

ORIGINAL ARTICLE**PREVALENCE OF *NEISSERIA GONORRHEA* AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AMONG SYMPTOMATIC WOMEN ATTENDING GYNECOLOGY OUTPATIENT DEPARTMENT IN HAWASSA REFERRAL HOSPITAL, HAWASSA, ETHIOPIA**

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ABSTRACT

BACKGROUND: *gonorrhoeae*, a sexually transmitted disease caused by *Neisseria gonorrhoea* for which humans are the only natural host. The causative organism is highly adapted to the genital tract and often causing asymptomatic and undetected infection in females in which Acquisition of gonococcal infection late in pregnancy can adversely affect labor and delivery as well as the well-being of the fetus. The aims of this study were to determine the prevalence and drug susceptibility pattern of *Neisseria gonorrhoea* among symptomatic women in Hawassa Referral Hospital.

METHODS: A cross-sectional study was conducted from December 1 2010 to February 30, 2011 at Hawassa Referral Hospital. All women who visited gynecology outpatient department (OPD) with suspected gonococcal infection were included. Endocervical swab was collected by the attending physician. The presence of gonorrhoea was confirmed by culture, gram staining and biochemical tests. Antimicrobial sensitivity test was performed using disc diffusion method and the result was interpreted accordingly.

RESULTS: Of the total 215 cases examined, 11 (5.1%) were confirmed to have gonococcal infection. Although not statistically significant, most of the cases 5/11 (45.5%) were in age group of 20-24 years and the identified organism had low level susceptibility to quinolones (ciprofloxacin 55%, ofloxacin 64% & lomefloxacin 64%).

CONCLUSION: Despite low rates of gonorrhoea infection, it is important to focus on high-risk populations (reproductive age group) because of the great physical and emotional costs of the disease. A high resistance for quinolones, the commonly used antibiotics was observed for this laboratory-based diagnosis is recommended.

Keywords: *Neisseria gonorrhoea*, drug susceptibility, symptomatic women, Southern Ethiopia

INTRODUCTION

Gonorrhoeae is a sexually transmitted disease caused by the bacteria *Neisseria gonorrhoea* for which humans are the only natural host (1). The advent of sulfonamide in 1936 and penicillin in 1943 antibiotic therapy for the treatment of gonococcal infection led to a rapid decrease in gonorrhoea prevalence. Then since the beginning of

the 20th century, peaks of reported cases of gonorrhoea occurred during World Wars I and II and following the "sexual liberation" of the late 1960s and early 1970s (2). In the late 1980s, with the onset of the HIV epidemic and a coincident widespread use of barrier contraceptives, the incidence of gonococcal infection again declined (1).

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In the absence of a national gonococcus screening program, little is known about the prevalence of gonococcal infection in women of reproductive age group in Ethiopia. To our knowledge the last gonococcal study in reproductive age group of women in Ethiopia was done before 20 years (3). The STI surveillance system in the country is weak. The Integrated Disease Surveillance Team of the Ministry of Health in 2005 reported that there is under reporting of STI cases including gonorrhoea in most part of the country. Except for the adult prevalence of HIV (4.7%) and syphilis (1.8%) there is no national estimate of other STIs including *gonorrhoea*. This is because the pattern of reporting from health institutions is not uniform. Some health institutions report using syndromic approach while others use etiologic approach (4).

The drug resistance varies greatly among countries. Therefore having prevalence's data as well as the drug susceptibility pattern within consecutive year is important especially for gonorrhoea, the highly drug resistant bacteria (5). There is no up to date data about the prevalence and resistance pattern of gonorrhoea in Ethiopia even if it is often incomplete due to; clinical presentation not specific enough for diagnosis based solely on symptoms also in lack of proper reporting mechanisms. The major objective of this study was to assess the prevalence of *Neisseria gonorrhoea* and their antimicrobial susceptibility patterns among symptomatic women attending a gynecology outpatient department in Hawassa referral hospital.

