

Prevalence, Antibiotic Resistance and Associated Factors of *Neisseria gonorrhoeae* Among Patients Attending Non-Profitable Private Clinics in Mekelle, Tigray, Ethiopia

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Background: Globally, *Neisseria gonorrhoeae* is the second most common cause of bacterial sexually transmitted diseases. The prominent predicament of this bacterium is its complications, non-susceptibility for many drugs, and aggravated transmission of other sexually transmitted infections. There is limited information about the prevalence, antibiotic resistance, and risk factors of *N. gonorrhoeae* in Tigray, Ethiopia. Therefore, we aimed to determine the prevalence, antibiotic resistance, and risk factors of *N. gonorrhoeae* among patients attending non-profitable private clinics in Mekelle, Tigray, Ethiopia.

Methods: A cross-sectional study among 229 patients was conducted from February to June 2018. The socio-demographic data and associated factors were gathered using structured questionnaire, and swabs were taken from urethra and cervix of males and females, respectively. Specimens were inoculated on standard bacteriological culture media and antibiotic susceptibility testing was performed using Kirby-Bauer disc diffusion technique following the Clinical and Laboratory Standard Institute. Data were analyzed using Statistical Package for Social Sciences Version 21. The level of significance at p-value <0.05 was considered statistically significant.

Results: The overall prevalence of *N. gonorrhoeae* was 23 (10.04%). High prevalence rates of *N. gonorrhoeae* were observed in females, urban residents and married ones. *N. gonorrhoeae* had shown statistically significant association with HIV positive, previous history of STIs, shisha users, Khat (*Catha edulis*) users, condom non-users and having more than two sexual partners. All isolates showed resistance to penicillin followed by tetracycline 16 (69.6%) and ciprofloxacin 8 (34.8%). Four isolates (7.4%) exhibited resistance to azithromycin with no resistance to ceftriaxone. Twelve (52.2%) isolates showed multidrug resistance (MDR).

Conclusions: The prevalence of *N. gonorrhoeae* and drug resistance, including multidrug resistance, was high in the study. Multiple factors were associated with the acquisition of *N. gonorrhoeae*. Therefore, behavioral change and communication should be strengthened.

Keywords: antibiotic resistance, associated factors, *Neisseria gonorrhoeae*, non-profitable private clinics, Tigray

Introduction

Neisseria gonorrhoeae is one of the major causes of global public health problems and it is the second most common cause of bacterial sexually transmitted infections.^{1,2} World Health Organization (WHO) predicted that the global incidence rate of *N. gonorrhoeae* among adults in 2012 was 78 million. Of these, 11.4 million cases were shared by the African region.³ The incidence rates of the bacteria reported from Russia and Poland were 1.9 up to 23.5 per 100,000 populations.^{4,5} Multiple sexual partners, sexually active age, unsafe sexual practice, lower socio-economic status, urban residency and substance use were described as predisposing factors and may increase the prevalence rate.⁶

The noticeable complications of *N. gonorrhoeae* are epididymitis, pelvic inflammatory disease, ectopic pregnancy, infertility, miscarriage and fetal deaths.^{2,7,8} Human Immunodeficiency Virus (HIV) infected persons are more susceptible to *N. gonorrhoeae* and pregnant mothers can transmit the bacteria to their newborn, and the newborn can acquire neonatal ophthalmia.^{9,10}

Due to the lack of appropriate laboratory diagnostic techniques, the management of sexually transmitted infections in Ethiopia is based on syndromic approach.¹¹ The syndromic approach management of STIs may lead to the emergence of multidrug-resistant *N. gonorrhoeae* in the region. The prevalence of *N. gonorrhoeae* among different groups of populations in Ethiopia ranges from 2.7% to 11.6%^{12–14} whilst the prevalence rate increased to 17.7% in HIV patients under antiretroviral treatment.¹⁵

The emergence of resistant *N. gonorrhoeae* to various antibiotics remains another principal public health threat. Today, the antibiotics that displayed non-susceptibility for *N. gonorrhoeae* are sulphonamides, penicillin, tetracycline, macrolides, fluoroquinolones, and early-generation cephalosporins.^{16–18} Hence, the WHO recommended that *N. gonorrhoeae* suspected patients should be treated using ceftriaxone plus azithromycin¹⁹ because countries in the WHO region reported that resistance rates for ceftriaxone, ciprofloxacin and azithromycin were significantly increased.²⁰

In the study area, there was limited data about the prevalence, antibiotic resistance and associated risk factors of *N. gonorrhoeae*. Therefore, the study was conducted to determine the prevalence, antibiotic resistance and associated risk factors of *N. gonorrhoeae* among patients attending non-profitable private clinics in Mekelle, Tigray, Ethiopia.

