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# Correlation between the Colony Phenotype and Amino Acid Sequence of the Variable Vaa Antigen in Clinical Isolates of *Mycoplasma hominis*

**M. A. Galyamina, K. V. Sikamov, D. R. Urazaeva, A. S. Avshalumov, M. V. Mikhaylycheva, O. V. Pobeguts, and A. Yu. Gorbachev**

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A new *Mycoplasma hominis* phenotype forming mini-colonies (MC) on agar and distinct from the phenotype forming typical colonies (TC) not only in size, but also in morphology, growth rate, and resistance to adverse factors, has been previously identified. In this study, the phenotype of colonies was determined and a comparative analysis of the amino acid sequence of the main variable antigen Vaa of the laboratory strain N-34 and seven clinical isolates of *M. hominis* was performed. It is demonstrated that the amino acid sequence of Vaa in clinical isolates forming TC (similar to the laboratory strain N-34) is entirely analogous to that of laboratory strain. Clinical isolates forming MC carry amino acid substitutions in the variable C-terminal region of Vaa, which can contribute to adhesion to eukaryotic cells and immune evasion. The connection between colony phenotype and amino acid sequence of Vaa is established.

**Key Words:** *Mycoplasma hominis; clinical isolate; variable antigen Vaa; colony phenotype; host organism adaptation*

*Mycoplasma hominis* is a human opportunistic pathogen capable of causing acute and chronic infections of the urogenital tract, meningitis and pneumonia in newborns through intrauterine fetal infection, as well as acute and chronic pyelonephritis [1-3]. It has been shown that mycoplasmas of this species can adhere to blood cells, thus spreading to organs and tissues, causing generalized mycoplasma infection [4]. Another characteristic of this bacterium is the prolonged persistence of the pathogen in the tissues of the infected organism [5]. It is hypothesized that *M. hominis* persisting in the human urogenital tract possesses oncogenic potential and contributes to the development of immortalization, migration, and invasion of tumor cells [6].

Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia. ***Address for correspondence:*** mrogova@gmail.com. M. A. Galyamina

*M. hominis* belongs to the class *Mollicutes* characterized by the absence of cell wall and reduced genome size (around 600 protein-coding genes). Despite genome reduction, it exhibits high adaptive potential allowing it to evade the host's immune system and cause chronic inflammation without apparent clinical symptoms. Adhesion and intracellular invasion are among its adaptive mechanisms [7,8]. The variable structure of surface membrane proteins-antigens P120, Lmp, and Vaa enabling immune evasion serves as another protective mechanism [9,10]. One of the key proteins involved in these two defense mechanisms is the lipoprotein Vaa. It is known that changes in the size and amino acid sequence of Vaa can affect adhesion of *M. hominis* and its ability to evade the immune response [11].

One of the research tasks was to conduct whole-genome sequencing and to compare the amino acid sequences of Vaa lipoprotein of the laboratory strain H-34 and seven clinical isolates of *M. hominis* ob-

tained from patients with urogenital infections. A new *M. hominis* phenotype forming mini colonies (MC) on agar, distinct from the phenotype forming typical colonies (TC) not only in size, but also in resistance to adverse factors, has been previously identified [10]. It is hypothesized that the MC phenotype is more adapted to living conditions in the host organism. The second task was to compare the colony phenotype of the laboratory strain H-34 and seven clinical isolates of *M. hominis*.

## MATERIALS AND METHODS

The laboratory strain *M. hominis* H-34 (MHOH34) was provided by Dr. K. H. Lemke (Lister Institute of Preventive Medicine, London, UK). Clinical isolates of *M. hominis* (MHO12, MHO7, MHO43, MHO11, MHO40, MHO45, and MHO1862) were obtained from biological material of patients with urogenital infections provided by FSBSI Research Institute of Obstetrics, Gynecology and Reproductology named after D. O. Ott, Ministry of Health of the Russian Federation. All isolates were cultured on Brain Heart Infusion (BHI) medium supplemented with 15% horse serum (Gibco, Thermo Fisher Scientific), 5% yeast extract, and 1% L-arginine in liquid medium and on agar dishes. The colonies were examined using a Letzlar light microscope.

Genomic DNA was extracted was performed using PureLink Genomic Mini Kit (Invitrogen) according to the manufacturer's instructions. For DNA library preparation for sequencing, the MGIEasy kit and the corresponding protocol (https://en.mgi-tech.com/products/reagents_info/8/) were used. Sequencing was carried out on the DNBSEQ-G400RS MGITECH sequencer. Genome assembly was performed using Unicycler Assembly software. Annotation of assembled genomes was done using Bakta tool. Multiple alignments of the amino acid sequences of the Vaa protein of the laboratory strain H-34 and clinical isolates were performed using Clustal W software. Phylogenetic analysis of the obtained alignments was conducted using Unipro UGENE 48.1 software. The Hamming distance matrix was obtained using code written in the R programming language.

