Development of a real-time PCR targeting the *yid*C gene for the detection of *Mycoplasma hominis* and comparison with quantitative culture

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Abstract

Mycoplasma hominis is an opportunistic human mycoplasma species that can be either commensal or pathogenic. Its detection by culture is considered to comprise the reference technique. Previously reported PCR techniques target the 16S rRNA or the *gap* gene, although sequence variations among clinical isolates may lead to variations in clinical sensitivity. The present study aimed to develop a specific TaqMan quantitative real-time PCR assay, targeting a gene conserved in all *M. hominis* isolates, and to compare it with quantitative culture. With the knowledge of the *M. hominis* PG21 genome sequence, the *yid*C gene, encoding a membrane protein translocase, was chosen as target. Its intraspecies heterogeneity was checked at the nucleotide level using 31 reference or clinical strains. The limit of detection, the analytical specificity and the reproducibility of the assay were assessed. Moreover, PCR and culture results were compared using 153 urogenital specimens. The limit of detection was seven copies/μL. The analytical specificity was 100%, with good inter- and intra-assay reproducibility. Among the 153 urogenital specimens, the *yid*C PCR and culture allowed detection of 55 and 45 *M. hominis*-positive samples, respectively. Comparison of the bacterial load among the 45 specimens found to be *M. hominis*-positive by both techniques revealed a statistically significant association between the quantitative results obtained. In conclusion, we developed a specific, sensitive and reproducible real-time PCR to detect all *M. hominis* clinical isolates. This PCR was shown to have higher sensitivity than culture, although both methods were correlated for quantification of *M. hominis* loads in urogenital specimens.

Keywords: Culture, detection, *Mycoplasma hominis*, quantitative real-time PCR, urogenital specimens, *yidC*

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Introduction

Mycoplasma hominis is an opportunistic human mycoplasma species that resides, as a commensal, in the lower urogenital tract. It can also be a causal agent in pelvic inflammatory disease and infections during pregnancy, and it has been associated with bacterial vaginosis [1]. In newborns, it can cause pneumonia, meningitis or abscesses [2]. It has also been involved in extragenital infections, especially in immunocompromised patients [1]. Direct detection techniques are the only methods adequate for genital mycoplasmas. Although culture is considered to be the reference technique for the detection of *M. hominis*, it requires specialized media and expertise and does not yield results before 2–5 days [3]. Most PCR techniques detecting *M. hominis* target the 16S rRNA gene or the 16S-23S intergenic spacer region by conventional or real-time assays [4–9]. However, minor sequence variations were observed in the 16S rRNA gene sequences [10] and may lead to a lower clinical sensitivity of these techniques. A quantitative real-time PCR using hybridization probes and targeting the glyceraldehyde-3-phosphate dehydrogenase gene (*gap*) was reported [11]. Variation in the *gap* gene was also described and the extent of variation was found to be larger than in the 16S rRNA gene [11,12]. Recently, a real-time PCR assay targeting the *fst*Y gene of *M. hominis* was reported, although the intraspecies heterogeneity within this gene was not assessed [13].

The present study aimed to develop a TaqMan quantitative real-time PCR assay, specific for the *M. hominis* species,

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targeting a gene conserved in all *M. hominis* isolates. The *yid*C gene was selected as a target, and the specificity, limit of detection and reproducibility of the assay were evaluated. In addition, the *M. hominis* detection by quantitative culture and by our real-time PCR assay was compared using 153 clinical urogenital specimens.

Materials and Methods

Organisms and specimens

The organisms used in the present study are listed in Table 1. Among mollicutes, all human species with a potential pathogenic role and some human and animal species phylogenetically close to *M. hominis* were tested. All isolates of mycoplasma or ureaplasma species were grown in Hayflick modified medium or Shepard medium, according to the species [3].

