P 612

Antimicrobial susceptibility data of *Mycoplasma genitalium* strains isolated in Japan

Ryoichi Hamasuna, Masahiro Matsumoto, Naohiro Fujimoto, Tetsuro Matsumoto
Department of Urology, Shin-Kokura Hospital
Department of Urology, University of Occupational and Environmental Health, Japan

Disclosures:
This study was funded by grants from the Japan Society for the Promotion of Science (JSPS) Kakenhi Grant Number 17K11193.
This study was approved by the Human and Animal Ethics review committee of the University of Occupational and Environmental Health, Japan (no. H29-104).

Background

The initial culturing of *Mycoplasma genitalium* from clinical specimens is still difficult and the antimicrobial susceptibility data of *M. genitalium* strains have not been enough. Multidrug-resistant *M. genitalium* strains including macrolide or fluoroquinolone-resistance are increasing worldwide and analysis of antimicrobial susceptibility data and genome mutation data of *M. genitalium* strains is important.

Material and methods

M. genitalium strains were isolated from urinary sediments of *M genitalium*-positive urine specimens from Japanese men. The details for isolation of *M. gentalium* strains were described previously (R. Hamasuna, *Journal of Clinical Microbiology* 45:847-850, 2007). The method for antimicrobial susceptibility testing of *M. genitalium* strains was the cell-culture method, described previously (R. Hamasuna, *Antimicrobial Agents and Chemotherapy.* 49:4993-4998, 2005). Clinical strains were cultured on Vero cells with Ultrocer G. For antimicrobial susceptibility testing, the strains were cultures with culture media plus several concentration of antimicrobials for 3 weeks. The *M. genitalium* DNA load after culturing was determined by TaqMan PCR assay. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial causing 99% inhibitions comparing to control DNA load (without antimicrobials) . The tested antimicrobials were azithromycin (AZM), clarithromycin (CLR), doxycycline (DOX), minocycline (MIN), ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF) and sitafloxacin (STFX).
The details of DNA sequencing of domain V of 23S rRNA and quinolone-resistance determining region (QRDR) of ParC and GyrA were described previously (R. Hamasuna, Plos One, https://doi.org/10.1371/journal.pone.0198355 June 8, 1-10, 2018).

Results

We isolated total 17 strains including 4 strains from patients at 2003 and 9 strains at 2017 and 4 strains at 2018-2019. The antimicrobial susceptibility data and genome mutation data were shown in **Table 1**. S199 and SO202 were isolated from same patients before and after the treatment and SO214 and SO215 were also isolated from same patients.

Table 1 MIC of *M. genitalium* strains isolated from Japanese men and the analysis of genomes such as 23S rRNA, ParC and GyrA.

Strain	Isolated year	MIC (mg/L)								Gene mutations (amino acid change*)		
		AZM	CLR	DOX	MIN	CIP	LVF	MXF	STFX	23S rRNA	ParC	GyrA
G37^T	1980	0.002	0.004	0.5	0.5	8	2	0.06	0.13	-	-	-
M6282	2003	0.001	0.004	0.5	0.5	1	0.5	0.03	0.03	-	-	-
M6283	2003	0.002	0.002	1	0.5	1	1	0.13	0.06	-	G205A(Ala69→Thr)	-
M6284	2003	0.001	0.004	0.13	0.13	2	1	0.06	0.06	-	-	-
M6287	2003	0.002	0.004	0.25	0.13	4	4	0.5	0.13	-	G259T(Asp87→Tyr)	-
IMC-1	2017	>16	>16	1	0.13	>16	>16	4	1	A2059G	G248T (Ser83→Ile)	G277T （Gly93→Cys)
OSSP35-2	2017	>16	>16	0.5	0.13	16	8	2	0.25	A2058G	G248T (Ser83→Ile)	G285T (Met95→Ile)
JMPP4	2017	0.002	0.03	0.25	0.13	4	1	0.25	0.06	-	G248A (Ser83→Asn)	-
28290	2017	>16	>16	0.5	0.13	2	2	0.13	0.03	A2058G	G248A (Ser83→Asn)	-
SO199	2017	>16	>16	0.5	0.25	>16	>16	2	0.5	A2059G	G248T (Ser83→Ile)	G285T (Met95→Ile)
SO202	2017	>16	>16	1	0.25	>16	>16	4	0.5	A2059G	G248T (Ser83→Ile)	G285T (Met95→Ile)
SO206	2017	>16	>16	1	0.25	2	1	0.25	0.04	A2058G	G248A (Ser83→Asn)	-
SO214	2017	>16	>16	1	0.5	8	4	1	0.13	A2059G	G248T (Ser83→Ile)	-
SO215	2017	>16	>16	2	0.5	16	8	1	0.13	A2059G	G248T (Ser83→Ile)	-
SO251	2019	>16	>16	1	0.13	8	16	8	1	A2059G	G248T (Ser83→Ile)	G285T (Met95→Ile) G295A (Asn99→Asp)
SO263	2019	>16	>16	1	0.25	16	16	16	1	A2059G	G248T (Ser83→Ile)	G285T (Met95→Ile) G295A (Asn99→Asp)
SO277	2019	>16	>16	0.25	0.25	>16	4	0.25	0.25	A2058G	-	-
SO284	2019	16	8	0.5	0.25	>16	8	2	0.5	A2059G	G248T (Ser83→Ile)	G285T (Met95→Ile)

*: *E. coli* numbering.

Discussion

Among 17 strains, 14 strains had some mutations of QRDR of ParC or GyrA. We tried to isolate *M. genitalium* strains from clinical specimens which had some mutations of M *genitalium* genomes and the mutations between the isolated *M. genitalium* strains and original clinical specimens were coincident.
The mutations of domain V of 23S rRNA such as A2058G or A2059G were completely related to high MICs of AZM or CLR.
Strains with high MIC of MXF such as IMC-1, OSSP35-2, SO199, SO202, SO214, SO215, SO251, SO263 and SO284 had the mutation G248T with amino-acid change Ser83→Ile on ParC. Strains such as JMPP4, 28290 and SO206 had the mutation G248A with amino-acid change Ser83→Asn on ParC, but the MIC of MXF to these 3 strains did not elevate. We think the mutation G248T with amino-acid change Ser83→Ile on ParC is closely related to MFLX-resistance, but mutation G248A with amino-acid change Ser83→Asn is not. The relationship between mutations on GyrA and high MIC of MXF is not unclear. SO251 and SO263 had double mutations on GyrA and the MIC of MXF of these strains were very high such 8 and 16 mg/L. We think the mutation on GyrA can add to additional resistance to MXF.
STFX had lower MICs to any *M. genitalium* strains than MXF even *M. genitalium* strains have the mutation G248T with amino-acid change Ser83→Ile on ParC. We make a hypothesis that STFX-resistance is related to addition mutations on GyrA plus ParC and are examining the clinical outcomes, mutations and MICs.

Conclusion

We isolated 17 M. genitalium strains from Japanese patients. Macrolide-resistant and fluoroquinolone-resistant strains were isolated.