## RESULTS

Figure 1 illustrates the phenotypes of colonies formed on agar by the laboratory strain MHOH34 and seven clinical isolates of *M. hominis*. In contrast to the laboratory strain that forms round colonies with a size ~200-400 μm, clinical isolates formed colonies of varying sizes that can be divided into two main groups: isolates forming typical *M. hominis* colonies (TC), similar in size and morphology to the laboratory strain (MHOH34, MHO12, and MHO7), and isolates forming mini-colonies (MC) not exceeding 50 μm (MHO43, MHO45, MHO40, MHO11, and MHO1862). Similar phenotypic differences in colonies are known for *M. hominis*: MC were first discovered in blood

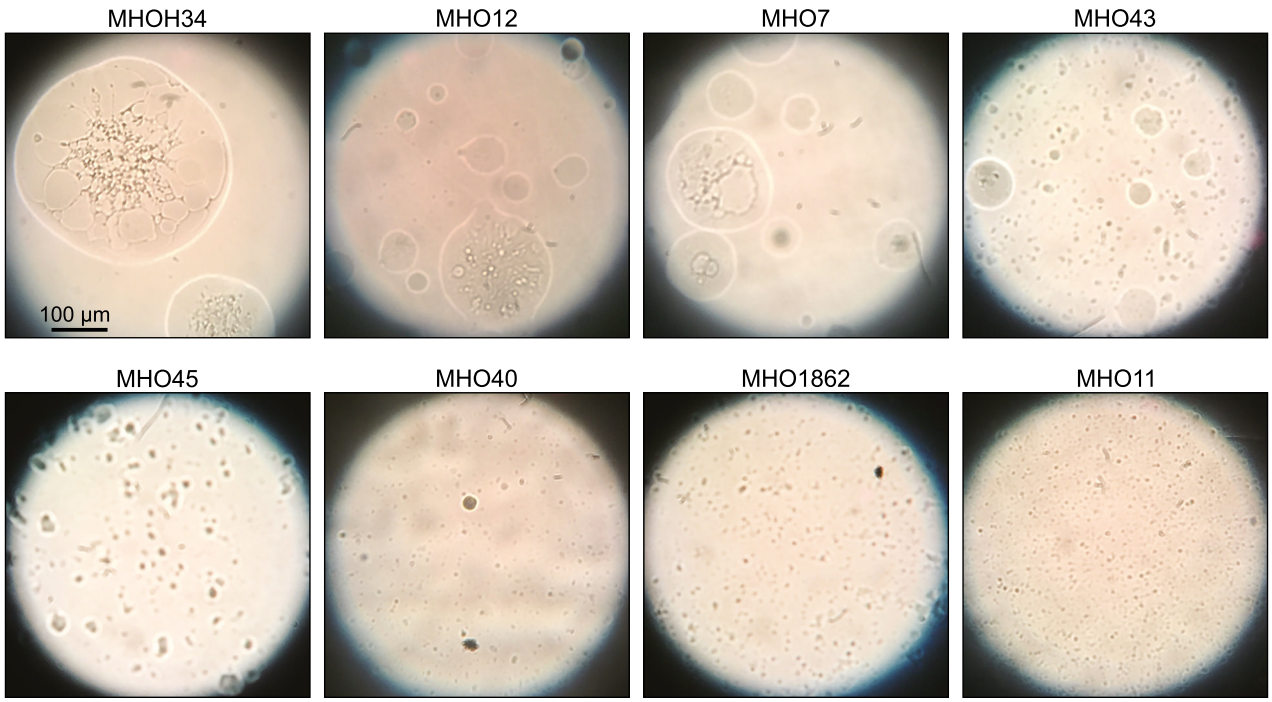


**Fig. 1.** Analysis of the morphology of colonies of laboratory strain H-34 (MHOH34) and clinical isolates MHO7, MHO12, MHO43, MHO45, MHO40, MHO1862, and MHO11 *M. hominis*. Light microscopy, ob. ×40.

