

Original Research Article

Antimicrobial susceptibility pattern of *Neisseria gonorrhoeae* isolated from fertile and infertile women

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Abstract

Purpose: To study the prevalence of *Neisseria gonorrhoeae* in the high vaginal swab samples of fertile and infertile women who were referred to Infertility and Sterility Hospitals, Tehran, Iran as well as investigate their antibiotic resistance pattern.

Methods: Three hundred and twenty high vaginal swabs were taken from fertile and infertile women. The samples were cultured and those that were *N. gonorrhoeae*-positive subjected to the disk diffusion susceptibility test.

Results: Prevalence of *N. gonorrhoeae* in fertile and infertile women were 5.26 and 16.92 %, respectively. All isolates harbored *orf1* gene. Patients in the age bracket > 40 years were at high risk. *N. gonorrhoeae* strains showed high level resistance against ampicillin (87.5 %), ciprofloxacin (75 %), tetracycline (71.87 %) and penicillin (68.75 %).

Conclusion: The possible role of resistant *N. gonorrhoeae* as pathogens causing female infertility constitutes an important finding of the study. The high levels of bacterial resistance against ampicillin, ciprofloxacin, tetracycline, penicillin and spectinomycin, should be taken into account in developing any new guidelines in the management of the infection.

Keywords: *Neisseria gonorrhoeae*, Female infertility Drug Resistance, Ciprofloxacin, Tetracycline, Spectinomycin

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INTRODUCTION

Infertility is one of the major serious concerns confronting medical practitioners. Despite all the advances in medical sciences, the main reasons of infertility especially in women are still unknown. Infertility means that couples have been trying to get pregnant with frequent interaction for at least a year with no achievement [1,2]. It has been estimated that about 72.4 million couples are infertile [3]. Majority of women with infertility problems manifest inflammatory changes of the oviduct or surrounding peritoneum. Most of these alterations result from infections [4].

Neisseria gonorrhoeae (the gonococcus) is a Gram-negative diplococcal bacterium which infects only humans. It causes gonorrhea, sexually transmitted infections (STI), Pelvic Inflammatory Disease (PID), ectopic pregnancy, chronic pelvic pain, infertility, acute urethritis, cervicitis and proctitis or pharyngitis [5]. *N. gonorrhoeae* infections represent 106 million of the estimated 498 million new cases of curable sexually transmitted infection that occur globally every year [5]. Previous studies have estimated 3.4 million cases of gonorrhea among adults in the European countries [5].

One of the best therapeutic options for treatment of the cases of gonorrhoea caused by this bacterium is antibiotic therapy. However, antibiotic-resistant *N. gonorrhoeae* have evolved and spread rapidly [6]. *N. gonorrhoeae* resistance is commonly observed with penicillin, tetracycline and fluoroquinolones antibiotics [6]. Resistance has also emerged against cefixime, an oral third generation cephalosporin, in recent years [7,8]. Since 2010, cefixime has not been recommended as first-line drug treatment [7,8] following WHO guidelines, stating that an antibiotic should not be used when more than 5 % of *N. gonorrhoeae* isolates are resistant [9]. Injectable ceftriaxone, in combination with oral azithromycin, are the recommended first line drug treatment [10]. Data regarding the epidemiology, prevalence of antibiotic resistance profile of *N. gonorrhoeae* in the cases of female infertility is scarce in Iran. Therefore, the present investigation was carried out to study the prevalence of *N. gonorrhoeae* in the high vaginal swab samples of fertile and infertile women as well as investigate the antibiotic resistance pattern of bacterial isolates.

EXPERIMENTAL

Ethical issues

The aspect of human studies in the present study was approved by the ethical committee of Department of Infertility and Sterility of Educational Hospitals, Iran (approval ref no. 20275 MD), and the Research Adjutancy of Hajar Hospital, Tehran, Iran (approval ref no. 45891 MD). The authors conformed to generally accepted scientific principles [11]. All samples were taken from volunteer women who were referred to Infertility and Sterility Hospitals, Iran. Written consents were obtained from the infertile and fertile women or their husbands.

