Ureaplasma gallorale, an Isolate from Chickens, Is Most Closely Related to the Human Isolate, *U. urealyticum*

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***Ureaplasma gallorale* is a urease-containing mycoplasma (a member of the *Mollicutes*) which is pathogenic for chickens, from which it was originally isolated. We amplified the 16S rRNA gene of this bacterium and then cloned and sequenced the amplicon. A phylogenetic analysis based on an alignment of the 16S rRNA sequences of *U. gallorale* and several other *Ureaplasma* species revealed that *U. gallorale* is more closely related to *Ureaplasma urealyticum* than to other *Ureaplasma* species.**

The members of the *Mollicutes* (trivial name, mycoplasmas) are eubacteria that lack cell walls. These minimal organisms have small genomes with low G+C contents and are considered phylogenetically related to the streptococcus-lactobacillus group of walled bacteria (8). Some members of the family *Mycoplasmataceae* contain the enzyme urease; these organisms are classified in the genus *Ureaplasma*. At this time, there are six named *Ureaplasma* species. The five *Ureaplasma* species which have been isolated from animals are *Ureaplasma urealyticum*, which was isolated from humans (7); *Ureaplasma diversum*, which was isolated from cattle (4); *Ureaplasma cati* and *Ureaplasma felinum*, which were isolated from cats (2); and *Ureaplasma canigenitalium*, which was isolated from dogs (3). The 16S rRNA sequences of the following two representatives of the two biovars of *U. urealyticum* have been published previously: strain T960 (GenBank accession number M23935) (8) and strain 27 (accession number L08642) (6). The sequences of the following species obtained from nonhuman animal hosts were recently deposited in the GenBank database by R. Harasawa: *U. diversum* (accession number D78650), *U. cati* (D78649), *U. felinum* (D78651) and *U. canigenitalium* (D78648). The sixth *Ureaplasma* species, *Ureaplasma gallorale*, was isolated from chickens (5), in which it is a significant pathogen. The 16S rRNA gene sequence of this organism was determined in this study.

We amplified most of the 16S rRNA gene of *U. gallorale* by using primers for conserved 5' and 3' regions of members of the *Mollicutes* (1) and incorporating the *Xho*I site in the 5' primer and the *Pst*I site in the 3' primer. After appropriate digestion the amplified ribosomal DNA was ligated to *Xho*I- and *Xba*I-digested pBlueScript-SK (Stratagene, La Jolla, Calif.) and selected on the basis of ampicillin resistance and plasmid size (6). The sequence of the insertion was determined by a combination of manual sequencing (double-stranded sequencing, using dideoxy terminators [Pharmacia, Uppsala, Sweden]) and automatic cycle sequencing (Applied Biosystems, Foster City, Calif.); both strands were sequenced. The 1,515-bp sequence between the primers which we determined represents about 95% of the total 16S rRNA. Using 1,200 nucleotides (a limitation of the computer program) of the *Ureaplasma* sequences and the *Mycoplasma pneumoniae* se-

quence (GenBank accession number M29061), we obtained a multiple alignment with the Clustal computer program and prepared a dendrogram (Fig. 1). The 16S rRNA sequences of all six *Ureaplasma* species exhibited high levels of homology (>94%) and thus are closely related.

The *Ureaplasma* species isolated from single host species (the biovar representatives *U. urealyticum* T960 and *U. urealyticum* 27 isolated from humans and *U. cati* and *U. felinum* isolated from felines) were phylogenetically related pairs. However, beyond these pairs, there was no correlation between strain and host relatedness. *U. gallorale*, the species that is most closely related to *U. urealyticum*, is found in poultry, the host that is the greatest evolutionary distance from humans. Colonization of hosts by mollicutes appears to have occurred independently more than once and not to have closely followed host evolution.

The very large database of 16S rRNA sequences allows rapid access to phylogenetic relationships. However, relationships based entirely on 16S rRNA sequences should be considered tentative until other conserved genes, such as those involved in DNA replication or the signature urease complex, are examined. In the meantime, it may be rewarding to seek ureaplasmas from more varied hosts, especially nondomesticated animals and birds.

FIG. 1. Dendrogram showing the relationships among the named species of the genus *Ureaplasma*, including the two biovars of *U. urealyticum*. Abbreviations: UU3, *U. urealyticum* 27; UREA, *U. urealyticum* T960; UG, *U. gallorale*; DIVERSUM, *U. diversum*; CATI, *U. cati*; FELINE, *U. felinum*; UDOG, *U. canigenitalium*; MPNEUMO, *M. pneumoniae*.

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Nucleotide sequence accession number. The nucleotide sequence of the 16S rRNA of *U. gallorale* determined in this study has been deposited in the GenBank database under accession number U62937.

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ADDENDUM IN PROOF

Since the submission of this manuscript, Harasawa and Cassell (1a) have shown the relatedness of the 16S rRNA genes of the five named *Ureaplasma* species from mammalian hosts. They were unable to amplify the rRNA gene for *U. gallorale* and hypothesized that it was genetically distant from *Ureaplasma* species from mammals.

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