Detection of Mutant *Mycoplasma hominis* Strains Resistant to 16-Membered Macrolide Antibiotic Josamycin in Clinical Samples

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The incidence of point mutations responsible for josamycin resistance was studied by PCR in 48 strains of *M. hominis* isolated from patients with bacterial vaginosis. Mutant *M. hominis* strains were detected in 48% cases.

Key Words: *Mycoplasma hominis; josamycin; resistance*

M. hominis is a component of microflora associated with bacterial vaginosis [7,9]. The presence of *M. hominis* is associated with a risk of preterm labor, spontaneous abortions, and respiratory diseases in newborns [6,10]. Antibacterial therapy of women of reproductive age is to be maximally safe. From this viewpoint, macrolide antibiotics are the drugs of choice. However, *M. hominis* is resistant to 14- and 15-membered macrolides (erythromycin, roxithromycin, clarithromycin, and azithromycin) [8]. Recent PCR studies showed that the mechanism of constructive resistance of *M. hominis* to erythromycin is associated with point mutation (G→A transposition) in site 2075 situated in the central loop of domain V of 23S rRNA [4]. A year later the same authors induced *in vitro* resistance of *M. hominis* to 16-membered macrolide josamycin [5], to which *M. hominis* is highly sensitive. The mechanism of resistance was linked with two point mutations (A2062G and A2062T) in domain V of 23S rRNA. Hence, macrolide resistance in *M. hominis* is caused by mutations in 23S rRNA gene. These studies are of particular interest, because there are no published data on the detection of such mutations in clinical samples of *M. hominis*.

We studied the incidence of mutations responsible for *M. hominis* resistance to 16-membered macrolide antibiotic josamycin in clinical samples.

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MATERIALS AND METHODS

Clinical strains of *M. hominis* were isolated from 48 patients with bacterial vaginosis (mean age 30±6 years). Scrapings from the urethra and/or cervical canal were collected during speculum examinations. The material was collected using disposable sterile urogenital tubes (DNC-med).

At the first stage of the study the agents were identified. DNA was isolated from biological material using DNA *Prep* 200 kit and protocol for DNA isolation from biological material (DIA*tom*™). PCR was carried out on a programmed MC2 thermocycler (DNK-Tekhnologiya) using *Termus aquaticus* DNA polymerase (Fermentas Institute of Applied Enzymology) and Biotaq DNA polymerase according to the following scheme: 0.1-1.0 μg genome DNA, 0.25 μM each oligoprimer, 250 μM each deoxynucleoside triphosphate were placed into 25 μl buffer for PCR (67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)SO_4$, 0.01% Tween-20), and 1.5 U thermophilic DNA polymerase and 20-30 μl mineral oil were added. The primers were selected at the Laboratory of DNA Diagnosis, Medical Genetics Center, and synthesized at Litekh Company; their sequences were as follows: F-TCTAGCAGAAGCTAGAGACTACGG and R-TACGTCCATTTCTACTAGTCCAACG.

At the second stage of the study mutations responsible for josamycin resistance were detected by allele-specific amplification. Primers homologous to

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the mutant sequence of *M. hominis* 23S RNA in position 2062 and to normal sequence (AF184237 and AF317663) [5] were synthesized. Amplification of wild and mutant type *M. hominis* 23S RNA fragments was carried out in two tubes using preserved reverse primer and specific direct primers:

FN-GACCCGCATCTAGACGAAAACA;
FM1-GAGACCCGCATCTAGACGAAAACG;
FM2-GAGACCCGCATCTAGACGAAAACT;
R-CACCTATCCTACACATGTTAAATC.

RESULTS

Mutant *M. hominis* strains were detected in 23 of 48 cases (48%). This incidence of josamycin-resistant *M. hominis* strains was appreciably lower than expected. According to some authors [1], sensitivity of clinical *M. hominis* strains to josamycin in culture is 94%. High sensitivity of *M. hominis* to josamycin *in vitro* was demonstrated in other studies [2,8].

These results can be interpreted from several viewpoints. First, point mutation in the *M. hominis* genome is not necessarily associated with antibiotic resistance phenotype (unambiguous data is available only for erythromycin [4]). According to the hypothesis proposed by P. M. Furneri *et al.* [5], *M. hominis* rRNA-operons are heterozygotic, and the mutant allele becomes dominant under the effect of antibiotic, which is paralleled by the development of phenotypical resistance. However, the mechanisms underlying appearance of resistant phenotype remain not quite clear. Second, it is possible that mutation in the A2062 site is caused by treatment with other 16-membered macrolides and streptogramin; cross-resistance to these drugs associated with A2062 mutation was described for *Streptococcus pneumoniae* [3]. Most patients with bacterial vaginosis in our study previously received oral or local antibiotic therapy. Moreover, we should remember that a sampling of 48 patients with bacterial vaginosis is small and the results can be different in a greater sampling.

Hence, point mutations associated with josamycin resistance are detected with high incidence (48%) in *M. hominis* strains associated with bacterial vaginosis. The relationship between these mutations and sensitivity to josamycin requires further studies.

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