EMERGENCE OF MULTI-DRUG RESISTANT *NEISSERIA GONORRHOEAE* IN THE AMHARA NATIONAL REGIONAL STATE, ETHIOPIA

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Abstract

Background: Occurrence of Neisseria gonorrhoeae isolates which are resistant to multiple antimicrobial agents is proving to be a serious public health problem in different corners of the globe. The main objective of the current study was to analyze the antimicrobial susceptibility pattern of N. gonorrhoeae isolates in Northwest Ethiopia.

Methods: This was a retrospective study of the susceptibility patterns of N. gonorrhoeae isolated from genital swabs of patients referred to the Amhara Regional Health Research Laboratory between September 2006 and June 2012 in Bahir Dar, Ethiopia. A structured check list was used to collect socio-demographic and laboratory data, which were analyzed using SPSS software version 16.

Results: Out of 352 genital specimens processed, 29 clinical strains of N. gonorrhoeae were isolated. Resistance against cephalosporin, ciprofloxacin, tetracycline, and penicillin G was observed in 27.8%, 40.9%, 92.6%, and 94.4% of the isolates, respectively. Twenty percent of the isolates showed dual resistance against ceftriaxone and ciprofloxacin. Multi-drug resistant N. gonorrhoeae were identified in 27.8% of the isolates. The level of resistance against tetracycline and penicillin G was maintained at a high level while resistance to fluoroquinolones and cephalosporins showed an increasing trend with time.

Conclusion and Recommendation: N. gonorrhoeae isolated from genital specimens in the Amhara National Regional State have high level of resistance to common and new generation antibiotics, some being multi-drug resistant. A change of diagnostic approach of gonorrhea to the etiologic approach coupled with determination of susceptibility patterns is recommended.
Key words: Multi-drug resistance, *Neisseria gonorrhoeae*, antibiotic resistance, cephalosporin resistance
Introduction

Gonococcal infections are the second most common prevalent sexually transmitted bacterial infections causing substantial morbidity worldwide each year [1]. In addition to causing serious complications, gonorrhea is a potent amplifier of the spread of sexually transmitted HIV [2] increasing the infective inoculums of HIV in persons with HIV and gonorrhea co-infection.

Resistance to commonly-prescribed antibiotics of *Neisseria gonorrhoeae* is an expanding global problem resulting in diminishing treatment options for gonorrhea. Loss of utility of several drugs such as sulfonamides, penicillin and tetracyclines, for treatment of gonorrhea, were reported in both developed and developing countries [3,4].

Until recently, quinolones and third-generation cephalosporins had activity against *N. gonorrhoeae* strains that were resistant to previously used antibiotics such as sulfonamides, tetracyclines. However, the emergence and spread of gonococci resistant to the quinolone group of antibacterials was reported from different corners of the world [5,6]. Failures of treatment with oral third-generation cephalosporins for infection with gonococci or raised minimum inhibitory concentrations to them have been documented in different continents including Europe, Asia and North America [7-10].

Although individual country-based intermittent analyses about drug resistant gonococci have been published from some countries, information about the exact magnitude of multidrug resistant (MDR) *N. gonorrhoeae* is generally lacking for most of African countries including
Ethiopia. The current treatment guidelines in Ethiopia, and some other African countries, utilize the syndromic case management for all sexually transmitted infections (STIs) including gonorrhea based on the WHO recommendation [11].

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 prescribing policy. This study was aimed to investigate antibiotic susceptibility pattern of *N. gonorrhoeae* isolated from genital swab of patients seen in the Amhara Regional Health Research Laboratory (ARHRL), Bahir Dar, Ethiopia.
Methods

Study design and Period: In this retrospective study, genital specimen analysis data from bacteriology laboratory registers which were documented between September 2006 and June 2012 in the ARHRL, Bahir Dar Center, Ethiopia was used.

Study area: The ARHRL Bahir Dar Center is the main referral laboratory in the Amhara National Regional State (ANRS). The laboratory is located in Bahir Dar, the capital of the ANRS. Patients visit the laboratory bringing laboratory requisition forms from the different health facilities located in the administrative divisions in the ANRS as well as from adjacent regions in the country.