Methods and Materials

Study Design, Settings and Study Period

A cross-sectional study among patients attending non-profitable private clinics was conducted from February to June 2018 in Mekelle, Tigray, Ethiopia. Mekelle is the capital city of Tigray regional state and is located 784km north of Addis Ababa.

Sample Size and Sampling Technique

The sample size was calculated using single population proportion formula, taking the prevalence rate of *N. gonorrhoeae* 11.3%.¹⁴ Expected margin of error (d) was 0.04 and the level of confidence was 95%. Thus, two hundred twenty-nine consecutive patients with 10% contingency were included in our study.

Socio-Demographic and Risk Factors Data Collection

Once secured the informed consent, information about the risk factors and socio-demographic characteristics were collected using questionnaire by trained health professionals.

Urethral and Cervical Swab Samples Collection

Two urethral or endocervical swab samples were collected from each patient aseptically following Standard Operating Procedure (SOP). The urethral sample was collected using sterile rayon swab by gentle massaging of the urethra from above downward. For those who did not have noticeable pus, the rayon swabs were inserted approximately 2 cm into the urethra and rotated gently before withdrawing. The cervical canals of female participants were opened using a well-disinfected vaginal speculum and a sterile rayon swab was rotated against the wall of the endocervical canal several times for 20–30 seconds and withdrawn without touching the vaginal surface. The specimens were inoculated on Modified Thayer Martin agar in the sample collection site and put in the candle jar having 5–10% CO₂ and transported to Mekelle University Medical Microbiology laboratory within 2 hours of collection and incubated immediately following the SOP.²¹

Isolation and Identification of *Neisseria gonorrhoeae*

The Modified Thayer Martin medium (OXOID, UK) plate contains vancomycin, colistin, nystatin and trimethoprim. The inoculated media were incubated at 37°C for 24–48 hrs. Then, *N. gonorrhoeae* was isolated and identified based on

colony morphology, gram staining, oxidase test, and carbohydrate utilization test. Small raised, grey shiny colonies, gram-negative diplococci, oxidase positive and glucose fermentation were considered as *N. gonorrhoeae*.

Antibiotic Susceptibility Testing

From a pure culture, 3–5 selected colonies of *N. gonorrhoeae* were transferred to a tube with a straight wire and prepared suspension of 2.5 mL normal saline and incubated at 36.5°C until the turbidity of the suspension equal to 0.5McFarland standard. The bacteria were inoculated evenly over the entire surface of MTM agar using a sterile cotton swab. The susceptibility to the following antimicrobial agents (OXOID, UK) was assessed: penicillin (10 IU), tetracycline (30 µg), ciprofloxacin (5µg), ceftriaxone (30µg), spectinomycin (100µg), and azithromycin (15µg).

The criteria used to select the antimicrobial agents tested were based on their availability and frequent prescriptions for the management of gonococcal infection and the national list of medicines by the Food, Medicine and Health-Care Administration and Control Authority (FMHACA) of Ethiopia.¹¹

Finally, antimicrobial susceptibility testing was performed for all isolates according to the criteria of Clinical and Laboratory Standard Institute (CLSI) by the modified Kirby-Bauer disk diffusion method on Thayer Martin medium plates.²²

Data Quality Control

Five percent of the questionnaire was pre-tested for comprehensiveness, effectiveness, reliability and validity. Culture media sterility was ensured by incubating 5% of each batch of the prepared media at 37°C for 24 hrs. The performance of prepared media was also checked by inoculating with control strains *N. gonorrhoeae* ATCC TM 49226. Moreover, the data collectors were trained and demonstrated for two days. The investigators conducted strict supervision on a daily basis.

