MgpB Types among Mycoplasma genitalium Strains from Men Who Have Sex with Men in Berlin, Germany, 2016–2018

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Abstract: Mycoplasma genitalium is a cell wall-less bacterium causing urethritis and other sexually transmitted diseases. Despite a strongly conserved genome, strains in clinical samples can be typed by different methods. To obtain data from the risk population of men having sex with men, we analyzed the typing region in the gene coding for the MgpB adhesin of M. genitalium first in 163 and 45 follow-up samples among patients of two specialized practices in Berlin, Germany. Strains belong to 43 different mgpB types emphasizing the diversity of the genome region. With respect to 133 types previously described, 27 new types were found. However, the majority of strains (64.4%) were assigned to types 4, 6, 113, and 108, respectively. A correlation between mgpB type and the occurrence of mutations associated with macrolide and quinolone resistance was not demonstrated. Investigation of follow-up samples from 35 patients confirmed the same mgpB and, additionally, MG_309 types in 25 cases. In 10 cases, differences between types in subsequent samples indicated an infection with a genetically different strain in the period between samplings. MgpB/MG_309 typing is a useful method to compare M. genitalium strains in samples of individual patients as well as those circulating in different populations.

Keywords: sexually transmitted infection; Mycoplasma genitalium; genotyping; MgPa; antimicrobial resistance

1. Introduction

Members of the Mollicutes class are bacteria that lack the classical cell wall. Among them, some species are important for human health because they colonize mucosal surfaces and cause long-lasting, common but mostly self-limiting infections. The extremely slow-growing species Mycoplasma genitalium is a sexually transmitted pathogen that causes urethritis in men and is associated with cervicitis and pelvic inflammatory disease in women [1,2]. The prevalence of M. genitalium in the general population ranges between 1% and 4% [3] but is found more frequently (up to 40%) in risk populations, such as men who have sex with men (MSM), urethritis, and HIV-positive patients, respectively. Eradication of M. genitalium is hampered by many asymptomatic cases and problems in treating confirmed infections. Besides the intrinsic resistance to all beta-lactams, the use of doxycycline is of limited clinical efficacy [4]. Thus, current guidelines recommend macrolides (azithromycin) as first choice antibiotics followed by quinolones (moxifloxacin) in cases of therapy.
failure [5]. Due to the rising occurrence of strains with resistance-associated mutations in 23S rRNA (macrolides) and the parC gene (quinolones) worldwide, as well as the lack of effective and approved therapeutic alternatives [6], further epidemiological information about the mechanisms of resistance development and possible correlations between resistance and genotypes of M. genitalium is needed.

Whole genome data of a limited number of M. genitalium strains showed a high level of recombination in particular regions and low overall nucleotide divergence between genomes [7]. Furthermore, previous studies investigated the usefulness of easy-to-use and comparable approaches to differentiate M. genitalium strains directly from PCR-positive clinical samples. These included the analysis of one of the variable regions of the gene coding for the MgpB adhesin (MG_191) of the microorganisms and of the variable number of tandem repeats (VNTR) in different regions [8,9]. MgpB typing is the most frequently used approach showing a high discrimination power that has led to a relatively great number of more than 100 types characterized to date [10], enabling comparisons of strains occurring in different populations or at different locations. Combination of mgpB typing and VNTR in gene MG_309 was described as useful for the investigation of sexual networks [8,9].

In the present study, we analyzed the mgpB types of first and follow-up samples of M. genitalium-positive patients in Berlin, Germany, to get an overview of circulating strains in a risk population characterized by MSM and HIV-positives in a metropolitan area. Information about the occurrence of mutations in 23S rRNA and the parC gene [11] allows the classification of resistance and types. The results confirmed a great spectrum of mgpB types. In addition, mgpB/MG_309 typing is a useful method for comparing first and follow-up samples of patients to distinguish between ongoing colonization with a strain of identical mgpB/MG_309 profile and probable new infection with a different strain.

