# 考察

- ➢ マイコプラズマ性尿道炎
- ● 細菌学的治癒率（本研究；93.8%）
- ✧ STFX (200mg)
  - ✓ 100% (11/11) (Ito S, et al. J Infect Chemother 2012)
- ✧ AZM (1g)
  - – 100% (3/3) (Takahashi S, et al. J Infect Chemother 2008)
  - – 87.0% (20/23) (Mena LA, et al. Clin Infect Dis 2009)
  - – 66.7% (30/45) (Schwebke JR, et al. Clin Infect Dis 2011)
- ✧ LVFX (500mg)
  - – 60% (3/5) (Takahashi S, et al. J Infect Chemother 2011)
- ✧ GFLX (400mg)
  - – 83.3% (15/18) (Hamasuna R, et al. Sex Transm Infect 2012)

# 結語

- ● STFX
  - – 非淋菌性尿道炎

✓標準治療として推奨できる

# Ⅲ．研究成果の刊行に関する一覧表

# 研究成果の刊行に関する一覧表

〈雑　　誌〉

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| 小野寺　昭一 | 性感染症の最近の動向 | 臨床婦人科産科 | 67(1) | 6 -12 | 2013 |
| Ryoichi Hamasuna | Identification of treatment strategies for *Mycoplasma genitalium*-related urethritis in male patients by culturing and antimicrobial susceptibility testing | Journal of Infection and Chemotherapy | 19 | 1 -11 | 2013 |
| Ryoichi Hamasuna, Satoshi.Takahashi, Shinya Uehara, Tetsuro Matsumoto | Should urologists care for the pharyngeal infection of *Neisseria gonorrhoeae* or *Chlamydia trachomatis* when we treat male urethritis? | Journal of Infection and Chemotherapy | 18 | 410-413 | 2012 |
| 濵砂　良一 | 特集　性感染症の現状と治療の問題点.　淋菌感染症 | 化学療法の領域 | 28(5) | 765-772 | 2012 |
| 余田　敬子 | 特集　性感染症　診断・治療ガイドライン2011を読んで淋菌の咽頭感染、クラミジアの咽頭感染に関する更新、改訂について | 泌尿器外科 | 25(9) | 1783-1787 | 2012 |
| Michinori Terada, Koji Izumi, Emiko Ohki, Yuka Yamagishi, Hiroshige Mikamo | Antimicrobial efficacies of several antibiotics against uterine cervicitis caused by *Mycoplasma genitalium* | Journal of Infection and Chemotherapy | 18 | 313-317 | 2012 |
| Ayumi Taguchi, Kei Kawana, Terufumi Yokoyama, Katsuyuki Adachi, Aki Yamashita, Kensuke Tomio, Satoko Kojima, Katsutoshi Oda, Tomoyuki Fujii, Shiro Kozuma | Adjuvant effect of Japanese herbal medicines on the mucosal type 1 immune responses to human papillomavirus (HPV) E7 in mice immunized orally with *Lactobacillus*-based therapeutic HPV vaccine in a synergistic manner | Vaccine | 30 | 5368-5372 | 2012 |

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Satoko Kojima, Kei Kawana, Kensuke Tomio, Aki Yamashita, Ayumi Taguchi, Shiho Miura, et. al | The prevalence of cervical regulatory T cells in HPV-related cervical intraepithelial neoplasia (CIN) correlates inversely with spontaneous regression of CIN | American Journal of Reproductive Immunology | 69 | 134-141 | 2013 |
| Satoshi Takahashi, Yuichiro Kurimura, Jiro Hashimoto, Teruhisa Uehara, Yoshiki Hiyama Akihiko Iwasawa, et. al | Antimicrobial susceptibility and penicillin-binding protein 1 and 2 mutations in *Neisseria gonorrhoeae* isolated from male urethritis in Sapporo, Japan | Journal of Infection and Chemotherapy | 19 | 50-56 | 2013 |
| 髙橋　聡 | 性感染症－尿道炎への対応－ | 腎と透析 | 72（増刊） | 492-494 | 2012 |
| 髙橋　聡 | 性感染症における診断キットの有用性と限界 | 小児科 | 53 | 467-472 | 2012 |

