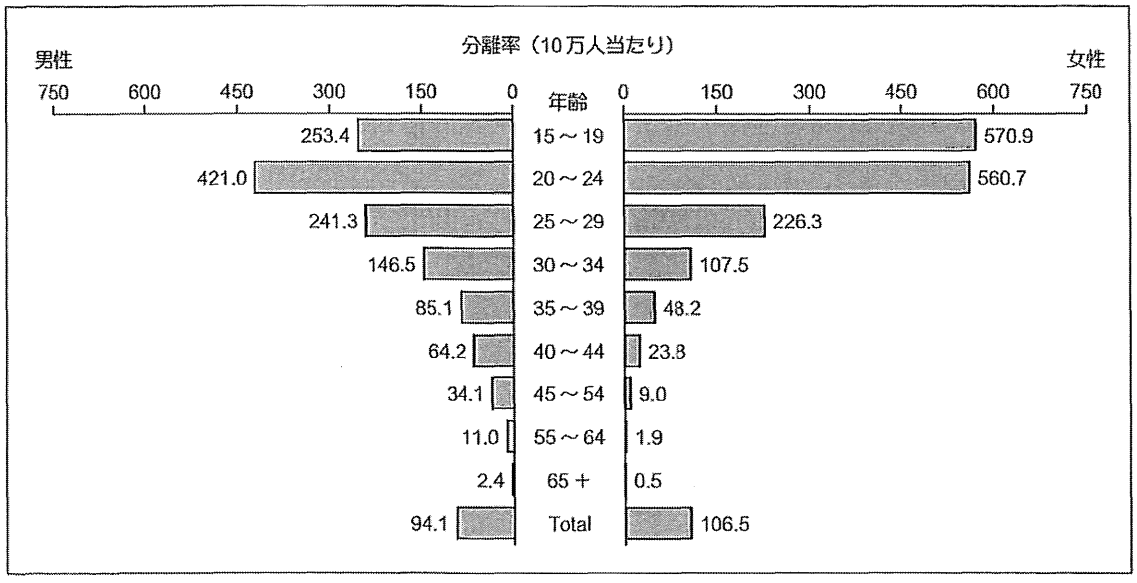

図1　2010年，米国における年齢・性別の淋菌感染症の発生（10万人当たり）
米国における淋菌感染症は若い女性に多く，わが国の報告と異なる。

（文献4より）

位に観察され，女性からの淋菌の分離率は低い[1)~3)]。しかし，CDC（米国疾病予防管理センター）における近年の統計では，淋菌もクラミジアと同じように若い女性から高い頻度で分離されることが報告された（図1）[4)]。

わが国では女性のSTI，特に子宮頸管炎ではクラミジアがおもな病原体であると考えられている。しかし，実際には淋菌に対する検査を行っていない例も多く，女性の淋菌感染症は見逃されている可能性もある。わが国の男性淋菌性尿道炎の感染源のほとんどは女性であると考えられるため（海外ではMSM：men who sex with men〔男性同性愛者〕の割合が高い），女性に対する淋菌検査を再検討する必要がある。

III　淋菌の咽頭感染

わが国で問題となるのは咽頭における淋菌の存在である[5) 6)]。咽頭から淋菌が分離されることは40年以上前から報告されている。1969年にThatcherらは，505名の軍人の咽頭の培養検査で1名より淋菌が検出されたと報告している[7)]。さらに，MSMの咽頭における淋菌・クラミジアの感染例が多くの論文に掲載されている。しかし，わが国の独特な状況は，オーラルサービスのみを行う性風俗嬢の咽頭を感染源とする男性尿道炎患者が劇的に増加したことにある。図2に我々が宮崎県で検討した咽頭からの感染例を示す[6)]。宮崎県では男性尿道炎のうち約40%が性風俗嬢より感染していた。特に2000年前後より，性風俗嬢のオーラルセックスが原因で感染した症例が急増した。この増加と，宮崎県にヘルス，ファッションマッサージと言われるオーラルサービスを行う性風俗店の出店が増加した時期とが重なることは興味深い。

実際に性風俗嬢の咽頭に淋菌が存在するかどうかは，保科らの検討で明らかとなった[8)]。京都市内

STI（sexually transmitted infection；性感染症）
PID（pelvic inflammatory diseases；骨盤内炎症性疾患）
CDC（米国疾病予防管理センター）
MSM（men who sex with men；男性同性愛者）

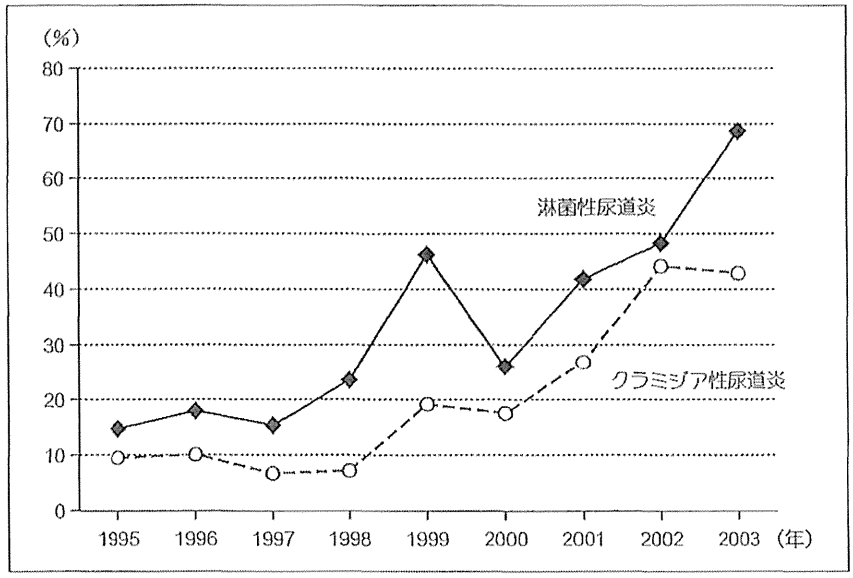

図2　性風俗嬢から感染した尿道炎患者のうち，性風俗嬢のオーラルセックスのみにより感染したと考えられる患者の割合の推移

性風俗嬢から感染した男性尿道炎のうち，オーラルセックスのみで感染したと考えられる症例の割合が1999年前後より急増した（宮崎市）。この時期，多くの性風俗店が同市内に出店し，尿道炎患者の急増と一致している。

（文献6より）

の婦人科において性風俗嬢の希望者に咽頭の淋菌・クラミジアの検査を行っている。ほとんどの症例でオーラルサービス時にはコンドームは使用していない。オーラルサービスのみを行う性風俗嬢の14%から淋菌が，7%からクラミジアが検出されていた（表1）。

また，伊与田らは男女（対象女性が一般女性か，性風俗女性か記載なし）における咽頭からの淋菌の検出を詳細に検討している[9]。STIクリニックを受診した男女127名の81%から，性器または咽頭から淋菌が分離されていた。検討を行った女性41名のうち，子宮頸管のみ，子宮頸管および咽頭の両方，咽頭のみからの淋菌分離率はそれぞれ22%，27%，7%であった。さらに，性器と咽頭の淋菌をパルスフィールドゲル電気泳動法にて比較しており，男女22症例のうち1例を除いてそのパターンが一致していた。性交時におそらく性交とともにオーラルセックスを行っており，同じ感染源から同時に淋菌が，性器，咽頭に感染していたことを示していると思われる。

また，余田らは2005～2008年のあいだの3期間において，多くの患者の咽頭から淋菌を検出している[10]。3時期で合計，男性335名，女性519名の検討を行い，男性53名（16%），女性79名（15%）の咽頭より淋菌が検出された。

