Clinical evaluation of *ResistancePlus*® MG FleXible for simultaneous detection of *Mycoplasma genitalium* and macrolide resistance.

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Introduction

- *Mycoplasma genitalium* (*Mgen*) is now a recognised cause of urethritis and other urogenital syndromes in men and women[1].
- *Mgen* has a disturbing capacity to develop antibiotic resistance and may soon become untreatable[2]. The macrolide azithromycin, has been the recommended first-line treatment for uncomplicated *Mgen* infections[3], however the widespread use of this antibiotic to treat a range of sexual health infections has likely enhanced the spread of macrolide resistance in *Mgen* worldwide[4-6].
- Mutations at positions 2058 and 2059 of the 23S rRNA gene (*E. coli* numbering) are strongly associated with macrolide resistance[5]. The development of rapid, simple, and accurate tests for the simultaneous detection of *Mgen* and macrolide resistance-mediating mutations (MRMMs) can significantly reduce the transmission and spread of *Mgen*, and decelerate the development of antibiotic resistance.
- Recently, a resistance-guided sequential therapy using a real-time polymerase chain reaction test that reports both the detection of *Mgen* and MRMM has been successfully evaluated[6,7]. This strategy has been recommended in recent BASHH[8] and IUSTI[9,10] guidelines.
- The aim of this study was to evaluate the clinical performance of the novel molecular, near-patient test ***Resistance****Plus*® MG FleXible (SpeeDx Pty Ltd, AU) on the GeneXpert Infinity-48s Platform (Cepheid, USA) for detection of *Mgen* and MRMMs.

GeneXpert® Infinity-48s (Cepheid)

Resistance*Plus*® MG FleXible (SpeeDx)

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Methods

- Between March 2019 and April 2019, a total of 146 samples (95 *Mgen* positive and 51 *Mgen* negative), were prospectively collected at the Vall d´Hebron University Hospital in Barcelona, Spain.
- Specimens consisted of the following:
  - 18 vaginal swabs
  - 32 endocervical swabs
  - 15 urethral swabs
  - 38 first-void urines
  - 43 rectal swabs
- Sample collection was performed according to routine practices, with urine samples collected in Vacumed® Urine (FL medical, Italy) and swab specimens collected with DeltaSwab ViCUM® (Deltalab, Spain). Of note, sample collection devices used in this study have not as yet been validated by the manufacturer for use with the ***Resistance****Plus*® MG FleXible test.
- Positive and negative specimens were initially selected based on the result of the Allplex™ STI Essential assay (Seegene, South Korea) for routine *Mgen* testing during the study period. Both primary samples and DNA extracts were stored at −20 °C and subsequently used for the current evaluation.
- In September 2019, primary samples were tested for *Mgen* and MRMM with the ***Resistance****Plus*® MG FleXible on the GeneXpert Infinity-48s instrument according to the manufacturer's instructions. The test detects *Mgen* via the *mgpB* gene and the MRMMs A2058G, A2059G, A2058T and A2058C in the 23S rRNA gene. Test results are available in approximately 2 hours.
- Sanger sequencing of the 23S rRNA gene was performed with the residual DNA extract from the Allplex™ STI Essential assay and used for confirmation of MRMMs.

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Results

- Results are displayed in Table 1. 84/90 (93.3%) of ***Resistance****Plus*® MG FleXible positives were suitable for subsequent Sanger sequencing.

Table 1.

ResistancePlus MG FleXible (n = 146)	**Allplex STI Essential**		**Positive Agreement % (95% CI)**	**Negative Agreement % (95% CI)**
	***Mgen* positive**	***Mgen* negative**		
Mgen positive	90	0	94.7 (88.1-98.3)	100.0 (93.0-100.0)
Mgen negative	5	51		
ResistancePlus MG FleXible (n = 84)	**Sanger sequencing**		**Positive Agreement % (95% CI)**	**Negative Agreement % (95% CI)**
	Non-mutant	**Mutant**		
23S rRNA Non-mutant	48	2[a]	94.1 (80.3-99.3)	96.0 (86.3-99.5)
23S rRNA Mutant	2[b]	32		

[a]Two rectal swabs reported as non-mutant by the ***Resistance****Plus*® MG FleXible assay but harbouring 23S rRNA mutations A2059G and A2058G, respectively, according to Sanger sequencing.

