# Manuscript title:

# The frequency of neisseria gonorrhoeae endocervical infection and its antibiotic resistance pattern in women referred to gynecology clinic of Fatemieh Hospital in Hamadan in 2020

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**Abstract**

**Background:** Gonorrhea is an infection caused by Neisseria gonorrhoeae that is mainly transmitted through sexual intercourse. This study was performed to evaluate the frequency of Neisseria gonorrhoeae and antibiotic resistance pattern to this infection in women referred to Fatemieh Gynecology Clinic in Hamadan.

**Methods:** In this cross-sectional study using availabe sampling method, 348 women referred to the gynecology clinic of Fatemieh Hospital in Hamadan in 2020 with complaints of vaginal discharge or vague pelvic pain were investigated. Patients were examined by a gynecologist, and if were eligible, a sample of endocervix is prepared and sent to the laboratory. Samples were cultured in a Thayer –Martin agar, then were examined by a cytopathologist for Neisseria gonorrhoeae and Chlamydia and the results were recorded in a researcher-made checklist. Data were analyzed using SPSS software version 16.

**Results:** The mean age of women was 34.93 ±7.57 years, and 72.7% lived in urban areas. Common complaints of women included pelvic inflammatory disease (56.9%), cervical discharge (73.6%), dysuria (25.6%) and dyspareunia (22.7%). None of the women had multiple sexual partners, hepatitis B or hepatitis C. one case (0.29%) of Neisseria gonorrhoeae was observed that be resistant to the antibiotics penicillin tetracycline, minocycline and cefazolin and was sensitive to the antibiotics chloramphenicol, rifampin, nitrosamine, cefoxitin and ceftazidime.

**Conclusion:** It seems that in women with cervicitis at reproductive age, without a history of high-risk sexual behaviors, Neisseria gonorrhoeae infection is less common and is not a significant problem.

**Keywords:** Neisseria gonorrhoeae, Chlamydia, Antibiotic resistance, Sexually transmitted infection

Introduction

Sexually transmitted infections (STIs) are caused by a wide range of bacteria, viruses, and parasites that are transmitted from person to person through vaginal, anal, or oral sex (1, 2). The World Health Organization (WHO) estimates that the prevalence and incidence of STIs in developing countries is about 20 times higher than in developed countries (3, 4). Chlamydia trachomaitis, Neisseria gonorrhoeae, Trichomonas vaginalis and Troponema pallidum are four common STIs that, in 2016, and were estimated caused of approximately one million new infections per day (5). In this year, the global prevalence of chlamydia was 3.8%, gonorrhea 0.9%, trichomoniasis 5.3% and syphilis 0.5% in the female population (6).

Gonorrhea is a set of clinical conditions caused by infection with a sexually transmitted bacterial pathogen caused by Neisseria gonorrhoeae. Gonorrhea is a disease known to be 2000 years old and human is its only natural reservoir (7, 8). Symptoms of Neisseria gonorrhoeae infection vary depending on the location of the infection. However about 10% of infected men and 80% of infected women are asymptomatic (9). Gonorrhea increases the risk of prostate cancer in men (10). The Centers for Disease Control and Prevention (CDC) estimates that there are more than 700,000 new cases in the United States each year, and only half of them are reported (11). Gonorrhea is the most common Safter chlamydial infection (12). The clinical presentation of this disease varies from asymptomatic to acute complications. This disease causes cervicitis, urethritis, and proctitis with involvement of the epithelium and if left untreated, it causes endometritis and salpingitis in women and urethritis and epididymitis in men (12-14). The main complications of gonorrhea in pregnant women are premature birth and neonate infection (12, 15).

Gonorrhea and syphilis are caused by bacteria and can generally be treated with antibiotics. However, these infections are often undiagnosed. The resistance of these STIs to the antibiotics has increased rapidly in recent years and reduced treatment options (16). Gonorrhea is the most resistant to antibiotics. Different types of drug-resistant gonorrhea infections that do not respond to any available antibiotics have already been identified (17). The formal surveillance system for STIs is a valuable public health tool that can monitor the extent and process of infection as well as the effectiveness of prevention strategies (18). Most developing countries do not have this formal surveillance system for STI and often obtain data from cross-sectional studies conducted separately in different population groups (19). Due to the fact that limited studies have been conducted in Iran to determine the prevalence of Neisseria gonorrhoeae and to diagnose antibiotic-resistant pattern in gonorrhoeae cases, this study was performed to evaluate the frequency of Neisseria gonorrhoeae and antibiotic resistance pattern in women referred to Fatemieh Gynecology Clinic in Hamadan in 2020.

