

The frequency of *Neisseria gonorrhoeae* endocervical infection among female carrier and changing trends of antimicrobial susceptibility patterns in Kashan, Iran

Shima Afrasiabi¹, Rezvan Moniri^{1,2*}, Mansooreh Samimi³, Seyed Gholamabbas Mousavi⁴

¹Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran. ²Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.

³Department of Gynecology and Obstetrics, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran. ⁴Truma Research center, Kashan University of Medical Sciences, Kashan, Iran.

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ABSTRACT

Background and objectives: *Neisseria gonorrhoeae* is the second most sexually transmitted diseases agents in developing countries. Antimicrobial resistance strains have created serious health concern. The aim of this study was to determine the frequency of endocervical gonococcal infection and antimicrobial susceptibility of *N.gonorrhoeae* in Kashan, Iran.

Materials and Methods: In this study, 294 endocervical swabs were collected from married women referred to the obstetrics and gynecology clinics in Kashan from December 2012 to May 2013. The samples were cultured in modified Thayer Martin in 37°C with 5-10% CO₂ for 72 hours. Gram staining, oxidase, catalase and carbohydrate utilization tests were used to identify the isolated species. All isolates were tested for their susceptibilities to antimicrobials using the Kirby Bauer-disk diffusion techniques.

Results: *N.gonorrhoeae* was detected in 2.38% of studied cases (95% confidence interval [CI] 1.5-3.26%). All isolates were resistance to ceftriaxone, penicillin G, ciprofloxacin, cefepime, and two isolate (28.5%) showed intermediate sensitivity to tetracycline.

Conclusion: Continued monitoring of prevalence of *N.gonorrhoeae* is important for preventing the dissemination of this microorganism. The present study emphasizes the importance of surveillance of antimicrobial resistance of *N.gonorrhoeae* in order to manage the rate of resistant strains and to revise the treatment policies.

Keywords: *Neisseria gonorrhoeae*, antimicrobial resistance, endocervix

INTRODUCTION

Neisseria gonorrhoeae is one of the important causes of sexually transmitted diseases (STD) (1).

The emergence of resistance to antimicrobial agents in *N. gonorrhoeae* is a major barrier in control of gonorrhea (2). Gonorrhea is a main cause of cervicitis in women and can lead to pelvic inflammatory disease (PID), infertility, ectopic pregnancy, and chronic pelvic pain (1). *N. gonorrhoeae* infections are often asymptomatic in women (1). According to the World Health Organization (WHO), the incidence rate of gonococci infection has increase by 21% since 2005 (3).

The isolation of *N. gonorrhoeae* from endocervical specimens by culture is suggested method for isolation of gonorrhea in women (4). The minimum inhibitory concentrations (MICs) of most commonly

*Corresponding author: Professor Rezvan Moniri
Address: Department of Microbiology and Immunology,
Faculty of Medicine, Kashan University of Medical Sciences,
Kashan, Iran.
Tel: +98-361-555 0021-25 Ext. 539
Fax: +98-361-555 1112
E-mail: moniri@kaums.ac.ir

used antimicrobial classes for treatment of gonorrhoea including penicillin, tetracycline, macrolides, and fluoroquinolones has increased (5). The percentage of resistance to ceftriaxone varied in extensive range from 1.3% to 55.8% (6). Recently, failure in treatment with the present suggested regimens for this disease has described (7).

The precise data collection focused on distribution of *N. gonorrhoeae*, demographic characteristics related with cervical gonococci infection and antibiotic susceptibility are vital for proposing the strategies to prevent and control this infection. On the other hand, in Iran, data about the prevalence of *N. gonorrhoeae* infection and related risk factors in women is scarce (8). The purpose of this study was to investigate the prevalence of *N. gonorrhoeae*, antibiotic susceptibility rate and determination of related risk factors in married women with or without symptoms in Kashan, Iran.

MATERIALS AND METHODS

In total, 294 married women from 17 to 55 years old with or without symptoms that referred to the obstetrics and gynecology clinics of the Kashan from December 2012 to May 2013, were investigated in this study. We excluded women who used antibiotics in the preceding 14 days. All subjects asked for filling a questionnaire to record demographic information, sexual history, condom use, history of abortion, and symptoms.

The studying of protocols were reviewed and approved by the medical ethics committee of Kashan University of Medical Sciences.

The endocervix was first cleaned with a sterile cotton swab to remove mucous and exudates then a sterile Dacron swabs were used to collect endocervical specimens. To isolate *N. gonorrhoeae*, endocervical swab was directly inoculated in modified Thayer Martin (Thayer Martin + laked sheep blood and the antibiotic supplement (BBL VCNT inhibitor) including vancomycin (300 µg), colistin (750 µg), nystatin (1250 units) and trimethoprim Lactate (500 µg). The inoculated culture plates were incubated at 37°C in a humid atmosphere containing 5-10% carbon dioxide for 24-72 hrs. Isolates were identified as *N. gonorrhoeae* on the basis of colony morphology, Gram staining, the oxidase, catalase and carbohydrate utilization tests. Antibiotic susceptibility pattern to ceftriaxone (30 µg), penicillin G (10 µg),

ciprofloxacin (5 µg), tetracycline (30 µg), cefepime (30 µg) [Mast Group Ltd., Merseyside, U.K.] were determined by using disk diffusion method according to recommendation of Clinical and Laboratory Standards Institute (CLSI) (9).

