Antimicrobial Susceptibility Profile of Gonococcal Isolates Obtained from Men Presenting with Urethral Discharge: Implication for National Syndromic Treatment guideline

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Abstract

Background: *Neisseria gonorrhoeae* (gonococcus) is one of etiologic agent for sexually transmitted diseases with high global significant public health importance. The treatment regimens for gonorrhea have changed frequently over the past few decades due to the organism's propensity for developing antibiotic resistance. This study was aimed to investigate quinolones, third-generation cephalosporins and other relevant antimicrobials susceptibility patterns of *N. gonorrhoeae* so that the national treatment regimen could be revised based on the information generated from this study. **Methods:** Urethral discharge specimens were cultured on Modified Thayer Martín media and suspected gonococcal colonies were confirmed using Oxidase, Superoxol tests followed by commercial identification kit (API-NHR). Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method using ciprofloxacin(5ug), ceftriaxone (30ug), cefixime(5ug), ceftiofur (30 ug), and spectinomycin (100 ug), on enriched GC agar. Minimum Inhibitory Concentration (MIC) was also done using concentration gradient strips (E-test) of the same antimicrobial agents. **Results:** The prevalence of gonococcal isolates from the current study was 69%. Out of the 361 gonococcal isolates, close to 68% were fluoroquinolone non susceptible with 60% resistant and 7% intermediate status. However, there was no detection of ceftriaxone non susceptible gonococcal isolates. The isolates also showed reduced non susceptible to spectinomycin and ceftiofur. **Conclusion:** The prevalence of gonococcal isolates from the current study was high. There was a high level of fluoroquinolone resistance in gonococcal isolates recovered in this study. **Key words:** *Neisseria gonorrhoeae*, Ciprofloxacin, Ceftriaxone MIC, Ethiopia

Background

Neisseria gonorrhoeae (gonococcus), is one of etiologic agents for sexually transmitted diseases (STD), with global high prevalence. Transmission occurs by direct contact with secretions of infected mucosal surface, and the incubation period can range from 1 to 10 days (1). Gonorrhea, as well as any other STD, can work as a gateway to HIV and other infections with microorganisms (2). Public health control of gonorrhea requires treatment with appropriate antimicrobials, as well as generalized and targeted prevention efforts (1, 3, 4, 5).

Treatment regimens for gonorrhea have been changed frequently over the past few decades due to the organism's propensity for developing antibiotic resistance. Over the past few years, gonococcus have become less susceptible to previously used antibiotics such as sulfonamides or tetracycline. Until recently, quinolones and third-generation cephalosporins are alternative to treat gonococcal infections. However, the emergence and spread of gonococci resistant to the quinolone group of antibacterial was reported from different corners of the world (4,6). This trend is concerning because no alternative antibiotic treatment options or combinations have been proven to be effective against the organism (4,6,7,8,9,10).

The treatment for gonococcal infection in sub-Saharan Africa countries including Ethiopia was based on syndromic approach using single dose fluoroquinolone treatment. The basis for such a regimen was

under the assumption that resistance to fluoroquinolones is considered to be low in Africa. However, with the occurrence of resistance to commonly prescribed antibiotics in both developed and developing countries, an updated knowledge of the prevailing susceptibility patterns of gonococci in Ethiopia is important for the proper selection and use of antimicrobial drugs as well as for the development of an appropriate prescription policy. This study was aimed to investigate the quinolones, third-generation cephalosporins and other related antimicrobials susceptibility patterns of *N. gonorrhoeae* isolated from urethral discharge swab of patients seen in Addis Ababa health Centers.

Methods

Study sites and design

This study was conducted in Addis Ababa City Administration. The city is geographically located in central part of the country. A cross-sectional facility-based study was conducted in eight health centers of Addis Ababa. The selected health facilities were Arada, Teklehaimanot, Addis Ketema, Kirkos, Kotebe, Akaki- Kaliti, Shiromeda and Kassanchis health centers. These health facilities were selected based on the assessment made purposively before this study on the flow of STI clients. There were eight study teams, one nurse and one overall study coordinator. Following training on study protocol, procedures, and research ethics, the study team stayed twelve months at the study site collecting samples.