亀岡らの報告[11]では，咽頭の検査を行った295名の女性のうち191名が性風俗嬢であると答えていた。性風俗嬢以外の女性101名のうち15名から淋菌が検出されていたこととなる。この検出率は余田らの報告と等しく約15%であり，一般女性の咽頭にも淋菌が感染している可能性が高い。

女性やMSMの場合，オーラルセックスと咽頭感染の関係を想像するのは容易である。オーラルセックスの際に男性淋菌性尿道炎患者の外尿道口が咽頭周囲に接し，淋菌は咽頭に付着するのであろう。しかし，ヘテロセクシュアルな男性の場合，どのように考えるべきであろうか？わが国で咽頭の淋菌検査を行った報告のうち，対象が「ヘテロセクシュアルな男性」と規定されていたものは

表1 咽頭からの淋菌の分離，検出（わが国のおもな報告）

対象		検出部位	検出率	検出方法	出典
女性	ファッションマッサージ嬢	咽頭	176/1,276 (13.8%)	LCR 法	保科（文献8）
	ソープランド嬢		2/39 (5.1%)		
女性	子宮頸管炎患者	子宮頸管および咽頭	11/41 (26.8%)	培養	伊与田（文献9）
男性		咽頭のみ	4/41 (7.3%)		
	男性淋菌性尿道炎患者	尿道分泌物および咽頭	14/103 (13.6%)		
女性	性感染症検査希望者	咽頭	79/519 (15.2%)	培養または核酸増幅法 (SDA 法)	余田（文献10）
男性			53/335 (15.8%)		
女性	性感染症の検査，または治療で来院した患者	咽頭（女性すべて）	45/295 (15.3%)	核酸増幅法 (TMA 法)	亀岡（文献11）
		（上記のうち性風俗嬢）	30/191 (15.7%)		
男性		咽頭	33/200 (16.5%)		
男性	男性淋菌性尿道炎患者	尿道分泌物および咽頭	4/18 (22.2%)	培養	Matsumoto（文献21）
男性	男性淋菌性尿道炎患者	尿道分泌物および咽頭	2/27 (7.4%)	培養	Muratani（文献26）
男性	男性淋菌性尿道炎患者	尿および咽頭	13/41 (31.7%)	核酸増幅法 (SDA 法)	Takahashi（文献30）

咽頭検査を行った男女の約15%より淋菌が検出される。特に性器から淋菌が分離される症例では，その分離率はさらに高くなる。

LCR：リガーゼ連鎖反応

（文献8～11，21，26，30より）

2論文であり，118名中19名（16%）から淋菌が検出されていた[12]。

このデータも余田らの報告と一致しており，STIとして医療機関を受診した患者の咽頭からの淋菌を検出すると，その検出率は約15%前後であることがわかってきた。したがってヘテロセクシュアルな男性の場合，オーラルセックスにより舌や口唇が子宮頸管炎の淋菌に汚染し，咽頭検体から淋菌が検出されると想像できる。咽頭に存在する淋菌がキスにて男女間を移行するかという問いもあるが，エビデンスがなく答えることはできない。咽頭に存在する淋菌すべてが，咽頭炎，扁桃炎といった疾患を引き起こすわけではないが，感染源として重要であることは間違いない

Ⅳ 性器外淋菌感染症

淋菌は男女の性器および咽頭以外にも感染しうる。近年わが国において，淋菌性腹膜炎，播種性淋菌感染症および淋菌性結膜炎の症例報告がなされている。表2に，淋菌性腹膜炎[13]～[16]，播種性

表2　性器外淋菌感染症（淋菌性腹膜炎，播種性淋菌感染症）のおもな症例報告（2000年以降）

年齢	性	疾患名	症状，経過	治療	出典
39	女	淋菌性腹膜炎	腹痛にて緊急開腹手術 腹水の培養にて淋菌検出	MINO, SBT/CPZ 2g/日 10日	赤羽（文献13）
53	男	播種性淋菌感染症	フィリピンから帰国後， 関節痛，発疹，発熱 血液培養にて淋菌検出	CEZ → CTRX, CFIX	矢部（文献17）
16	女	淋菌性腹膜炎	腹痛，高熱，下痢， 4名以上のパートナーあり 抗菌薬治療無効で，緊急手術 ドレナージ排液の核酸増幅法で 淋菌陽性	FMOX, MINO	Baba（文献14）
27	女	淋菌性腹膜炎	急性腹症にて腹腔鏡手術， 不正性器出血 腹水の核酸増幅法にて淋菌陽性	FMOX, MINO → CTRX 1g/日　4日	古元（文献15）
40	女	淋菌性腹膜炎	腹痛，発熱，腸閉塞にて開腹術 尿の核酸増幅法で淋菌陽性	MINO＋IPM CTRX 2g/日 AMPC 1,000mg/日	塩川（文献16）
49	男	播種性淋菌感染症	海外から帰国後，発熱， 左足関節痛 関節液，尿の培養にて淋菌検出 交際相手の外国人と性交渉	CTRX　1g/日 → 2g/日約50日	横田（文献18）

淋菌性腹膜炎，播種性淋菌感染症の多くは偶然に淋菌が分離されていた。このため，初期治療で用いられた抗菌薬のほとんどで無効である。

MINO：ミノサイクリン，SBT：スルバクタム，CPZ：セフォペラゾン，CEZ：セファゾリン
CTRX：セフトリアキソン，CFIX：セフィキシム，FMOX：フロモキセフ，IPM：イミペネム
AMPC：アモキシシリン

（文献13～18より）

淋菌感染症[17][18]の報告症例の一部を列記した。

女性における急性腹症としてまれに淋菌性腹膜炎が起こりうる。これらはPIDから波及したFitz-Hugh-Curtis症候群として取り扱われる。クラミジア感染症に合併すると考えられているが，この症候群の元々の報告は淋菌性のものである。急性腹症として手術が行われることも多く，腹水の検査にて淋菌が検出されることが多い。淋菌は培養条件により増殖しないこともあり，性的活動期の女性の急性腹症の症例では，淋菌・クラミジアの核酸増幅法や，淋菌に対応した培養検査が求められる。

播種性淋菌感染症は非常にまれな病態である。関節痛をともなうことが多く，関節液や血液から淋菌が検出される。しかし，最初から播種性淋菌感染症が疑われることはほとんどなく，偶然，培養検査にて淋菌が検出されることが診断となる。海外からの帰国者やMSMなど，難治性や原因不明の発熱症例などでは播種性淋菌感染症を疑うことが診断のきっかけとなるかもしれない。

淋菌性結膜炎はかなり多くの症例が報告されている。非常に強い眼瞼の腫脹，結膜充血や膿性眼脂が特徴である。しかし，淋菌が多くの抗菌薬に感受性であった時期には一般の細菌性結膜炎として経過していたものと思われる。後述するが，わが国の淋菌は多剤耐性であり，特に結膜炎に頻用

されるキノロン系点眼薬は無効である。難治性の細菌性結膜炎症例の眼脂の培養より淋菌が検出される。多くは性器の膿などにより手指が汚染され，その手指で眼を触れることにより発症すると考えられる。

V 淋菌感染症の治療

わが国の淋菌は多剤耐性化している。ペニシリンが開発された当時，ペニシリンは淋菌に対して強力な抗菌作用を有し，ほぼ100%の細菌学的効果を示した。しかし，ペニシリンを分解するpenicillinaseを産生する一部のpopulation (penicillinase-producing *N. gonorrhoeae*：PPNG) が世界に広がり，現在もアジア，アフリカでは蔓延している[19]。このPPNGの遺伝子はプラスミド上にあり，菌株間で伝搬していったと考えられる。しかし，わが国ではPPNGの分離頻度は一時15%程度となるも現在は数%である[20]。