〈書　　籍〉

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者名 | 書　籍　名 | 出版社名 | 出版地 | 出版年 | ページ |
|---|---|---|---|---|---|---|---|
| 余田　敬子 | 第8章 アレルギー・感染症の検査 実戦的STI検査 | 小林　俊光 | 実戦的耳鼻咽喉科検査法（ENT「耳鼻咽喉科」臨床フロンティア） | 中山書店 | 日本 | 2012 | 181-189 |

# Ⅳ．研究成果の刊行物・別刷

性感染症

# 性感染症の最近の動向

小野寺 昭一


- 性感染症定点調査：1987年から厚生省（現厚生労働省）結核・感染症サーベイランス事業として行われている．診療科の内訳はおおよそ産婦人科系（産科，婦人科，産婦人科の合計）49%，泌尿器科41%，皮膚科9%，性病科1%の比率である．
- 性感染症診断・治療ガイドライン：日本性感染症学会では，2002年から性感染症の診断・治療のためのガイドラインを発行している．原則として2年ごとに改訂し，2004年版，2006年版，2008年版，2011年版まで発行されている．
- セフトリアキソン高度耐性淋菌：2009年2月にわが国の風俗関係の女性の咽頭からセフトリアキソンのMICが2 μg/mLと高度耐性の淋菌が分離され，世界に衝撃を与えた．幸いに現時点で，その後にこのような高度耐性淋菌が分離されたとの報告はない．


## はじめに

わが国では，1987年から国立感染症研究所感染症情報センターが性感染症の発生動向調査を行っており，定点把握疾患として，性器クラミジア感染症，性器ヘルペス，尖圭コンジローマ，淋菌感染症の4疾患のサーベイランスが行われている．定点数は当初，全国約600の医療機関であったが，現在では960前後の医療機関から報告されている．

本稿では，定点サーベイランスと全数把握の梅毒の報告を中心にわが国の性感染症の最近の動向について述べ，合わせて2012年1月に告示された新たな「性感染症に関する特定感染症予防指針」の主な改正点についても述べることとする．

