Emergence of High Level Azithromycin-Resistant *Neisseria gonorrhoeae* Strain Isolated in Argentina

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Abstract: One *Neisseria gonorrhoeae* strains highly resistant to azithromycin AzHLR (MIC >2048 mg/L) was isolated in Argentina in 2001 and it has been characterized by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) as ST696, suggesting a different event to other isolates in Europe. Neither, *mtrR* mutations or presence of *mef* gene were detected.

Key Words: Azithromycine resistance, Gonococci, mtrR

Single oral dose (1 g) of azithromycin is a recommended therapy for chlamydial infections. However, this dose is not enough effective for gonorrhea and because the use of 2 g (highly effective again *Neisseria gonorrhoeae*) has been reported as being associated with gastrointestinal intolerance, is not widely recommended for gonorrhea.[1] However, several countries might have been using this antibiotic both for the primary treatment of gonococcal infections and for the simultaneous treatment of chlamydial infections,[2] and it is strongly recommended to monitor gonococcal resistance to azithromycin in countries where this drug is used.[1]

The National Reference Center for STDs in Argentina include azithromycin since 1997 on the routine surveillance susceptibility patterns for gonococcal strains isolated in hospitals belonging to the National Gonococcal Susceptibility Surveillance Program. As result of this, 2 azithromycin-resistant gonococcal strains were detected over the period between 1997and 2004: the first one in November 2001 and the second one in May 2003, with MICs of >2048 and 8 mg/L, respectively.[3] Susceptibility to azithromycin was determined by the agar dilution method according with the CLSI guidelines,[4] using 0.03 to 2048 mg/L as range of concentrations, and was confirmed by the Spanish Reference Laboratory for *Neisseria*, using both agar dilution and Etest method.

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Recently the emergence of high-level azithromycin resistance (AzHLR; >256 mg/L) in gonococci has been described in Scotland[5] and England and Wales.[6] In Scotland, they have been isolated since 2004 and most of them belong to either the sequence type (ST) 470 or the ST649. In England and Wales, a cluster of six AzHL isolates was reported all of them belonging to the ST649. The gonococcal strain with AzHLR isolated in Argentina was characterized by *N. gonorrhoeae* multiantigen sequence typing[7] (NG-MAST) as belonging to the ST696 by sequencing internal regions of the genes encoding 2 variable outer membrane proteins, *Por* and *TbpB* (available at: http://www.ng-mast.net). The 3 mentioned STs (ST470, ST649, and ST696) present a common *TbpB* allele (29) but 3 different *Por* sequences (339, 442, and 105 respectively). The *Por* alelle 105 present in the Argentinean strain shows 27 and 28 bases pair difference with *Por* 339 and 442, respectively, indicating different genetic lineages and suggesting independent events to produce gonococcal strains with AzHLR both in Latin America and Europe. The long passed time between the descriptions of this type of strains might suggest not easy transmission and/or survival for this type of strains. However, the finding of a cluster of isolates from heterosexual patients in England and Wales and the continuous increase notice in Scotland most probably show a real potential for spreading.

The Argentinean strain with an MIC to azithromycin of 8 mg/L showed a new ST with a *Por/TbpB* allelic combination of 1389/4. Both strains, with azithromycin resistance (MICs of 8 and >2048 mg/L) were characterized as auxotype/serovar classes Proline-/IByust and Proline-/IBrpyst, respectively, using the methodology already described.[8,9] The analysis of both strains by Pulsed Field Gel Electrophoresis (PFGE) using *Bgl*II (data not showed) presented two different and unrelated pattern profiles (7 band differences), concluding that both gonococci belong to 2 different clones that might have acquired the resistance in 2 different and independent genetic events.

Decreased susceptibility to azithromycin in *N. gonorrhoeae* is mainly due, to an overproduction of *mtr*(CDE)-encoded efflux pump determined by *mtr*R mutations.[7]

These changes have been described affecting different sites, reflected on Table 1. For the characterization of the mechanism for resistance in both Argentinean gonococcal strains with azithromycin resistance, 2 gonococci with intermediate resistance (MIC 1 mg/L) and 1 susceptible strain (MIC 0.06 mg/L), all of them isolated in Argentina, were also included (Table 1).

The *mtrR* sequence of the strain with AzHLR (n° 1782) showed one of the alterations already associated with decreased susceptibility to azithromycin. The strain n° 2498 with an MIC of 8 mg/L presented a dinucleotide (TT) insertion which has been also described previously.[10] Finally, those gonococcal strains (2516 and 2518) showing intermediate resistance to azithromycin appeared with changes that have been also asso-

TABLE 1. Analysis of Changes in the *mtrR* Gene Associated With Decreased Susceptibility to Azithromycin on the Gonococcal Strains Included in This Study

Strain N°	MIC mg/L	Year of Isolation	City of Isolation	Gly-45 K → Asp-45	Deletion in 13 bp Inverted Repeat Region	Additional Changes
1782	>2048	2001	Buenos Aires	+	−	—
2498	8	2003	Buenos Aires	−	−	Insertion of 2 T bases
2516	1	2003	Neuquén	−	+	—
2518	1	2003	El Palomar	−	+	—
3783	0.06	2005	Resistencia	−	−	—

ciated with this level of resistance. Those mutations appearing on strains 2498 and particularly 1782 did not explain the high level of resistance to this antibiotic, because they have been already described in strains only showing intermediate level of resistance.

The *mef* gene code for an efflux pump conferring also resistance to macrolides, particularly in Gram-positive but also described in Gram-negative species.[11] The presence of the *mef* gene was checked by PCR either in the strain 1782 and 2498 and none of them presented the gene.

Mutations in the peptidyl transferase loop in domain V of 23S rRNA have been also associated with macrolide resistance in *N. gonorrhoeae.*[12] To identify mutations within domain V (of the peptidyltransferase loop) for each of the 4 copies of the 23S rRNA gene, a 2-step PCR method is ongoing for checking the presence of mutations in Argentinean strains showing azithromycin resistance.

Further studies are in progress trying to characterize the mechanisms for high level of resistance to azithromycin in these gonococcal strains.

The very high MIC levels recorded here would make the suggestion of using new formulations of azithromycin,[13] showing less problems of gastrointestinal intolerance untenable, if strains of this type were to emerge elsewhere and spread. Decreased susceptibility to azithromycin in Latin America and the Caribbean region has been already detected through GASP network surveillance[14] and, particularly in Argentina, gonococcal strains showing an MIC of 1 mg/L to azithromycin ranged from 9 to 17% during 1999 to 2001, and an average of 5.6% until 2006, falling to 2.8% in 2007. Besides the 2 strains presented in this study, 3 additional isolates with MICs of 16 mg/L were detected in 2004 from different provinces[15] and 1 of 2 mg/L in each year during 2004, 2005, and 2006. The fall in incidence of azithromycin resistance might be reflecting either a success in recommendations to restrict the use of the antibiotic or a temporal and natural change in the prevalence of these types of strains. Otherwise, the finding of isolates with high level of resistance proves the relevance of azithromycin susceptibility surveillance in order to define the real utility of this antibiotic for the treatment of gonococcal infections in Argentina.

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