これに対し，染色体性にβ-ラクタム系抗菌薬に耐性を示す株の分離頻度の増加が著しく，現在，ペニシリンに対する淋菌の感受性率は1%以下である[20][21]。さらに，第三世代経口セファロスポリンに対し耐性を示す淋菌が報告され[22]，急速に蔓延していった。CDC[23]や他の多くの国で推奨治療薬とされるcefiximeに対してもわが国では効果が低い[24]。これらの淋菌は口腔内に存在する他の*Neisseria*属の遺伝子の一部を取り，淋菌自体がキメラ化し，β-ラクタム系抗菌薬に耐性を示すと考えられている。

現在わが国で95%以上の細菌学的効果が期待されるβ-ラクタム系抗菌薬はceftriaxoneとcefodizimeの2薬剤である[25]。また，1980年代後半からクラミジアと淋菌に対する同時治療として使用されてきたキノロン系抗菌薬に対する耐性率はわが国では70%を超す（表3）[20][21][26]。

2009年，わが国ではついにceftriaxoneに対する高度耐性株が分離された。この菌は京都の性風俗嬢の咽頭から分離されたもので，多剤耐性菌である[27][28]。現時点までは，この耐性菌の蔓延は認められていないが，今後，注意深い観察が必要である。

また，淋菌，クラミジアに有効であるとされる

表3 淋菌の薬剤感受性率およびβ-lactamase産生菌の割合の推移（東京地区）

抗菌薬	Breakpoint MIC (μg/mL)*	感受性率 (%)			
		1999年 (41)**	2003年 (58)	2006年 (47)	2009年 (38)
Penicillin G	0.06	N.D.	1.7	4.3	10.5
Cefixime	0.25	100	96.6	100	47.4
Ceftriaxone	0.25	100	100	100	100
Spectinomycin	32	100	100	100	100
Levofloxacin	0.125†	41.5	17.2	17.0	5.3
β-lactamase産生株の割合		2.4	5.2	4.3	0

*CLSIによるbreakpont MIC，**菌株数，†Ofloxacinより推定したbreakpoint MIC
東京地区の検討では2009年にcefixime低感受性株の増加が認められたが，2010年には感受性率は96.7%と回復していた。
CLSI：Clinical Laboratory and Standards Institute，MIC：最小発育阻止濃度
（文献20より改変）

PPNG (penicillinase-producing *N. gonorrhoeae*)　AZM (azithromycin)
MIC（最小発育阻止濃度）　CLSI (Clinical Laboratory and Standards Institute)

azithromycin (AZM) 2g徐放製剤の耐性化が問題となっている。AZMに対する淋菌の耐性化はイギリスにて報告され，近年，多くの国でその分離頻度が上昇していると言ってよい[29]。わが国においてもAZMに対するMIC(最小発育阻止濃度)の上昇が認められる。ただし，AZMに対するbreak-point MICがCLSI (Clinical Laboratory and Standards Institute)で決定されていないため，その耐性に対する考え方があやふやになっていると言わざるを得ない。CDCによるガイドラインではAZMの使用を限定している[23]。

わが国では他国と比較して，淋菌・クラミジアに対する核酸増幅法の使用が保険で認められており，その検査頻度が高い。このため，淋菌，クラミジア感染症の診断は確実にできると考えられる。したがって，わが国の保険体制を考慮すると，あえて1剤による治療法を推奨せずとも，それぞれの病原菌に対して確実に治療を行うことが適切な治療法であると個人的には考える。

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J Infect Chemother (2013) 19:1–11
DOI 10.1007/s10156-012-0487-3

REVIEW ARTICLE

Identification of treatment strategies for *Mycoplasma genitalium*-related urethritis in male patients by culturing and antimicrobial susceptibility testing

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Received: 30 August 2012 / Published online: 18 October 2012
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Abstract *Mycoplasma genitalium* was first isolated from urethral swab specimens of male patients with non-gonococcal urethritis. However, the isolation of *M. genitalium* strains from clinical specimens has been difficult. Co-cultivation with Vero cells is one available technique for the isolation of *M. genitalium*. The strains that can be used for antimicrobial susceptibility testing by broth dilution or agar dilution methods are limited. Macrolides, such as azithromycin (AZM), have the strongest activity against *M. genitalium*. However, AZM-resistant strains have emerged and spread. Mutations in the 23S *r*RNA gene contribute to the organism's macrolide resistance, which is similar to the effects of the mutations in macrolide-resistant *Mycoplasma pneumoniae*. Of the fluoroquinolones, moxifloxacin (MFLX) and sitafloxacin have the strongest activities against *M. genitalium*, while levofloxacin and ciprofloxacin are not as effective. Some clinical trials on the treatment of *M. genitalium*-related urethritis are available in the literature. A doxycycline regimen was microbiologically inferior to an AZM regimen. For cases of treatment failure with AZM regimens, MFLX regimens were effective.

Keywords *Mycoplasma genitalium* · Isolation · Antimicrobial susceptibility · Clinical trials

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Introduction

Male urethritis is a syndrome that causes discharge from the urethral meatus, and urethral pain, and it is an important sexually transmitted infection (STI). Urethritis is classified as either gonococcal urethritis or non-gonococcal urethritis (NGU) by the presence or absence of *Neisseria gonorrhoeae* in the urethral or urine specimens. In Japan, NGU in which *Chlamydia trachomatis* is detected by any methods is called "chlamydial urethritis", and urethritis in which *C. trachomatis* is not detected is called "non-chlamydial non-gonococcal urethritis" (NCNGU). In females, an inflammatory condition in the cervical canal is called cervicitis. When *N. gonorrhoeae* or *C. trachomatis* are detected from the cervical specimens of patients with symptoms of cervicitis, the condition may be called gonococcal cervicitis or chlamydial cervicitis, respectively. However, when specific pathogens are not detected, the condition may be called simply "cervicitis" or "non-gonococcal cervicitis".

Mycoplasma genitalium is one species of Mycoplasma that was isolated from urethral swabs from patients with NGU in 1980 [1]. The pathogenicity of *M. genitalium* has been the focus of epidemiological studies, studies in animal models, antimicrobial susceptibility testing in vitro, clinical response to antimicrobials, and transmissibility in males. Taylor-Robinson [2] proposed the modified Henle–Koch postulates for establishing the pathogenicity of *M. genitalium* in disease, and *M. genitalium* is now considered an established cause of male NGU [3, 4] and of female cervicitis [3–6]. It has been suggested that *M. genitalium* can cause other diseases, such as acute epididymitis [7, 8] chronic prostatitis [9, 10], and balanoposthitis [11] in males and pelvic inflammatory disease [12–14], urethritis [15, 16], and adverse pregnancy events [17–19] in females. However,

more studies are needed regarding the pathogenicity of *M. genitalium* in such diseases [6, 12, 20].

There are various reports and studies that discuss both the prevalence of *M. genitalium* and tests, such as nucleic acid amplification tests (NAATs), that are used for detecting *M. genitalium* in diseases of males and females. However, there have been few reports investigating the basic culturing methods or antimicrobial susceptibility testing of *M. genitalium*. In this paper, I will discuss culturing methods and antimicrobial susceptibility testing of *M. genitalium* and further propose possible treatment strategies for *M. genitalium*-related urethritis.

Culturing of *M. genitalium*

Discovery of *M. genitalium*

M. genitalium strains were first isolated from the urethral swabs of two patients with NGU [1]. The presence of unknown pathogens in NCNGU was recognized by clinicians before the discovery of *M. genitalium*. Male patients with urethritis, in whom neither *N. gonorrhoeae* nor *C. trachomatis* were detected, had symptoms of urethritis and an inflammatory response, such as the presence of white blood cells in the urinary sediment or in urethral smears. In addition, some patients were cured of symptoms with tetracycline-based therapy [3, 4].