Sample collection

Samples were collected from June to November 2015; a total of 130 high vaginal swab specimens were taken from infertile women. In a primary view, a woman after a year of regular intercourse without prevention was defined as infertile. Vaginal specimens were collected from the ventral fornix without any contact with urine and external parts of reproductive system using speculum and commercial sterile cotton-tipped swabs. All specimens were collected by an expert midwife. One hundred and ninety vaginal swab specimens were also collected from fertile women with no history of infertility.

Culture-based identification of *N. gonorrhoeae*

All the samples were inoculated directly onto modified Thayer Martin (Thayer Martin + laked sheep blood and the antibiotic supplement (BBL VCNT inhibitor) including colistin (750 µg), nystatin (1250 units), vancomycin (300 µg) and trimethoprim Lactate (500 µg)) and incubated immediately at 34 to 36 °C in an atmosphere of 5 % CO₂ for 72 h. Morphologically suggestive colonies were screened by Gram staining and by their positive responses to the oxidase and catalase tests. Positive colonies were subcultured on chocolate supplement agar (Chocolate Polyvitex/Biomérieux®), and their identification confirmed by fluorescent-antibody staining (Baxter Scientific, McGaw Park, Ill).

DNA extraction and PCR confirmation of positive colonies

Genomic DNA was extracted from the bacterial colonies using the commercial genomic DNA extraction kit (Fermentas, Germany) according to the manufacturer's instruction. The DNA concentration has been determined by measuring absorbance of the sample at 260 nm using spectrophotometer [12]. Presence of *N. gonorrhoeae* was confirmed with the PCR-based amplification of *orf1* gene. Reaction was performed in a final volume of 50 µL containing 5 µL 10 × buffer + MgCl₂, 2 mM dNTP, 2 unit Taq DNA polymerase, 100 ng genomic DNA as a template, and 25 picomole of each primer (5'-CAACTATTCCCGATTGCGA-3' and 5'-GTTATACAGCTTCGCCTGAA-3') (amplify the 221–480 bp region of *orf1* gene). PCR was performed using a thermal cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) under the following conditions: an initial denaturation for 10 min at 94 °C and 40 cycles including 94 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, and a final extension at 72 °C for 5 min. *S. aureus* ATCC 19424 was used as a positive control and distilled water was used as a negative control. PCR amplification products (15 µl) were subjected to electrophoresis in a 2 % agarose gel in 1X TBE buffer at 80 V for 30 min, stained with SYBR Green.

Evaluation of antimicrobial susceptibility pattern of *N. gonorrhoeae*

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique. Antibiotic resistance of the *N. gonorrhoeae* strains against 9 commonly used antibiotics was determined using the Clinical and Laboratory

Standards Institute guidelines [20]. Susceptibility of *N. gonorrhoeae* isolates were tested against ampicillin (10 µg/disk), azithromycin (15 µg/disk), cefixime (5 µg/disk), ceftriaxone (30 µg/disk), ciprofloxacin (5 µg/disk), chloramphenicol (30 µg/disk), penicillin (10 u/disk), tetracycline (30 µg/disk) and spectinomycin (100 µg/disk) antibiotic agents (Oxoid, UK). The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards [13]. *N. gonorrhoeae* ATCC 49226 was used as a standard reference strain for quality control of susceptibility testing.

Statistical analysis

The results were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Statistical analysis was performed using SPSS/18.0 software (SPSS Inc, Chicago, IL) for significant relationship between the prevalence of bacteria and their antibiotic resistance pattern. The chi-square test and Fisher's exact 2-tailed test analysis were performed in this study. Statistical significance was regarded at $p < 0.05$.