Source population, study participants

The source populations were patients in the selected health facilities of Addis Ababa who were participated in the study. All urethral discharge specimens analyzed between August 2013 and August 2014 were included in this study.

Laboratory methods

Specimen Collection: Men presenting to the selected health facilities with urethral discharge syndrome were recruited in the study following their consent. A sterile cotton-tipped swab were used to obtain a swab specimens. Then sterile Dacron swabs tipped applicator were used to collect urethral secretions. The swabs were inoculated on in-house prepared Modified Thayer Martin Agar plates made of Gonococcal agar base supplemented with isovitalex (vitox); vancomycin, colistin, nystatin, and trimethoprim (VCNT); and synthetic hemoglobin (Oxoid and BBL). The inoculated plates were incubated on the site using candle jar and transported to the Ethiopian Public Health Institute (EPHI), Clinical Bacteriology and Mycology Reference Laboratory within the same day of collection. Swab was rolled onto a microscopy slide, labeled, heat fixed, placed in a slide box and sent to EPHI for Gram-stain analysis.

Culture and identification: In the clinical bacteriology laboratory, inoculated plates were incubated at 35oC in carbon dioxide enriched environment (5-8 % CO₂) for 72 hours inspecting every day for the growth of small, translucent and non-pigmented colonies. Gram negative diplococcic convex, glistening, elevated,

muroid colony characteristics and oxidase, catalase and supercool (30% H₂O₂) positive were considered as probable *N. gonorrhoeae* and further confirmed by carbohydrate and enzymatic tests using API-NHR. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method using ciprofloxacin (5 ug) ceftriaxone (30 ug), cefixime (30 ug), cefoxitin (30 ug), and spectinomycin (100 ug), on enriched GC agar (Oxoid Ltd) plus 1% BBL Isovitallex Enrichment. Minimum Inhibitory Concentration (MIC) was done using concentration gradient strips (E-test) of the same antibiotics. The range of inhibition zones and MIC for each type of antibiotic disk were interpreted according to Clinical Laboratory Standard Institute (CLSI) guideline (11). *N. gonorrhoeae* reference strain ATCC 49226 was used as a positive control.

Data extraction methods

A structured check list was used to collect socio-demographic, clinical history, sexual behaviors, pro antibiotics taken and laboratory data such as the antibiotic susceptibility results.

All data were double entered to Cespro 8 soft by two individuals and data analysis were done using SPSS version 20.

Operational definitions

Non-susceptible *N. gonorrhoeae* isolates were defined as those that are not sensitive to the antibiotic tested for susceptibility, i.e., those isolates exhibiting resistance or intermediate resistance.

Dual non-susceptibility: non-susceptibility to any two of the antibiotics tested for susceptibility.

Multi Drug Non-susceptibility: Combined non-susceptibility to an injectable cephalosporin and any two of quinolones, penicillins or tetracyclines.

Declaration

Ethics and Consent To Participate

This study was ethically cleared by Scientific and Ethical Review Office (SERO) of Ethiopian Public Health Institute and the IRB of CDC-Atlanta.

At the enrollment visit, all men with urethral discharge (UD) were given written consent diagnosed according to the syndromic treatment guidelines currently approved in Ethiopia. Those who are eligible (> 18 years of age) and willing to participate in the study were asked using structured questionnaire for their demographic and behavioral data. All data were kept confidential anonymously. Brief counseling on the importance of adherence to STI medications, not having sex while taking medications, HIV/STI prevention, and recommendations to use condoms to reduce STI/HIV acquisition and transmission was also given.

Results

A total of 599 urethral discharge specimens were collected from male patients presenting at eight health centers and the specimens were microbiologically analyzed at the clinical bacteriology reference laboratory of the Ethiopian Public Health Institute through bacterial culture and antimicrobial susceptibility testing following the standard operating procedures. The mean age of the study participants was 27 years (SD + 7.2), with all of them being male.

Of all the specimens analyzed, 415 (69.3%) gonococcal isolates were identified (Table 1).

Discussion

The prevalence of gonococcus in this study was relatively higher than other studies conducted in Ethiopia (12,13,14). The difference might be due to the nature of participants in the current study that all of them were males. The general notion is that naturally, males tend to be more symptomatic for gonococcal infection and hence can have increased level of health care seeking behavior which in turn make them more represented in the statistics (15).