Source population, study participants: The source populations were patients in the ANRS visiting both private and government health facilities, who were referred to ARHLBC for laboratory work up. All genital specimen analyses reports September 2006 and June 2012 were included in this study.

Laboratory Methods

As part of the routine bacteriologic investigations at ARHRL, all genital specimens were plated onto modified Thayer Martin media immediately after collection in the laboratory and incubated at 37°C in 5% CO₂. The plates were read daily for up to 72 hours and isolates were identified by conventional phenotypic and biochemical methods. Gram negative diplococcic which were oxidase and catalase positive were considered as N. gonorrhoeae. Susceptibility
testing was performed by Kirby-Bauer disc diffusion method using penicillin G (10ug), tetracycline (30ug), ciprofloxacin (5ug) and ceftriaxone (30ug). CLSI guidelines were used for interpretation of zones of inhibition and quality control.

**Data extraction methods:** A structured check list was used to collect socio-demographic and laboratory data such as the antibiotic susceptibility test results and year of test. The laboratory records do not specify the type of symptoms and other clinical information.

In order to analyze trends of antibiotic resistance by *N. gonorrhoeae*, the study period was divided into three categories, i.e., Sept. 2006 – Aug. 2009, Sept. 2009 – Aug. 2011 and Sept. 2011 - June 2012.

**Data Quality Management, Processing and Analysis:** Data were double-entered using Epi info version 3.5 and analyzed using SPSS software version 16.

**Operational Definitions:**

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

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

Multi Drug Resistance: Combined resistance to an injectable cephalosporin and any two of quinolones, penicillins or tetracyclines.
**Ethical considerations:** Ethical approval to conduct the study was obtained from the research and technology transfer core process of the ANRH bureau. Permission to conduct the study was granted from the ARHRL, Bahir Dar center.
Results

Between September 2006 and June 2012, 352 genital specimens were analyzed in ARHRL, Bahir Dar Center by bacterial culture following the standard operating procedures. The mean age of the participants was 28.1 years (SD=8.4), with female to male ratio of 1.1. As indicated on table 1, there were 29 clinical strains of *N. gonorrhoeae* isolated and processed for susceptibility testing during the study period.

Table 2 shows the antibiotic susceptibility pattern of *N. gonorrhoeae* isolates. In toto, as many as 27.8% of the isolates were resistant against ceftriaxone. Resistance to ciprofloxacin was noticed in 40.9% of the isolates. High level of resistance was observed against penicillin G (94.4%) and tetracycline (92.6%). Cephalosporin resistance coupled to any two of penicillins, ciprofloxacin or tetracycline, i.e., multi-drug resistance, was identified in 27.8% of the isolates. Dual resistance to ceftriaxone and ciprofloxacin was noticed in 20% of the isolates, all of which occurred between Sept. 2011 and June 2012.

Ciprofloxacin resistance between Sept. 2009 to Aug. 2011 indicated a proportion of 11.1%. Between Sept. 2011 to June 2012, the levels of resistance against ciprofloxacin was 77.8%. Resistance against ceftriaxone was demonstrated by 20% of the isolates between Sept. 2006 and Aug. 2009 while it was 80% between Sept. 2011 to June 2012. The trend of drug resistance over the past 6 years to tetracycline and penicillin G as well as to the newer generation of antibiotics is illustrated on Figure 1.
Discussion

For the first time, this study reports an evidence of multi-drug resistant *N. gonorrhoeae* isolates in ANRS of Ethiopia. It also revealed increasing trend in cephalosporin and fluoroquinolone resistance. Resistance to old, inexpensive antibiotics such as penicillin and tetracycline was maintained at a high level.

Although the mainstay of management of discharge syndromes in the study area is the syndromic approach, the number of isolates being sent for bacteriological analysis showed an increasing tendency with time. As opposed to the etiological approach which uses laboratory tests to identify the causative agents of STIs, the syndromic approach leads to immediate treatment for possible causes of one of seven STI syndromes based on symptoms only [12]. This indicates a gradual shift to the etiologic approach of diagnosis of gonorrhoea. The occurrence of drug resistant *N. gonorrhoeae* isolates could be the underlying factor to the increased request for the exact identification of etiologic agents and determination of their susceptibility patterns.

