Enferm Infecc Microbiol Clin. 2020;38(6):293–298

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Scientific letter

Mycoplasma genitalium and fluoroquinolone resistance detection using a novel qPCR assay in Barcelona, Spain

Detección de Mycoplasma genitalium *y resistencia a fluoroquinolonas empleando una nueva técnica de qPCR en Barcelona, España*

Mycoplasma genitalium is now a well-recognized sexually transmitted infection (STI), known to cause urethritis and other adverse reproductive tract conditions in both men and women.[1] Antimicrobial resistance has become a major concern in treating *M. genitalium* infections. The macrolide azithromycin, given as an extended 1.5 g-dose (500 mg day 1, 250 mg days 2–5), has been the recommended first-line treatment against uncomplicated infections.[2] However, macrolide resistance has rapidly emerged, exceeding 50% in many countries,[3] likely enhanced by the widespread use of this antibiotic. The fourth-generation fluoroquinolone moxifloxacin is currently the second-line antibiotic used to treat macrolide resistant infections.[2] Even so, *in vivo* resistances are increasingly being reported in many countries, including Spain.[4,5] Fluoroquinolone resistance is associated with single nucleotide polymorphisms (SNPs) in the quinolone determining region (QRDR) of the *parC* gene affecting amino-acids S83 and D87.[6,7]

Since data regarding fluoroquinolone resistance in *M. genitalium* remains limited in local population, the aim of this retrospective study was to estimate the rate of fluoroquinolone resistance-associated mutations among a 2016–2017 series of individuals in Barcelona, Spain, using the recently validated commercial MG + parC (beta) assay (SpeeDx, Australia).[8]

Specimens were collected at the Microbiology Department of the Vall d'Hebron University Hospital, as a part of a previous study,[9] between December 2016 and February 2017. Demographic and clinical data are published elsewhere.[9] A total of 82 baseline samples from 80 patients, coming from different clinical settings (hospitals, primary care centers and STI clinics), were included.

Briefly, the samples were processed as follows. First, samples were tested for *M. genitalium* by real-time PCR (qPCR) using the Allplex™ STI Essential Assay (Seegene, South Korea). Then, positive samples were retrospectively screened for macrolide resistance genotypic markers using the commercial qPCR ResistancePlus® MG assay (SpeeDx, Australia) and Sanger sequencing. DNA extracts were finally stored at −20 °C for subsequent analyses. For the current study, samples were tested for fluoroquinolone resistance-associated mutations using the MG + parC (beta) assay.[8] The MG + parC (beta) test is a novel two-well qPCR assay that detects *M. genitalium* through the *mgpB* (MG191) gene and five *parC* mutations linked with moxifloxacin treatment failure: G248T (S83I) in well 1, and A247C (S83R), G259A (D87N), G259T (D87Y) and G259C (D87H) in well 2. Ethical approval for the study was obtained from the Vall d'Hebron University Hospital Ethics Committee (351/2018).

Among the 80 infections studied, fluoroquinolone resistance-associated mutations were detected in eight cases (10.0% [95% confidence interval (CI), 4.4–18.8%]). Results are described in Table 1. Of them, three infections (37.5% [95% CI, 8.5–75.5%]) harbored mutation G248T (S83I), individually reported by the MG + parC (beta) assay. Detection of the remaining *parC* mutants were reported through a single channel in well 2. Furthermore, macrolide resistance was significantly more prevalent among *parC* mutants (75.0% [95% CI, 34.9–96.8%]) compared to wild-type (WT) infections (33.3% [95% CI, 22.7–45.4%]); (OR 6.0 [95% CI, 1.1–32.0]), $p = 0.036$.

Table 1
Prevalence of *parC* fluoroquinolone resistance-associated mutations in *M. genitalium*.

Characteristics of patients/infections	*parC* Mutant[a] MG *N.*; %	Non-mutant MG *N.*; %
Sexual preference		
WSM	2; 25.0	18; 25.0
MSW	2; 25.0	19; 26.4
MSM	4; 50.0	35; 48.6
Macrolide resistance status		
Resistant	6; 75.0	24; 33.3
Susceptible	2; 25.0	48; 66.7
Location		
Genital	6; 75.0	58[c]; 80.6
Rectum	2; 25.0	16; 22.2
Total = 80	8[b]; 10.0	72; 90.0

Abbreviations: MG, *Mycoplasma genitalium*; MSW, men who have sex with women; MSM, men who have sex with men; WSM, women who have sex with men.

