Antibiotic Susceptibility of *Neisseria gonorrhoeae* in Bacolod City, Philippines

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors ACJ and JPTL designed and proposed the study. Authors CTG and DGMJ wrote the protocol. Authors JPTL, CTG and MADA did the literature search and statistical analysis. Authors ACJ and MADA managed the analyses and discussions of the study. Authors ACJ and DGMJ wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Resistance of *Neisseria gonorrhoeae* against clinically recommended antibiotics continues to rise globally. This retrospective study aimed to describe the antimicrobial resistance and resistance trend of *N. gonorrhoeae* in Bacolod City from February 2010 to January 2015. A total of 99 isolates (97 males and 2 females) from ages 15 to 65 were included in the study. The highest incidence was observed between 25 - 29 age bracket. Resistance rate against the tested antibiotics were as follows: penicillin (100%), ciprofloxacin (75.3%), tetracycline (69%), spectinomycin (8%), cefixime (0%) and ceftriaxone (0%). Production of beta-lactamase was also found in all isolates; while presumed plasmid mediated tetracycline resistance was found in 34 isolates. Resistance to ciprofloxacin and tetracycline were also seen to be increasing during the five year study, but

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resistance to third generation cephalosporins, namely cefexime and ceftriaxone remains unchanged.

Keywords: Neisseria gonorrhoeae; antimicrobial resistance; bacolod city.

1. INTRODUCTION

Gonorrhea caused by Neisseria gonorrhoeae is a sexually transmitted infection, brought about by unsafe sex practices, which has medically significant implications if not treated early [1]. These include infertility, inflammation leading to acute and chronic abdominal pain in women, ectopic pregnancy and maternal death, first trimester abortion, severe neonatal eye infection that may lead to blindness and fivefold increase in HIV transmission [1]. According to the CDC [2], gonorrhea continues to be of major health concern having a rate of 98.1 (2009), 106.7 (2009-2012), and 106.1 (2013) cases per 100,000 population in the United States. Additionally, the rate of infection in males is higher than females, especially among individuals aged 20 – 24 years.

Treatment of gonococcal infection is often made with the administration of ceftriaxone in combination with azithromycin [3,4]. This is because increasing resistance to penicillin, tetracycline and fluoroquinolones were observed over the years [5,6,7]. The antibiotic cefixime was also noted to be an effective antibiotic for gonorrhea; however, resistance to cefixime is already observed in various countries such as in Canada [8], United States [9], Brazil [10], Switzerland [11], Iran [12] and Hungary [13].

Gonorrhea poses a potential public health dilemma because of its capability to exhibit extensive resistance to medications used for treatment of infection like gonorrheal strains like H041 and F89 that was isolated in Japan and France [14]. Although country-wide data from ARSP is readily available, it is still important to describe the latter within the locality. Thus, it is imperative that surveillance on the resistance and antimicrobial trend of N. gonorrhoeae should be monitored and updated because of its public health importance and it is crucial in guiding empiric therapy in individual setting.

2. MATERIALS AND METHODS

2.1 Inclusion / Exclusion Criteria

This retrospective study made use of N. gonorrhoeae isolates that were obtained from patients who were suspected of having gonorrhea and have had medical examinations in two tertiary hospitals, namely, Dr. Pablo O. Torre Memorial Hospital and Corazon Locsin Montelibano Memorial Regional Hospital. Also, these medical facilities have the capability of culturing and isolating the organism in Bacolod City, Philippines. The patient’s data was collected i.e., age, sex and anatomical site of infection. The study covered a period of five years from February 01, 2010 to January 31, 2015.

2.2 Isolation and Identification of N. gonorrhoeae

Cervical or urethral discharges were collected and cultured to non-selective chocolate agar plate and were incubated at 36.5°C for 24-48 hours with 5% carbon dioxide. Purification of mixed isolates was also done by subculturing suspected colonies to another chocolate agar. Identification was then made using gram stain, oxidase, superoxol, sugar fermentation and resistance to colisin that is based on the algorithm of Ng and Martin [15] and that of Perilla et al [16]. N. gonorrhoeae is observed as gram negative diplococci when gram stained, positive for oxidase and superoxol, ferments only glucose, and is resistant to colisin.

2.3 Antibiotic Susceptibility Testing

Susceptibility testing was performed based on the methods described by Perilla et al. [16] utilizing the Kirby-Bauer disc diffusion method using ceftriaxone (30 µg), cefixime (30 µg), ciprofloxacin (5 µg), spectinomycin (100 µg), tetracycline (30 µg) and penicillin g (10 units) in a GC agar with 1% Isovitalex or Vitox [17]. Results as to susceptible or resistant were based on the M100-S24 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement of CLSI [18].