SUBJECTS AND METHOD

Study Design and Area: A prospective cross-sectional study was conducted from December 1, 2010 to February 30, 2011 at Gynecology outpatient department (OPD) and Microbiology Laboratory of Hawassa Referral Hospital, Hawassa, Ethiopia.

Sample size was calculated based on the highest prevalence of gonorrhoea estimate, 5-15% for all Africa and we used the highest 15% for this study (6). Expected margin of error (d) was 0.05 and confidence interval (z) was 95%. The calculated sample size was 215 (10% non-response rate inclusive) (7). All women of reproductive age group who attended gynecology out patient at Hawassa Referral Hospital with suspected STIs were included in the study.

Women of reproductive age group (15-44 years) with any one of the sign and symptom for STIs such as pain during sexual intercourse, a painful or burning sensation when urinating and abnormal vaginal discharge were included. Others with symptoms indicating development of Pelvic Inflammatory Disease (PID) like cramps and pain, bleeding between menstrual periods, vomiting, and fever were also included in the study.

Women who have no sign and symptom for STIs, women on recent antibiotic treatment, and those who were outside the reproductive age group were excluded from the study. Clinical examination was done by physician to all patients who were attending gynecological OPD.

All relevant data were obtained by attending physician and was transferred to the questionnaire prepared for this study. Two swabs were collected from each patient from endocervical canal by the physician one for gram stain and the other for culture. The samples were immediately delivered and inoculated to appropriate media in Microbiology Laboratory of Hawassa Referral Hospital. Amies transport media (Oxoid, Basingstoke, and Hampshire, UK, England) was used at times of delay.

While one of the two swabs taken from individual patient was used for gram stain the other was inoculated on to nonselective chocolate agar and selective agar modified Thayer-Martin medium (Oxoid, Basingstoke, and Hampshire, UK, England). Some fastidious strains, such as the arginine-, hypoxanthine- and uracil-requiring strains, are more susceptible to the concentrations of vancomycin or trimethoprim used in the selective media which can grow in nonselective chocolate agar. The inoculated plates were incubated at 37°C in a moist atmosphere enriched with CO₂ 5% using candle jar. *N. gonorrhoeae* produces small raised, grey shiny colonies on modified Thayer-Martin medium after overnight incubation (8).

Neisseria gonorrhoea is differentiated from other *Neisseria* species, *Moraxella* species, *Kingella* species and other commensals based on the production of acid from glucose only and not from maltose, lactose, sucrose and fructose. Accordingly, the carbohydrate utilization test was done using API NH identification kit strips (Oxoid, Basingstoke, and Hampshire, UK, England). In general all positive cultures were identified by their characteristic appearance on the media, Gram staining reaction and confirmed by

the pattern of biochemical reactions using the standard method.

Antimicrobial susceptibility was assessed by using Kirby-Bauer disk diffusion test, according to NCCLS (9). Gonococcal specimens were sub cultured from the selective primary medium to a chocolate agar to obtain a pure culture of the specimen. From a pure culture of 3-5 selected colonies of bacteria were transferred to a tube with a straight wire and prepared a suspension in 2.5 ml normal saline and incubated at 37°C until the turbidity of the suspension become adjusted to a McFarland 0.5. Sterile swab was used to distribute the bacteria evenly over the entire surface of chocolate agar. The susceptibility to the following antimicrobial agents (Oxoid) were assessed: penicillin (P 10 IU), tetracycline (TE 30µg), ciprofloxacin (CIP 5µg), ceftriaxone (CRO 30µg), cefixime (CFM 5µg), Cefoxitin (FOX 30µg), cefpodoxime (CPD 10µg), spectinomycin (SPT 100µg). The criteria used to select the antimicrobial agents tested were based on their availability and frequent prescriptions for the management of gonococcal infection.