M. genitalium was isolated by Tully et al. [1] by the direct inoculation to SP4 Mycoplasma medium (SP4 medium) [21] with urethral swabs. Taylor-Robinson [4] brought urethral swab specimens from 13 male patients with NGU to Tully's laboratory, where the SP4 medium had been developed shortly before. SP4 was the best artificial medium for the growth of Spiroplasma [21] and it was also efficient for the recovery of other mycoplasmas. In the initial study, 2 strains of *M. genitalium*, strain G37^T and strain M30, grew in SP4-medium approximately 50 days after inoculation. When *M. genitalium* grows in SP4 medium, the color of the medium changes to yellow from red owing to the production of acid from the breakdown of glucose. A frequently passaged *M. genitalium* strain can change the color of the medium in approximately 1 week.

In the early decades following the discovery of *M. genitalium*, attempts were made to isolate other *M. genitalium* strains, but these attempts failed [22], with the exception of the isolation of several strains from respiratory or synovial specimens (R32, TW10-5G, TW10-6G, TW48-5G, and UTMB-10G) [23, 24]. However, these strains were mixed cultures with *M. pneumoniae* and were genetically similar to G37^T [25, 26], and there is concern that the ATCC strains, including these 5 strains and the late-passaged M30 strain (not the initially recovered M30), are contaminants of G37^T [25]. In China, 8 *M. genitalium* strains have been recovered from urogenital tract specimens by direct inoculation into SP4 medium [27], but details were not known about the isolation.

Isolation of *M. genitalium* strains by co-culture with Vero cells

In 1996, new *M. genitalium* strains were isolated from urogenital tract specimens by a newly developed method of co-culturing specimens with Vero cells reported by Jensen et al. [28]. This co-cultivation with living cells has been shown to be a reliable way of culturing *M. genitalium*. They reported that 4 *M. genitalium* strains were isolated from the urethral swabs of male patients with NGU. These authors were able to grow 9 strains in Vero cells, but 5 strains were lost after attempts to passage the cultures for adaptation for growth in Friis broth medium (FB medium). Thus, the most difficult aspect of culturing *M. genitalium* is adaptation for stable axenic cultures.

In the literature, the clinical specimens from which *M. genitalium* has been successfully isolated are urethral swabs [1, 27–29], urinary sediments [30], and cervical swabs [31]. When urinary sediments are used in the initial culture, some special procedures are necessary [30]. Some components in urine can destroy monolayers of Vero cells, and the urine sediments should therefore be washed in culture media. Furthermore, prolonged storage of urine specimens before culturing is detrimental to the successful isolation of *M. genitalium* strains. The infectivity of *M. genitalium* to Vero cells is lost after storage for more than 2 days under room temperature conditions. The recommended procedure for the storage of urinary sediments for the Vero cell culture method is to centrifuge the urine at $>10{,}000 \times g$ for 15 min and to re-suspend the pellet of the urine specimen in mycoplasma medium as soon as possible after urine collection.

An observable color change in axenic culture medium, such as that seen in SP4 medium or FB medium, is a convenient method for monitoring the growth of *M. genitalium*. However, the number of strains that can be grown in axenic culture medium and thus be monitored by color change are limited, and color change cannot be used to monitor growth in cell culture. In the study by Jensen et al., which was the first report of co-cultivation with Vero cells and clinical specimens, the polymerase chain reaction (PCR) was used to monitor growth [28]. The authors diluted the supernatant of the cell culture by tenfold serial dilutions, detected the MgPa adhesin gene of *M. genitalium* by PCR, and generated *M. genitalium* growth curves. In our subsequent studies, quantitative PCR (qPCR) was used to monitor growth [30, 32]. This qPCR method was based on

the report by Jensen et al. of the detection of the MgPa adhesin gene of *M. genitalium* [33]. *M. genitalium* has the smallest genome of any self-growing organism and has only one *MgPa* gene. Therefore, growth curves of *M. genitalium* DNA loads, as determined by qPCR using the TaqMan assay, could be used to estimate the growth of *M. genitalium*. By this method, several new *M. genitalium* strains, including the azithromycin (AZM)-resistant strains described below, have been isolated [29].

By using qPCR to monitor the growth of *M. genitalium*, it was discovered that qPCR could also be used for antimicrobial susceptibility testing [32]. This idea was developed based on an interesting observation that the growth of certain *M. genitalium* strains was inhibited by high concentrations of ampicillin, penicillin G, amphotericin B, and polymyxin B, which had been added to the culture medium to prevent contamination by other bacterial or fungal species [30]. It had previously been reported that high concentrations of ampicillin or penicillin G could inhibit the growth of other mycoplasmas, including *Mycoplasma neurolyticum*, *Mycoplasma hyopneumoniae*, *Mycoplasma flocculate*, and *Mycoplasma dispar* [34]. When high concentrations of antimicrobials were added to the culture medium at the initial cultivation, the growth of *M. genitalium* was also inhibited. In our experiments, 200 IU/ml of penicillin G and 500 μg/ml of polymyxin B were shown to be the maximal amounts of antimicrobials that could prevent contamination but not inhibit the growth of *M. genitalium* in the initial cultivation. It was interesting that strain $G37^T$ was insensitive to penicillin G [minimum inhibitory concentration (MIC) >3,000 IU/ml] and ampicillin (MIC >2,000 IU/ml) [35], which allowed for the recovery of $G37^T$ from culture. The mechanism by which penicillins or antifungal agents inhibit the growth of *M. genitalium*, which does not have a cell wall, is unclear, but it may be an unspecific toxic effect.

Adaptation of *M. genitalium* strains in axenic culture medium

After confirmation of the growth of *M. genitalium* with Vero cells in culture, we moved on to the next steps of adaptation to axenic culture medium and cloning of the newer strains of *M. genitalium* [36]. The supernatant of the Vero cell culture in which *M. genitalium* grew was re-suspended in FB medium and incubated, and when good growth was observed, the so-called cloning procedure was initiated. To obtain a pure culture of the *M. genitalium* strain, the FB medium, at the time when the color changed to yellow from red, was aspirated and expelled with a syringe through a small-bore cannula several times in order to disrupt micro-colonies and clumps of *M. genitalium* cells and obtain single cells. Normally, in mycoplasmology, the broth is passaged through a 0.45-μm filter, but for unknown reasons this procedure did not produce growth of our strains. The single-cell suspension in 0.1 ml of FB medium was then plated onto FB agar. After several days to weeks, small fried-egg-shaped colonies were observed. A single colony on the FB agar was then cut away under the microscope, and the piece of agar that included one colony was inoculated into FB medium and incubated. This procedure using FB medium and FB agar was repeated 3 times, and finally a new strain of *M. genitalium* was obtained. This entire process took more than 6 months. Thus, the isolation of *M. genitalium* from clinical specimens still remains difficult and very time-consuming.