N. gonorrhoeae ATCC 49226 was used as a standard reference strain for quality control of susceptibility testing of gonococcal isolates. Data were analyzed using SPSS (version 16.0 for Windows; SPSS Inc., Chicago, IL) and outcome variables include prevalence rates and their 95% confidence intervals (CIs) were calculated. The Fischer's exact test and/or χ^2 -test were used for analysis of categorical data. A *P*-value of <0.05 was considered statistically significant.

RESULTS

The age of participants in this survey ranged from 17–55 years with a mean of 32.3 years \pm 8.6. Gonorrhoea was diagnosed in 7 out of 294 women (2.38%). We found an odds ratio of 2.38 (95% confidence interval 1.5-3.26) for *N. gonorrhoea* infection. No significant relationship observed between symptoms and *N. gonorrhoea* infection. All of the infected women were symptomatic vaginal discharge. The highest resistance rate (100%) was observed for ciprofloxacin, penicillin G, ceftriaxone and cefepime, but 28.5% of isolates showed intermediate sensitivity to tetracycline.

DISCUSSION

This result suggests a high frequency rate of *N. gonorrhoeae* infection in Kashan, Iran. These results are similar to the incidence reported by Akya *et al* for *N. gonorrhoeae* infection in Kermanshah-Iran (10). In this study the detection rate of *N. gonorrhoeae* was relatively higher than the results of the other studies in Iran. Bakhtiari *et al* reported the prevalence rate of 0.2% positive culture of *N. gonorrhoeae* in Babol, Iran (11). In another research were done on 328 pregnant and non-pregnant women in Zanjan-Iran, the prevalence of *N. gonorrhoeae* was 0.9% (12). In another study performed in Sabzevar-Iran, the reported prevalence of gonococci infection by Triplex PCR techniques was 1.25% (13). The prevalence of gonococcal infection in other countries is relatively high. In one of these studies performed in Italy, Trevisan *et al.* found *N. gonorrhoeae* positivity in 1.9% of the symptomatic population (14). Rahman

et al. found 3.8% prevalence of *N. gonorrhoeae* in health clinic attendees complaining of vaginal discharge in Bangladesh (15). Gonorrhoea is a global disease with tremendous public health importance. In women, it is often chronic, presenting with unclear or no symptoms, but may lead to severe complications. Because of the lack of diagnostic and treatment facilities, limited opportunity for seeking medical care, and poor health-care-seeking behavior, the impact of gonococci infection on ill health trends is more severe among women (16).

The results of our study showed that the younger aged women are at higher risk for *N. gonorrhoeae*, because 85.7% of the infected women were between 17 and 30 years old. It is clear and is not surprising that as women at this age have higher sexual promiscuity and consequently are at higher risk for STD (17). Other reasons for the racial discrepancy in gonorrhoea rates are not well understood, while probably include differences in access to health services and utilization, geographic clustering of populations, other interrelated social and economic factors, and sexual partner choices along both socioeconomic and racial lines (18).

The likely reasons might be due to lack of differential diagnosis which can lead to increase number of untreated patients. As resistance was developed for most of the drugs ordered in syndrome management and consequently increase rate of transmission also lead to drug resistance (19).

Penicillins are no longer active and fluoroquinolone-resistance has increased severely in the past decade globally (20). Although rising resistance to ceftriaxone is reported (21). Unemo *et al.* found high-level resistance to cefixime, ceftriaxone and all fluoroquinolones, macrolides, trimethoprim-sulfamethoxazole, and tetracycline in France (22). In another study high rates of extended-spectrum cephalosporins resistance was observed in India (23).

In this study the high resistance rates observed in women in contrast with other studies performed in Iran (24, 25). Zargooshi debated that one of the reasons for high resistance rate could be related to self medication by patients (26).

N. gonorrhoeae has a well-recognized potential to rapidly develop resistance to antibiotics. The organism's ability for genetic recombination and phenotypic diversity increases transmission and evasion of host immune systems which is necessary for its survival in human host. This tendency for

genetic transformation and recombination also results in rapid spread of antibiotic resistance genes that have provided ineffective treatment in many parts of the world. This includes the penicillins and early-generation cephalosporins, tetracyclines and, more recently, the quinolones. It has been known for a long time that a logic procedure which is effective on decreasing disease burden is required for control of gonococcal disease. Sexual behavior change, improved diagnostic ability, sufficient surveillance, the condition of appropriate antibiotic treatment, may leads to successful disease control and prevention.

To our knowledge, this is the first study done on antibiotic susceptibility of *N. gonorrhoeae* isolates in Kashan, Iran. However, more *N. gonorrhoeae* isolates from Kashan are needed to investigate the regional differences in resistance rates. Moreover, clinical data such as sexual behavior were not consistently recorded and were thus not included into the study results. Future studies will carefully record sexual partner and provide additional epidemiologic information.

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