Standard reference strain of *Neisseria gonorrhoeae* ATCC 49226 was used as recommended by the Clinical and Laboratory Standards Institute (CLSI) for QC of susceptibility testing of gonococcal isolates. Reference strain from the EHNRI laboratory stock was also used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

Data entry and analysis was done using SPSS for Windows version 16.0. Prevalence rate was calculated for the positive cases of examined subjects and separately by age groups. Logistic regression and Fisher exact test analysis were used to estimate adjusted odds ratios. The level of significance was set at 0.05 in order to consider a p-value <0.05 as indicator of a statistically significant difference with 95% confidence.

This research project was approved by the Department of Medical Microbiology, Parasitology and Immunology Research and Ethical Review Committee, Faculty of Medicine; Addis Ababa University. Official permission from the study site was obtained. Written informed consent was obtained from study participants. The laboratory test results were given for participants and proper treatment was also delivered for positive individuals.

RESULTS

Socio-Demographic Characteristics of The Study Population: Out of 1342 patients visited the Hawassa University Referral Hospitals gynecology OPD between December 1, 2010 and February 30, 2011, a total of 215(16.0%) patients were included in the study. On their visit, patients were interviewed and examined clinically. One hundred sixty three (75.8%) of the 215 cases were from urban and 52 (24.2%) from rural. The age ranged from 15 to 44 years with a mean of 25.51 years \pm 7.45. Most of the study subjects, 118 (54.9%) were married, 72 (33.5%) were students in occupation, 92(44.2%) Protestants in religion and 68(33.5%) Sidama by ethnic group (Table 1).

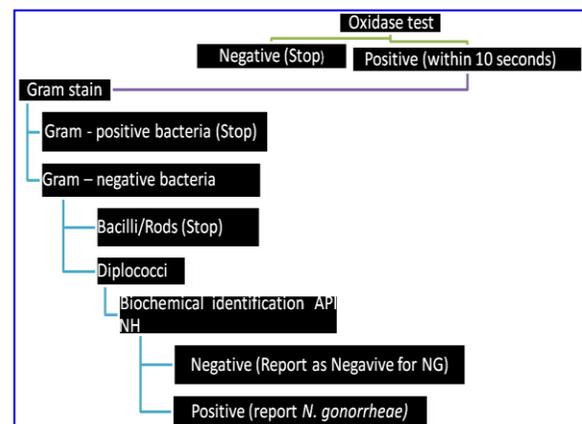


Figure 1. Flow chart of Laboratory identification of bacteria

Overall Prevalence of Gonococcal Infection: Among 215cases, 11 (5.1%) were confirmed to have gonococcal infection. Of the 11 patients who were positive for gonococcal infection, 6(54.5%) were from rural, and 5(45.5%) were from urban setting. There is significant statistical association with living area (p= 0.026) and the odds of having gonorrhoea infection for women living in rural was four-times higher than urban counter parts (OR = 4.12, (95% CI, 1.203, 14.121). Gonococcal infection was observed in 6/11 (54.5%) of married

women and 5/11 (45.5%) of students by occupation. Of the 25 pregnant women included in the study, one (4.0%) was positive for *Neisseria gonorrhoeae*. Five of the 11 patients who were confirmed to have gonococcal infection were in age group of 20-24 years though not statistically significant ($p>0.05$) (Table 2 and fig 2).

The macroscopic examination of swab showed that 8/11 (72.7) positives were from mucopurulent swab and 3/11 were from bloody swab, however, no statically significance association was observed (OR =2.409, 95% CI (0.619, 9.378)).

Table 1. Description of the demographic data of 215 patients investigated for gonococcal infection at Hawassa University Referral Hospitals, Hawassa, Ethiopia (Dec2010- Feb 2011).

