



PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *NEISSERIA GONORRHOEAE* AMONG PATIENTS ATTENDING GYNECOLOGY AND SEXUAL TRANSMITTED INFECTION CLINICS AT BAHIR DAR TOWN, NORTH WEST ETHIOPIA

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ABSTRACT

Background:- *Neisseria gonorrhoeae* infection is the second most common prevalent sexually transmitted bacterial infection. Currently, the increasing prevalence of antimicrobial-resistant *N. gonorrhoeae* isolates is a concern. The aim of this study was to determining the proportion and antimicrobial susceptibility pattern of *N. gonorrhoeae* isolates among patients attending Gynecology and Sexually transmitted infection clinic in the selected health institution at Bahir Dar Town, Northwest Ethiopia. **Methods:** Across sectional study was done at the selected health institution in Bahirdar town from February 01 to July 30, 2017. Data on socio-demographic characteristics and possible associated factors were collected by face to face interview using structured pre-tested questionnaire. Urethral and endo-cervical swab sample were collected by trained nurses. The collected samples were transported to microbiology laboratory and processed within 15min following standard microbiological techniques. Antimicrobial susceptibility profile of the isolates was performed using Kirby-Bauer disk diffusion method. Descriptive statistics were used to present relevant variables. Binary logistic regression was used and significant variables were further adjusted using multivariate analysis. A *p*-value <0.05 was considered as level of significance. **Results:** Of the total 169 STI symptomatic patients screened, 8(4.7%) were positive for *N. gonorrhoeae* infection. The highest proportion of *N. gonorrhoeae* infection occurred in the age group of 15-29 years at 7(6.1%). The number of *N. gonorrhoeae* infection isolate was seven in females and one in males. However, the difference was not statistically significant (*P* = 0.76). Alcohol intake (*p*=0.035) and khat chewing (*p*=0.011) were significantly associated with *N. gonorrhoeae* infection. All *N. gonorrhoeae* isolates were resistant to Penicillin and Tetracycline. Whereas three isolates showed resistance to Doxycycline, Cefoxitin, Cefixime, Cefpodoxime, Ofloxacin, Spectinomycin but all isolates were sensitive to Ceftriaxone. This study concludes that drinking alcohol and chewing chat were the identified associated variables. Thus, awareness on substance use should be created in the community. Emphasis should be given among health professionals in minimizing misuse of antibiotics.

KEYWORD: *N. Gonorrhoeae*, Drug Susceptibility Patterns, STI, Bahirdar, Ethiopia.

BACKGROUND

Sexually transmitted infections (STI) are a major public health problems in most part of the world [MMWR, 2015]. Gonorrhea is a type of STI caused by *Neisseria gonorrhoea* [WHO, 2016] and it is the second most common causes of STI [Viscidi and Demma, 2003; Gonorrhea CDC Fact Sheet, 2014; WHO, 2015, 2016].

N. gonorrhoea is a gram negative cocci, micro-aerophilic and fastidious bacterium. It preferentially colonizes and multiplies easily in the warm, moist mucosal surface of the reproductive tract [Merz & So 2000]. These bacteria have natural transformation and antigenic variation

capacity. Its virulence factors include: pili, IgA proteases, endo-toxin and porin proteins (ProB), antibiotic resistance gene (*gyrA* and *parC*) [Edwards and Apicella, 2004; Stathi *et al.*, 2006; Junior *et al.*, 2007; Low *et al.*, 2014; Lin1 *et al.*, 2015]. The primary site of infection is the epithelium of reproductive tracts. It also infects rectum, joints, throat and eyes [Stathi *et al.*, 2006; Low *et al.*, 2014; Lin1 *et al.*, 2015]. Infection of the vagina or penis cause burning/itching sensation, urgency and pain during urination. If infection left untreated, it may lead to serious complications like pelvic inflammatory disease, epididymitis, ectopic pregnancy,

infertility, tenosynovitis and blindness [Merz and So, 2000; Bala, 2011].

About 87.7 to 106.1 million people suffer from gonorrhea worldwide and 53% of them were male [Gewirtzman, Bobrick, Conner and Stephen K Tying, 2011; MMWR, 2015; Newman *et al.*, 2015; WHO, 2016]. According to World Health Organization (WHO) report, the burden of gonorrhea is high especially in Africa and other developing countries where the prevalence is >8.2% [WHO, 2012]. In Ethiopia, the prevalence of gonorrhea disease varies from place to place. Most of these data were from simple cross sectional study. In Ethiopia gonorrhea and other STI are managed by syndromic approach [Hailemariam *et al.*, 2013; Ali *et al.* 2016].