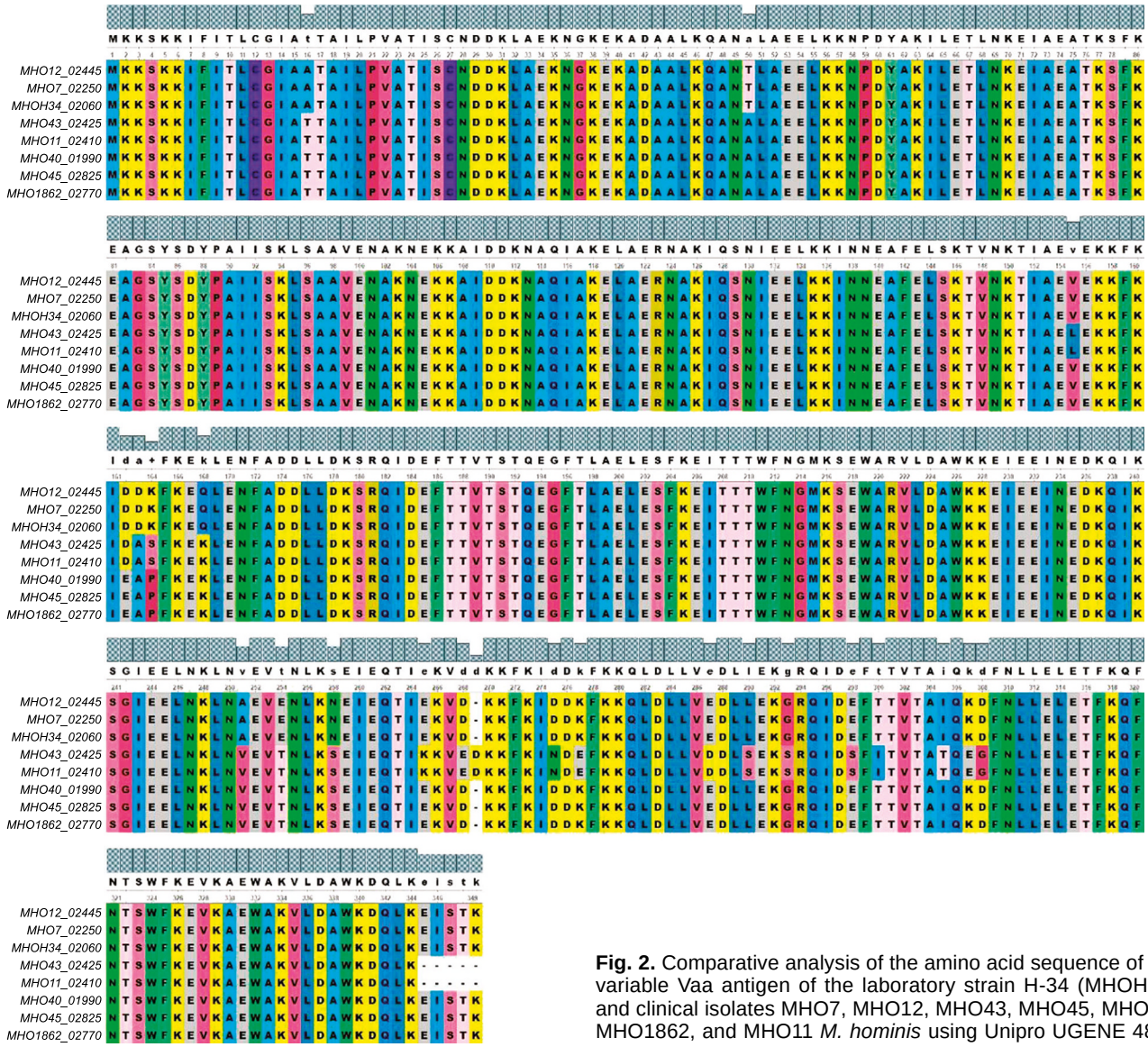


**Fig. 2.** Comparative analysis of the amino acid sequence of the variable Vaa antigen of the laboratory strain H-34 (MHOH34) and clinical isolates MHO7, MHO12, MHO43, MHO45, MHO40, MHO1862, and MHO11 *M. hominis* using Unipro UGENE 48.1.

serum agar cultures from patients with various inflammatory diseases [12]. MC differed not only in size, but also in exceptional resistance to adverse factors. This led to the assumption that they are adapted to life in the competitive and unfavorable environment within the host organism. It was also demonstrated that the formation of MC is associated with restructuring of the energetic metabolism, contributing to the development of a persistent phenotype [13].