Antimicrobial susceptibilities of *M. genitalium*

Broth dilution method and agar dilution method

There are two methods recommended for antimicrobial susceptibility testing for Mycoplasma species [37, 38]—the broth dilution method and the agar dilution method. In the broth dilution method, antimicrobials are diluted twofold with SP4 medium in the wells of a microtiter plate, and known concentrations of the *M. genitalium* strains are added to the wells. The plates are sealed and incubated. The color of the medium is observed, and the MIC is defined as the minimum concentration of the antimicrobial that can inhibit the color change. The agar dilution method involves the use of agar plates that contain increasing concentrations of antimicrobials. Diluted *M. genitalium* strains are inoculated onto each of the agar plates, and colonies are counted after incubation. Taylor-Robinson and Bebear [38] determined that the MIC was the lowest concentration of antimicrobials completely preventing colony development after incubation at 37 °C. In contrast to the findings in that study, Hannan et al. [37, 39] showed a modified method in which the MIC was defined as the lowest concentration of an antimicrobial that causes more than 50 % inhibition of growth compared with the number of colonies on the control plate without antimicrobials. However, this concept may be regarded as the minimum bactericidal concentration. In a recent document reporting standardized methods for antimicrobial susceptibility testing for *Mycoplasma pneumonia*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* [40], it was determined that the MIC was read as the lowest concentration of the antimicrobial agents that prevented colony formation when examined under a stereomicroscope. The broth dilution method is convenient for determining the antimicrobial susceptibilities of *M. genitalium*. However, the limitation of these conventional methods is that very limited numbers of strains are available, because

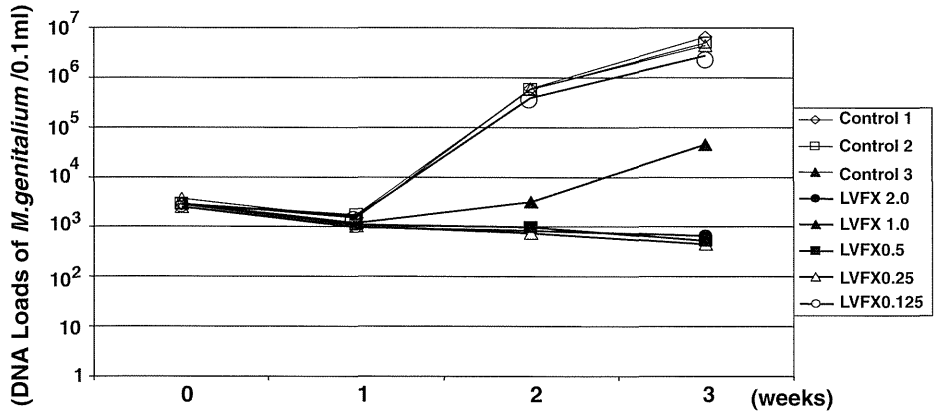
obtaining *M. genitalium* strains that can grow in axenic culture medium takes a long time.

"Cell culture method" by using quantitative PCR

To resolve this culturing dilemma, a "cell culture method" using qPCR was developed [32]. The growth of *M. genitalium* in Vero cell cultures can be monitored by qPCR. When *M. genitalium* strains were cultured with culture media containing any concentration of antimicrobials, it was found that the growth of the bacteria was inhibited (Fig. 1). Culture media with twofold dilutions of antimicrobials were prepared by determining the appropriate final concentration after inoculation with a suspension of Vero cells and *M. genitalium*. Then, *M. genitalium* was cultured in Vero cells with culture media containing antimicrobials in a 24-well tissue culture plate. The supernatant of the culture media was harvested 3 weeks later, and the DNA loads of *M. genitalium* were calculated. The MIC value was defined as the lowest concentration of an antimicrobial that caused >99 % inhibition of growth compared with DNA loads of *M. genitalium* cultured in Vero cells on culture medium without antimicrobials. When the growth of *M. genitalium* on Vero cells is observed, antimicrobial susceptibilities can be measured using this method. The results of the antimicrobial susceptibility testing were comparable for the cell culture method and the broth dilution method [32, 41]. Some macrolide-resistant strains were identified by the cell culture method [29]. Antimicrobial susceptibility testing using qPCR has also been used for several intracellular bacteria, such as *Rickettsia*, *Coxiella,* and *Tropheryma* species [42–44]. The major drawback of this method is cost. In addition, non-cloning strains can be used for antimicrobial susceptibility testing. Therefore, it is possible that the results of this method were involved with MICs of multiple strains, but the possibility was lower because the growth of *M. genitalium* is still difficult with any culture methods.

Antimicrobial susceptibilities of *M. genitalium* strains

Earlier reports showed that *M. genitalium* strains were sensitive to tetracyclines, macrolides, and fluoroquinolones, with the exception of nalidixic acid [35, 45]. In addition, the MIC range of each antimicrobial for $G37^T$, R32G, TW48-5G, TW10-5G, TW10-6G, and UTMB was very narrow [41, 45]. In this sense, these strains were genetically extremely homogeneous [25]. Our study [41]



Inhibition rate/control (%)	1w	2w	3w
LVFX 2.0 mg/l	37.4	99.9	>99.9
LVFX 1.0 mg/l	36.7	99.9	>99.9
LVFX 0.5 mg/l	31.4	99.8	>99.9
LVFX 0.25 mg/l	26.3	99.5	99.1
LVFX 0.125 mg/l	-3.5	32.3	47.2

Fig. 1 Growth curves of *Mycoplasma genitalium* with or without levofloxacin (*LVFX*) generated by monitoring DNA loads using the quantitative polymerase chain reaction (PCR) method. The growth curves were generated by measuring DNA loads of *M. genitalium* cultured with or without levofloxacin. *M. genitalium* was co-cultured with Vero cells. The culture medium, Eagle's minimum essential medium (MEM) with 2 % Ultroser G serum substitute (Ciphergen, Cergy-Saint-Christophe, France), contained increasing concentrations of levofloxacin. As a control, *M. genitalium* was cultured without any antimicrobials. Copy numbers of the *MgPa* gene were determined using TaqMan quantitative PCR methods [30, 32, 33]. The inhibition rates of the antimicrobials were calculated by the following formula: inhibition rates (%) = [(average of DNA loads in control wells-DNA loads in test well)/(average of DNA loads in control wells)] × 100

Table 1 Minimum inhibitory concentrations (MICs) of 23 *Mycoplasma genitalium* strains, as determined by the broth dilution method [41]

Antimicrobials	MICs (mg/l)			
	MIC_{50} (23 strains)	MIC_{90} (23 strains)	MIC ranges	
			ATCC strains (7 strains)	Non-ATCC strains (16 strains)
Sitafloxacin	0.063	0.125	0.063–0.125	0.008–0.125
Moxifloxacin	0.063	0.125	0.063–0.25	0.016–0.25
Gatifloxacin	0.25	0.25	0.125–0.5	0.031–0.5
Levofloxacin	1	2	1–2	0.125–2
Ciprofloxacin	4	8	4–8	0.063–4
Norfloxacin	32	64	32–64	1–32
Minocycline	0.125	0.25	0.031–0.125	0.063–0.25
Doxycycline	0.125	0.25	0.063–0.25	0.063–1
Tetracycline	0.125	0.5	0.063–0.25	0.063–2
Azithromycin	0.001	0.002	0.001–0.002	0.0002–250
Clarithromycin	0.004	0.008	0.002–0.008	0.0005–128

showed broader MIC ranges in antimicrobial susceptibilities among the newer clinical isolates from urogenital specimens (Table 1). The macrolides, including AZM and clarithromycin (CAM), had the strongest activities against *M. genitalium*, but some strains were resistant to the macrolides. Among the fluoroquinolones, there were large differences in the anti-*M. genitalium* activities. Newer fluoroquinolones, such as moxifloxacin (MFLX) and sitafloxacin (STFX), had the strongest activities, but the activity of norfloxacin was weak. The activities of levofloxacin (LVFX) and ciprofloxacin (CPFX), which are used worldwide, are not strong, and the MIC_{90} values were 2 and 8 mg/l, respectively. The activities of the tetracyclines are of intermediate strength, falling between those of the fluoroquinolones, such as LVFX and those of the newer fluoroquinolones, such as MFLX and STFX.