2. Results

The mgpB type of M. genitalium strains was identified in 163 first samples. In Figure 1A, the similarity of sequences of samples obtained from both practices (practice S: n = 43, practice G: n = 120) is summarized in dendrograms. Overall, the occurrence of 43 different mgpB types was confirmed, resulting in a discriminatory index of 0.827. Most of the strains (64.4%) belong to four types: 4 (38.6%), 6 (11.0%), 113 (8.6%), and 108 (6.1%), respectively (Figure 1B). Only one strain could be assigned to the predominant number of types (n = 29, 67%). Strains showing mutations associated with macrolide or quinolone resistance were detected in 70% and 30% of types, respectively. However, regarding the four most common mgpB types, the rates of resistant strains are different (type 4: 96.8% macrolide and 1.6% quinolone resistance; type 6: 72.2 and 11.1%; type 113: 76.9 and 15.4%; type 108: 90.0 and 30.0%, respectively). In comparison with the 133 mgpB types described to date (Supplementary Table S1), 27 new sequences were found. The derived amino acid sequences of the part of the MgpB adhesin (aa 78 to 140) demonstrated 36 differences from the sequence of reference strain G37 (Supplementary Figure S1). For two types (133 and 160), the insertion of two amino acids was confirmed. In comparison to G37, 73% of differences are limited to four amino acids: Ser107 (42 of 43 types detected in the present study), Ser101 (33/43), Asp96 (20/43), and Ala117 (15/43), respectively. Some mgpB types showed differences of nucleotide sequences from all other types but are identical with respect to the amino acid sequence (type 3 = types 7, 113, 143; type 4 = 62; type 6 = 153; type 15 = 111, 137, 141).
Figure 1. Similarity and distribution of partial mgpB sequences of 163 Mycoplasma genitalium strains. (A) Dendrograms of sequences from samples from practice S and G based on alignment by CLUSTALW (M—strain with macrolide resistance-associated mutation, F—strain with fluoroquinolone resistance-associated mutation). Bar—nucleotide substitution per 100 residues. (B) Percentage distribution of the 43 mgpB types confirmed in the study (underlined: new types in comparison with types described in Supplementary Table S1).
The mgpB types in first and follow-up specimens were compared in 35 patients (Table 1). In seven men, two or more follow-up samples were analyzed. The same mgpB type in first and follow-up sample was confirmed in 25 patients (71.4%). The time between samplings varied from 8 (patient no. 34) up to 392 days (patient no. 25). In contrast, specimens from 10 patients (time between samplings: 7 to 324 days) showed different types. Interestingly, among these follow-up samples, further new types were not found. Using VNTR typing of MG_309 gene as an additional approach for discrimination, the change of mgpB type in 8 of 10 cases was combined with different VNTR types of M. genitalium in the first and follow-up specimens. Changes of mgpB types in two patients (no. 18 and 28) were not associated with alterations of MG_309 types. Of note, the emergence of mutations associated with quinolone resistance occurred exclusively in follow-up samples that differed in both mgpB and VNTR types. Furthermore, a change in macrolide resistance-associated mutations (A to G or A to G/T at position 2071 or 2072 of 23S rRNA) or the occurrence of an SNP of 23S rRNA further suggests an infection of these patients with genetically different strains during the time between samplings. In contrast, mutations of 23S rRNA were detected in follow-up strains of two patients (no. 12 and 30, respectively) without a change in their mgpB and VNTR type, supporting the hypothesis that resistance develops during treatment with azithromycin. Among the remaining 33 cases, strains without macrolide resistance-associated mutations in first and follow-up specimens of two patients were demonstrated. In one patient (no. 19), the mgpB/MG_309 profile of the strain in both samples was identical, indicating failure of azithromycin treatment. Typing in a second patient (no. 26) resulted in different mgpB and MG_309 types, making a re-infection with a macrolide-susceptible strain probable. All other patients (89%) carried macrolide-resistant strains in first and follow-up specimens.
Table 1. Comparison of mgpB and MG_309 genotypes of M. genitalium in first and follow-up samples from the same patient (grey highlighted: difference between mgpB and/or MG_309 types).