Even though gonorrhea infection is primarily affects people whose age is between 15 and 49 year; low socio economic status, unmarried, past history of infection and low education are the most risk factors documented so far [Edwards and Apicella, 2004; Who, 2012; Newman *et al.*, 2015].

The emergence of resistance to antimicrobial agents is a global public health problem, a according to different countries stated that emerging resistance of *N.gonorrhoeae* to antimicrobial agents, resulting from both wide dissemination of resistant clones and strains with novel resistance mechanisms [Junior *et al.*, 2007]. *N. gonorrhoeae* develops resistance to B-lactam antibiotic by producing , Penicillinase, B-lactamase, acquiring resistance plasmid and chromosomally mediated [Gong *et al.*, 2016]. CDC advocated the use of expanded generation cephalosporin or fluoroquinolones as first line therapy for uncomplicated gonorrhea [Byarugaba, 2009; Bala, 2011]. Currently, strains exhibiting decreased susceptibility to ciprofloxacin or ofloxacin have been reported in Asian and European countries [Tapsall, 2001; Junior *et al.*, 2007; Gong *et al.*, 2016; Magnus *et al.*, 2016].

Few available reports on prevalence of *N. gonorrhoeae* in Ethiopia showed significant variation in distribution among the regions. Due to unavailability of culture-facility in health institutions in the country, the treatment of gonorrhea is almost empirical and antibiotics are generally given without considering sensitivity report. Recently in any health facilities the STI case are routinely managed by syndromic approach [Health Sector DevelopmentProgramme, 2014; STI, 2015] where patients suspected of *N. gonorrhoeae* infection are treated empirically by giving antibiotics recommended in the package. The objective of this study was to determine the proportion of *N. gonorrhoeae* and its antimicrobial susceptibility profile, and associated factors for *N. gonorrhoeae* infection among people with STI complaints presented to health facilities in Bahir Dar.

MATERIALS AND METHODS

Study Area: This study was conducted in Bahirdar town. In 2015, Bahirdar has a population of 256,156, of whom 97,160 males and 158,996 females [CSA, 2015]. Patient flow in selected health institution for more than 5 million people of which 560 were STI cases. STI cases can be seen at two (gynecology and STI) clinics in the Bahirdar Health institution.

Study Design and Period

A facility based cross sectional study was conducted from February 01 to July 30 2017 in selected health institution found in Bahirdar town.

Source Population

All men and women STI symptomatic patients attended STI clinic and gynecology outpatient department at FHRH, FGA, and MSIE at Bahirdar town.

Study Population

All men and women STI suspected patient who attended in STI clinic and gynecology outpatient department at FHRH, FGA, and MSIE at Bahirdar town from February-2017-to July-30-2017.

Inclusion Criteria

All men and women who had sign and symptom of STI and any age group and who had no history of abnormal uterine bleeding (AUB) and antibiotic treatment in the preceding one week were included in the study.

Exclusion Criteria

- All men and women who had history of antibiotic treatment in the preceding one weeks at the time of data collection
- Severely ill patients
- Abnormal uterine bleeding (AUB)
- Pregnancy
- women who were on menstruation at the time of examination

Sample Size Determination

The sample size was calculated using single proportion formula by taking 11.3% of *N. gonorheae* infection taken from previous study [Ali *et al.* 2016], 95% confidence interval, 5% margin of error using kish's formula [Kish.L,1995]

$$n = \frac{(Z_{\alpha/2})^2 p(1-p)}{d^2}$$

Where

n = Sample size

α = level of significance

z = at 95% confidence interval Z value ($\alpha = 0.05$) => $Z_{\alpha/2} = 1.96$

p = Proportion of occurrence of the gonorrhea to be studied 11.3%.

d = Margin of error at (5%) (0.05)

Accordingly the sample size calculated as 154, and adding 10% non-response rate (15 individual) i.e. $154+15 = 169$

Sampling Technique

First lottery method was used to select three representative health institutions found in Bahirdar city. Then the calculated sample size was proportionally allocated to Feleg-Hiwot Referral Hospital (FHRH) (N=100), Family Guidance Association (FGA) (N=40), and Bahirdar health center (BDHC) (N=29) according to their patient flow. Patients eligible for this study were included using systematic sampling technique until the required sample size was achieved.