2.4 Determination of Plasmid-Mediated Tetracycline Resistant N. gonorrhoeae (TRNG) and Beta-lactamase Production

Plasmid mediated tetracycline-resistant N. gonorrhoeae can be presumed if the disk zone diameter to tetracycline is ≤19 mm [18].
Beta-Lactamase production was also determined using a cefinase (nitrocefin-based) disc [18].

2.5 Quality Control

All culture media and antibiotic discs used were subjected to quality control using *N. gonorrhoeae* ATCC 49226.

3. RESULTS

A total 99 *N. gonorrhoeae* were isolated and identified from February 2010 to January 2015, of which 98% were from male and 2% from females. Among these patients, the youngest were 15 years of age (3, 3.03%) while the oldest was 65 years old (1, 1.01%) with a mean of 29 (SD 9.98). Age range with the most number of cases was from 25 – 29 years old (26, 26.26%) followed by 20 – 24 years old (25, 25.25%). Majority of the organisms were isolated from urethral (97, 97.8%) and the remaining from cervical discharges (2, 2.02%).

Antibiotic resistance of the 99 isolates are summarized in Fig. 1 as these are the antibiotics that should be considered for routine testing as indicated by the CLSI (2014). It is also interesting to note that all isolates were β-lactamase producing, 59 out of 99 isolates were found to be resistant to tetracycline (TRNG) and out of this 59, 34 had a zone of inhibition of less than 19 mm.

For five years, resistance to penicillin is 100%, while resistance to both ciprofloxacin and tetracycline are more than 60%. On the other hand, spectinomycin was lower than 15% and there was no non-susceptibility observed for both cefixime and ceftriaxone as shown in Table 1.

![Fig. 1. Five-year antibiotic resistance of *N. gonorrhoeae*](image)

**Table 1. Yearly Percent Resistance of *N. gonorrhoeae* from February 2010 to January 2015**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Total # of isolates</td>
<td>20</td>
<td>12</td>
<td>18</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>(%R) Penicillin G</td>
<td>100 (20/20)</td>
<td>100 (12/12)</td>
<td>100 (18/18)</td>
<td>100 (29/29)</td>
<td>100 (20/20)</td>
</tr>
<tr>
<td>Beta-lactamase Producing</td>
<td>100 (20/20)</td>
<td>100 (12/12)</td>
<td>100 (18/18)</td>
<td>100 (29/29)</td>
<td>100 (20/20)</td>
</tr>
<tr>
<td>(% &lt; 19 mm in Penicillin G)</td>
<td>100 (20/20)</td>
<td>100 (12/12)</td>
<td>100 (18/18)</td>
<td>100 (29/29)</td>
<td>100 (20/20)</td>
</tr>
<tr>
<td>(%R) Ciprofloxacin</td>
<td>65 (13/20)</td>
<td>83.3 (10/12)</td>
<td>66.7 (12/18)</td>
<td>75 (21/28)</td>
<td>94.4 (17/18)</td>
</tr>
<tr>
<td>(%R) Tetracycline</td>
<td>63.2 (12/19)</td>
<td>66.7 (8/12)</td>
<td>66.7 (12/18)</td>
<td>75 (18/24)</td>
<td>69.2 (9/13)</td>
</tr>
<tr>
<td>(% &lt; 19 mm in Tetracycline)</td>
<td>26.3 (5/19)</td>
<td>33.3 (6/18)</td>
<td>50 (12/24)</td>
<td>40 (12/30)</td>
<td>30.8 (4/13)</td>
</tr>
<tr>
<td>(%R) Spectinomycin</td>
<td>13.3 (2/15)</td>
<td>9.1 (1/11)</td>
<td>0 (0/17)</td>
<td>10.7 (3/28)</td>
<td>6.7 (1/15)</td>
</tr>
<tr>
<td>(%R) Cefixime</td>
<td>0 (0/20)</td>
<td>0 (0/12)</td>
<td>0 (0/18)</td>
<td>0 (0/29)</td>
<td>0 (0/20)</td>
</tr>
<tr>
<td>(%R) Ceftriaxone</td>
<td>0 (0/20)</td>
<td>0 (0/12)</td>
<td>0 (0/18)</td>
<td>0 (0/29)</td>
<td>0 (0/20)</td>
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</tbody>
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The numbers on each cell of the table are: percent (Number of resistant isolates/Total isolates tested for the antimicrobial agent in that year band)
4. DISCUSSION