| Patient no. | Samples     | Time between samples (d) | MgpB type | MG_309 type | Profile (mgpB+MG_309) | Comments |
|-------------|-------------|---------------------------|-----------|-------------|-----------------------|----------|
| 1           | S7/S17      | 29                        | 4         | 9/9         | 4-9                   |          |
| 2           | S10/S27     | 41                        | 134       | 10/10       | 134-10                |          |
| 3           | S11/S23     | 30                        | 108       | n.d.        | -                     |          |
| 4           | S12/S25     | 27                        | 12        | n.d.        | -                     |          |
| 5           | S36/S48     | 23                        | 108       | 10/10       | 108-10                |          |
| 6           | S45/S56     | 35                        | 108       | 10/10       | 108-10                |          |
| 7           | G1/G14      | 27                        | 4         | 12/12       | 4-12                  |          |
| 8           | G4/G32      | 48                        | 4         | 10/10       | 4-10                  |          |
| 9           | G5/G28      | 43                        | 145       | 11/11       | 145-11                |          |
| 10          | G8/G46      | 81                        | 113       | 9/9         | 113-9                 |          |
| 11          | G9/G60      | 85                        | 4         | 12/12       | 4-12                  |          |
| 12          | G11/G35     | 27                        | 7         | 11/11       | 7-11                  | development of MRAM |
| 13          | G12/G42     | 42                        | 4         | 8/8         | 4-8                   |          |
| 14          | G17/G40/G68 | 30/46                     | 133       | 10/10/11    | 133-10/137-11         |          |
| 15          | G18/G44     | 36                        | 4         | 10/10       | 4-10                  |          |
| 16          | G20/G30     | 12                        | 4         | 10/10       | 4-10                  |          |
| 17          | G22/G48/G71 | 49/56                     | 138       | 12/12/12    | 138-12                |          |
| 18          | G25/G55     | 53                        | 6/136     | 11/11       | 6-11/136-11           | difference in MRAM, emergence of QRAM |
| 19          | G43/G58     | 50                        | 136       | 13/13       | 136-13                |          |
| 20          | G56/G93     | 166                       | 4/136     | 9/13        | 4-9/136-13            | difference in MRAM, emergence of QRAM |
| 21          | G70/G164    | 324                       | 151/7     | 11/10       | 151-11/7-10           | difference in MRAM |
| 22          | G80/G111/G122 | 154/24              | 4/134/134 | 9/10/10    | 4-9/134-10            | SNP A2200G of 23S rRNA between G80 and G111 |
| 23          | G81/G98     | 97                        | 4         | 11/11       | 4-11                  |          |
| 24          | G86/G107    | 96                        | 152/4     | 11/10       | 152-11/4-10           | difference in MRAM |
| 25          | G91/G114/G128/ G168/G204 | 91/64/87/150 | 108       | 10/10/10/10 | 108-10 |          |
| 26          | G99/G104    | 14                        | 108/113   | 9/11        | 108-9/113-11          |          |
| 27          | G101/G105   | 7                         | 6/108     | 11/10       | 6-11/108-10           |          |
| 28          | G106/G113   | 36                        | 137/4     | 10/10       | 137-10/4-10           |          |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 29 | G121/G124 | 13 | 113 | 14/14 | 113-14 |
| 30 | G115/G127 | 35 | 113 | 11/11 | 113-11 | development of MRAM |
| 31 | G130/G132/G156 | 11/63 | 6/6/7 | 11/11/9 | 6-11/7-9 | difference in MRAM, emergence of QRAM |
| 32 | G131/G140 | 21 | 159 | 9/9 | 159-9 |
| 33 | G147/G166/G174 | 39/59 | 4 | 9/9/9 | 4-9 |
| 34 | G161/G163 | 8 | 6 | 11/11 | 6-11 |
| 35 | G154/G169/ | 48/91/ | 2 | 9/9/9/9 | 2-9 |
|   | G186/G202 | 50 |   |   |   |

1—Description according to supplementary Table S1; 2—number of tandem repeats in MG_309; 3—not determined (sequencing results not evaluable); 4—MRAM: macrolide resistance-associated mutation.; 5—QRAM: quinolone resistance-associated mutation.
3. Discussion

In this retrospective study, the mgpB types of *M. genitalium* strains from outpatients in Berlin, Germany, were determined. The data give a detailed picture of circulating types in a single location and during a relatively short time period of sixteen months. Furthermore, most of the patients belong to the group of MSM and/or HIV-positives considered as risk populations for infections with this pathogen. In recent studies, the prevalence of *M. genitalium* in both groups ranged between 3% and 17% [3,12–15]. To our knowledge, this is the first study determining mgpB types among patients strongly predominated by MSM. With respect to the 133 types characterized in previous studies (Supplementary Table S1), we confirmed the occurrence of 16 known and 27 new types among 163 patients. Including these new types detected in the present report, a total of 160 mgpB types have been described to date, confirming the extent of possible nucleotide changes and insertions in the relatively small part of the mgpB gene of around 200 bases. The discriminatory index of the method was calculated to be 0.85 [9], 0.94 [8], and 0.95 [10,16], respectively, and mgpB typing was recommended for general studies of *M. genitalium* epidemiology. In previous reports, mgpB types were found to be identical in many of the investigated follow-up samples collected over long time periods from the same patient [8,9,16–18] and to be stable after a cultural passage of a limited number of isolates [9].