Data collection Techniques

Demographic data, clinical profiles and factors related to STI were collected through face to face interview using pre-tested structured questionnaires. Nurses attending STI and gynecology OPD in selected clinics were assigned as data collectors. Written consent was obtained from study participant patients prior to data collection then information on socio-demographic and other variables of interest was collected by trained data collectors using pre-designed questioners.

Swab Sample collection and processing

Endo-Cervical and urethral swab were collected using Dacron or rayon swabs of aseptically following standard procedures by trained nurses obtained discharge from Cervix and urethra sites before the discharge collected cleaned by antiseptic solution. The collected specimen was transported to the Amhara Public Health institution (APHI) within 15-minute using Amies charcoal transport media [Khanam *et al.*, 2014].

The Dacron or rayon swabs were inoculated in to Modified Thayer-Martin Media (MTMM) (OXOID transport swabs Italia SpA and Brescia Italy). The swab was strike in four quadrants to allow growth of pure colonies. The inoculated plates were incubated at 35°C in a moist atmosphere enriched with 5% CO₂ for 24-48 hours. Negative plates were incubated for additional 24 hours. Then the growth was inspected macroscopically and detected for *N. gonorrhoeae* [CLSI, 2016; Janda, 2004].

Isolation and Identification of *N. gonorrhoeae*

Identification of the culture isolates was done according to the standard bacteriological methods [CLSI, 2016]. Colony morphology, gram reaction and microscopic features were used as primarily identification criteria. Further identification was done by biochemical tests such as Oxidase and catalase tests. Bacteria that showed growth checked by gram staining. Gram negative Intracellular diplococci further checked by Oxidase and catalase test. Those positive for Oxidase and catalase were considered as species *N. gonorrhoeae*.

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing was done on GC-chocolate agar with 1% Iso-Vitale-X supplement for all isolates according to the criteria of Clinical and Laboratory Standard Institute (CLSI) by the Kirby-Bauer disk diffusion method [CLSI, 2016]. Bacterial suspensions with turbidity standard equivalent to 0.5 McFarland were swabbed evenly on plate. A set of ten antibiotic discs (Oxoid Ltd., Basingstoke, Hampshire, England) with the following concentrations: The drug choose for *N. gonorrhoeae* was use, Penicillin (P, 10µg), Tetracycline (TE, 30µg), Ciprofloxacin (CIP-5µg), Ceftriaxone (CRO-5µg), Doxycycline (DOX-100), Cefoxitin (FOX-30µg), Cefixime (CFM-5µg); Cefpodoxime (CPD-30µg) OFX (ofloxacin-5µg), Spectinomycin (SPT-100µg). These were placed on the surface of the plate. Then, the plates were incubated at 37 °C in candle jar, generating 5 % CO₂, for 20–24 h. Zone of inhibition was measured and interpreted as sensitive, intermediate and resistant according to the principles established by CLSI [CLSI, 2016]. In order to monitor quality (potency) of disks, a standard strain of *N. gonorrhoeae* American Type Culture Collection (ATCC) 49226 was tested weekly. These antibiotic disks were selected based on the national drug list and recommendation of *N. gonorrhoeae* treatment [CLSI, 2016].

DATA ANALYSIS

Data were sorted, coded and entered in to computer by using Epi Data version 3.02 after checked for completeness and accuracy. Then data were exported to Statistical Package for Social Sciences (SPSS) database program 20 version for further analysis. Univariate analysis and appropriate graphic presentations was used for describing data. Bivariate analysis of demographic and other possible variables associated with *N. gonorrhoeae* infection was done. Adjusted odds ratio along with 95% Confidence Interval (CI) was estimated to identify factors associated with *N. gonorrhoeae* culture positivity using multivariate logistic regression analysis. A p-value < 0.05 was considered as statistically significant.

RESULTS

Socio-Demographic characteristics of the study participants

A total of 169 STI symptomatic patients were screened at FHRH, FGA and BDHC. Of the total participants, 153(90.5%) were females. The median age of participants was 26 years. Majority (78.7%) of participants were urban dwellers. Similarly, 133 (78.7%) were from urban area, 93(55%) were unmarried, and 75(44.5%) were unemployed as shown in Table 1.

