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Brief report

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Prevalence of mutations associated with macrolide and fluoroquinolone resistance in *Neisseria gonorrhoeae* with Allplex™ NG&DR Assay (Seegene®) in a tertiary hospital from Madrid, Spain

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ABSTRACT

Background. The prevalence of drug-resistant *Neisseria gonorrhoeae* (NG) infections is increasing. Studies report the prevalence of NG strains presenting A2059G/C2611T (rRNA 23S) and S91F (*parC*) mutations conferring resistance to azithromycin and ciprofloxacin.

Material and methods. We conducted a prospective cohort study evaluating first void-urine urines, rectal, and oropharyngeal swabs collected from a cohort of patients in a tertiary hospital in Madrid between October 2022 and January 2023. Samples were screened by Allplex™ 7-STI Essential Assay (Seegene®). Drug resistances were performed by Allplex™ NG&DR Assay (Seegene®).

Results. A total of 1,415 patients were included, of which 112 had a positive sample for NG infection. One patient had a C2611T mutation (0.9%) and neither patient showed A2059G mutation. We found 67 (59.8%) S91F-positive patients. Forty-four patients (39.3%) not had any mutations.

Conclusions. We report a low-prevalence of mutations A2059G/C2611T to macrolides and a high-prevalence to S91F in NG infections. Molecular methods for the detection of NG resistance could be useful in direct non-culturable samples.

Keywords: *Neisseria gonorrhoeae*; azithromycin; ciprofloxacin; antimicrobial resistance; mutations

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Prevalencia de mutaciones asociadas a resistencia a macrólidos y fluoroquinolonas en *Neisseria gonorrhoeae* con el kit Allplex™ NG&DR Assay (Seegene®) en un hospital terciario de la Comunidad de Madrid, España

RESUMEN

Introducción. La infección por *Neisseria gonorrhoeae* (NG) resistente está aumentando. Se ha descrito la prevalencia de cepas de NG con mutaciones A2059G/C2611T (rRNA 23S) y S91F (*parC*) que confieren resistencia a azitromicina y ciprofloxacino.

Material y métodos. Realizamos un estudio prospectivo evaluando orinas de primera micción, hisopos anales y faríngeos recogidos de una cohorte de pacientes en un hospital terciario de Madrid entre octubre de 2022 y enero de 2023. El cribado de las muestras se realizó mediante Allplex™ 7-STI Essential Assay (Seegene®). Las resistencias a macrólidos y fluoroquinolonas se realizaron mediante Allplex™ NG&DR Assay (Seegene®).

Resultados. Se incluyeron 1.415 pacientes, de los cuales 112 fueron positivos para NG. Un paciente presentaba una mutación C2611T (0,9%) y en ningún paciente se detectó A2059G. Encontramos 67 pacientes (59,8%) positivos para S91F. Cuarenta y cuatro pacientes (39,3%) no presentaban mutaciones.

Conclusiones. Reportamos una baja prevalencia de mutaciones A2059G/C2611T a macrólidos y una alta prevalencia de S91F en NG. Los métodos moleculares para la detección de resistencias en NG podrían ser útiles en muestras directas no cultivables.

Palabras clave: *Neisseria gonorrhoeae*; azitromicina; ciprofloxacino; resistencia antimicrobiana; mutaciones

INTRODUCTION

Antimicrobial resistance in *Neisseria gonorrhoeae* (NG) is increasing worldwide. Mechanisms of NG resistance to macrolides include chromosomal mutations, genes encoding rRNA methyltransferases, and overexpression of efflux pumps [1]. Resistance to fluoroquinolones is mainly due to the presence of mutations in the *gyrA* or *parC* genes [2]. The first-line treatment for NG infections is third-generation cephalosporins, such as intramuscular ceftriaxone or oral cefixime. Macrolides (azithromycin) are a second-line treatment that should not be used in monotherapy. Treatment with fluoroquinolones (ciprofloxacin) may be an option in allergic or intolerant patients to third-generation cephalosporins, provided that a *gyrA* mutation is not detected following the sexually transmitted infections guidelines [3,4]. The resistance rates to macrolides and fluoroquinolones are well known for *Mycoplasma genitalium* infections due to the large supply of commercially available assays [5-7]. However, new commercial kits are emerging for the detection of mutations associated with therapeutic failure of these second-line treatments using nucleic acid amplification tests (NAAT) for NG infection. [8]. We have researched the epidemiology and prevalence of macrolide and fluoroquinolone resistances in patients with *N. gonorrhoeae* sexually transmitted infection collected at Hospital Universitario La Paz (HULP), a tertiary hospital from Madrid, Spain.