RESULTS

Table 1 represents the total prevalence of *N. gonorrhoeae* in fertile and infertile women of Iranian infertility and sterility hospitals. Results showed that 32 out of 320 high vaginal swab samples (10 %) were positive for *N. gonorrhoeae*. Besides, the prevalence of bacteria in the high vaginal swab samples of fertile and infertile women were 5.26 and 16.92 %, respectively. Those older than 40 years old fertile (20 %) and infertile (50 %) women had the highest prevalence of *N. gonorrhoeae*. Significant statistical differences were seen for the prevalence of *N. gonorrhoeae* between fertile

and infertile women ($p < 0.01$). Statistical difference was also seen between the age of women and prevalence of *N. gonorrhoeae* ($p < 0.05$). All the *N. gonorrhoeae* strains were confirmed using the PCR amplification of *orf1* gene (Figure 1). The *N. gonorrhoeae* isolates were positive for the *orf1* gene (Table 1).

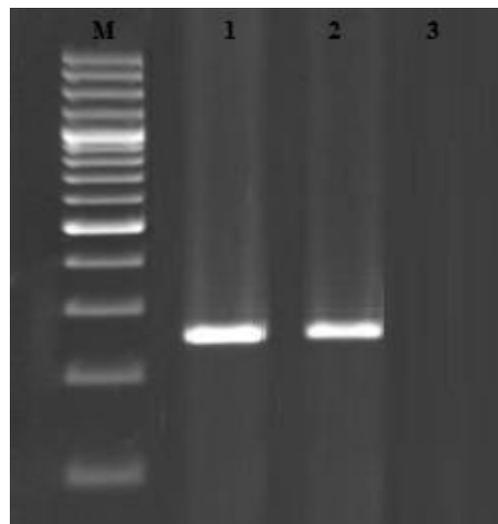


Plate 1: Gel electrophoresis of PCR products for *orf1* gene (260 bp). M: 100 bp ladder, 1: Positive sample for the *orf1* gene, 2: Positive control and 3: Negative control

Table 2 represents the antimicrobial resistance pattern of *N. gonorrhoeae* strains isolated from fertile and infertile women of Iranian infertility and sterility hospitals. We found that the *N. gonorrhoeae* strains showed high level resistance against ampicillin (87.5 %), ciprofloxacin (75 %), tetracycline (71.87 %) and penicillin (68.75 %). *N. gonorrhoeae* isolates of infertile women were more resistant against commonly used antibiotic than those of fertile women ($p < 0.05$).

Table 1: Total prevalence of *Neisseria gonorrhoeae* in fertile and infertile women in infertility and sterility hospitals in Tehran, Iran

Type of sample	No. of samples collected	<i>N. gonorrhoeae</i> (N, %)	<i>Orf1</i> gene-positive (%)
Fertile women	< 20 years	55	1 (1.81)
	20-30 years	65	1 (1.53)
	30-40 years	45	3 (6.66)
	> 40 years	25	5 (20)
	Total	190	10 (5.26)
Infertile women	< 20 years	30	4 (13.33)
	20-30 years	50	3 (6)
	30-40 years	30	5 (16.66)
	> 40 years	20	10 (50)
	Total	130	22 (16.92)
Total	320	32 (10)	32 (10)

Table 2: Antimicrobial resistance pattern of *Neisseria gonorrhoeae* strains isolated from fertile and infertile women of Iranian infertility and sterility hospitals

Type of sample (no. positive)	Antibiotic resistance pattern (%)								
	AM10	AZ15	CFX5	CTX30	CIP5	C30	P10	TET30	SPCT100
Fertile women (10)	7 (70)	1 (10)	2 (20)	-	6 (60)	-	5 (50)	6 (60)	4 (40)
Infertile women (22)	21 (95.45)	5 (22.72)	3 (13.63)	2 (9.09)	18 (81.81)	1 (4.54)	17 (77.27)	17 (77.27)	14 (63.63)
Total (32)	28 (87.5)	6 (18.75)	5 (15.62)	2 (6.25)	24 (75)	1 (3.12)	22 (68.75)	23 (71.87)	18 (56.25)