Prevalence of *N. gonorrhoeae*

Out of the total 169 participants whose urethral and or endo-cervical swabs investigated, *N. gonorrhoeae* was isolated from 8(4.7%) patients. The highest proportion 7(6.1%) of *N. gonorrhoeae* infection isolated among people whose age range between 15-29 year-olds

compared to above 30year-olds participants. However, the difference was not statistical significant ($P=0.234$). The number of *N. gonorrhoeae* isolates was higher in females 7(4.3%) than males 1(6.2%). Moreover, it was higher among urban 6(75%) than rural dwellers, unmarried 6(75%) than married, unemployed 6(75%) than employed, and among those attained less than primary school 6(75%) than their counter parts. However, none of these variables showed significant differences (Table 2).

When assessed the prevalence of *N. gonorrhoeae* infection with regard to risk sexual behavior, it was found that among people who had multiple sexual partners, 8(100%), while only 1(9.1%) and 1(8.3%) identified among those who had history of abortion and

rape, respectively. Similarly, about 3(37.5%) and 5(62.5%) of was isolated among those who have been chewing khat for the last 6 months and who consume alcohol, respectively.

However, the proportion of *N. gonorrhoeae* isolates was significantly higher among alcohol drunker $p=0.035$ and khat chewers $p=0.011$ than their counters (Table 2).

Antimicrobial Susceptibility Testing:

All the eight isolates were sensitive to Ceftriaxone. Only three isolates showed resistance Doxycycline, Cefoxitin, cefpodoxime, ofloxacin, cefixime and spectinomycin. On other hand, all eight isolates showed resistance against Penicillin and Tetracycline.

Table. 1: Socio-Demographic characteristics and sexual risk behavior of patient attending Gynecology and STI clinic at Bahir Dar town Ethiopia (Feb-July 2017)

Variables	Categories	Frequency	Percent
sex	Male	16	9.5
	Female	153	90.5
Age ranges (year)	15-29	114	67.5
	≥ 30	55	32.5
Residence	Urban	133	78.7
	Rural	36	21.3
Monthly Income in ETB	≤ 1000 ETB	42	24.9
	> 1000 ETB	127	75.1
Educational status	$\leq$ Primary School	87	51.5
	$>$ primary school	82	48.5
Marital status	Married	76	45.0
	Unmarried	93	55.0
Occupation	Unemployed	75	44.4
	Employed	94	55.6
Drinking Alcohol	yes	46	27.2
	No	123	72.8
Chewing chat	Yes	15	8.9
	No	154	91.1
Smoking cigarette	Yes	8	4.7
	No	161	95.3
Number of sexual partner	single	76	45.0
	multi-sexual	53	31.4
	No sexual partner	40	23.7
Use of condom	Yes	49	29.0
	No	120	71.0
History of abortion	Yes	11	6.5
	No	142	84.0
History of Rape	Yes	12	7.1
	No	141	83.4

Table. 2: Association of *N. gonorrhoeae* infection with socio-demographic and sexual risk behaviors of patient attending Gynecology and STI clinic at Bahir Dar town Ethiopia (Feb-July 2017)

Variables		<i>N. gonorrhoeae</i>		Total	p- value
		Positive No (%)	Negative No (%)		
Sex	Male(1)	1 (6.2)	15 (93.8)	16	0.76
	Female(2)	7 (4.3)	146(95.4)	153	
Age	15-29	7(6.1)	107(94)	114	0.234
	≥30	1(2)	54(98)	55	
Residence	Urban	6(4.5)	127(95.5)	133	0.79
	Rural	2(5.6)	34(94.4%)	36	
Marital Status	Married	2(2.6)	76(97.4)	78	----
	Unmarried	6(6.6)	85(93.4)	91	
Educational Status	≤Primary School	6 (6.9)	81 (93.1)	87	0.191
	>primary school	2 (2.4)	80 (97.6)	82	
Occupation	Un employed	6 (8)	69 (92)	75	0.096
	Employed	2 (2.1)	92 (97.9)	94	
AMI income (ETB)	≤1000	0(0)	42 (100)	42	-----
	>1000	8 (6.3)	119 (93.7)	127	
Alcohol drinking	Yes	5(10.9)	41(89.1)	46	0.035
	No	3(2.4)	120(97.6)	123	
Chewing khat the last 6-month	Yes	3(20)	12(80)	15	0.011
	No	5(3.2)	149(96.8)	154	
use Condom	Yes	2(4.1)	47(95.9)	49	0.799
	No	6(5)	114(95)	120	
Cigarette smoking	Yes	1(12.5)	7(87.5)	8	0.314
	No	7(4.3)	154(95.7)	161	
Number of sexual partner	Single	0(0)	76(100)	76	0.445
	Multiple-sex partner	8(15.1)	45(84.9)	53	
	No-partner	0(0)	40(100)	40	1
Abortion History	Yes	1(9.1)	10(90.9)	11	0.468
	No	6(4.2)	136(95.8)	142	
Rape History	Yes	1(8.3)	11(91.7)	12	0.525
	No	6(4.2)	135(95.8)	141	