MATERIAL AND METHODS

In a prospective observational cohort study design, we collected the demographic, clinical, and analytical data of patients with NG infection (as confirmed by RT-PCR) from the hospital database and laboratory informatics systems from October 2022 to January 2023. Only one NG sample by patient was included in the study. In addition, sexually transmitted coinfections and serological data were collected from patients during the NG infection episode.

Genitourinary samples were screened for NG by real-time PCR Allplex™ 7 STI Essential Assay (Seegene®, Seoul, South Korea), which includes *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Mycoplasma genitalium*, among others STI pathogens. Positive samples for NG were analyzed with the new kit Allplex™ NG & DR Assay (Seegene®) to study mutations associated to macrolides and fluoroquinolones resistance. This is a new commercial kit authorized for in vitro diagnostic use (IVD). The assay includes the A2059G mutation (23S rRNA) associated with high-level macrolide resistance and the C1126T mutation (23S rRNA) associated with moderate-level macrolide resistance. Resistance associated with fluoroquinolones was detected by the S91F (*gyrA*) mutation including in the assay. The RT-PCR was performed in combination with automated DNA extraction and PCR setup using a Microlab STARlet Liquid Handling robot (Hamilton®), according to the manufacturers' instructions. Genitourinary samples were amplified by CFX96™ Touch Real-Time PCR Detection System thermal cycler (BioRad®, Hercules, California). Seroprevalence data about patients were collected for HIV (Anti-HIV/Ag-p24), syphilis (IgG), HBV (Anti-HBc IgG), and HCV (IgG). Serological analysis was performed by Atellica (Siemens Healthcare Diagnostics®, Germany).

RESULTS

A total of 1,415 (765 males and 650 females) patients were screened for NG infection of which in 112 patients (8%) NG was detected. The proportion of males (n=101, 90.2%) with NG infection was higher than females. The median age of the patients was 29 years-old (IQR: 23-37). In the seroprevalence study, twenty-five out of 96 patients (26%) were positive for HIV, 32/98 (32.7%) had syphilis-positive, 10/84 (11.9%) were HBV-positive, and 5/96 (5.2%) had HCV-positive. Thirteen (11.6%) out of the 112 patients had no serological records at our center.

Specimens collected were mainly first void-urines (75 samples) following by rectal (26 samples), and oropharyngeal swabs (11 samples). Twenty-one out of 112 patients (18.8%) had some sexually transmitted coinfection during the study with *Chlamydia trachomatis* (n=20) or *Trichomonas vaginalis* (n=1) (Table 1).

In 44 patients were not detected any mutation associated

Table 1 **Distribution of mutations associated with macrolide and fluoroquinolone resistance by sample and sexually transmitted coinfections (STCs).**

Sample	Total	STCs (n)	NM (n)	%	Macrolide resistance (n)		%	Fluoroquinolone resistance (n)	%
					A2059G	C2611T		S91F	
First-void urine	75	15 (1CT, 1TV)	25	33.3%	0	1	1.33%	48	64%
Anal Swab	26	6 CT	13	50%	0	0	0%	13	50%
Oropharyngeal swab	11	0	5	45.4%	0	0	0%	6	54.6%
Total	112	21	44	39.3%	0	1	0.9%	67	59.8%

NM: Non-mutations detected, CT: *Chlamydia trachomatis*, TV: *Trichomonas vaginalis*

with macrolide or fluoroquinolone resistance. A single mutation C1126T (23S rRNA) associated with moderate-level macrolide resistance was only detected in one patient from first-void urine. Neither A2059G (23S rRNA) mutation associated with high-level macrolide resistance was detected. No macrolide resistance-associated mutations in *N. gonorrhoeae* were detected in 99.2% of the samples. However, in 67 strains of NG (59.8%) the S91F mutation associated with fluoroquinolone resistance in *gyrA* gene was detected.