[b]Two first-void urines reported as 23S rRNA mutant by the ***Resistance****Plus*® MG FleXible assay but reported as wild-type by Sanger sequencing.

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Vall d'Hebron Barcelona Hospital Campus

Results

- Overall, the rate of MRMM in *Mgen* in this study population was 41.8% (95% CI, 30.8–53.4%).
- Refined data of macrolide resistance estimates are provided in Table 2.

Table 2.

Sexual behaviour/ Location	WT	MRMM	Total
	No.; % (95% CI)	No.; % (95% CI)	No.
MSM	15; 38.5 (23.4-55.4)	24; 61.5 (44.6-76.6)[c]	39
- Genital	4; 36.4 (10.9-69.2)	7; 63.6 (30.8-89.1)	11
- Rectum	12; 41.4 (23.5-61.1)	17; 58.6 (38.9-76.5)	29
MSW[a]	5; 83.3 (35.9-99.6)	1; 16.7 (0.4-64.1)	6
Men - unknown behaviour[b] - Genital	5; 71.4 (29.0-96.3)	2; 28.6 (3.7-71.0)	7
Women	21; 77.8 (57.7-91.4)	6; 22.2 (8.6-42.3)	27
- Genital	19; 76.0 (54.9-90.6)	6; 24.0 (9.4-45.1)	25
- Rectum	2; 100.0 (15.8-100.0)	0; 0.0 (0.0-84.2)	2
Total	**46; 58.2 (46.6-69.2)**	**33; 41.8 (30.8-53.4)[d]**	**79**

[a]All MSW had *Mgen* positive detections in urethra.

[b]All individuals were men with *Mgen*-positive detections in urethra.

[c]One MSM had concurrent genital and rectal *Mgen* detection. While *Mgen* in rectum was resistant to macrolides (mutation A2058T; *E. coli* numbering), the strain in urethra was susceptible to macrolides.

[d]Mutants corresponded to 11 A2058G, 19 A2059G and 3 A2058T.

Abbreviations: MSM, men who have sex with men; MSW, men who have sex with women; WT, wild-type; MRMM, macrolide resistance-mediating mutations in the 23S rRNA gene; CI, confidence interval.

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Vall d'Hebron Barcelona Hospital Campus

Discussion

- This is a clinical evaluation of the ***Resistance****Plus*® MG FleXible test, the first near-patient assay for simultaneous detection of *Mgen* and MRMM, conducted on the fully automated GeneXpert® Infinity-48s system.

- The ***Resistance****Plus*® MG FleXible test performed well for the detection of *Mgen* compared to the Allplex™ STI Essential assay with good positive and negative agreement percentages. Results categorized as false-negative were not evaluated with a third reference technique, but were re-tested with the Allplex™ STI Essential assay for discrepant analyses. The high Ct values of these discordant samples (median Ct 37.05) suggested low bacterial loads that may be close to the limit of detection for these commercial assays.

- The ***Resistance****Plus*® MG FleXible test also performed well for the detection of MRMM when compared with Sanger sequencing of the 23S rRNA gene. In this study, 2 first-void urines were reported as mutants by the ***Resistance****Plus*® MG FleXible assay but were reported as wild type with Sanger sequencing, slightly affecting the specificity of the technique for MRMM testing (96.0% in this study). Overall, the performance results are similar to recent evaluations of this test[11,12].

- ***Resistance****Plus*® MG FleXible offers a simplified laboratory workflow, when compared to Sanger sequencing and many other technically challenging molecular methods, such as the Allplex™ STI Essential assay. ***Resistance****Plus*® MG FleXible can be run on any of the fully automated GeneXpert® systems. Since these instruments are now available in many laboratories globally, the ***Resistance****Plus*® MG FleXible is a simple and easy-to-implement assay for the detection of *Mgen* and MRMM in routine sexual health diagnoses.

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Conclusions

- The ***Resistance****Plus*® MG FleXible assay is a rapid, simple and accurate assay for simultaneous detection of *Mgen* and MRMMs.
- This novel test may facilitate the implementation of a resistance-guided therapy patient treatment algorithm for *Mgen* infections in a wider range of clinical settings, significantly improving clinical management and limiting antibiotic resistance selection.

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