Materials and Methods:

The present study is a cross-sectional study that was performed on 348 women with complaints of vaginal discharge or vague pelvic pain referred to the gynecology clinic of Fatemieh Hospital in Hamadan in 2020. Samples were selected by available sampling method from patients referred to Fatemieh Gynecology Clinic in Hamedan in 2020.

Inclusion criteria:

 • Age in the range of sexual activity (15 to 50 years)

• Declare consent to participate in the study

Exclusion criteria

• Antibiotics consumption for the past two weeks

• Treated with immunosuppressive drugs

• Having vaginal bleeding

• History of malignancy

The ethics committee of Hamadan University of Medical Sciences was approved the study (Ethics code: IR.UMSHA.REC.1399.746). The method of data collection was as follows: after obtaining informed consent, demographic characteristics (age, age of marriage, age of sexual activity), occupation and income level, level of education, place of residence, history of preterm delivery, history of abortion, history of sexually transmitted disease, time of sexual intercourse, dysuria, as well as history of HIV or hepatitis were questioned and recorded in a researcher-made questionnaire.

In order to prepare an endocervix sample for in vitro culture, after placing a spleen moistened with warm water into the vagina, the cervical mucus sample was swabbed and transferred to a Thayer–Martin agar and sent to the laboratory for culture of Neisseria gonorrhea.

For culture, 39 gr of the Thayer–Martin agar was dissolved in one liter of distilled water and placed in a snake pan to boil, then placed in an autoclave at 120 ° C. After removing from the autoclave, it was allowed to reach room temperature. Fill about 2/3 of the plate with the prepared material and place it in the refrigerator when the plate cools down. After culturing, all samples were interpreted and evaluated by a cytopathologist and the results were recorded in a checklist.

Antibiogram method: In GC agar containing growth factor of 1% of bacterial colonies in physiological serum, a concentration of 0.05 McFarland was prepared and cultured on the above culture (maximum of 6 antibiotics in 100 mm plates). The samples were placed at 36 ° C and 0.05% CO2 for 20-24 hours. To interpret the results (sensitive, semi-sensitive and resistant) we judged according to Table 1.

Diagnosis of gonococcal colonies: Suspected colonies that were positive for oxidase and catalase were cultured in Thayer–Martin agar and a 10 mg penicillin disk was inserted at the same time; After 24 hours, the undeveloped edges were removed and stained.

Table 1. Method of interpreting the result of Neisseria gonorrhoeae antibiogram

|  |  |
| --- | --- |
| Antibiogram result | Antibiotic |
| Resistant | Semi-sensitive | Sensitive |
| 26≥ | 27-46 | ≥47 | Penicillin |
| - | - | ≥35 | Cefteriaxon |
| - | - | ≥31 | Copotrime |
| - | - | ≥28 | Ceferitine |
| - | - | ≥31 | Cefirime |
| 30≥ | 31-37 | ≥38 | Tetracycline |
| 27≥ | 28-40 | ≥41 | Ciprofloxacin |
| 24≥ | 25-30 | ≥31 | Ofloxacine |

Descriptive statistics including frequency, percentage, mean and standard deviation were used for describing the data. Data were analysed using Stata 14.

**Results:**

In this study, 348 women referred to Fatemieh Gynecology Clinic in Hamadan were studied. The mean age of women was 34.93 ± 7.57 years, minimum 15 and maximum 58 years and their mean age of marriage was 18.43± 4.00 years. In terms of education, 45.5% had less than diploma education. According to the findings of Table 2, most of the patients lived in the city (72.7%) and were housewives (91.1%). The most common method of contraception in women under study was oral contraceptive pills (40.2%) and natural method (35.1%), respectively. History of preterm delivery, abortion, ectopic pregnancy and infertility in the studied women were 7.2%, 14.1%, 2% and 4.3%, respectively. Cervical discharge (73.6%), pelvic pain (56.9%), burning urine (25.6%) and dyspareunia (22.7%) were the most common symptoms and complaints of patients, respectively. In terms of risk factors, none of the women surveyed had multiple sexual partners or a history of HIV, hepatitis B, or hepatitis C.

