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Detection of alarming increase in antibiotic resistant neisseria gonorrhoeae isolates in karaj, Iran

Abstract

Background: Of the most common bacteria causing Sextually Transmitted Diseases (STD) is Neisseria gonorrhoeae. Rising gonorrhea rates, growing antimicrobial resistance, and increasingly limited antimicrobial options globally have made gonorrhea an urgent antibiotic-resistant threat.

Materials and methods: Two-hundred vaginal swabs were collected from non-pregnant women patients in Karaj, Iran during 2015-2016. The bacterial isolates were identified by gram-staining and biochemical tests. The antibiotic susceptibility of isolates was performed and the MIC and MBC concentration of azithromycin and co-trimoxazole were determined by the micro-broth dilution test according to the CLSI 2016.

Results: The mean age of participants was 35.85%. The abundance of married people was 97%, with 148 people having completed post-secondary education. Of the total 200 cervisitis samples, 12 (6%) of them were able to grow in vitro on the Thayer martin agar medium. all the isolates were resistant to cephixime and the majority of them (11/12 or 91.7%) were resistant to ceftriaxone, cefotaxime, ciprofloxacine, ceftizoxime, ceftizoxime, spectinomycin, and gentamicin. most of N. gonorrhea isolates were susceptible to azithromycin and co-trimoxazole in the MIC determination test.

Conclusion: In our study, given that the majority of gonococci had multiple resistances and showed at least three antibiotic classes in the society, this level of multiple resistance seems to be alarming. It is possible that the availability of antibiotics and self-treatment has increased the resistance of N. gonorrhea to antibiotics, which may be due to the lack of education from health care personnel, and more attention to the education provided by health care personnel is helpful.

Keywords: Neisseria gonorrhea, antibiotics, sexually transmitted diseases

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Introduction

Of the most common bacteria causing Sextually Transmitted Diseases (STD) is Neisseria gonorrhoeae. Rising gonorrhea rates, growing antimicrobial resistance, and increasingly limited antimicrobial options globally have made gonorrhea an urgent antibiotic-resistant threat.[1,2] Similarly, the Centers for Disease Control and Prevention identified Neisseria gonorrhoeae antimicrobial resistance as being an urgent threat.[3] Therefore, it has a substantial economic burden for health care system globally. The prevalence of STD among women may be significant because women often suffer silently from this kind of infection; as many as 70% to 80% of women who are infected do not experience any symptoms and therefore, they cannot be distinguished from infected women by clinical examination.[3] WHO estimated that 106 million cases of STD occur annually with approximately 2/3rd of these cases reported from developing countrie,[4] however, although this kind of infection is easily curable by a single dose of antibiotic but resistance also emerged against various antibiotics in recent years.[5] In Zahedan, Iran, during 2007 - 2010, the percentage of resistance to antibiotics likes penicillin and co-trimoxazole 79.2% and 93.5% respectively. Similarly another study reported that the highest resistance rate (100%) was observed for ciprofloxacin, penicillin G, ceftriaxone and cefepime; therefore, the emergence of resistance to various antibiotics is of great concern.[6,7]

Materials and methods

Clinical samples

The vaginal swabs were collected from 200 non-pregnant women patients admitted to the hospital in Karaj, Iran during 2015-2016. All samples from patients with servisitis which took part in the questionnaire form were included in this study. The demographic data of them were obtained including Age, education, occupation, marital status, smoking, number of sexual partners, contraceptive methods, having a history of pregnancy, abortion, preterm labor, infertility, receiving antibiotics in a recent month, history of genital infection, history of venereal infection in a spouse, the results of direct observation of the cervix (the presence or absence of erythema, ulcers, secretions, volume and type of discharge).

Bacterial isolates

The bacterial isolates were identified by gram-staining, biochemical tests and culture on Thayer-martin specific rich medium under micro-aerophilic conditions.

The antibiotic susceptibility test

The susceptibility pattern of isolates was determined by disk diffusion and culture of 0.5 Mc Fahrland suspension on the Mueller Hinton agar containing 5% sheep blood according to the clinical and laboratory standards institute (CLSI) guidelines. The disks ciprofloxacin (CIP), Ceftriaxone (CRO), Cefixime (CFM), Tetracycline (TE), Azithromycin (AZM), Co-trimaxazole (SXT), Gentamicin (GM), Cefotaxime (CTX), Ceftriaxime (CT), Ceftazidime (CAZ), Spectinomycin (SCM), Cefuroxime (RX), Penicillin (PG) and amoxicillin (AMMOX) were purchased from Padtan Teb Co, Iran. The Neisseria gonorrhea ATCC49226 and E. coli ATCC 25922 were used as the quality control of the disks. The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentration. The MIC and MBC concentration of azithromycin and co-trimoxazole were determined by the micro-broth dilution test in MHA with 10 percent sheep blood agar according to the CLSI 2016.