Socio-demographic characteristics	Category	Frequency	Percent
Age- group	15-19	50	23.3
	20-24	71	33
	25-29	34	15.8
	30-34	25	11.6
	35-39	23	10.7
	40-44	12	5.6
Address	Urban	163	75.8
	Rural	52	24.2
Marital status	Single	81	37.7
	Married	118	54.9
	Divorced	9	4.2
	Widowed	7	3.3
Occupation	Student	72	33.5
	Gov.employees	48	22.3
	Farmers	42	19.5
	House wife	18	8.4
	Daily workers	13	6
	Merchants	11	5.1
	Others	11	5.1
Ethnic group	Sidama	72	33.5
	Amhara	42	19.5
	Oromo	38	17.7
	Wolayta	33	15.3
	Gurage	11	5.1
	Tigre	10	4.2
	Others	9	4.2

®= Kembata, Yem, Silte, Gedio. Etc, Ω = Employee In Private Sector, No Occupation

Bacteriologic Examination: Of the 215 endocervical specimens examined by Gram stain, 21 (9.8%) were positive for either intra cellular or extra cellular gram negative diplococci (GNDC).

Among the 21 gram stain positive for GNDC, 19 were showed growth on media (Table 3).

Table 2. Association between prevalence of gonococcal infection and demographic characteristics of 215 patients investigated for gonococcal infections at Hawassa University Referral Hospital, Hawassa, Ethiopia (Dec 2010-Feb 2011).

Socio-demographic characteristics	Category	NG	No NG	OR(95%CI)	Fisher's Exact Test
Age- group	15-19	3	47	1	0.894
	20-24	5	66	1.187 (0.27, 5.21)	
	25-29	2	32	0.979 (0.155 , 6.195)	
	30-34	0	25	0.000 (0.000)	
	35-39	1	22	0.712 (0.070, 7.240)	
Address	40-44	0	12	0.000 (0.000)	0.026
	Urban	5	158	1	
Marital status	Rural	6	46	4.122(1.203, 14.121)	0.565
	Single	5	76	1	
	Married	5	113	0.673 (0.188, 2.403)	
	Divorced	1	8	1.900(0.197, 18.341)	
Occupation	Widowed	0	7	0.000 (0.000)	0.644
	Student	5	67	1	
	Gov. employees	1	47	0.285(0.032, 2.520)	
	Farmers	2	40	0.670(0.124, 3.616)	
	House wife	2	16	1.67(0.298, 9.430)	
	Daily workers	1	12	1.11(0.120,10.418)	
	Merchants	0	11	-	
	Others	0	11	-	
Religion	Protestant	4	92	1	0.188
	Orthodox	3	53	2.314(0.499, 10.738)	
	Muslim	2	36	1.704 (0.273, 10.623)	
	Catholic	2	8	7.667(1.113, 52.796)	
	Others	0	15	0	
Ethnic group	Sidama	4	68	1	0.995
	Amhara	3	39	1.308 (0.278, 6.148)	
	Oromo	2	36	0.944 (0.165, 5.407)	
	Wolayta	2	31	1.097 (0.191, 6.310)	
	Gurage	0	11	0	
	Tigre	0	10	0	
Macroscopic examination of swab	Others	0	9	0	0.042
	Muco purulent	8	101	1	
	Bloody swab	3	87	2.409 (0.619, 9.378)	
	Whitish	0	27	0	

NG = *Neisseria gonorrhoeae*, No NG = no *Neisseria gonorrhoeae*, @= Kembata, Yem, Silte, Gedio. Etc, Ω = Employee In Private Sector, No Occupation

Of the 215 endocervical specimens cultured on chocolate and MTM, 16(7.4%) were positive on MTM and 23(10.7%) were positive on chocolate. Oxidase tests were done for all culture positives

and for colony resembling *Neisseria gonorrhoea* in chocolate agar even in the absence of growth in MTM. Among the 23 Oxidase tested, 15 (65.2%) were Oxidase positive and further gram staining

was done from the colony and finally the confirmatory biochemical tests were done. In general, from a total of 16 culture positive on MTM and from 15 of Oxidase positives only 11

were isolated as *Neisseria gonorrhoea* by further biochemical tests (Oxidation or utilization of carbohydrates) and reported as *Neisseria gonorrhoea* (Table 4).

Table 3 Gram stain, Culture and Biochemical tests result of patients investigated for gonococcal infections at Hawassa University Referral Hospitals, Hawassa, Ethiopia (Dec2010-Feb 2011).