In this study, high level of resistance to new generation antibiotics such as ciprofloxacin and ceftriaxone was noticed. The high proportion of fluoroquinolone resistance by *N. gonorrhoeae* are coherent with reports from many countries including South Africa, Kenya and the Southeast Asia region [13-15]. The reason for this consistency may be the global nature of gonorrhea. Those infected in one part of the world will often spread the infection to those living elsewhere if ineffective treatment is used. However, the roles of spontaneous mutation and the abilities of bacteria to adapt to different environmental situations to the occurrence of MDR *N. gonorrhoeae* in the study area cannot be ruled out.
However, low levels of resistance to ciprofloxacin were reported from a multi-centered study conducted in Central African Republic, Cameroon and Madagascar [16]. The inconsistency of our finding with these reports may be the difference in time frame between the studies.

With the introduction of syndromic management for STIs [12], the use of fluoroquinolones and cephalosporins is practiced in a standardized fashion. However, this creates a selective advantage for the organisms resistant to these medications [17]. As a result, *N. gonorrhoeae* resistant to such drugs would occur. Use of fluoroquinolones and cephalosporins for treatment of other conditions may lead to sensitization of the *N. gonorrhoeae isolates* in asymptomatic patients, gradual selection of drug resistant strains and eventually clinically significant resistance. The high levels of tetracycline and penicillin resistance reported in this study parallel earlier experience of other countries such as Malawi and Kissumu, Kenya [3,4]. As Gedebou M and Tasew A had reported the presence of a relatively lower level of resistance of *N. gonorrhoeae* to tetracycline (8%) and Penicillin (41%) in Ethiopia three decades ago[18], the current magnitude of resistance to these drugs seems to have developed gradually. Also a study to determine the susceptibility patterns of *Neisseria gonorrhoeae* isolates in Northwest Ethiopia had reported in 2001 lower resistance rates to penicillin G (85.2%), tetracycline (29.6%) and ceftriaxone (4.2%) [19], thus supporting the gradual nature of the condition.

The finding of the presence of MDR *N. gonorrhoeae* in the study area is coherent with the reports from other areas also. Combined resistance to oral cephalosporins and any two of quinolones, penicillins or tetracyclines was reported from Western Pacific region [20].
Based on the WHO recommendation that an antimicrobial associated with a resistance of ≥ 5% of strains should be abandoned[11], cephalosporins, ciprofloxacin, penicillin G or tetracyclines, should no longer be recommended for the treatment of gonorrhoea in the general population in the ANRS.

Currently, the Centers for Disease Control and Prevention (CDC) recommends dual therapy with ceftriaxone (250 mg intramuscularly as a single dose plus either azithromycin 1 gram orally as a single dose or doxycycline 100 mg orally twice a day for 7 days as the most effective treatment for uncomplicated gonorrhea [21]. Even though it is not possible to have data about the susceptibility patterns of \textit{N. gonorrhoeae} isolates for doxycycline in the study area, it is theoretically known that microorganisms that have become insensitive to one tetracycline invariably exhibit cross resistance to other tetracyclines due to transmissible plasmids [22]. Therefore, based on the finding that there is high ceftriaxone-tetracycline dual resistance rate, the use of ceftriaxone-doxycycline combination for the treatment of gonorrhea in the study area would be questionable.

The results from this study are alarming because the level of resistance is being reported in a setting where there are only few alternatives for the management of gonorrhea. Since antibiotic treatment is the foundation of gonorrhea management, the emergence of MDR \textit{N. gonorrhoeae} can be associated with an increase in the risk of complications of infection, hence a challenge to clinicians. The occurrence of MDR I in a country that has diseases such as tuberculosis, HIV and malaria[23] as the public health priorities is an additional burden. It threatens effective disease control because there are no effective antimicrobial resistance
surveillance programs for *N. gonorrhoeae* in the ANRS as well as in other parts of Ethiopia. The impact of untreated gonorrhea on HIV transmission could be very serious as in the ANRS as the prevalence of HIV is high [24].