TABLE 1. Microorganisms used to assess the specificity of the real-time PCR assay

Mycoplasma and ureaplasma strains
Mycoplasma agalactiae
Mycoplasma amphoriforme
Mycoplasma arginini
Mycoplasma arthritidis
Mycoplasma fermentans
Mycoplasma genitalium
Mycoplasma orale
Mycoplasma penetrans
Mycoplasma pneumoniae
Mycoplasma pulmonis
Mycoplasma salivarium
Ureaplasma urealyticum
Ureaplasma parvum
Other bacterial or fungal strains
Acinetobacter baumannii
Candida albicans
Chlamydia trachomatis
Clostridium perfringens
Corynebacterium striatum
Enterobacter aerogenes
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Gardnerella vaginalis
Haemophilus influenzae
Haemophilus parainfluenzae
Klebsiella oxytoca
Lactobacillus acidophilus
Moraxella catarrhalis
Morganella morganii
Neisseria perflava
Neisseria gonorrhoeae
Prevotella bivia
Pseudomonas aeruginosa
Pseudomonas putida
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus xylosus
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus constellatus
Streptococcus bovis
Streptococcus oralis
Streptococcus pneumoniae

A total of 153 urogenital samples, collected between September 2007 and March 2008 and conserved frozen in 2 mL of 2SP transport medium [3], were retrospectively selected from the Department of Bacteriology, University Hospital of Bordeaux (France). They included 110 cervico-vaginal swabs, 20 peritoneal liquids, 15 urethral swabs, five anal swabs, two endometrial specimens and one of the fallopian tube. Among them, 45 were positive by a quantitative culture technique in Hayflick modified broth medium supplemented with arginine [3]. The bacterial load was measured in colour-changing units (CCU) per mL. DNA was extracted from 190 μL of clinical specimens or bacterial cultures supplemented with 10 μL of internal control (see below) using the MagNA Pure LC DNA isolation kit I (Roche, Meylan, France) in accordance with the manufacturer's instructions.

Taqman assay

The *M. hominis* PG21 genome [14] was compared with other available sequenced mycoplasma genomes using a bidirectional best hit method from the multiproteome differential query tool of the MolliGen 2.0 database (http://www.cbib.u-bordeaux2.fr/outils/molligen/home.php). Primers and a probe were designed for a fully-conserved gene fragment of the *yid*C gene (MHO_0010, Genbank FP236530) using the PRIMER EXPRESS software (Applied Biosystems, Foster City, CA, USA), and their specificity was checked by BLAST analysis.

Our assay, targeting a 94-bp fragment of the *M. hominis yid*C gene, was performed using primers MHyidCfwd (5′-TCACTAAACCGGGTATTTTCTAACAA-3′, nucleotides (nt) 1642–1667) and MHyidCrev (5′-TTGGCATATATTGCGATAGTGCTT-3′, nt 1735–1712), and the TaqMan probe MHyidC (5′-FAM-CTACCAATAATTTTAATATCTGTCGGTATG-BHQ1-3′, nt 1681–1710). To monitor the efficiency of DNA extraction and PCR inhibition, a DNA extraction and inhibition internal control (IC) labelled with the Cy5 dye was used (Diagenode, Liège, Belgium). The PCR reaction mixture consisted of 10 μL of 2× LightCycler 480 Probes Master (Roche), 0.6 μL of each primer (10 μM), 0.8 μL of the probe (5 μM), 2 μL of IC primers/probe (Diagenode) and 1 μL of template DNA including the extracted IC DNA. Amplifications were performed using a LightCycler 480 thermocycler (Roche) under the conditions: initial denaturation at 95°C for 10 min, followed by 45 cycles of 10 s at 95°C and 1 min at 60°C. Each sample was tested in duplicate and negative results were validated if the internal control gave a fluorescent signal.

Specificity, limit of detection and reproducibility

To determine the analytical specificity of the assays, DNA extracts from 11 mycoplasma and two ureaplasma species

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