Test	Gram stain	Growth in chocolate	Growth in MTM	Oxidase test	Oxidation of glucose only in API NH kit	Isolated <i>Neisseria gonorrhoeae</i>
Positive	21	23	16	15	11	11
Negative	194	192	199	8	4	204
Total	215	215	215	23	15	215

*MTM = modified Thayer-Martin medium, *API NH = Analytical profile index for identification of neisseriae and hemophilia

Antimicrobial Susceptibility Testing: The susceptibility patterns of isolated bacteria (n=11) was done against 11 antimicrobial agents by the agar disc diffusion technique. The sensitivity of gonococcal isolates ranges from 100% to Ceftriaxone and cefixime to 0 % to Penicillin and Tetracycline. The lowest susceptibility was observed for penicillin and Tetracycline. No resistance was found to Ceftriaxone and cefixime. However, low level of susceptibility to quinolones (ciprofloxacin 55.0%, ofloxacin 64.0% &

lomefloxacin 64.0%), recommended in the national protocol as first-line antibiotics for gonorrhea treatment was observed. There was decreased susceptibility to spectinomycin as well (82%). As shown in Table 4, most of the isolates haven't shown multiple drug resistance 9/11 (81.8%) and none of the isolates were sensitive to all antibiotics. In this finding high level of resistance (82%) to Penicillin and (55%) to Tetracycline was observed.

Table 4. Antimicrobial Susceptibility Patterns of *Neisseria gonorrhoeae* from patients who visited gynecologic OPD at Hawassa University Hospitals, Hawassa, Ethiopia (Dec2010-Feb 2011)

Organism		CRO	FOX	CIP	SPT	CFM	CPD	CTX	OFX	LOM	P	TE
NG n=11	S	11(100%)	9(82%)	6(55%)	9(82%)	11(100%)	10(91%)	10(91%)	7(64%)	7(64%)	0	0
	I	-	2(18%)	3(27%)	2(18%)	-	-	-	2(18%)	3(27%)	2(18%)	5(45%)
	R	-	0	2(18%)	0	-	-	-	2(18%)	1(9%)	9(82%)	6(55%)

S= Sensitive, I=Intermediate, R=Resistant, P: Penicillin, TE: Tetracycline, CIP: Ciprofloxacin CRO: Ceftriaxone; CTX: cefotaxime; FOX: Cefoxitin; CFM: cefixime; CPD: cefpodoxime; OFX: ofloxacin; LOM: lomefloxacin; SPT: spectinomycin

DISCUSSION

Gonococcal infection has a disproportionate impact on the health of women. In women, it is often chronic, presenting with vague or no symptoms, but may lead to severe complications such as chronic pelvic inflammatory disease, ectopic pregnancy, and infertility. Because of the lack of diagnostic and treatment facilities, limited opportunity for seeking medical care, and poor

health-care-seeking behavior. The impact of gonococcal infection on ill health tends to be more severe among women (17).

In Ethiopia, twenty years back the prevalence of *Neisseria gonorrhoea* was done on women attending gynecologic, obstetric and family planning clinics to correlate the serological diagnosis of gonorrhoea with clinical evidence of pelvic infection. So comparison with results from

this study is not easy as the methodology of the studies were unrelated (3).

The overall prevalence of Gonococcal infection among reproductive age group of women in this study is almost similar with findings from Laos 3.7% (10) and it is within the range of sub-Saharan African estimated report which is 2-15% (6).

The prevalence in this study is higher compared to other reports like Jordan 2.2% (11) and Vietnam 0.7% (12). The likely reason might be due to lack of differential diagnosis which can lead to increase number of untreated patient. As resistance was developed for most of the drugs ordered in syndromic management and consequently increase rate of transmission also lead to drug resistance.

On the other hand, the prevalence of gonococcal infection in our study was lower compared southern Mozambique (13), India (14) and Nigeria (15). This Variation In the prevalence of gonococcal infections in different area might offer an explanation for this difference. Besides these, today's treatment is almost universal, making the rigorous exclusion of gonorrhoeae, up to one-third of female gonorrhoeae contacts eventually found to be negative (16). Also it is known gonorrhoeae and drug resistance vary greatly among countries and in regions even in sub region of the developing world, because of socio-demographic factors, the treatment algorithm and the way the case diagnosed and treated varies in every region.