Data Analysis

Data related to *N. gonorrhoeae* were analyzed using SPSS version 21, and descriptive statistics were presented using tables and percentages. Crude odds ratio (COR) and adjusted odds ratio (AOR) at 95% level of confidence were calculated. A p-value less than 0.05 indicated that the association between dependent and independent variables existed.

Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board (IRB) of Mekelle University, College of Health Sciences with reference number ERC 1195/2017. The study was carried out in accordance with relevant national, international and scientific guidelines' along with our study was conducted in accordance with the Declaration of Helsinki. After briefing the objectives of the study and before collecting the data, informed consent and assent were collected from adult participants and minors' guardians, respectively. Furthermore, the study participants tested culture positive for *N. gonorrhoeae* were consulted. The data and samples were kept confidential and used for the specified objectives only and finally, the specimens were discarded following the infection prevention guideline.

Results

Socio-Demographic Characteristics and Risk Factors

A total of 229 patients were participated in this study. Of which, the proportion of females, 25–34 years old, urban residences, secondary school level of education, married and employees were 128 (55.9%), 123 (53.7%), 190 (83%), 84 (36.7%), 139 (60.7%) and 76 (33.2%), respectively.

Nineteen (8.3%) of the study participants were HIV positive and twenty (8.7%) had previous history of sexually transmitted infections. The proportion of shisha users and Khat (*Catha edulis*) chewers were 13 (5.7%) and 26 (11.4%), respectively. There were also 36 (15.7%) participants who do not use condom during sexual intercourse and other 36 (15.7%) participants who had more than 2 sexual partners. About 90 (39.3%) of the participants had the habit of alcohol intake, Table 1.

Table 1 Socio-Demographic Characteristics of Patients Attending Non-Profitable Private Clinics of Mekelle, Tigray, Ethiopia

Variables	Frequency, n (%)	Prevalence, n (%)
Sex		
Male	101 (44.1)	8 (3.5)
Female	128 (55.9)	15 (6.6)
Age groups in years		
15–24	59 (25.8)	2 (0.9)
25–34	123 (53.7)	16 (7)
35–44	38 (16.6)	3 (1.3)
>44	9 (3.9)	2 (0.9)
Residence		
Rural	39 (17)	5 (2.2)
Urban	190 (83)	18 (7.9)
Educational status		
Illiterate	12 (5.2)	1 (0.4)
Primary	59 (25.8)	5 (2.2)
Secondary	84 (36.7)	9 (3.9)
Diploma and above	74 (32.2)	8 (3.5)
Marital status		
Single	54 (23.6)	1 (0.4)
Married	139 (60.7)	17 (7.4)
Divorced	25 (10.9)	4 (1.7)
Widowed	11 (4.8)	1 (0.4)
Occupation		
Employees	76 (33.2)	6 (2.6)
Housewives	31 (13.5)	3 (1.3)
Drivers	13 (5.7)	4 (1.7)
Merchants	66 (28.8)	5 (2.2)
Students	20 (8.7)	3 (1.3)
Others	23 (10)	2 (0.9)
HIV status		
Positive	19 (8.3)	6 (2.6)
Negative	210 (91.7)	17 (7.4)
History of STI		
Yes	20 (8.7)	5 (2.2)
No	209 (91.7)	18 (7.9)
Shisha use		
Yes	13 (5.7)	7 (3.1)
No	216 (94.3)	16 (7)
Alcohol intake		
Yes	90 (39.3)	7 (3.1)
No	139 (60.7)	16 (7)
Chewing Khat		
Yes	26 (11.4)	10 (4.4)
No	203 (88.6)	13 (5.7)
Condom use		
Yes	193 (84.3)	14 (6.1)
No	36 (15.7)	9 (3.9)
Number of sexual partners		
1–2	193 (84.3)	17 (7.4)
>2	36 (15.7)	6 (2.6)

Prevalence of *N. gonorrhoeae*

The overall prevalence of *N. gonorrhoeae* in the current study was 23 (10.04%). The prevalence of *N. gonorrhoeae* among females, 25–34 years old, urban residences, secondary school educational status, married and employee participants were 15 (6.6%), 16 (7%), 18 (7.9%), 9 (3.9%), 17 (7.4%) and 6 (2.6%), respectively. Similarly, the prevalence of *N. gonorrhoeae* among HIV positive persons, persons having previous history of STIs, shisha users, alcohol drinkers, Khat chewers, condom non users and having more than two sexual partners were 6 (2.6), 5 (2.2), 7 (3.1), 7 (3.1), 10 (4.4), 9 (3.9) and 6 (2.6), respectively, [Table 1](#).