Despite the great diversity of mgpB types among the *M. genitalium* strains of the present study, around 50% of all samples belong to two types (four and six, respectively). Both types were first described after investigation of strains in a collection of *M. genitalium*-positive samples from different countries [16]. Remarkably, type four was also found in 32% of samples investigated in a small German study that characterized *M. genitalium* strains between 2015 and 2016 [19]. Belonging of many strains to few mgpB types might be caused by relations between patients, like sexual networks, explaining the occurrence of clusters. This suggestion is supported by the relatively low discriminatory index of 0.83 calculated in the present study. It is speculative to decide whether this dominance of particular types is the result of the more probable transmission of frequently occurring strains or of the more virulent properties of some strains. Consistent with the sequence-variable P1 protein of the phylogenetically related species *Mycoplasma pneumoniae* that causes community-acquired infections of the human respiratory tract, the MgpB adhesin of *M. genitalium* can be considered as important for host-pathogen interaction [20]. Great parts of this membrane protein were found as cell surface-exposed, but the near N-terminal region, including the typing sequence, showed no reaction with sera of infected animals, and antibodies to this protein part had no influence on hemadsorption [21]. The occurrence of identical mgpB types over long time periods in many follow-up samples of our study and other reports suggests that the host immune system has limited influence on changes in the MgpB typing region during colonization.

Interestingly, the *M. genitalium* strains in samples from the four women included in this report belong to different mgpB types (4, 8, 31, and 74, respectively). Because of the low number of women, further studies will have to clarify whether significant differences of mgpB types between both gender and between homo- and heterosexual patients in a defined region can be demonstrated.

Here, the typing of *M. genitalium* was performed among samples demonstrating relatively high resistance rates of 79.9% (macrolides) and 13.0% (quinolones), respectively [11]. A clear correlation between distinct mgpB types and the occurrence of resistance-associated mutations was not found. Macrolide- and quinolone-resistant strains were confirmed in 30 and 13 of the 43 types determined. Despite the fact that knowledge about the genotype of a clinically relevant strain has had no consequences for therapy up to now, the relations between resistance and typing markers are of epidemiological interest. Regarding the related species *M. pneumoniae*, results of different studies indicated a relationship between the occurrence of macrolide resistance as well as the clinical severity of infections with particular sequence or VNTR type strains [22–24].

Combination of mgpB and MG_309 typing is suitable to discriminate *M. genitalium* strains colonizing different patients as well as strains in follow-up samples from the same patient [8,9]. In this context, further data about differences of types are important in investigating whether the emergence of resistance-associated mutations is caused by the development of resistance during treatment or by the acquisition of a genetically different strain in the period between samplings [10].
Here, the mgpB types of 71% of strains in follow-up samples correspond to the type in the first sample. As expected, differences of type between first and follow-up sample(s) seem to not depend on the time between samplings, as the type remained unchanged in many patients, confirming the long-term stability of this typing marker, as already reported in other studies [8,9,16–18]. In contrast, the mgpB type of M. genitalium in the follow-up specimens from 10 of 35 patients differed in comparison with previous sample(s). This rate is relatively high in comparison with previous investigations, which analyzed mainly strains from heterosexual patients [8,17]. Sexual behaviour (number of contact persons, condom use, participation in sexual networks) can be assumed as important for these differences and should be further investigated in controlled studies. The change of mgpB type in the follow-up samples of the present report comes along with differences in the number of VNTR in MG_309, supporting the hypothesis of newly acquired infections in these patients with genetically different strains. The occurrence of mutations associated with quinolone resistance in the follow-up samples of three patients was linked with a change of mgpB type and a different number of repeats in MG_309. Importantly, quinolones were not prescribed between sampling. Conversely, the development of macrolide resistance in the follow-up specimens of two patients was combined with an identical mgpB/MG_309 profile, supporting the emergence of resistance during therapy with azithromycin [10,11]. It should be noted that the colonization of a patient with strains showing different mgpB types cannot be excluded. In the present study, two locations (urethra and rectum) of four patients were sampled simultaneously. In one case, the mgpB types differed (data not shown), which complicates the evaluation of the results of typing and may explain differences between samples to some extent.