Key: AMI= average monthly income, 1= Reference

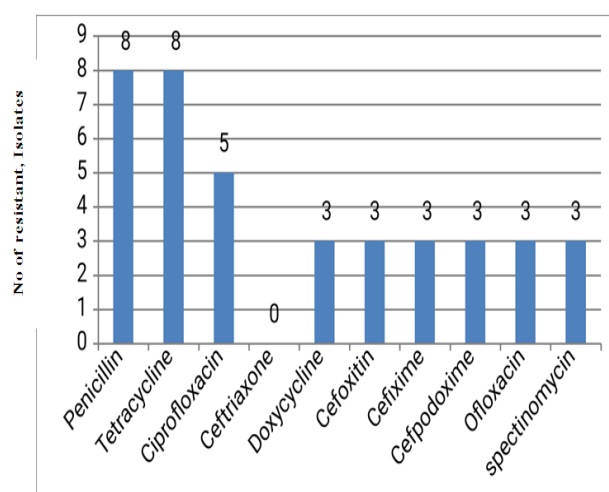


Figure. 1: Resistance profile of *N. gonorrhoeae* isolates, Bahirdar, Ethiopia, 2017.

DISCUSSION

N. gonorrhoeae is considered as one of the global public health problem. It remains a major cause of morbidity in

sexually active age groups, and people who were dependent on alcohol [GSTIS 2015].

In this study, the prevalence rate of *N. gonorrhoeae* infection among symptomatic patients visiting gynecology and sexual transmitted infection clinic in Bahirdar town was 8(4.7%). This figure might be considered as relatively low prevalence as this study included all patients with symptomatic to general STI not *N. gonorrhoeae* related symptoms. However, comparing with similar studies, which ranges the present finding is consistent with studies done in Hong Kong 4.4% [Mayaud et al., 1995], Burkina Faso 4.5% [Naidoo S, Wand H, Abbai NS, 2014], Côte d'Ivoire 5.1% [Men et al., 2010], Hawassa, Ethiopia 5.1%, [Hailemariam et al. 2013]. However, it was higher than studies from 0.3% in Melbourne, 0.5% in British, 0.6% in African Napdo, 1.6% in Yaounde, 1.9% in Mexico, 2% in Central African Republic, 2.2% in Jordan, 2.3% in Nepal, 2.6% in African city, 3.2% in Nairobi, Kenya and 3.8% at Global level [Luo et al. 2016; Bowle et al. 1981; GSTIS 2015, WHO 2016; Gewirtzman et al. 2011, Christian et al. 2005; Daly et al. 1994; Newman et al. 2015].

On the other hand, this finding was lower than studies from Ghana 6%, South Africa 8%, Bahirdar 8.2%, Gambella 11.3%, Jimma 17.7%, other countries like Bangladesh 42%, Uganda 59%, Taiwan 65% and Malawi 80% [Shimelise 2012; Bowle et al.1981; Buve et.al, 2001; Yu et al. 2005; Tibebe et al. 2013; Khanam et al. 2014, Tumwesigye et al. 2015; Newman et al. 2015; Ali et al., 2016, Duplessis et al, 2017]. The observed discrepancy might be due to difference in the target population, sample size and cultural practices.

In this study, drinking alcohol and chewing chat showed statistical significant association with *N. gonorrhoeae* infection. Association between drinking alcohol and *N. gonorrhoeae* infection have been reported from Melborn, Nepal, Hong Kong, Gambella, and Jimma, Ethiopia, Uganda and Malawi. It was also suggested that substance use particularly alcohol consumption and khat chewing are cofactors as loss of inhibition and involvement in risky sexual behaviors such as multiple sexual partners and unprotected intercourse [WHO, 2011; Kahsay et al. 2015].

Unlike our study, some other study reported association between *N. gonorrhoeae* infection and age [Luo et al., 2016], residency [Hailemariam et al., 2013], commercial sex work [Christian et al., 2005; Shimelise,2012; Yu et al., 2005; Zachariah et al., 2005].