Associate Factors of *N. gonorrhoeae*

Our study analyzed the factors associated with *N. gonorrhoeae* using binary logistic regression. HIV-positivity, previous history of STIs, shisha usage, Khat users, condom non-users and having more than 2 sexual partners were significantly associated with the prevalence of *N. gonorrhoeae*, [Table 2](#).

Antibiotic Susceptibility Testing of *N. gonorrhoeae*

Twenty-three isolates of *N. gonorrhoeae* were tested with six antibiotics. All isolates were resistant to penicillin, 69.6% isolates to tetracycline and 34.8% isolates to ciprofloxacin and 30.4% isolates to spectinomycin. No isolate showed resistance to ceftriaxone, [Table 3](#).

Multidrug-Resistant *N. gonorrhoeae*

Multidrug-resistance is defined as the resistance of an isolate in one or more than one agent to three or more classes of antibiotics. Based on the definition, the multidrug-resistant *N. gonorrhoeae* was encountered in 12 isolates (52.2%). Four isolates of *N. gonorrhoeae* showed resistance to ciprofloxacin, penicillin and tetracycline; three isolates to penicillin, spectinomycin and tetracycline; and one isolate to penicillin, azithromycin and spectinomycin. Three isolates showed resistance to ciprofloxacin, penicillin, azithromycin, and tetracycline. No *N. gonorrhoeae* isolate was identified as susceptible to all antibiotics tested in our study, [Table 4](#).

Table 2 Univariate and Multivariate Analysis of Associated Factors of *N. gonorrhoeae*

Variables	COR (95% CI)	p-V	AOR (95%CI)	p-V
HIV status				
Positive	0.191 (0.064–0.566)	0.003	0.177 (0.032–0.979)	0.047
Negative			1	
History of STI				
Yes	0.283 (0.092–0.868)	0.027	0.149 (0.028–0.798)	0.026
No			1	
Shisha use				
Yes	0.069 (0.021–0.228)	0.000	0.042 (0.007–0.258)	0.001
No			1	
Alcohol intake				
Yes	1.008 (0.417–2.437)	0.986	1.815 (0.550–5.988)	0.328
No			1	
Chewing Khat				
Yes	0.232 (0.085–0.635)	0.004	0.446 (0.093–2.136)	0.312
No			1	
Condom use				
Yes			1	
No	0.097 (0.038–0.245)	0.000	0.535 (0.122–2.344)	0.000
Number of sexual partners				
1–2			1	
>2	4.262 (1.682–10.802)	0.002	4.307 (1.139–16.293)	0.031

Table 3 Antibiotic Susceptibility Patterns of *N. gonorrhoeae*

Antibiotics	<i>N. gonorrhoeae</i> (n=23)	
	Resistant	Sensitive
Penicillin	23 (100)	0 (0)
Ciprofloxacin	8 (34.8)	15 (65.2)
Ceftriaxone	0 (0)	23 (100)
Azithromycin	4 (17.4)	19 (82.6)
Spectinomycin	7 (30.4)	16 (69.6)
Tetracycline	16 (69.6)	7 (30.4)

Table 4 Multidrug-Resistant *N. gonorrhoeae*

Antimicrobial Agents		MDRs (%)
Number	Type	
R ₀	Zero	None
R ₁	P	3 (13.04)
R ₂	P, S	3 (13.04)
R ₃	P, TTC	6 (26.1)
	CIP, P, TTC	4 (17.4%)
	P, S, TTC	3 (13.04)
R ₄	P, Az, S	2 (8.6)
	CIP, P, AZ, TTC	3 (13.04)

Abbreviations: Az, azithromycin; CIP, Ciprofloxacin; P, penicillin; R₀, Resistant to none of the classes of antibiotics; R₁, Resistant of one agent to one class of antibiotics; R₂, resistance of one agent to two class of antibiotics; S, Spectinomycin; TTC, Tetracycline; MDR, Multidrug resistance.

