Antimicrobial Resistance in Neisseria Gonorrhoeae Isolates from Ribeirão Preto, São Paulo, Brazil

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ABSTRACT

Introduction: gonorrhea is sexually transmitted, with a high incidence worldwide. Occurrence of resistance and difficulties in treatment is often reported. Penicillin is not used anymore, and quinolones or cephalosporins are the remaining therapeutic options. However, there are resistance reports to these drugs as well. Objective: to evaluate the occurrence of resistance to penicillin, tetracycline, ofloxacin, ciprofloxacin, azithromycin and ceftriaxone in Neisseria gonorrhoeae (gonococcus). Methods: ninety-three endocervical and urethral secretion samples, suspected of gonorrhoea, were cultured from September 2008 to May 2012. Samples were collected at the STD/Aids Reference Center and processed at Instituto Adolfo Lutz in Ribeirão Preto, SP, Brazil. Antimicrobial susceptibility tests were performed by the E-test (Oxoid). Beta-lactamase was determined by the ceftaxine disk method (BD BBL). The susceptibility study included a gonococcus isolated from a case of conjunctivitis. Results: gonococcus was isolated in 41.9% (35) of the cases in the study. Male patients were predominant in 92.3% of samples, with ages ranging from 14 to 62 years, and the conjunctivitis isolate was recovered from a 1 month old patient. Isolates were resistant to penicillin (44.4%); tetracycline (55.5%); ofloxacin (36.1%); and ciprofloxacin (36.1%). All isolates were susceptible to ceftriaxone, and 80.6% were susceptible to azithromycin. The beta-lactamase test was positive for 31.0% of isolates. Conclusion: in vitro results showed that tetracycline was less effective, and ceftriaxone the most effective antibiotic against gonococcus. The resistance to different drugs limits the options of gonorrhoea effective treatment.

Keywords: Neisseria gonorrhoeae, resistance, antimicrobials, gonorrrhoea, STD

INTRODUCTION

Gonorrhea, which etiologic agent is the bacterium Neisseria gonorrhoeae (gonococcus), is one of the oldest sexually transmitted diseases (STD), with worldwide high incidence. Transmission occurs by direct contact with secretions of infected mucosal surface, and the incubation period can range from 1 to 10 days⁶. The most common clinical manifestations of the infection are urethritis and cervicitis, although it can affect the anal mucosa, oropharynx, conjunctiva, among others⁷.

About 30 to 80% women and 5 to 85% men can remain asymptomatic carriers of gonococcus, making it difficult to break the transmission chain of the disease. Therefore, along with the assessment of sexual partners and health education, the detection of carriers are among the main control strategies of gonorrrhoea⁸. In Brazil, the absence of a notification system of STD cases makes information scarce. In 2003, the Ministry of Health National STD/Aids Program estimated the occurrence of 1.5 million cases of gonorrrhoeae⁹.

The high demand for medical care, the cost of treatment and the socioeconomic impact of STDs represent an economic loss of 17%, mostly in developing countries⁵.

Gonorrhea, as well as any other STD, can work as a gateway to the HIV and other microorganisms. According to Wasserheit⁶ and Cohen⁷, STDs increase the risk of acquiring HIV infection from three to five times.

Direct bacterioscopy of secretions stained by Gram and culture are the traditional methods used for diagnosis of gonococcal infections⁷. Bacterioscopy has 90% sensitivity and 98% specificity in the detection of purulent gonococcal infections, although this sensitivity becomes reduced in asymptomatic patients. In this context, culture is an indispensable method for the diagnosis, allowing the isolation of gonococcus and the subsequent determination of antimicrobial sensitivity profile⁵.

Molecular techniques have been made available through commercial kits for the diagnosis of gonococcus, using nucleic acid amplification tests, which show high sensitivity and specificity. Gonococcus intolerance in the transportation of the sample and the rapidity of the result are advantages of these techniques on the culture⁶.

The gonococcus is a highly fastidious bacterium, which success in isolation and identification depends on the adequate collection and transportation of the sample, in addition to the quality of inputs used and trained personnel⁹. Few laboratories carry out this methodology routinely due to the specificity concerning the cultivation of gonococcus. The difficulty in the infection diagnosis, coupled with inadequate use of antimicrobials, promotes the development of resistance to available drugs, which is the biggest obstacle to the control of the disease, by limiting the therapeutic options for effective treatment. When not carried out correctly, especially in women, treatment can cause serious sequelae, such as infertility, miscarriage and ectopic pregnancy⁹.