Data analysis

The analysis of data obtained was done with the Graph Pad Prism 6.1. The Chi-square and T-test was used for the data analysis. The p value<0.05 and 95% confidence interval (95% CI) was considered as significant result.

Results

Patients and bacterial isolates

The mean age of participants was 35.85%. The abundance of married people was 97%, with 148 people having completed post-secondary education. Most of the patients under study had a natural prevention method (31.51%). Most of the partners had a partner and six did not have sex partners. 87% of women had no history of infertility. 93 of participants had taken antibiotics in the last month. Of the objects, 171/200 had genital tract infections and 33 cases had a history of genital tract infection. 73.5% of women had erythema symptoms, 68.5% wounds and 98.5% of discharge, of which 167 had yellow or green discharge, smell, and 65 cases had high levels of discharge of the total 200 cervisitis samples, 12 (6%) of them were able to grow in vitro on the Thayer martin agar medium.

Antibiotic susceptibility test

The antibiotic susceptibility of isolates was conducted with Kirby Bauer disc diffusion method on agar medium on the basis of Clinical and Laboratory Standards Institute (CLSI) guidelines using 14 antibiotics. The results showed that all the isolates were resistant to cephixime and the majority of them (11/12 or 91.7%) were resistant to ceftriaxone, cefotaxime, ciprofloxacine, ceftizoxime, ceftizoxime, spectinomycin, and gentamicin (Table1).

Table 1 The antibiotic susceptibility test of 12 N. gonorrhea isolates

Antibiotic	Susceptible	Intermediate	Resistant
Ciprofloxacine	1 (8.3%)	-	11 (91.7%)
Ceftriaxone	1 (8.3%)	-	11 (91.7%)
Cefixime	-	-	100%
Tetracycline	1 (8.3%)	1 (8.3%)	10 (83.3%)
Azithromycin	1 (8.3%)	3 (25%)	8 (66.7%)
Co-tromoxazole	12 (100%)	-	-
Gentamicin	1 (8.3%)	-	11 (91.7%)
Cefotaxime	1 (8.3%)	-	11 (91.7%)
Ceftizoxime	1 (8.3%)	-	11 (91.7%)
Ceftazidime	1 (8.3%)	-	11 (91.7%)
Spectinomycin	1 (8.3%)	-	11 (91.7%)
Cefuroxime	1 (8.3%)	1 (8.3%)	10 (83.3%)
Amoxicillin	-	2 (16.7%)	10 (83.3%)
Penicillin	2 (16.7%)	-	10 (83.3%)

Detection of co-trimoxazole and azithromycin MIC and MBC

The results of co-trimoxazole and azithromycin MIC and MBC has been shown in (Table 2). As shown in (Table 2), most of N. gonorrhea isolates were susceptible to azithromycin and co-trimoxazole in the MIC determination test.

Table 2 The results of co-trimoxazole and azithromycin MIC and MBC of 12 N. gonorrhea isolates

N. gonorrhoeae isolates	Azithromycine		Cotrimoxazole	
	MIC(µg/ml)	MBC(µg/ml)	MIC(µg/ml)	MBC(µg/ml)
1	./5	100%	./125	./25
2	./062	./25	./031	./125
3	./125	./5	./015	./125
4	1	1	./125	./125
5	./25	1	./062	./125
6	./062	./062	./015	./062
7	./125	./25	./031	./031
8	./062	./062	./25	./25
9	1	2	./062	./062
10	./125	./25	./25	./5
11	./031	./031	./125	./125
12	./5	./5	./062	./125

Discussion

Among the patients, the natural method (N=60) was the most commonly used prevention method, of which more than half (N=35) were infected with N. gonorrhoeae, which is similar to the studies of the use of physical prevention methods such as condom and insists on the use of chemical methods to prevent the spread of sexual diseases. Based on the demographic data, more than half (n= 40) of those infected with gonorrhea had yellow-greenish discharge, which confirmed the association of cervicitis and gonorrhea with similar studies. Moreover, the majority (n=8) of those infected with gonorrhea had a sexual partner. Three of the people who said they were lacking sexual partners were suffering from gonorrhea, possibly indicating a risk factor in the community, and also point out that some people, like other studies, cannot be honestly answerable for various reasons. Antibiotics were expected to be negative for cultivation, but more than half of the people who had received antibiotics had been taking antibiotics, which probably have led to the resistance to these antibiotics. Although commercially designed alkyd-based assays to detect N. gonorrhoeae have a higher sensitivity to tests such as cultivation, the specificity of these tests is discussed because of recombination occurring abundantly among the members of the Neisseria genus and crossover reactions with many target sequences, including 16S rRNA, and also, when cppB gene sequences were used to detect N. gonorrhoeae, false positive results were observed among N. meningitidis and N. lactamica.[8]