Discussion

Neisseria gonorrhoeae is not only the cause of second leading bacterial sexually transmitted infection, but also it is resistant to the commonly used antibiotics as reported from different regions of the globe.^{16–18,20} Hence, this study described the prevalence, antibiotic resistance and associated factors among patients attending non-profitable clinics in Mekelle, Tigray, Ethiopia.

The overall prevalence of culture-confirmed *N. gonorrhoeae* was 23 (10.04%), which was comparable with studies from Ethiopia^{13,14} and Mongolia.²³ However, it was lower than reports from Ethiopia,¹⁵ Egypt,²⁴ Nigeria²⁵ and Mozambique,²⁶ but higher than the finding reported from Ethiopia,¹² Nigeria,²⁷ Colombia,²⁸ and the United States of America.²⁹ The difference in prevalence may be due to the differences in sexual behavior of societies in different countries and knowledge and use of prevention methods for sexually transmitted infections. The prevalence of *N. gonorrhoeae* may also be varied in different countries because of other various reasons such as lack of laboratory diagnosis methods, the difference in the accuracy of laboratory methods, and the difference in the skill of professionals and *N. gonorrhoeae* had a statistically significant association with HIV positive, previous history of STIs, shisha users, Khat users, condom non-users and having more than two sexual partners. These associated factors may enhance the transmission of *N. gonorrhoeae* and are supported by other studies.^{14,30,31} The proportions of *N. gonorrhoeae* in females, 25–34 years old and urban residents were high, which was different from studies in Ethiopia.^{14,31}

N. gonorrhoeae isolate in this study was 65% susceptible to ciprofloxacin, which is higher than a study in Ethiopia,³¹ but lower than another report from Ethiopia¹² and Thailand.^{17,18} Likewise, about 70% are susceptible to spectinomycin which is lower than a report from Ethiopia.¹² On the other side, four (17.4%) isolates of *N. gonorrhoeae* were resistant to azithromycin, which is higher than the study reported from Brazil.³² This may be due to syndromic approach of treatment

which, in turn, is due to the lack of an antimicrobial susceptibility test. In our study, all isolates of *N. gonorrhoeae* remained susceptible to ceftriaxone which is different from other reports that showed resistance from Thailand¹⁷ and Spain.³³ Hence, high multidrug resistant isolates of *N. gonorrhoeae* were observed in the study area.

This study may be contributed to the local policy makers in prevention and control of the infection and its drug resistance in the study area. It is also a regional input to the national and international scientists. However, there are some limitations, including the inability to determine the prevalence of other STIs because of the budgetary resources for acquiring chemicals and reagents. The study was also unable to determine resistance at the genetic level because of the lack of instrumentations and the necessary budgets.

Conclusions

High prevalence of *N. gonorrhoeae* isolates and drug resistance including multidrug resistance were observed in the study. These were associated with HIV positivity, having a previous history of STIs, shisha users, Khat users, condom non-users and having more than two sexual partners. Moreover, the highest rates of *N. gonorrhoeae* were identified in urban residences, married and employees. Furthermore, significant amount of *N. gonorrhoeae* isolates showed resistance to tetracycline, ciprofloxacin and spectinomycin. All of the isolates of *N. gonorrhoeae* showed resistance to penicillin, but no isolate was resistant to ceftriaxone. More than.

Abbreviations

CLSI, Clinical and Laboratory Standard Institutes; HIV, Human Immunodeficiency Virus; STI, Sexually Transmitted Infections; SOP, Standard Operating Procedures; SPSS, Statistical Package for Social Sciences; WHO, World Health Organizations.

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Author Contributions

All authors made a significant contribution in the conception, study design, execution, acquisition of data, or analysis, and interpretation. Moreover, all authors took part in drafting, revising or in critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

We declared that we have no competing interests.

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