[a] Mutant category includes the mutants targeted in the MG + parC (beta) assay – A247C (S83R), G248T (S83I), G259T (D87Y), G259C (D87H) and G259A (D87N).

[b] Mutants included three G248T (S83I), mutation individually reported by the MG + parC (beta) assay in well 1. The remaining five *parC* mutants were reported in well 2.

[c] Two MSM had both genital and rectal *M. genitalium* infections.

The research provides further data regarding fluoroquinolone resistance in *M. genitalium* in Spain, where estimates remain limited. In the present study fluoroquinolone resistance-associated mutations were detected in 10% of infections, similar to previous investigations reporting a prevalence of 5%-9%.[4,5,8] Additionally, the presence of fluoroquinolone resistance-associated mutations was strongly associated with macrolide resistance in our series ($p = 0.036$). In fact, the prevalence of resistance to both classes of antibiotics was (7.5% [95% CI, 2.8–15.6%]) in the study population. Consequently, multi-drug resistant infections may gradually appear in our settings against which therapeutic options are very scarce.

Recently, a resistance-guided sequential treatment utilizing the novel ResistancePlus® MG assay has been successfully evaluated with promising results in terms of infection eradication and antibiotic resistance selection control.[10] In this scenario, the novel commercial MG + parC (beta) assay, demonstrating to be rapid, simple and accurate, could complement this resistance-guided therapy approach with the additional detection of fluoroquinolone resistance-associated mutations in *M. genitalium*, optimizing and refining antimicrobial stewardship. Nevertheless, despite the test detects known fluoroquinolone resistance-associated SNPs, further

studies are required to fully establish the contribution of other newly *parC* mutations in fluoroquinolone resistance.[6,7] Moreover, more investigations on new antibiotics and novel combinations with existing treatments are imperative to fight against *M. genitalium* that may soon become the next sexually transmitted superbug.

Funding

SpeeDx Pty Ltd. supplied all the reagents for molecular testing of *M. genitalium* and fluoroquinolone resistance.

Conflicts of interest

J.S.P and J.E. employed by the "Vall d'Hebron University Hospital", have received remuneration for contract work from SpeeDx Pty Ltd. M.E. employed by the "Corporació Sanitària Parc Taulí", has received remuneration for contract work from SpeeDx Pty Ltd. M.F.H. and M.E. have participated in symposiums organized by SpeeDx Pty Ltd.

References

1. Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to multicolored butterfly. Clin Microbiol Rev. 2011;24:498–514.
2. Jensen JS, Cusini M, Gomberg M, Moi H. 2016 European guideline on *Mycoplasma genitalium* infections. J Eur Acad Dermatol Venereol. 2016;30:1686–93.
3. Lau A, Bradshaw CS, Lewis D, Fairley CK, Chen MY, Kong FY, et al. The efficacy of azithromycin for the treatment of genital *Mycoplasma genitalium*: a systematic review and meta-analysis. Clin Infect Dis. 2015;61:1389–99.
4. Barberá MJ, Fernández-Huerta M, Jensen JS, Caballero E, Andreu A. *Mycoplasma genitalium* macrolide and fluoroquinolone resistance: prevalence and risk factors among a 2013–2014 cohort of patients in Barcelona, Spain. Sex Transm Dis. 2017;44:457–62.
5. Piñeiro L, Idigoras P, de la Caba I, López-Olaizola M, Cilla G. Guided antibiotic therapy for *Mycoplasma genitalium* infections: analysis of mutations associated with resistance to macrolides and fluoroquinolones. Enferm Infecc Microbiol Clin. 2019;37:394–7.
6. Murray GL, Bradshaw CS, Bissessor M, Danielewski J, Twin J, Jensen JS, et al. Increasing macrolide and fluoroquinolone resistance in *Mycoplasma genitalium*. Emerg Infect Dis. 2017;23:809–12.
7. Couldwell DL, Tagg KA, Jeoffreys NJ, Gilbert GL. Failure of moxifloxacin treatment in *Mycoplasma genitalium* infections due to macrolide and fluoroquinolone resistance. Int J STD AIDS. 2013;24:822–8.
8. Fernández-Huerta M, Bodiyabadu K, Esperalba J, Bradshaw CS, Serra-Pladevall J, Garland SM, et al. Multicenter clinical evaluation of a novel multiplex real-time (qPCR) assay for detection of fluoroquinolone resistance in *Mycoplasma genitalium*. J Clin Microb. 2019 [Epub ahead of print].
9. Fernández-Huerta M, Vall M, Fernández-Naval C, Barberá MJ, Arando M, López L, et al. *Mycoplasma genitalium* macrolide resistance update: rate among a 2016–2017 cohort of patients in Barcelona, Spain. Enferm Infecc Microbiol Clin. 2019 [Epub ahead of print].
10. Read TRH, Fairley CK, Murray GL, Jensen JS, Danielewski J, Worthington K, et al. Outcomes of resistance-guided sequential treatment of *Mycoplasma genitalium* infections: a prospective evaluation. Clin Infect Dis. 2018;68:554–60.