In the susceptibility test, most of isolates were sensitive to azithromycin and cotrimaxazole, and most of them were resistant to other antibiotics. In a study by Ghaznavi Rad, 100% of strains were sensitive to penicillin.[9] In 1999, penicillin resistant strains were 4.5% (84%) and it was raised to 68.5% in 1997 and 68.5% in 2000.[9] In the study of Naderi in Mashhad in 2008, resistance to penicillin was

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71.8%.[10] This resistance rate in Japan was reported at 10.3%.[11] In Brunner' study, 77% of strains were resistant to penicillin[12] from 2010 to 2013, in a four-year period. In our study, penicillin resistant strains were reported in 83.33% and intermediate strains were 16.7%.[12] Strains resistant to tetracycline have been reported in Ghaznavi Rad.[9] and in Naderi Nasab et al.[10] 36% and 59%, respectively. This resistance rate in Japan was 18.4%[11] and Hungary (86%).[12] In contrast to the results obtained in this study, isolated bacteria were more resistant and only 16.66% of the species were susceptible and intermediate-resistant to this antibiotic. This figure was 10% in Hong Kong and the Philippines.[13] In a study conducted in Hungary over a four-year period, it was found that 66% of N. gonorrhea strains were resistant to this antibiotic.[12] In our study, strains resistant to this antibiotic were 91.66% and sensitive strains were reported as 33.3%. Tabasi found two N. gonorrhea isolates from women with cervicitis in Kashan which were resistant to ceftriaxone.[14] In our study, eleven resistant and one sensitive strain were reported. In a study (2009) among endocervical samples from 400 patients in Zahedan, antibiotic resistance to penicillin was 79.2%, and ciprofloxacin 53.2%, ceftriaxone 3.8%, spectinomycin 2.5%, Cefixime 12.9%, Co-trimoxazole 93.5%, Tetracycline 88.3%, and Gentamicin 29.8%, being lower than our study except for co-trimoxazole and tetracycline.[15] This high prevalence of antibiotic resistance to these antibiotics in the present study and in other countries may be indicative of the development of antibiotic resistance among N. gonorrhoeae. In the vast majority of studies, self-treatment has the major role in increasing the resistance of the Neisseria gonorrhoeae. On the other hand, this high antibiotic resistance may be due to a lack of completion of the course of antibiotic treatment. Given that the MIC of two antibiotics azithromycin and co-triaxazole are in sensitive range for most specimens, it is still possible to use them for treatment, but observation of a resistant strain and three intermediate strains to azithromycin suggests the need for quantitative and qualitative evaluation antibiotic susceptibility tests. In our study, given that the majority of gonococci has multiple resistances and showed at least three antibiotic classes, this level of multiple resistances seems to be alarming. Resistant strains of the population are rising, which may increase the resilience of the availability of drugs and self-treatment. Following the use of antibiotics, strains of reduced sensitivity have been diagnosed in several countries worldwide. But the number of cases of refractory cases has been reported from one country to another.[16]

Conclusion

In our study, given that the majority of gonococci had multiple resistances and showed at least three antibiotic classes in the society, this level of multiple resistances seems to be alarming. It is possible that the availability of antibiotics and self-treatment has increased the resistance of N. gonorrhea to antibiotics, which may be due to the lack of education from health care personnel, and more attention to the education provided by health care personnel is helpful. On the other hand, high antibiotic resistance can be due to the lack of completion of the course of antibiotic treatment. Therefore, it is imperative that the treatment staff emphasize the length of the treatment period and, take the necessary training and follow-up. In addition, in order to prevent the increase of drug resistance, appropriate suspicious cases should be considered for proper treatment of patients and, if diagnosis is confirmed, antibiotic susceptibility testing should be performed. Also, due to the high prevalence of N. gonorrhea among natural prophylaxis, it seems that replacing physical methods such as condoms instead of chemical methods such as pills and ampoules, etc. are effective ways.

Acknowledgment

None.

Conflicts of interest

None.

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