Table 2. Demographic and clinicsl characteristics of the study population

|  |  |  |
| --- | --- | --- |
| **Percentage** | **Frequency** | **Variable** |
| 72.7 | 223 | Urban | Location |
| 24.1 | 84 | Rural |
| 3.2 | 11 | Sub-urban |
| 91.1 | 16 | House keeper | Job |
| 4.6 | 317 | Employee |
| 4.3 | 15 | Self-employee |
| 40.2 | 140 | OCP | Contraception method |
| 35.1 | 122 | Natural  |
| 9.5 | 33 | IUD |
| 6.3 | 22 | Condom  |
| 3.7 | 13 | Tl |
| 5.2 | 18 | Other |
| 7.2 | 25 | Preterm delivery | Previous pregnancy complications |
| 14.1 | 49 | Abortion |
| 2 | 7 | Ectopic pregnancy |
| 4.3 | 15 | Infertility |
| 73.6 | 256 | Cervical discharge | Signs and symptoms  |
| 56.9 | 198 | pelvic pain |
| 25.6 | 89 | Burning urine |
| 22.7 | 79 | dyspareunia |

OCP: Oral contraceptive pills, IUD: intrauterine device, TL: [Tubal Ligation](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwjGlOnKzun3AhWSQuUKHcfCCbEQFnoECAUQAQ&url=https%3A%2F%2Fwww.familyplanning.org.nz%2Fadvice%2Fcontraception%2Ftubal-ligation&usg=AOvVaw3WNtgUYQ2A_oE8DjFclq6d)

According to the findings of Table 3, the frequency distributions of Staphylococcus coagulase, Escherichia coli, Staphylococcus aureus and Neisseria gonorrhea in the studied women were 19.83%, 9.77%, 1.45% and 0.29%, respectively.

Table 3. Frequency distribution of Neisseria gonorrhea, Chlamydia and Trichomonas in the study population

|  |  |  |
| --- | --- | --- |
| **Percentage** | **Frequency** | **Microorganism** |
| 19.83 | 69 | Staphylococcus coagulase |
| 9.77 | 34 | Escherichia coli |
| 3.45 | 12 | Staphylococcus aureus |
| 0.29 | 1 | Neisseria gonorrhea |

According to the antibiogram results, the patient with gonorrhea positive culture was resistant to penicillin, tetracycline, minocycline and cefazolin antibiotics and sensitive to chloramphenicol, rifampin, nitrosphin, cefoxidine and ceftazidime antibiotics (Table 4).

Table 4. Antibiogram results related to the drug resistance status of a patient with gonorrhea

|  |  |
| --- | --- |
| Antibiogram results | Antibiotic |
| Resistant | Semi-sensitive | Sensitive |
| 1 |  |  | Penicillin Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
|  |  | 1 | Chloramphenicol Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
| 1 |  |  | Minoscline Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
| 1 |  |  | Tetracycline Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
|  |  | 1 | Nitrosphine Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
|  |  | 1 | Ciprofloxacin Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
| 1 |  |  | Cefazolin Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
|  |  | 1 | Cefoxytin Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
|  |  | 1 | ceftazidime Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |
|  |  | 1 | Rifampin Chloramphenicol Minoscline Tetracycline Nitrosphine Ciprofloxacin Cefazolin Cefoxytin سفتازیدیم Rifampin |

Discussion

This study was conducted to evaluate the frequency of Neisseria gonorrhoeae and antibiotic resistance pattern to gonorrhoeae in women referred to Fatemieh Gynecology Clinic in Hamadan. The results showed a very low prevalence of Neisseria gonorrhoeae in the study population. In the present study, the women had not risk factors for Neisseria gonorrhea infection. In our study, one case of gonorrhea positive culture was resistant to penicillin tetracycline, minocycline and cefazolin antibiotics and was sensitive to chloramphenicol, rifampin, nitrosphine, cefoxitin and ceftazidime antibiotics. In this study, cervical discharge, pelvic pain, burning urine and dyspareunia were the most common complaints of patients.