Clinical trials of treatment for *M. genitalium*-related urethritis

Some clinical trials have been performed regarding the treatment of *M. genitalium* infections. In these trials, the microbiological effect of treatment was evaluated by the results of NAATs. However, there is no consensus regarding when the response of antimicrobials should be evaluated after their administration. In the *Japanese guideline for clinical research of antimicrobial agents on urogenital infections* [46] the response is assessed 2–4 weeks after the completion of therapy, and the primary outcome measured in cases of male NGU is the microbiological response. In addition, the relationship between microbiological efficacy and antimicrobial susceptibility is unclear. We have not identified breakpoint MICs for any antimicrobials against *M. genitalium* infection.

Clinical trials of AZM or doxycycline (DOXY) treatment for *M. genitalium*-related urethritis

The clinical trials of various agents for the treatment of *M. genitalium*-related urethritis in males are described in Table 2. I selected studies in which the eradiation rates of *M. genitalium* after treatment were shown [47–63]. In early clinical trials for the treatment of *M. genitalium*-related urethritis, efficacies of the tetracyclines and the macrolides were compared. Gambini et al. [47] reported that the eradication rates of *M. genitalium* by DOXY and AZM were comparable, but other reports showed that DOXY was ineffective compared with AZM [50, 52, 55]. Two recent randomized studies clearly demonstrated that the efficacy of DOXY against *M. genitalium*-related urethritis was inferior to that of AZM, as measured by the microbiological response [58, 59]. It is interesting to note that there is a remarkable discrepancy between the relatively good in vitro MIC of DOXY in most *M. genitalium* strains and the poor treatment efficacy of this drug, which cured, in general, only 30 % of the infected patients.

There has been some discussion concerning the dosage of AZM, which was used either as a single dose of 1 g of AZM or in an extended AZM regimen, such as an initial dose of 500 mg followed by 250 mg/day for 4 days, mainly suggested by Scandinavian researchers [50, 52, 55, 56]. Jernberg et al. [56] reported the microbiological results after any of the following methods of administration: a 1 g single dose of AZM; AZM stat plus repeated doses of 1 g of AZM for 5–7 days after the initial administration; or extended AZM treatment. The eradication rates of *M. genitalium* in these regimens were similar, at 78.7, 73.7, and 79.6 %, respectively. When we use AZM regimens, a single 1 g dose of AZM is recommended. We do not have

Table 2 Microbiological efficacies of antimicrobials against *M. genitalium* in clinical trials of treatment for male non-gonococcal urethritis

Author and country	Year	Design of study	Initial regimens	Eradication rates (initial regimen)	Second regimens used in cases of treatment failure	Eradication rates (second regimen)
Gambini et al. [47]	2000	Open label	DOXY 200 mg/day	94.3 % (33/35)		
Italy			7 days AZM 1 g stat	82.4 % (14/17)		
Johannisson et al. [48]	2000	Open label	TC 500 mg × 2/day, 10 days	38.5 % (5/13)		
Sweden						
Maeda et al. [49]	2001	Open label	LVFX 100 mg × 3/day, 7 days	33 % (4/12)		
Japan						
Falk et al. [50]	2003	Open label	DOXY 200 mg stat + 100 mg/day, 8 days	37.5 % (6/16) (DOXY or Lymecycline)	AZM 500 mg stat + 250 mg/day, 4 days	100 % (8/8)
Sweden			Lymecycline (tetracycline) 300 mg × 2/day, 10 days			
			AZM 500 mg stat + 250 mg/day, 4 days	100 % (8/8)		
Dupin et al. [51]	2003	Open label	MINO 100 mg/day, 7 days	42.9 % (3/7)	MINO 100 mg/day, additional 7 days	0 % (0/1)
France			SPCM 2 g stat + MINO 100 mg/day, 7 days	100 % (1/1)		
			DOXY 100 mg/day, 7 days	0 % (0/1)		
Wikstrom and Jensen [52]	2006	Open label	DOXY 200 mg stat + 100 mg/day 8 days	0 % (0/6)	AZM 1 g stat or 500 mg stat + 250 mg/day, 4 days	100 % (14/14)
			EM 500 mg × 2/day, 10 days	18.2 % (2/11)		
Sweden			AZM 1 g stat or 500 mg stat + 250 mg/day, 4 days	100 % (7/7)		
Bradshaw et al. [53]	2006	Open label	AZM 1 g stat	71.9 % (23/32)	AZM 1 g weekly, 3 weeks	0 % (1/3)
Australia					MFLX 400 mg × 1/day, 10 days	100 % (9/9)
Stamm et al. [54]	2007	Double-blind multi-site controlled	Rifalazil 2.5 mg stat	0 % (0/5)		
USA			Rifalazil 12.5 mg stat	0 % (0/7)		
			Rifalazil 25 mg stat	0 % (0/5)		
			AZM 1 g stat	85.7 % (6/7)		
Bjornelius et al. [55]	2008	Open label	DOXY 200 mg stat + 100 mg/day, 8 days	9.2 % (7/76)	AZM 500 mg stat + 250 mg/day, 4 days	95.7 % (45/47)
Sweden			AZM 1 g stat	84.6 % (33/39)	DOXY 100 mg/day, 15 days	66.7 % (2/3)
Jernberg et al.[a] [56]	2008	Open label	AZM 1 g stat	78.7 % (144/183)	AZM 500 mg stat + 250 mg/day, 4 days	34.8 % (8/23)
Norway			AZM 1 g stat + repeated AZM 1 g	73.7 % (28/38)		
			AZM 500 mg stat + 250 mg/day, 4 days	79.6 % (78/98)	OFLX 200 mg × 2/day, 10 days	58.3 % (21/36)
			OFLX 200 mg × 2/day, 10 days	44.4 % (4/9)	MFLX 400 mg/day, 7 days	100 % (24/24)
			MFLX 400 mg/day, 7 days	100 % (3/3)		

Table 2 continued

Author and country	Year	Design of study	Initial regimens	Eradication rates (initial regimen)	Second regimens used in cases of treatment failure	Eradication rates (second regimen)
Takahashi et al. [57] Japan	2008	Open label	AZM 1 g stat	100 % (3/3)		
Mena et al. [58] USA	2009	Randomized	DOXY 100 mg × 2/day, 7 days	45.2 % (14/31)	AZM 500 mg stat + 250 mg/day, 4 days	60 % (3/5)
			AZM 1 g stat	82.7 % (19/23)		
Schwebke et al. [59] USA	2011	Randomized controlled double-blind	DOXY 100 mg × 2/day, 7 days (with or without tinidazole)	30.8 % (12/39)		
			AZM 1 g stat (with or without tinidazole)	66.7 % (30/45)		
Takahashi et al. [60] Japan	2011	Open label	LVFX 500 mg/day 7 days	60 % (3/5)		
Hamasuna et al. [61] Japan	2011	Open label	GFLX 200 mg × 2/day, 7 days	83.3 % (15/18)		
Hagiwara et al. [62] Japan	2011	Open label	AZM 1 g stat	83.3 % (25/30)		
Ito et al. [63] Japan	2012	Open label	STFX 100 mg × 2/day, 7 days	100 % (11/11)		

AZM azithromycin, *DOXY* doxycycline, *EM* erythromycin, *GFLX* gatifloxacin, *LVFX* levofloxacin, *MFLX* moxifloxacin, *MINO* minocycline, *OFLX* ofloxacin, *SPCM* spectinomycin, *STFX* sitafloxacin, *TC* tetracycline

[a] Combined male urethritis and female cervicitis

data regarding the use of a 2 g single dose of AZM in the treatment of *M. genitalium*-related urethritis.