The production of enzymes, the acquisition of plasmids, and genetic mutations are among the various resistance mechanisms of antimicrobial gonococcus⁹. The β-lactamase enzyme gives the gonococcus the ability to inactivate β-lactam antibiotics. The isolates of gonococcus should be investigated concerning the pro-

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duction of this enzyme, which can be detected by iodometric and acidimetric methods, and also through chromogenic cephalosporin (nitrocefin)\(^{[11]}\).

Due to the acquisition of resistance to antimicrobials, the treatment of gonococcus has evolved through time. The sulfonamide was the first indication for the treatment of gonorrhea, and subsequently penicillin followed by tetracycline. The fluoroquinolones (ciprofloxacin, ofloxacin and levofloxacin) were introduced more recently, but there are already reports of emergence of resistance to this class of antibiotics in many countries\(^{[12]}\).

Because the constant changes of antimicrobial sensitivity profile of the gonococcus, the monitoring of circulating strains is essential to detect the emergence and spread of drug-resistant strains, helping the institution of effective measures in the control of gonococcal infections\(^{[13]}\).

For the treatment of urethritis, specialized clinics in the city of Ribeirão Preto, use penicillin, azithromycin, ciprofloxacin and ceftriaxone. However, clinicians have noted some cases of treatment failure with the use of such antibiotics.

**OBJECTIVE**

Evaluate the occurrence of isolates of gonococcus and its *in vitro* susceptibility to antibiotics: penicillin, tetracycline, ofloxacin, ciprofloxacin and ceftriaxone, and determine its production of \(\beta\)-lactamase.

**METHODS**

This is a retrospective study of 93 patients of both genders, clinically suspected urethritis and/or gonococcal cervicitis, which appeared spontaneously in STD/AIDS Reference Center in the city of Ribeirão Preto, São Paulo State, Brazil, from September 2008 to May 2012. These patients signed an informed consent, agreeing to participate in the study, which was reviewed and approved by the opinion of CONEP number 5.071/2008.

A gonococcus isolate was included in the evaluation of antimicrobial susceptibility, in a culture recovered eye secretion of a newborn from a hospital in the city of Ribeirão Preto, which was sent to the Instituto Adolfo Lutz-RP (IAL-RP) for identification.

The culture method for the isolation and identification of *Neisseria gonorrhoeae* were according to the Brazilian Ministry of Health Manual recommendations\(^{[14]}\). The urethral secretion or endocervical swabs were collected from the patients and immediately spread in Thayer-Martin (selective) and chocolate agar (non-selective) culture media, and incubated in 5-10% of CO\(_2\) atmosphere. Part of secretion was collected with bacteriological loop for direct bacterioscopy. Samples were forwarded to the IAL-RP, where plates were incubated at 35-37\(^\circ\)C for at least 48 hours, and then observed the growth of colonies suspected of gonococcus. The phenotypic identification has taken into consideration the colony morphology, the cell morphology (Gram-negative diplococci), the oxidase proof (positive), and the carbohydrates utilization in the Cystine Tryptic Agar - CTA medium: dextrose (positive), lactose (negative), sucrose (negative) and maltose (negative)\(^{[15]}\).

Direct bacterioscopy were realized by Gram stain and observed in immersion (1,000 x), in common optical microscope for the presence of Gram-negative intracellular diplococci characteristic of gonococcus.

Antimicrobial sensitivity was evaluated by Minimum Inhibitory Concentration (MIC), using the episolometric test or E-test (Oxoid). The interpretation of results and reference values used for penicillin, tetracycline, ciprofloxacin, ofloxacin and ceftriaxone was based on the recommendations of the Clinical Laboratory Standards International (CLSI, 2009)\(^{[16]}\). For azithromycin the values considered were described in literature\(^{[17,18]}\). The *Neisseria gonorrhoeae* ATCC 49226 strain reference was used for the quality control of culture media and the CIM, as recommended by CLSI\(^{[19]}\).

The research of beta-lactamase enzyme was determined by the cefinase disk method (Becton Dickinson), described by Swenson et al.\(^{[19]}\). In contact with cefinase disk beta-lactamase enzymes producing strains break the beta-lactam ring and produce a red color pigment.

The following control strains were used: *Staphylococcus aureus* (ATCC 29213): positive, and *S. aureus* (ATCC 25923): negative.

**RESULTS**

Of the 93 suspected cases of gonorrhea, 52 were men and 41 women, ranging from 14 to 62 years of age. Among the suspected cases 43% (\(n = 40\)) were diagnosed as positive for gonorrhea, and in two of them there was no growth in culture, and the diagnosis was made only by bacterioscopy. In three cases it was not possible to carry out the sensitivity test, due to the loss of viability of strains for the achievement of the MIC.