In this study all *N. gonorrhoeae* isolates were sensitive to Ceftriaxone. However, three isolates showed resistance towards Doxycycline, cefoxitin, cefpodoxime, ofloxacin, cefixime and spectinomycin. This was in line with reports from Hawassa and Gambella, Ethiopia, Nigeria, Bangladesh, Brazil ,Taiwan, MMWR [Hailemariam et al.2013; WHO 2015; Luo et al 2016; Yu et al.2005; Khanam et al.2014; MMWR, 2014; Ali et al. 2016]. However, due to frequent and improper uses of these antibiotics without laboratory test might pose this pathogen to develop resistance in near future [Allen et al.2011; STD, 2012; WHO, 2014]. A recent WHO report showed widespread resistance to older and cheaper antibiotics. Moreover, there are finding cases of the infection that are untreatable by all known antibiotics.

In this study *N. gonorrhoeae* isolates showed 62.5% of resistance against ciprofloxacin which is much higher than reports from Hawassa and Gambella, Ethiopia Nepal, Brazil and Uganda [Hailemariam et al. 2013; WHO, 2015; Luo et al.2016]. Such relative high rate of resistance might be associated with self-prescription, indiscriminate and intensive use of this antibiotic in health facilities found in Bahirdar. Moreover, ciprofloxacin is included in the syndromic management package, it is often used in combination with doxycycline or azithromycin for infections concomitant with *Chlamydia trachomatis*. It is also widely used both for other STDs and various bacterial infections. The alarming percentage of resistance against ciprofloxacin observed in this study challenges the current use of this drug in the syndromic management package of *N.*

gonorrhoeae infections. Hence, switching from ciprofloxacin to ceftriaxone was optimal [Roy et al., 2005].

When comparing this finding with previous studies, comparable resistance profile is being reported across the countries. This report confirmed that early generation of Beta lactam antibiotics and fluoroquinolones became useless antibiotics for treatment of Gonococcal infection [Tibebe et al., 2013; Khanam et al., 2014; MMWR, 2014; Newman et al., 2015; Ali et al., 2016]. Notably, high levels of ciprofloxacin >90% resistance have been reported in a majority of countries globally, especially in the WHO southeast Asia region, the western pacific region and Africa region [Newman et al., 2015; Manuscript, 2011]. The variation in rate of penicillin resistance *N. gonorrhoeae* isolates in the same country might be an indication of the overtime period rising in resistance trend of the bacteria to this drug.

The cephalosporin drugs such as ceftriaxone found to be effective antibiotics for the treatment of *N. gonorrhoeae* which are responsible to cause Endo-cervical or urethral infections. This might be because these agents are expensive and not commonly used on the study area. This is in agreement with study done in USA, Australian and Romania [Tapsall, 2001; Toskin, 2014; Newman et al., 2015].

CONCLUSIONS

The proportion of *N. gonorrhoeae* infection was comparably higher in the study area. Higher proportion was identified among urban dwellers, female, unmarried individuals, unemployed, those having multiple sexual partners, and inconsistent condom users.

All isolates were resistant against penicillin and tetracycline. On the other hand, all isolates were found to be sensitive to ceftriaxone.

Drinking alcohol and chewing chat were the identified associated factors exposing individual for *N. gonorrhoeae* infection.

RECOMMENDATIONS

- ✓ Penicillin and tetracycline should be avoided for treating *N. gonorrhoeae* infection
- ✓ Ceftriaxone can be used effectively for treating *N. gonorrhoeae* infection
- ✓ The current syndromic management approach with ciprofloxacin treatment should be rechecked for the resistance profile.
- ✓ Regular behavior and sexuality education is mandatory for those dependency substances users.
- ✓ Large-scale community based survey should be done to identify the true prevalence and other possible associated factors hidden in the community.

AUTHORS' CONTRIBUTIONS

TM was major contributor designed the study, participated in data collection, analysis, interpretation and write-up, and drafted the manuscript. RM participated in analysis, interpretation and write-up, drafted the manuscript and critically revised the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

ETHICAL APPROVAL

Ethical clearance was obtained from ethical approval committee of College of Medicine and Health Sciences, Bahirdar University. Formal letter of cooperation was written for Bahirdar Regional Health Bureau, Amhara public health institution, Felelege Hiwot Referral Hospital, Family Guidance Association, and Bahirdar Health Center. Each respondent was informed about the objective of the study and written consent was signed by each study participant. To ensure confidentiality, data was kept anonymous whereby names of the study subjects was not be written on the questionnaire using codes instead of any personal identifiers. Only positive laboratory test results were given for patient's respective physician for any beneficiary measure.

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