AZM is used worldwide in the treatment of NGU. The eradication rates of *M. genitalium* in the clinical trials of treatment with AZM regimens reached almost 100 % [50, 52, 57]. However, Bradshaw et al. [53] in Australia reported treatment failure after a regimen of AZM. They treated patients with NGU with a single 1 g dose of AZM. In their study, *M. genitalium* was detected in 34 patients, and NAATs results of *M. genitalium* from the urethral swab specimens of nine patients after treatment with AZM remained positive. Three patients were treated with a repeat dose of 1 g of AZM, but *M. genitalium* was not eradicated. Finally, these patients were treated with 400 mg/day of MFLX for 10 days, which was a microbiologically effective treatment. Macrolide-resistant *M. genitalium* strains were isolated from the urethral swab specimens obtained from four of these patients [29] and in 5 out of 7 evaluable sample sets, macrolide-susceptible genotypes were detected in pre-treatment specimens, suggesting that the treatment failure was due to resistance selected for by the treatment with 1 g single-dose AZM. In recent studies, the eradication rates of *M. genitalium* treated with AZM were 68–86 % in clinical trials [54–56, 58, 59].

Clinical trials of fluoroquinolone treatment of *M. genitalium*-related urethritis

The first clinical trial that used a fluoroquinolone to treat *M. genitalium* infection was reported by Maeda et al. [49] in Japan. A regimen of LVFX (100 mg, 3 times a day for 7 days) was not effective against *M. genitalium*. MFLX was administered to patients that experienced treatment failure with an AZM regimen [53, 56]. Other fluoroquinolone-based regimens were introduced in Japan, including LVFX 500 mg [60], gatifloxacin (GFLX) [61], and STFX [63]. The microbiological efficacies of LVFX (300 mg/day), GFLX, MFLX, and STFX were 33, 84, 100, and 100 %, respectively. The MIC values of these antimicrobials were 2, 0.25, 0.125, and 0.125 mg/l, respectively, in our study [41]. Agents with MIC values of <0.125 mg/l are suitable if we use fluoroquinolones, such as MFLX and STFX, against *M. genitalium*-related urethritis. However, STFX is used only in Japan.

Antimicrobial resistance in *M. genitalium* strains

Resistance to azithromycin

Antimicrobial resistance has become a problem in the treatment of *M. genitalium* infections. The most important issue is macrolide resistance. The presence of macrolide-resistant *M. genitalium* was first reported by Bradshaw et al. [53], as described above. This group sent the urethral swab specimens from patients that experienced clinical failure with an AZM regimen to Jensen for culture, and 7 macrolide-resistant strains were isolated, 4 from Australia and 3 from Scandinavia [29] (Table 3). These resistant strains had mutations in region V of the 23S *r*RNA gene, which were similar to mutations detected in macrolide-resistant *M. pneumoniae* strains [64, 65]. The mutations in the 23S *r*RNA gene in *M. genitalium* were also detected in specimens collected after treatment failure with a single dose of AZM in Japan [66]. It is concerning that macrolide resistance in *M. genitalium* is spreading throughout the world. In fact, AZM-resistant *M. genitalium* is spreading in Australia [29, 53], Northern Europe [29], and France [67], and in Greenland, where the *C. trachomatis* incidence is very high and where AZM 1 g is the standard treatment, a resistance rate of 100 % has been reported [68]. Recently, a study of *M. genitalium*-positive patients from Australia showed that almost half of those patients failing AZM 1 g single-dose treatment had susceptible genotypes present in the pre-treatment specimens, strongly suggesting that the single-dose treatment leads to the selection of resistant strains [69].

Resistance to fluoroquinolones

Fluoroquinolone resistance is typically found in rod-shaped gram-negative bacterial species, and it is known that mutations in the gyrase genes *gyrA* or *parC* contribute to

Table 3 MICs (mg/l) of macrolide-resistant *M. genitalium* strains, as determined by quantitative polymerase chain reaction (PCR) [29]

Strain	Origin	Moxifloxacin	Levofloxacin	Ciprofloxacin	Doxycycline	Tetracycline	Azithromycin	Clarithromycin
M6257	Sweden	0.25	1	1	1	4	≥8	≥16
M6270	Australia	0.125	2	4	0.25	0.5	≥8	≥16
M6271	Australia	0.125	1	4	0.25	0.25	≥8	≥16
M6302	Sweden	0.125	1	4	0.125	0.5	≥8	≥16
M6303	Norway	0.25	2	8	1	1	≥8	≥16
M6320	Australia	0.063	0.5	2	0.25	1	≥32	≥16
M6321	Australia	0.063	2	2	0.125	0.25	≥32	≥16

resistance. Deguchi et al. [70] reported that mutations in the *gyrA* gene were found in purified *M. genitalium* DNA obtained from the urine specimens of patients that had treatment failure with LVFX. In our studies of GFLX treatment for NGU [61], genetic mutations in the *gyrA* or *parC* gene of *M. genitalium* were also detected [71]. In these studies, *M. genitalium* was detected from 18 patients with NGU. The eradication rate of GFLX with 200-mg doses twice a day for 7 days was 83 % and *M. genitalium* remained in the specimens from 3 patients after treatment. Mutations of both *gyrA* and *parC* were found in *M. genitalium* DNA purified from the urinary sediments of the 3 patients with treatment failure. In addition, only one *M. genitalium* strain with fluoroquinolone resistance was isolated from the genital specimens (unpublished data). This strain has high MICs against GFLX (32 mg/l) and MFLX (16 mg/l) and has mutations of both the *gyrA* and *parC* genes. These mutations in gyrase genes were not found in fluoroquinolone-sensitive strains. By these findings, it was suggested that the fluoroquinolone resistance of *M. genitalium* was closely related to mutations of gyrase genes, similar to findings in fluoroquinolone-resistant gram-negative rods.

Conclusion and future studies

M. genitalium is an important pathogen causing male urethritis. However, a broader knowledge of the antimicrobial susceptibilities of *M. genitalium* has been missing because of the difficulty in isolating *M. genitalium* strains from clinical specimens. Male patients with urethritis from whom *M. genitalium* is isolated are regarded as having NGU, and treatment regimens with AZM have been recommended. However, macrolide-resistant *M. genitalium* strains have emerged and are spreading throughout the world. Reconsideration of the treatment strategies for NGU will be necessary in the not-too-distant future, so as to treat *C. trachomatis* and *M. genitalium* infections individually.

The development of a newer and less expensive rapid-test kit is imperative for the treatment of *M. genitalium* infections. In addition, the isolation and culture of *M. genitalium* strains is still complicated and time-consuming work. However, the culture of microorganisms is a basic and important issue in the field of microbiology. Genetic analyses developed for screening antimicrobial resistance are important strategies for the guidance of treatment. These screening methods should be based on the analysis of isolated strains. The isolation and culture of *M. genitalium* remains important and should be continued. In addition, improved techniques and/or a better medium for the more effective isolation of *M. genitalium* should be developed.