AM10: ampicillin (10 µg/disk), AZ15: azithromycin (15 µg/disk), CFX5: cefixime (5 µg/disk), CTX30: ceftriaxone (30 µg/disk), CIP5: ciprofloxacin (5 µg/disk), C30: chloramphenicol (30 µg/disk), P10: penicillin (10 u/disk), TET30: tetracycline (30 µg/disk), SPCT100: spectinomycin (100 µg/disk)

DISCUSSION

Infections of the genital tract may be predisposing factors in unexplained infertility. Hormonal disorders that occurred in infertile women can lead to reduced levels of immunity and increase bacterial colonization of vaginal epithelium [14]. Women follicular fluid is not sterile and the levels of cytokines, chemokines and growth factors which are essential for ovarian function [15], secretion of ovarian steroid hormones [15] and development and regression of corpus luteum [16] in follicular fluid of infertile women have been decreased. Therefore, it is not surprising the 16.92 % of high vaginal swab samples of infertile women were infected with resistant strains of *N. gonorrhoeae*. The present study found high levels of *N. gonorrhoeae* resistance against ampicillin, ciprofloxacin, tetracycline, penicillin and spectinomycin.

Several investigations have been done in this field all-around the world. Afrasiabi *et al* [17] reported that gonorrhea was diagnosed in 7 out of 294 (2.38 %) Iranian women which was lower than our results. They showed that the highest levels of resistance was observed for ciprofloxacin (100 %), penicillin G (100 %), ceftriaxone (100 %) and cefepime (100 %), but 28.5 % of isolates showed intermediate sensitivity to tetracycline. Significantly lower prevalence of *N. gonorrhoeae* were also found among Iranian previously published research [18]. Brunner *et al* [19] reported that among the 9097 sexually active patients tested, 582 had an *N. gonorrhoeae* infection (6.39%). The isolates were all sensitive to ceftriaxone and spectinomycin and 581/582 strains were sensitive to cefixime (99.82 %). They also showed a high percentage of detected resistance to penicillin (77 %), tetracycline (86 %), and ciprofloxacin (66 %) which are similar to our findings.

In Iran, as is the case in many other countries, the control of gonococcal infections mostly depended on an effective single-dose antibiotic therapy given at the first clinical presentation of

the patient. Prior knowledge of the antimicrobial susceptibility pattern of the particular strain infecting the patient was routinely not established however. With increasing evidence of antimicrobial resistance in gonococcal infections there was a real risk of treatment failure and development of further resistance to antibiotics.

Unfortunately, irregular and indiscriminate prescription of some routine antibiotics including ampicillin, ciprofloxacin, tetracycline, penicillin and spectinomycin in Iranian infertility and sterility hospitals caused high level resistance against them. High prevalence of gonococcal resistance against these antibiotics has been reported previously from United States [20] and United Kingdom [21]. Wit was found that 3.12 % of *N. gonorrhoeae* strains were resistant against chloramphenicol. Chloramphenicol is only used in the final stages of treatment when other therapeutic agents are not effective, hence a low level of resistance against this antibiotic.

CONCLUSION

The results of this study highlight the role of *N. gonorrhoeae* as a possible pathogens of infertility in women, and are a significant contribution to current knowledge on antimicrobial susceptibility of *N. gonorrhoeae* strains isolated from cases of infertility in Iran. A high level of antimicrobial resistance has been found in the isolated strains. The emergence of resistance against currently recommended antibiotics is worrisome.

Thus, rapid identification of cases of gonococcal female infertility, and also primary treatment with ceftriaxone, cefixime, and azithromycin, and in severe cases with chloramphenicol, may reduce the risk of further complications. Monthly check-up of fertile women and treatment with appropriate antibiotics may reduce the risk of infertility. Changes in the pattern of antibiotic resistance should be taken into account in developing any new treatment guidelines for the infection.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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