In sub-Saharan Africa, gonococcal treatment practice is based on syndromic approach using single dose fluoroquinolone treatment. It is hypothesized that resistance to fluoroquinolones is low in Africa, but there has been limited systematic data collection and analysis to verify this notion. A multicounty antimicrobial resistance study on gonococcal strains isolated in 2004-2006, indicated low rates of fluoroquinolone resistance with 0% ,1.3and 4.0% in Central African Republic, Cameroon, and Madagascar, respectively (16). Similarly, study conducted in a Maputo and Mozambique, in 2005, suggested that there was no resistance to fluoroquinolone by gonococcal isolates (17).

In contrast, the findings from several other countries in sub-Saharan Africa suggested increasing levels of fluoroquinolone resistance in gonococcal isolates. According to a study done in South Africa, in 2004, 7% of the gonococcal isolates from Pretoria region, 8% from Western Cape and 17% from Johannesburg were found to be resistant to antibiotics from the class of fluoroquinolone. In addition, another study conducted in same country and the same study populations, in 2007, indicated that; 27% of the gonococcal isolates from Cape Town and 32% from Johannesburg isolates were found to be resistant to ciprofloxacin (18). This represents 2.9 fold and 1.9 fold increases, respectively within a 3-year time period. Similarly, a 2-year prospective study carried out among STI patients, from 2004 to 2006, in Johannesburg indicated an increase in ciprofloxacin resistance from 13% in the first year to 26.3% in the second year (19). Study conducted in Kenya and Uganda also showed that gonococcal resistance level to fluoroquinolone has reached up to 53% and 83%, respectively (20,21).

The present study has revealed that *N. gonorrhoeae* isolates recovered from this study have shown high level of resistance to commonly prescribed fluoroquinolone class of antibiotics in Ethiopia. This finding is in agreement with other studies which reported a resistance level of 53% and 83% in the East African region Kenya and Uganda. Reports from South Africa also indicate that the resistance level reached up to

32% (19,20,21,22). High proportion of quinolone resistance in this study might be due to prior treatment using ciprofloxacin as indicated in table 4. Gonococcal syndromic treatment using oral fluoroquinolone has become very problematic due to the emergence of high proportion of resistant isolates as witnessed from the current study. Non-susceptibility to ceftriaxone was not detected in all of the isolates tested during the study period. This finding is not in agreement with other studies conducted in different part of Ethiopia (12, 13, 14). However, our finding was in agreement with other studies conducted elsewhere (23,24,25,26,27). This might be due to exposure of participants for specific group of antimicrobial agents during the study period. As indicated in figure-1, the existence of certain segment of isolate population with MIC value close to the cutoff point may mean that there are concerns over the possibility of minority non-susceptible bacterial population to potentially replacing the susceptible majority ones. Therefore, investigating the molecular mechanism of resistance in these group of isolates may be imperative to fully understand the epidemiology (26).

Penicillinase test was done by chromogenic test and almost more than half of the isolates were positive for beta lactamase. Table 2, Table 3.. Most of the isolates in the current study were resistant to Benzyl penicillin even though the antibiotic is not used for the national gonococcal treatment algorithm. This finding from our study was in line with other studies (24, 25,27, 28,29).

Formerly, the STI treatment practice in Ethiopia was based on the syndromic approach (treating individuals immediately for possible causes of STI syndromes based on symptom). However, because of the finding from this study, the national guideline for treatment has been changed. Besides this, the etiologic approach of diagnosis of gonorrhea is important at least for revisiting clients in order to identify non susceptible isolates for the current candidate of antimicrobial agents in practice.

Conclusion

In the current study, the proportion of *N. gonorrhoeae* were found to be high. The most worrisome thing was that these gonococcal isolates were highly resistant to the new generation of antibiotics from fluoroquinolones (ciprofloxacin) which was indicated as a treatment of choice as per the previous guideline. The information generated from this study was used as in put to revise the national syndromic guideline for management of patients presenting with urethral discharge due to gonorrhea infection and therefore, ciprofloxacin was replaced by ceftriaxone which was found to be effective in terms the in vitro susceptibility result. In conclusion, the diagnostic approach, which is syndromic based management, needs to be periodically validated and modified based on determination of susceptibility patterns of *N. gonorrhoeae* isolates.