DISCUSSION

We have reported the prevalence of mutations associated with macrolide and fluoroquinolone resistance in our population with *Neisseria gonorrhoeae* infection. Most of the patients were sexually active males with a positive serological status (HIV, syphilis, HBV, or HCV). The sexually transmitted coinfections were mainly by *C. trachomatis*.

The therapeutic use of azithromycin represents a useful therapeutic line always in combination with other drugs for NG infection. Sanchez-Busó *et al.* reported an overall prevalence in Europe of 8% of macrolide resistance [9]. In Spain, azithromycin sensitivity studies of NG have been reported by the gradient diffusion methods. The prevalence of azithromycin resistance contrasts between 2-12% [10–13]. However, no studies using NAAT methods have been described. The prevalence of mutations A2059G and C1126T associated with high/moderate-level macrolide resistance in our study population was low according with other reports [9,14]. However, there are other mechanisms of macrolide resistance that imply therapeutic failure of NG infections and are not detected by the assay, such as A2058G high macrolide resistance, or resistances associated with overexpressed of efflux system, among others [1,14]. Kandinov *et al.* reported an overexpression of efflux pumps by mutations in the MtrCDE genes causing more than 90% of azithromycin resistance.

We found a high prevalence of S91F mutation associated with failure to fluoroquinolones treatment in NG infection according with another report in Europe [9]. The highest prevalence of fluoroquinolone resistance was detected in urine due to maybe to the higher representation of gonococcal urethritis. According to this high prevalence, STI therapeutic guidelines recommend prior screening for mutations in the *gyrA* gene before treatment with fluoroquinolones [3]. In the same way, other mutations associated with fluoroquinolones resistance such us D95A, D95G, or D95N (*gyrA*) or D86N (*parC*) are not included in the Allplex assay although these mutations are less prevalent [9]. Secondly, the highest prevalence of fluoroquinolone resistance was found in pharyngeal samples. In addition, fluoroquinolones resistance was detected in the half of the rectal samples. However, the presence of commensal *Neisseria* species with resistance to fluoroquinolones in clinical samples (such as pharyngeal or rectal swabs) could lead to false-positive results for the S91F mutation. The culture would be necessary to select *N. gonorrhoeae* in this type of samples.

As a limitation of our study, no data were collected on the sexual practices of patients. Moreover, these detection of a little number of mutations could imply underdiagnoses of macrolide and fluoroquinolone resistance. Therefore, it would be necessary to evaluate the correlation between the detection of these resistances by NAAT and the MICs values obtained by standard resistance methods from EUCAST/CLSI breakpoints or next generation sequencing (NGS) in other studies.

In conclusion, the Allplex™ NG&DR Assay kit (Seegene®) has some limitations in clinical use due to the empirical use of third-generation cephalosporins as first-line treatments for NG infections and the possibility of detecting resistances from commensal species. However, the assay could be useful in cases where targeted therapy with fluoroquinolones (if no resistance mutations are detected) is established in patients who have not received empirical treatment. In addition, the useful in joint fluid for gonococcal arthritis diagnoses should be studied due to the use of fluoroquinolones in this clinical entity [15]. Moreover, the detection of macrolide or fluoroquinolone resistance-associated mutations in NG by NAAT methods could be useful in direct clinical samples such as first-void urine or when NG strains cannot be isolated due to lack of growth. Furthermore, mainly the assay could be useful epidemiologically to monitor the emergence of resistant NG without the need for positive culture or NGS studies.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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