Introduction The condition of antimicrobial resistance in *Mycoplasma genitalium* has been becoming serious in the world. The macrolide-resistance is closely related to mutation on region of 23S rRNA gene. The fluoroquinolone-resistance is probably related to mutation on gyrase or Topoisomerase IV genes such gyrA or parC, like as other fluoroquinolone-resistant bacteria. In our study, we analysed the mutations related to antimicrobial resistance among *M. genitalium* genes which collected in Japan and compared with mutations of M6489, the multidrug-resistant strain.

Methods The *M. genitalium* genomes were collected from the urine specimens of Japanese males with urethritis during the period between 2005 and 2016. In addition, the genomes of *M. genitalium* strain which can grow in the culture media, included M6489, the multidrug-resistant strain. The region V of 23S rRNA and quinolone-resistance determining region (QRDR) on gyrA and parC genes were sequenced and the mutations related to macrolide- or quinolone-resistance were analysed.

Results The *M. genitalium* genomes from 157 Japanese males and 10 *M. genitalium* strains were analysed. Among the genomes from Japanese males, mutations related to macrolide-resistance such as A2058G or A2059G were detected in 4.4% (4/90) genomes at 2005–2009 and in 40.3% (27/67) at 2010–2016. Two types of mutations on the gyrA gene with amino-acid change and 11 types of mutations on the parC gene with amino-acid change were found. These mutations were detected in 26.6% (24/90) at 2005–2009 and 53.7% (36/67) at 2010–2016. Most frequent mutations were Pro69→Ser in 18 genomes and Ser80→Ile in 16 genomes. M6489 had A2059G on 23S rRNA and Asp87→Asn on gyrA and Ser80→Ile on parC gene. If these mutations on M6489 were related to fluoroquinolone-resistance, the fluoroquinolone-resistant *M. genitalium* increased 3.4% (3/87) to 16.4% (11/67).

Conclusion The mutations related to macrolide-resistance and fluoroquinolone-resistance genes increased in Japan.