*N. gonorrhoeae* is already evolving into a super bug because of its resistance to antimicrobials and is of global concern [19]. In the period of five years, the susceptibility of *N. gonorrhoeae* to cefixime and ceftriaxone remains to be unchallenged since 100 percent susceptibility was picked-up on the disc diffusion method used by this study in Bacolod City. This means that these two, 3rd generation cephalosporins can still be given as an empiric therapy for gonococcal infection. These results were also comparable with the Annual Summary Progress Report of ARSP [7] in the Philippines, Jabeen et al. [5] in Pakistan and Costa [10] in Brazil. Unfortunately, even though there was no reported cefixime resistance in Bacolod City as shown neither in this study nor in the report given by ARSP [7], resistance to cefixime and treatment failure were already presented in many foreign studies [6,8,14]. Moreover, according to the Morbidity and Mortality weekly report of CDC [9], routine use of cefixime as a first line regimen for treatment of gonorrhea is no longer recommended. Even though the CDC recommends dual therapy using ceftriaxone coupled with azithromycin for the treatment of gonorrhea [17] the latter antibiotic was not tested because there were no breakpoints to interpret its susceptibility in the M100-S24 of CLSI [18].

Spectinomycin, an aminocyclitol was recommended as drug of choice to patients who are allergic to cephalosporins [20]. However, even though with low resistance, it can almost certainly be discounted as a single-agent therapy as a first line treatment and is not effective in eradicating pharyngeal gonorrhea [14] and it exceeds the 5% cut-off of the WHO for precluding its use [5]. It is also imperative to note that pharyngeal infections due to *N. gonorrhoeae* are asymptomatic and is rarely screened [10].

Resistance to Ciprofloxacin was noted to be high with a cumulative percentage of 75.3%. Though the resistance dropped from 83.3% in the 2nd year of study to 66.7% in the 3rd year, a rise to 75% in 4th year and 94.4% in the 5th year were also observed. The increase in resistance may have been facilitated by the use of fluoroquinolones for treating gonorrhea and other conditions [21]. The cumulative percentage of this study is higher than the study of Brunner et al. [13] in Hungary et al. with 66%, Costa et al. [10] in Brazil with 11.9%, and ARSP [7] with 74%. However this result is lower than that of Li et al. [4] in China with 98.8%.

*N. gonorrhoeae* in this study was consistent for its resistance to Penicillin G which is 100%. This finding even exceeds the percent resistance that was reported by the ARSP [7] for the past years, so as with the data of Li et al. [4], Brunner et al. [13], Costa et al. [10], and Tibebu et al. [17] with resistance of 67.7%, 77%, 71.1%, and 94.4% respectively. Additionally, all isolates had zones of inhibitions to penicillin which were below 19 mm and all of them were able to produce the enzyme beta-lactamase recognizing this plasmid-mediated penicillin resistance [18].

A persistently high tetracycline resistance (TRNG) was observed in this study having more than 60% of resistance for 5 consecutive years. Moreover, 34 isolates (34.34%) were found to have zones of inhibitions below 19 mm against tetracycline. These findings were also noted to be higher than that of Costa et al. [10] with 11.25%, Martin et al. [22] with 33.3% and ARSP with 55% [7], however the results generated by this study is lower compared to that of Brunner et al. [13] (86%) in Hungary and Bokaelian et al. [12] (88.3%) in Iran. Although penicillin and tetracycline are obsolete for the treatment of gonorrhea, these antibiotics are still monitored because they are still used in other parts of the world and its worldwide prevalence of resistance remains to be high [15].

The development of antibiotic resistance among *N. gonorrhoeae* may be due to a number of factors that includes self-medication, uncontrolled sale of antimicrobials or lack of medical care for treatment [10]. Also, according Lal Patel et al. [20] the problem of antibiotic resistance is greatly influenced by poverty and the factors related to it like self-medication, non-compliance to treatment and financial capacity. Additionally, according to Brunner et al. [13], resistance may initially emerge in the commensal Neisseria species inhabiting the human body as these are exposed frequently to antimicrobials than the transiently acquired gonococcus. The commensal Neisseria can act as a reservoir of resistance gene that can be transferred to gonococci through transformation.

Surveillance in the antimicrobial resistance of gonococci in the locality was difficult primarily because most clinicians only request gram staining which has more than 99% specificity and more than 95% sensitivity among symptomatic
men [23] and 99.6% correlation with molecular methods [24]. Also, due to the cost involved, treatment of gonorrhea relies on syndromic management in developing countries [20], making culture and sensitivity test impractical that can be used for AMR surveillance is scarce.

5. CONCLUSION

The data gathered by this study indicates that it is still best to consider ceftriaxone to be the first line of therapy against gonococcal infections as it showed no resistance against this antibiotic as of this time. The variations of resistance with the administration of spectinomycin cannot also justify having its practical use as an empiric therapy based on WHO guidelines and its limitation to site of infection. Furthermore, high resistance to penicillin, ciprofloxacin and tetracycline suggests that it is not anymore suitable for therapy against N. gonorrhoeae since it exceeds resistance of more than 50% and is of increasing trend of resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

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