おのでら しょういち：富士市立中央病院（〒417-8567　静岡県富士市高島町50）


0386-9865/13/¥500/論文/JCOPY

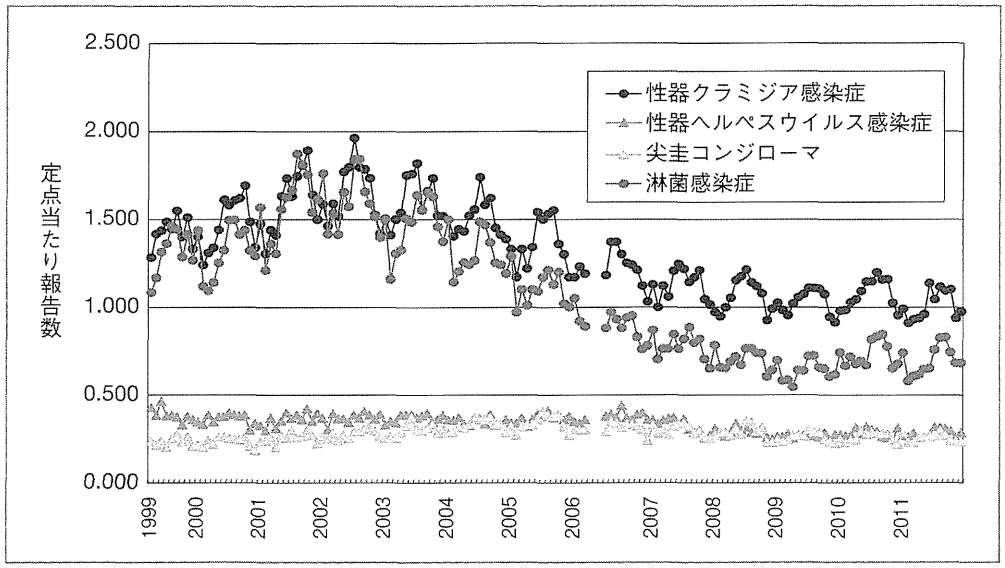



**図1　定点把握4性感染症の年次推移：1999～2011（男性）**

（感染症発生動向調査，2012年1月13日現在）

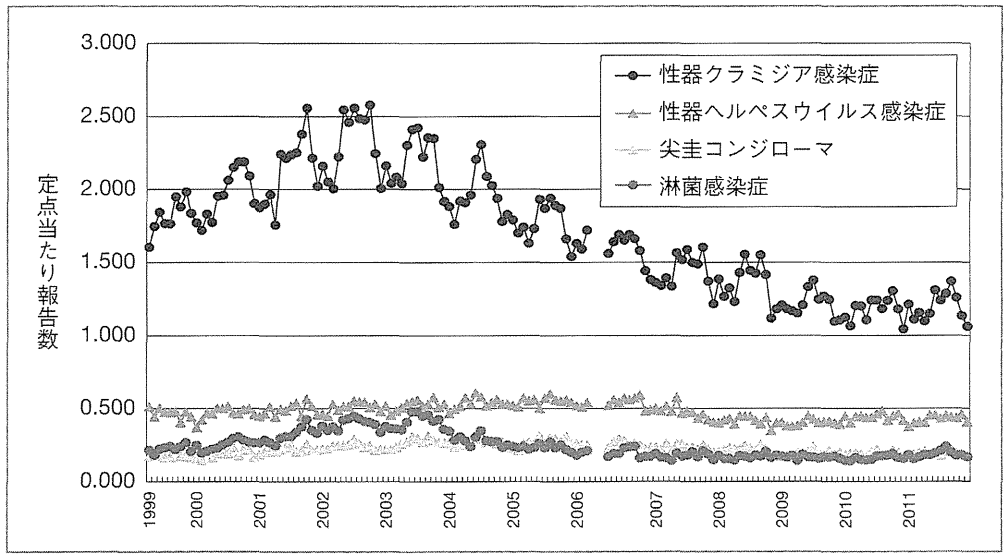



**図2　定点把握4性感染症の年次推移：1999～2011（女性）**

（感染症発生動向調査，2012年1月13日現在）

## 定点把握4性感染症の年次推移

1999年から2012年1月までの性感染症の動向を**図1～3**に示した[1]．

男性では性器クラミジア感染症が最も多く，2番目に多いのが淋菌感染症で，性器ヘルペス，尖圭コンジローマはほぼ同数となっている．一方，女性では，性器クラミジア

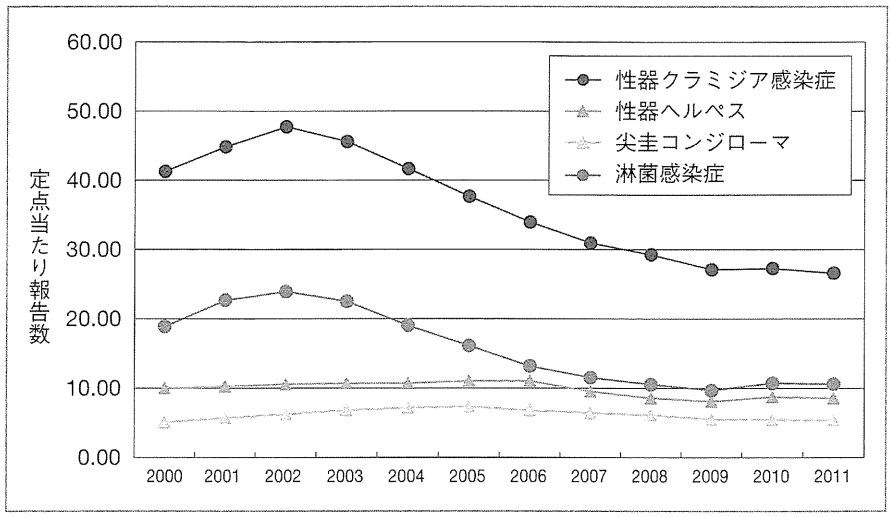



**図3　定点把握4性感染症の年次推移：定点当たり報告数，2000～2011（男女合計）**
（感染症発生動向調査，2012年1月13日現在）

感染症が全体の約60％と突出して多く，第2位が性器ヘルペスで，淋菌感染症と尖圭コンジローマの報告数はほぼ同数となっている．

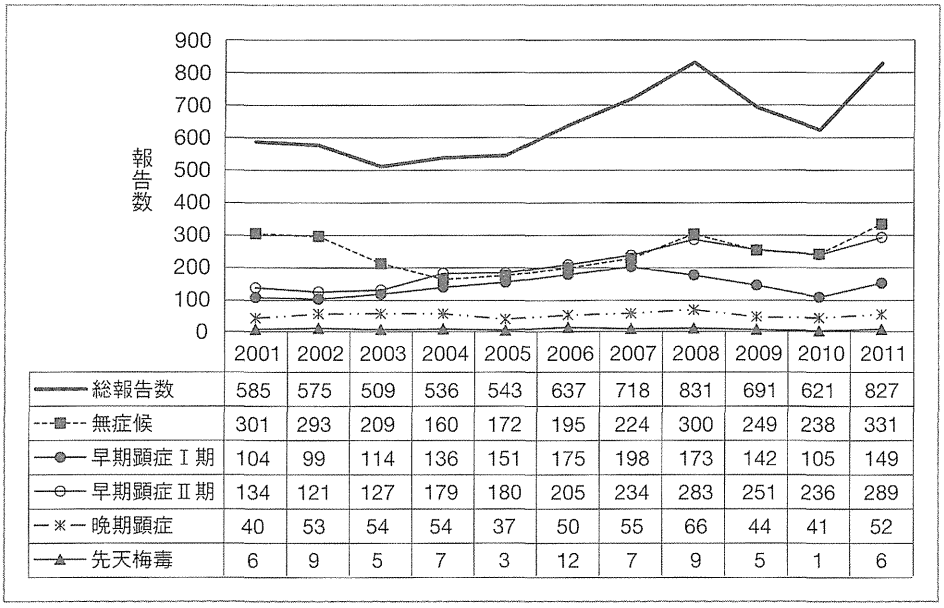
性器クラミジア感染症は，男性，女性とも2003年頃から減少傾向がみられ，2009年まで続いたが，2010年，2011年はほぼ横ばいの状態となっている．淋菌感染症の動向は，男性では性器クラミジア感染症とほぼ同じであるが，女性では1年遅れの2004年から減少し，2010年以降は横ばいか微増の状態となっている．性器ヘルペス，尖圭コンジローマは男女とも全体を通して横ばい傾向が続いていたが，2010年以降，男女ともやや増加傾向がみられている（**図1～3**）．