The diversity of Vaa is determined by a varying number and composition of homologous variable cassettes located in the C-terminal part of the protein [12]. Each individual cassette has an average length of 110 amino acid residues (a.a.), and each cassette contains a helix motif. Cassette organization of Vaa is a result of combination of duplications and deletions of cassettes within a single *M. hominis* isolate and cassette recombination between isolates. All studied strains have a Vaa protein sequence size of 348 a.a., except for isolates MHO43 and MHO11 with a size of 344 a.a. All of them have two variable cassettes. The first 27 a.a. constitute a signal peptide, followed by a highly conservative module (105 a.a.) among *M. hominis*, and then a variable part consisting of variable cassettes. In the Vaa sequence, isolates MHO12 and MHO7 are identical to the laboratory strain MHOH34. The remaining isolates differ from it, but share some similarities. Similar Vaa sequences are found in isolates MHO43 and MHO11: they have 20 identical amino acid substitutions that distinguish them from MHOH34 (A16T, T50A, V273L, D281A, K282S, Q286K, A369V, E372T, N376S, E383K, D386E, D394N, K396E, E406D, L409S, E417S, T419I, I424T, K426E, and D427G). These substitutions are mostly located in the variable C-terminal part of the protein (Fig. 2). Isolates MHO40, MHO45, and MHO1862 also have identical

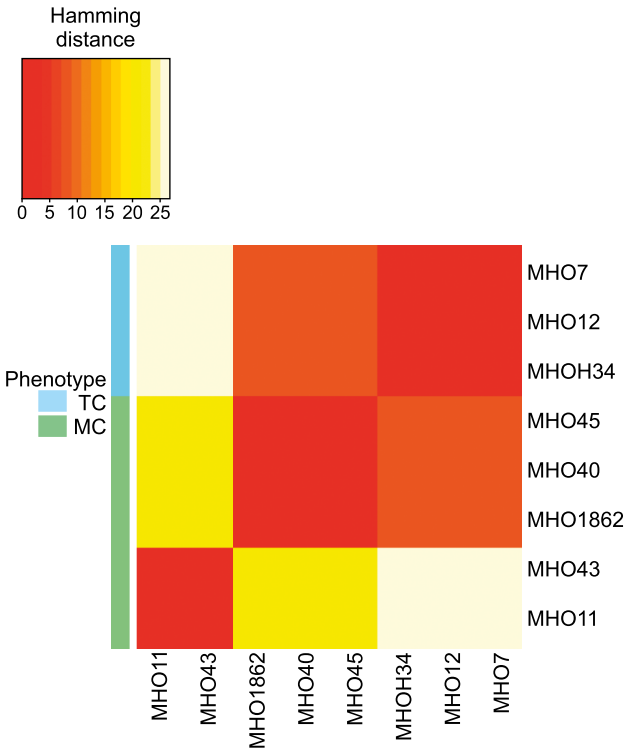


**Fig. 3.** Cluster analysis using the Hamming distance matrix taking into account amino acid substitutions in the sequence of the variable Vaa antigen of the laboratory strain H-34 (MHOH34) and clinical isolates MHO7, MHO12, MHO43, MHO45, MHO40, MHO1862, and MHO11 *M. hominis*.

amino acid substitutions compared to the laboratory strain: some common with MHO43 and MHO11 (A16T, T50A, D281A, Q286K, A369V, E372T, and N376S) and one unique (K282P).

It is known that the diversity of surface lipoproteins encoded by the *vaa* gene depends on the number of repeats located in the central part of the gene encoding the alpha-helix region, frame shift, as well as deletions or nucleotide substitutions in the variable C-terminal part of the gene [15,16]. Changes in the size and amino acid sequence of Vaa affect adhesion and the ability to resist the immune response [11]. We found that all isolates can be categorized into groups by the colony phenotype and amino acid sequence of Vaa (Fig. 3). The first group comprises isolates MHOH34, MHO12, and MHO7 forming TC and having an identical Vaa sequence. The second group consists of isolates MHO43, MHO45, MHO40, MHO11, and MHO1862 forming MC on agar and having amino acid substitutions in the Vaa sequence. Within the second group, isolates can also be further categorized based on Vaa sequence similarity – these are the subgroups of isolates MHO43, MHO11 and MHO45, MHO40, and MHO1862. Thus, the colony phenotype corresponds precisely to changes in the Vaa sequence: clinical isolates forming TC have amino acid sequence of Vaa similar to the laboratory strain MHOH34, while clinical isolates forming MC have amino acid substitutions in the variable C-terminal region of Vaa. We hypothesize that these substitutions are associated with adaptation to life within the host organism.

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**Conflict of interest.** The authors have no conflicts of interest to declare.

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