Among the suspected cases of gonorrhea 90% (\(n = 31\)) were male. The predominant age group (55%) was 21 to 30 years (Figure 1). The gonococcus isolated from conjunctival secretion was of a 1 month of age child, whose sex was not reported.

MIC was held in 36 samples of gonococcus, being 31 urethral secretions, four endocervical secretions and one conjunctival secretion (Table 1). Resistance to at least two classes of antimicrobials was observed in 44.4% (\(n = 16\)) of these samples, 25% (\(n = 9\)) were only sensitive to ceftriaxone. A total of 29 isolates (80.6%) was sensitive to azithromycin, and seven (19.4%) showed decreased sensitivity to this antibiotic.

The beta-lactamase test was positive for 34.4% of cases.

Among the 40 patients with suspicion of gonorrhea, 15% (\(n = 6\)) were already in use of antibiotics and 17.5% (\(n = 7\)) showed some sort of associated pathology (Table 2).

**DISCUSSION**

In developing countries, STDs are among the five major diseases for which medical attention is sought\(^{[20,20]}\). STDs' control has been a concern for health authorities, mainly due to their increase in the young and adolescents\(^{[21,22]}\). In this study, it was observed that the 14 to 20 years age group was the second most incident among cases of gonorrhea.

Blocking of the transmission chain is among the main ways of control and prevention of STDs, as well as the appropriate treatment of infections and the proper management of partners\(^{[23]}\). Gonorrhea’s control is hampered by the large number of asym-
Table 2 – Associated pathologies and previous use of antibiotics in 40 suspected cases of gonorrhea

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associated pathologies</td>
<td></td>
</tr>
<tr>
<td>HPV/condyloma</td>
<td>5% (n = 2)</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>5% (n = 2)</td>
</tr>
<tr>
<td>HCV</td>
<td>2.5% (n = 1)</td>
</tr>
<tr>
<td>Ulcerated lesion</td>
<td>5% (n = 2)</td>
</tr>
<tr>
<td>Previous antibiotic therapy</td>
<td></td>
</tr>
<tr>
<td>Benzetacil</td>
<td>5% (n = 2)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>5% (n = 2)</td>
</tr>
<tr>
<td>Azitromycin</td>
<td>2.5% (n = 1)</td>
</tr>
<tr>
<td>Ciprofloxacin + benzetacil</td>
<td>2.5% (n = 1)</td>
</tr>
</tbody>
</table>

HPV: human papillomavirus, HCV: hepatitis C virus.
The gonococcus isolates of this study were different from those studied in the city of Manaus. The results found in both locations were, respectively, related to the production of beta-lactamase enzyme (34.4% and 14.5%), resistance to penicillin (44.4% and 21.8%) and ciprofloxacin (36.1% and no fully resistant strain)(39).

Barreto et al. (2004), in Rio de Janeiro, alert to the use of penicillin, tetracycline and azithromycin in the treatment of gonorrhea because found that 8.7% of gonococcal strains were beta-lactamase producing, to penicillin, 76.5% had intermediary resistance, 20.0% with reduced susceptibility to azithromycin and only 33.9% were fully sensitive to tetracycline(40).

For azithromycin, appropriate results in vitro have been recommended by Mehaffey et al.(27), considering the MIC with ≤ 2 μg/mL breakpoint. In this study it was observed that sensitivity decreased 19.4% to this antibiotic, following the recommendations of Dillon et al.(31), who have established the MIC with breakpoint = 0.25 to 1 μg/mL. Studies of Ferreira et al.(34) in 2004, found a resistance of 21.9%.

Azithromycin has been employed in the treatment of gonorrhea as well as in the standardized treatment urethritis and cervicitis of uncertain cause or chlamydial infection. However, for treatment of chlamydial infection is recommended regimen azithromycin 1 g orally in a single dose, inadequate in cases of gonococcus infection, which may be favoring the emergence of resistance(34).

Due to the resistance of Neisseria gonorrhoeae to penicillin, tetracycline and quinolones (ofloxacin and ciprofloxacin) found in the present study, is not advised the use of these antimicrobials as first choice drugs for the treatment of gonorrhea. The CDC(15) and the World Health Organization(25) not recommend use of drugs whose strains exceed 5% resistance.

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Conflict of interest

Authors declared there is no conflict of interest.

REFERENCES


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