Declarations

Consent for Publication

Not Applicable

Availability of data and material

Data are available, we can share if necessary

Conflict of Interest

Authors declared no conflict of interest

Funding

This study was funded by CDC through the sexually transmitted infection surveillance program granted to Ethiopian Public Health Institute.

Authors' contributions

SF wrote the proposal, Laboratory work, analyzed the data and drafted the paper. **RA, NA**, and **MA** participated in data collection, entry and analysis. **ET** participated in the analysis and reviewed the manuscript. All authors participated in the preparation of the manuscript and approved the final manuscript.

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Table

Table 1: Gram stains and culture laboratory results of Urethral discharge specimen from patients

GC confirmation	Method	Result	Frequency	Percent
Gram stain		Positive	449	75%
		Negative	150	25
Culture		Positive	415	69%
		Negative	184	31%
Total			599	100

Table 2: percent of Antimicrobial susceptibility pattern of gonococcal isolates from Urethral discharge patients (N=361)

Antimicrobial agent	Class	Susceptibility profile			
		Resistant	Intermediate	Susceptible	Non susceptible
Penicillin	Penicillins	52.9			
Spectinomycin	Aminocyclitols	3	1.1		
Ciprofloxacin	Flouroquinolone	60.2	7.1		
Ceftriaxone	Cephalosposrin	-		100	
Cefixime	Cephalosposrin			85	15
Cefoxitin	Cephalosporin	1.1			
Azithromycin	Macrolides	10		90	
Cefoxitin + Ciprofloxacin		-			
Cefoxitin + Spectinomycin		-			
Ciprofloxacin + Spectinomycin		0.6			
Ciprofloxacin + penicillin		7			

MIC Ranges for Resistance and Intermediate Susceptibility as defined by CLSI Guidelines:

Ciprofloxacin: Resistant > 1 ug/mL, Intermediate 0.12-0.5 ug/mL

Spectinomycin: Resistant ≥ 128 ug/mL, Intermediate 64 ug/mL

Ceftriaxone Sensitive ≤ 0.25

Cefixime Sensitive ≤ 0.25

Cefoxitin Resistant ≥ 8 , Intermediate 4

Azythromycin Sensitive ≤ 1

Table 3. MIC Antimicrobial susceptibility pattern of gonococcal isolates from Urethral discharge patients (N=361)

Penicillin			Ciprofloxacin			Spectinomycin			Ceftriaxone			Cefixime			Azithromycin		
I	S	R	I	S	R	I	S		R	I	S	R	I	S	R	I	S
-	170	-	-	78	-	-	-		-	-	245	-	-	323	-	-	-
-	-	-	-	41	-	-	-		-	-	47	-	-	-	-	-	156
-	-	-	-	-	-	-	-		-	-	12	-	-	-	-	-	-
-	-	-	-	-	-	-	-		-	-	53	-	-	-	-	-	140
-	-	-	23	-	-	-	-		-	-	4	-	-	-	-	-	4
-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	46
20	-	24	-	-	-	-	127		-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	39		-	-	-	-	-	-	8	-	-
1	-	37	-	-	-	-	43		-	-	-	-	-	-	6	-	-
-	-	-	-	-	-	-	57		-	-	-	-	-	-	1	-	-
-	-	-	-	-	-	-	38		-	-	-	-	-	-	-	-	-
-	-	158	-	-	11	4	42		-	-	-	-	-	-	-	-	-
1	20	170	219	23	119	11	4	346	-	-	361	-	-	323	15	-	346

Table 4: Medical treatment history of patients with Urethral discharge from health centers in Addis Ababa, August 2013- August 2014

Medication history	Response	Number	Percent(%)
Taking medication	Yes	123	20.5
	No	476	79.5
	Total	599	100
Know the type of medication	Yes	107	87
	No	16	13
	Total	123	100
Ciprofloxacin	Yes	91	85
	No	16	15
	Total	107	100
Doxycycline	Yes	93	86.9
	No	14	13.1
	Total	107	100
Metronidazole	Yes	12	11.2
	No	95	88.8
	Total	107	100

Figures

Figure 1

Minimum inhibitory concentration of ceftriaxone against *N. gonorrhea*

Supplementary Files

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- [gonococcus.rar](#)