In our study, patients who came from rural areas had 4 fold increase risk of developing infections. This is because they lack treatment facilities, limited opportunities for seeking medical care and as they have poor health-care-seeking behavior are less likely to be diagnosed, and treated effectively for gonococcal infection. Although socio-demographic factors have great influence in the prevalence of STDs in which most studies confirmed (18), in our finding the distribution of gonococcal infection to most socio-demographic factors have no statistical significant association ($p>0.05$).

Regarding age group, there is no statistically significant difference in the frequency of gonococcal infections among different age groups but, the highest prevalence was observed in age group 20-24 years. This might be because of the

sexual active age groups are at risk of STIs and the unsafe sex practice might be higher in rural area where in our study the dominating study groups were living. The other reason for the highest burden among the young women could be due to the fact that young is at greater disadvantage due to the absence of information necessary for early recognition of disease symptoms (19). The other possible explanation, they might have practice of unsafe sex or they might be victim for rape, even though, our questionnaire hasn't addressed these factors are important for acquiring the infection.

The high level of resistance to penicillin and tetracycline found in our study has been widely reported throughout the world, USA (20), Australia (18) and Romania (21) due to emergence of penicillin resistant beta-lactamase producing strains. There was no resistance to ceftriaxone and cefixime. The possible explanation for this might be these drugs are expensive, not intensively used and not easily available outside the hospitals beyond this these drugs are newer compared to the others. The absence of resistance to third-generation cephalosporines (cefixime and ceftriaxone) in our study make these drugs excellent choices as first-line treatment.

According to syndromic case management principle set by Ministry of health (22) the drugs (ciprofloxacin, Tetracycline and spectinomycin) prescribed for patients suspected for gonococcal infections have shown resistance. Low level of susceptibility to quinolones (ciprofloxacin, ofloxacin & lomefloxacin), recommended in the national protocol as first-line antibiotics for gonorrhea treatment was almost similar with other studies like, USA (20) and Australia (18). And this is also in agreement with study done in other part of Ethiopia where most of the isolates were resistant to commonly used antibiotics (23, 24). This may be because of the intensive use of antimicrobial agent, easy availability and indiscriminate use of these drugs outside the hospitals, and many antibiotics are available over the counter for Self-medication.

The cephalosporin drugs; Cefixime, Ceftriaxone and cefpodoxime were effective antibiotics for the treatment of *Neisseria gonorrhoea* which are responsible to cause endocervical infections. This might be because these agents are expensive and not commonly used. This is in agreement with study done in

USA (25), Australian (18) and Romania (21). However, the present study showed a high level of resistance to ciprofloxacin compared to the study in Central African Republic, Cameroon, and Madagascar (26). It will be real that Scientists are worried gonorrhea will soon become untreatable with these antibiotics (25). This leads to conclusion that if the problem won't be attended to on time, it will become very difficult to treat the infection.

In conclusion despite low rates of gonorrhea infection, it is important to focus on high-risk populations (reproductive age group) because of the great physical and emotional costs of the disease. Future studies should focus on identifying behavioral or environmental factors to address differences in predictors within groups. Future studies to assess the resistance trends in Ethiopia and to allow timely revision of treatment protocols are highly recommended.

ACKNOWLEDGEMENTS

We wish to thank the staff of gynecology OPD for their support in identifying the target patients with careful clinical examination and sample collection also for their dedicated follow up and treatment of infected women. We thank EHNRI especially Mr. Surafel who supplied us control strains and other kits.

We greatly acknowledge the financial assistance of Addis Ababa University for covering the research.

We gratefully acknowledge Mr. Getahun Hailemeskel, head of Department of medical laboratory of Hawassa Referral Hospital and other staff of Hawassa referral hospital for their unreserved technical support and in facilitating good working environment.

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