In conclusion, the results of the present study confirm a great diversity of mgpB types among MSM in Berlin, Germany. A clear correlation of the type with macrolide and quinolone resistance-associated mutations was not found. However, mgpB typing is useful for monitoring the circulation of strains among different populations, as well as in combination with VNTR characterization in MG_309, to compare first and follow-up samples from patients. Thus, typing of M. genitalium strains is a valuable and reliable tool for studies to further understand the epidemiology, treatment failures, and development of antimicrobial resistance of an emerging sexually transmitted pathogen.

4. Material and Methods

Primarily, to test the prevalence of macrolide and quinolone resistance [11], samples investigated in this study were collected in Berlin, Germany, between September 2017 and December 2018 in two practices (designated as S and G) specialized in the treatment of STIs. The included patients are predominately MSM with a relatively high portion of HIV-positives (Table 2). The retrospective study was approved by the Institutional Review Board of the TU Dresden (no.: EK 473122017).
Table 2. Characteristics of M. genitalium-positive patients (n = 163) and specimens included in the study.

| Variable                  | Value                          |
|---------------------------|-------------------------------|
| Mean age (years; range)   | 36.1 (20–61)                  |
| Males (%)                 | 97.5                          |
| MSM (%)                   | 92.6                          |
| HIV-positive (%)          | 46.6                          |
| First specimens           |                               |
| Rectal swabs (%)          | 57.7                          |
| First-void urine (%)      | 38.6                          |
| Vaginal swabs (%)         | 2.4                           |
| Urethral swab (%)         | 0.6                           |
| Pharyngeal swab (%)       | 0.6                           |
| Follow-up specimens (n = 45) |                            |
| Number of patients (% of all patients) | 35 (21.5)                  |
| Rectal swabs (% of follow-up specimens) | 30 (66.7)            |
| First-void urine (%)      | 14 (31.1)                     |
| Urethral swab (%)         | 1 (2.2)                       |

As described recently [11], the DNA of the samples was extracted by the EZ1 DNA tissue kit and EZ1 Advanced XL (Qiagen) automated extraction system and tested for M. genitalium by real-time PCR using a commercial assay (Anyplex™ STI-5 Detection Assay; Seegene). DNA of positive samples was stored at −20 °C until the determination of genotypes. Overall, 163 M. genitalium-positive first specimens from 163 different patients were included in the present study. In addition, 45 follow-up samples of 35 different patients were investigated. All samples of the study were pretreated regarding the occurrence of resistance-associated mutations in the 23S rRNA (macrolides) and parC gene (quinolones) of M. genitalium [11].

MgpB types were determined after amplification of the typing region (nt 180–460) of MG_191 gene by a nested PCR approach (35 cycles each; Eppendorf personal cycler) and sequencing as described [19]. PCR products were purified using the MSB Spin PCRapace kit (Stratec). Nucleotide and derived amino acid sequences were compared with the corresponding part of the reference genome of type strain G37 (GenBank accession no. NC_000908.2) and with the 133 mgpB types published to date (Supplementary Table S1). The discriminatory index of mgpB typing was calculated as reported in [25]. Phylogenetic tree prediction was performed by using CLUSTALW (DNASTAR lasergene).

To further discriminate first and follow-up samples, the variable number of tandem repeats in the MG_309 gene of M. genitalium was identified as reported in [19].

The sequences of 27 new mgpB types described in this study were deposited in GenBank (accession numbers: MN387712-MN387714, MN387716-MN387739).

Supplementary Materials: The following are available online at www.mdpi.com.xxx/s1, Table S1: Designation of types of M. genitalium strains based on mgpB typing. Sequences described in supplemental material of the publications (1) or deposited in GenBank (2–11), as well as the types found in the present study, were aligned with MegAlign (DNASTAR lasergene), Figure S1: Alignment of partial MgpB amino acid sequences of type strain G37 and of the 43 mgpB types detected in the present study. Positions according to the sequence of G37. For designation of types see supplementary Table S1.

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