The findings in this report are subject to the following limitations. First, the data presented here are based on results reported on laboratory registers due to the retrospective nature of the study, where documentation problems are an issue. Second, susceptibility testing was done by the Kirby-Bauer disc susceptibility testing method, which is unsuitable for the laboratory differentiation of partially resistant from fully sensitive strains of *N. gonorrhoeae* [25]. Third, data available only include results from genital gonococcal isolates among patients who were referred for laboratory analysis. As patients who respond to empiric treatment are not sent for laboratory analysis, subjects included in this study are those who were already exposed for different antibiotics. That individuals at risk for the occurrence of antibiotic resistance are the study subjects makes selection bias unavoidable.

The other is the small number of isolates documented and analyzed over the years. This was mainly related to the policy in Ethiopia which recommends syndromic case management of STI cases as opposed to the etiologic approach which would have lead to laboratory analysis of more isolates and determination of drug susceptibility patterns. However, in light of similar trends in other regions of the world, the patterns observed in this study coupled to the ability of *N. gonorrhoeae* to develop resistance are concerning enough.

**Conclusions and recommendations**
High proportion of *N. gonorrhoeae* isolated from genital specimens in the ANRS are resistant to old and new generation antibiotics. Some isolates are multi-drug resistant. In view of this, the national guideline for management of gonorrhoea needs to be urgently reviewed. The diagnostic approach needs to be modified to include determination of susceptibility patterns of *N. gonorrhoeae* isolates. Clinicians need to consider treatment of gonorrhea based on individual susceptibility patterns instead of the routine empirical treatment of discharge syndromes. A strategy should be established to rapidly detect patients diagnosed with gonorrhoea who experience a clinical treatment failure following treatment with recommended antibiotics. Current treatment recommendations for gonorrhea in the study area need to be validated and updated. Protocols for management of gonorrhea treatment failures need to be in place. Antimicrobial resistance surveillance program needs to be launched in ANRS in order to obtain antimicrobial resistance profiles in a timely manner and with sufficient clinical and epidemiological information.
Conflict of interest: There is no conflict of interest.

Authors’ contribution

MT wrote the proposal, analyzed the data and drafted the paper. AS participated in data collection, entry and analysis. GM and AK 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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References


**Table 1. Number of Specimens and N. gonorrhoeae Isolated from September 2006 to June 2012, ANRS, Ethiopia**

<table>
<thead>
<tr>
<th>Year Category (Duration)</th>
<th>Total Number of Genital Specimen Processed</th>
<th>Total Number of N. gonorrhoeae Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 2006 to Aug. 2009 (36 Months)</td>
<td>111</td>
<td>9</td>
</tr>
<tr>
<td>Sept. 2009 to Aug. 2011 (24 Months)</td>
<td>119</td>
<td>7</td>
</tr>
<tr>
<td>Sept. 2011 to June 2012 (10 Months)</td>
<td>122</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>352</strong></td>
<td><strong>29</strong></td>
</tr>
</tbody>
</table>
**TABLE 2. ANTIBIOTIC SUSCEPTIBILITY PROFILE OF N. GONORRHOEAE ISOLATES FROM SEPTEMBER 2006 TO JUNE 2012, ANRS, ETHIOPIA**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Percent Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>27.8</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>94.4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>92.6</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>40.9</td>
</tr>
<tr>
<td>Ceftriaxone and Penicillin G</td>
<td>35.7</td>
</tr>
<tr>
<td>Ceftriaxone and Tetracycline</td>
<td>31.3</td>
</tr>
<tr>
<td>Ceftriaxone and Ciprofloxacin</td>
<td>20</td>
</tr>
<tr>
<td>Penicillin G and Tetracycline</td>
<td>87.5</td>
</tr>
<tr>
<td>Penicillin G and Ciprofloxacin</td>
<td>66.7</td>
</tr>
<tr>
<td>Tetracycline and Ciprofloxacin</td>
<td>38.1</td>
</tr>
<tr>
<td>Multi drug resistant</td>
<td>27.8</td>
</tr>
<tr>
<td>Other Triple resistance</td>
<td>63.6</td>
</tr>
<tr>
<td>All four drugs resistance</td>
<td>27.3</td>
</tr>
</tbody>
</table>
Figure 1. Trend and Antibiotic Resistance patterns of N. gonorrhoeae isolates in ARHRL center between September 2006 and August 2012 Bahirdar, Northwest Ethiopia.

CE=Ceftriaxone, PE=Penicillin G, TE=Tetracycline, CI=Ciprofloxacin, MDR=Multi-drug resistant