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https://doi.org/10.1016/j.eimc.2019.10.003

Application of a new method for the detection of catheter colonization and catheter-related bacteraemia in newborns

Aplicación de un nuevo método para la detección de colonización y bacteriemia relacionada con catéter en neonatos

Healthcare associated infections (HAIs) in neonatal units are increasing due to advances in invasive therapeutic and diagnostic procedures and increased survival of preterm babies being bloodstream infections (BSIs) one of the most common type of HAIs.[1,2] The drawing of paired blood samples from the catheter and a peripheral vein is considered the best approach to reduce suspicion of colonization of the device, contamination of the sample or true bloodstream infection caused by common commensal microorganisms.[3]

Current guidelines for the diagnosis of catheter-related bloodstream infections (CRBSI) recommend culture of catheters only when clinical suspicion are present to avoid unnecessary antimicrobial treatment.[4] Catheter colonization can be diagnosed either by rolling the catheter tip on the agar surface (Maki's roll-plate technique, MT) or by sonicating the distal 4–5 cm of the catheter.[5] However, MT is not fully reliable in the case of silicone neonatal peripherally inserted central catheter (SN-PICC) tips based on the study of Martín-Rabadan et al. due to the low capacity of this method for microbial detection inside catheters. Culture results may improve after catheter fragmentation.[6] In fact, the new protocol of 'Microbiological diagnosis of infections associated with intravascular catheters' elaborated by the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) includes roll-plate after slicing (RPS) as a good option in CRBSI diagnosis.[7]

Our objective was to compare the yield of MT and RPS for the detection of colonization and CRBSI. For this purpose, tips of neonatal catheters, all of them SN-PICCs, were prospectively cultured in the Hospital Miguel Servet of Zaragoza for 2 years (January 1, 2017 to December 31, 2018). Once the MT was performed, the catheter was opened longitudinally and the contents were extended with swab, leaving the catheter in the culture plate. They were incubated 4 days, issuing a preliminary report at 48 h. In all cases, culture was carried out for *Malassezia furfur*, adding a drop of olive oil in the initial area of the seed preventing it from spreading.

It was considered that the catheter was colonized when either of the two methods found ≥15 colony forming units (CFU). CRBSI was defined when the same microorganism was isolated in the catheter culture and peripheral blood cultures, without another source of infection.

During the study period, 79 sPICCS were processed. The number of colonized catheters was 24 (30.4%), with TM detected 15/24 and with RPS 21/24 ($p = 0.146$). The microorganisms isolated in colonized catheters were 18 Gram positive, 4 Gram-negative and 7 yeast, 5 of them *M. furfur*. BRC was diagnosed in 11 patients (13.9%), in 6 of them the diagnosis was made only with RPS ($p = 0.031$). Isolated microorganisms are shown in Table 1, in which the CFUs are expressed as: negative, >15 when the CFUs are between 15–100 and >100.