group	15-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55
Number of patients	14	31	36	20	11	9	2	4
Positives	1	7	10	3	3	0	0	2

number of cases as the social norms and stigma associated may discourage them from seeking medical help. Furthermore, the lack of knowledge related to STIs may contribute to this age group being the most vulnerable. The highest affected age group was 26–30 years (38% of the total infections).

Conclusion

The WHO's goal is to reduce the incidence of gonorrhea by 90% by the year 2030. For this, regional monitoring of epidemiology and transmission needs to be done. The historic judgment of the Honorable Supreme Court of India that decriminalized homosexuality has reduced discrimination and improvement in the health-seeking behavior of this subgroup may be expected in future. Screening of high-risk populations like MSM would play a key role in achieving the goal by dampening the transmission rate as the prevalence is disproportionally high in this subset.

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Conflicts of interest

There are no conflicts of interest.

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