In a study by Muthusamy et al. in India on 130 women referred to an outpatient clinic for STIs, 3 cases were positive for gonorrhea (20). The sample size of our study was higher and the prevalence of Neisseria gonorrhoeae was lower than that of Muthusamy et al. In another study conducted in India by Sonkar et al. in 2017, the number of 11,000 women were examined for Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis. 55% had abnormal vaginal discharge and 48% had lower abdominal pain. Based on PCR diagnosis, the prevalence of gonorrhea was reported to be 7% (21). In our study, the presence of abnormal vaginal discharge was one of the inclusion criteria, so the frequency of vaginal discharge was higher than the study of Sonkar et al. The frequency of pelvic pain in our study was similar to the findings of Sonkar et al. In our study, the study population was selected of the referring cases to the gynecological clinic instead of the surveillance system information, and the prevalence of gonorrhea was lower than the findings of the study by Sonkar et al.

In a 1970 study by Catterall et al. In the United Kingdom on women with vaginal discharge, the prevalence of Neisseria gonorrhoeae was 31.6% (22). The reason for the huge difference between the results of our study and this study is the improvement of people's health and hygiene over time.

In the study of Abdolahian et al. in Rasht, the prevalence of gonorrhea in 1951 women of reproductive age was 0.6%. In this study, no significant relationship was observed between their age, age of first sexual contact, method of contraception and socioeconomic status and gonorrhea infection; however, there was a significant relationship between the type of purulent mucus discharge at the time of referral and gonorrhea infection (23). Also in the study by Tabasi et al. in Kashan in 2002 on determining the frequency of gonococci in patients with cervicitis and its drug resistance in patients referred to the gynecology clinic in Kashan, out of 352 women with cervicitis, only 2 (0.63%) had gonococcal cervicitis, both of which were less than 25 years old. Both began having sex under the age of 20, and both had urinary symptoms such as burning and frequent urination (24). The sample size, study population, cultivation method and culture agar used in our study was similar to the study by Tabasi et al. and the findings of the two studies were almost consistent. One of the reasons for the low rate of positive gonorrhea is that gonorrhea is a hard-to-grow bacterium. On the other hand, in our study, none of the women surveyed had a history of high-risk behavior and multiple sexual partners. Evidence indicating the key rol of commercial heterosexual sex networks in the transmission dynamics of STIs (25).

In a 2017 study by Muthusamy et al. In India, all Neisseriagnore isolates were sensitive to spectinomycin, 33% resistant to ciprofloxacin, and sensitivity to other antibiotics was 100% (20). In the Naderi et al. study on 71 patients with Neisseria gonorrhoeae, out of 1348 samples, the sensitivity to penicillin was 16.9%, tetracycline 25.3%, erythromycin 67.6% and ciprofloxacin 70.3% (26). As can be seen, Neisseria gonorrhoeae is highly resistant to first-line drugs, so before prescribing and choosing the type of antibiotic treatment for patients, their antibiogram results should be considered.

The use of culture method in terms of low cost and the lack of specific methods and more accurate diagnosis of infection, including molecular methods may be one of the limitations of the study.

In order to know the epidemiological status of the prevalence of Neisseria gonorrhoeae infections in other population groups, it is suggested that a similar study be performed on high-risk groups, including female sex workers and addicted women as well as HIV positive women.

**Conclusion:**

It appears in women with cervicitis at reproductive age, who do not have a history of high-risk sexual behaviors and multiple sexual partners; Neisseria gonorrhoeae infection is less common. However, according to the selection of research samples from patients referred to the gynecology clinic of Fatemieh Hospital in Hamadan, which may not represent the entire population of women, caution should be exercised in generalizing the results. Neisseria gonorrhoeae is highly resistant to first-line drugs, so before prescribing and choosing the type of antibiotic treatment for patients, their antibiogram results should be considered.

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**Conflict of interest**

The author claimed no conflict of interest.

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