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INTERNATIONAL JOURNAL OF UROLOGY
International Journal of Urology (2013) **20**, 676–684 doi: 10.1111/iju.12152

Review Article

Mycoplasma genitalium in male urethritis: Diagnosis and treatment in Japan

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Abbreviations & Acronyms
FVU = first voided urine
GU = gonococcal urethritis
MIC = minimum inhibitory concentrations
NCNGU = non-chlamydial non-gonococcal urethritis
NGU = non-gonococcal urethritis
PCR = polymerase chain reaction
STI = sexually transmitted infections
WBC = white blood cells

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Received 17 December 2012; accepted 5 March 2013. Online publication 16 April 2013

Abstract: Male urethritis is a common disease for urologists, with the most common pathogens being, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. When the tests fail to detect these pathogens, the presented urethritis is called non-chlamydial non-gonococcal urethritis. *Mycoplasma genitalium* is one of the pathogens for non-chlamydial non-gonococcal urethritis. The test for detecting *M. genitalium*, which is commercially available in Japan, is not accepted by the Japanese insurance system now. The detection rate of *M. genitalium* from patients with non-gonococcal urethritis is 10–20% in Japan. Antimicrobial susceptibility testing for *M. genitalium* showed that macrolide has the strongest activity and the minimum inhibitory concentrations of tetracyclines were not substantially lower. Some kinds of fluoroquinolones, such as sitafloxacin and moxifloxacin, have stronger activities against *M. genitalium*. For non-gonococcal urethritis, macrolides and tetracycline are recommended in some guidelines. In clinical studies, tetracyclines are less effective against *M. genitalium* than azithromycin, and azithromycin regimens including 1 g stat or 2 g stat are now recommended for urethritis with *M. genitalium*. However, macrolide-resistant *M. genitalium* strains have recently emerged and are spreading worldwide. This macrolide-resistance is closely related to mutations on the 23S *r*RNA gene. Sitafloxacin and moxifloxacin have shown good efficacies for *M. genitalium* in some clinical studies. If the azithromycin regimens fail, we must consider the use of fluoroquinolones, such as sitafloxacin, in Japan. The most important issues include the acceptance of *M. genitalium* examinations by the national insurance system and the individual treatment of *C. trachomatis* and *M. genitalium* in the not-too-distant future.

Key words: antimicrobial susceptibility, infection, *Mycoplasma genitalium*, treatment, urethritis.

Introduction

Male urethritis is a commonly encountered disease for Japanese urologists, with the most common pathogens being *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. However, there are many cases in which no pathogens are detected by the microbiological tests that are accepted by the Japanese insurance system; that is, the nucleic acid amplification tests for *C. trachomatis* or *N. gonorrhoeae*. When the tests fail to detect these pathogens, the presented urethritis is known as NCNGU. Other previously reported potential pathogens for NCNGU include *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Niesseria meningitidis*, *Haemophilus* species, herpes simplex virus, adenovirus and others.[1] Among these microorganisms, *T. vaginalis* and *M. genitalium* have been confirmed to exhibit pathogenicity to the male urethra, and treatment strategies for these pathogens are described in some guidelines for STI.[2–5]

In many countries, urologists diagnose and treat male urethritis as STI. The clinical state of NCNGU in which *N. gonorrhoeae* or *C. trachomatis* are detected by known tests are well understood by urologists. However, *M. genitalium* is not recognized, because the commercially available tests to detect this pathogen are not accepted by the national insurance system, especially in Japan. In the present review, to understand the diagnosis and treatment of male urethritis with *M. genitalium* for urologists, literature from peer-reviewed journals

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and conference proceedings that show evidence regarding *M. genitalium*-infection were searched.

How or when was *M. genitalium* discovered?

Before the discovery of *M. genitalium*, clinicians knew that some types of microorganisms besides *C. trachomatis* or *N. gonorrhoeae* participated in male urethritis, because male patients testing negative for the two bacteria had symptoms of urethritis, such as urethral discharge and urethral pain. The patients also suffered from inflammatory responses including an increase in WBC in the urinary sediment or urethral smears. These patients responded to antimicrobials, such as tetracyclines, and their findings suggested the presence of unidentified bacterial pathogens in the urethra.[6]

Taylor-Robinson, who worked at St. Mary's Hospital in London, UK, observed motile spiral forms in the urethral smears of patients with NGU using dark-field microscopy.[6,7] He thought that these forms resembled spiroplasmas, which can infect plants or insects. In 1980, urethral swabs were collected from 13 male patients with NGU. These specimens were transported to Tully's laboratory at the National Institutes of Health in Bethesda, MD, USA, and Tully developed a new medium, called SP4 medium, in which any spiroplasma or mycoplasma could grow.[8] The specimens from the urethral swabs were inoculated into SP4 medium and two new mycoplasma strains were identified from the two specimens approximately a month after the initial incubation. These strains were named G-37^T and M-30, and were found to be closely related to, but serologically different from, all other known mycoplasma.[9] Finally, these strains were confirmed as a new species, *M. genitalium*, the 11th species of human originated mycoplasma.[7]

What is *M. genitalium*?

As shown through electron microscopy, *M. genitalium* is shaped like a flask or bottle, measures 0.6–0.7 μm in length and has tip-like structures that attach to the surface of cells.[7,10] This bacterium can grow on axenic culture media, such as SP4 medium or Friis's medium, or on agar to make small colonies called "fried-egg colonies". However, its isolation from clinical specimens has proven extremely difficult. G-37^T and M30 could grow in SP4 medium at the initial cultivation. After the recovery of *M. genitalium*, many researchers have tried to isolate *M. genitalium* from genital specimens, but all failed to directly inoculate the genital specimens to the SP4 medium.[11]

Jensen reported a new method for the isolation of *M. genitalium* from the clinical specimens using co-cultivation with Vero cell in Eagle's minimal essential medium supplemented with serum substitute and glutamine.[12] This method was reasonable, because the *M. genitalium* attaches to animal cells by adhesin and invades them. After *M. genitalium* grew adequately, an adaptation for the axenic culture medium was attempted. Through this method, more than 30 strains were reported to be isolated from clinical specimens.[12–15] However, the success rates for their initial growth in Vero cell culture were not high; the success rate for adaptation to the axenic culture medium was even lower. The isolation of *M. genitalium* from clinical specimens has been obviously difficult and time-consuming.

M. genitalium has the smallest known genome size of a self-replicating organism, at 580 kbp. The full sequence of the *M. genitalium* gene was reported in 1995,[16] and the synthesis of the complete genome was carried out in 2008.[17] It is thought that *M. genitalium* possesses the minimum functional genes to maintain life.

Pathogenicity of *M. genitalium*

To establish that *M. genitalium* is a cause of disease in humans, Taylor-Robinson proposed a modification to the Henle-Koch postulates.[18] This proposal focused on epidemiological studies, studies in animal models, antimicrobial susceptibility testing *in vitro*, clinical responses to antimicrobials and transmissibility. In a chimpanzee animal model, *M. genitalium* grew at the urethra or vagina, induced inflammatory responses and caused an elevation of serum antibodies.[19] The sexual transmission of *M. genitalium* between couples was shown using DNA sequence typing.[20] Many epidemiological studies have shown a prevalence of *M. genitalium* among patients with male urethritis, cervicitis and other diseases. Now, *M. genitalium* is considered an established cause of male NGU[7,10,21] and female cervicitis.[7,10,22,23] Furthermore, acute epididymitis,[24,25] chronic prostatitis[26,27] and balanoposthitis[28] in males, in addition to pelvic inflammatory disease,[29–31] female urethritis[32] and adverse pregnancy events[33] in females, are considered to be related to *M. genitalium* infections; however, further studies are required to determine that *M. genitalium* can cause these diseases.[23,29,34]

Prevalence of *M. genitalium* in male patients with urethritis in Japan

Nucleic acid amplification tests have been used to detect *M. genitalium* in the clinical specimens of patients. Taylor-Robinson analyzed 38 papers on male urethritis between 1993 and 2010 that showed the prevalence rates of *M. genitalium* in male urethritis.[7] *M. genitalium* was detected from the urethras of 15–25% of male patients with the NGU symptoms of discharge and urethral pain. The prevalence of *M. genitalium* among males without symptoms was 5–10%. The odds ratios and 95% confidence intervals of the association between *M. genitalium* and male NGU or NCNGU were 5.5 (95% CI 4.3–7.0) and 7.6 (95% CI 5.5–10.5), respectively. These data support that *M. genitalium* is a pathogen for male urethritis, including cases of NGU and NCNGU.

In Japan, the test to detect *M. genitalium* in patients has not been accepted by the national insurance system. There-

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