次に，疾患ごとに年齢別の動向をみてみる．

性器クラミジアの2003年以降の減少は，男女とも10歳台後半から20歳台までの若い世代で目立っていた．ただ，男性では2010年以降に20歳台後半から上の世代でやや増加する傾向がみられている．

淋菌感染症では男女とも20歳台が発症のピークとなっているが，女性では10歳台後半でも20歳台後半と同程度の発症者がみられている．若い世代で2003年以降に明らかな減少傾向がみられたのは性器クラミジアと同様の傾向であったが，2011年には男女とも多くの年齢層で再増加の傾向がみられ，今後の動向に注意をする必要がある．

性器ヘルペスは，ここ数年間20歳台から30歳台の年齢層で男女とも減少傾向がみられていたが，2010年以降，多くの年齢層で増加傾向がみられている．なお，性器ヘルペスは男女とも年齢が上がるにつれて発生頻度が高くなり，特に女性では，40歳台後半以降は最も多い性感染症となっている．男性では女性ほど極端ではないにしても，やはり60歳台以降になると性器ヘルペスが最も頻度が高い性感染症となっている．このように性器ヘルペスがほかの性感染症と異なり高齢者にも初発例の報告がある理由として，その多くは，若い年齢で感染して潜伏していた性器ヘルペスウイルス（HSV）




| | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 総報告数 | 585 | 575 | 509 | 536 | 543 | 637 | 718 | 831 | 691 | 621 | 827 |
| 無症候 | 301 | 293 | 209 | 160 | 172 | 195 | 224 | 300 | 249 | 238 | 331 |
| 早期顕症Ⅰ期 | 104 | 99 | 114 | 136 | 151 | 175 | 198 | 173 | 142 | 105 | 149 |
| 早期顕症Ⅱ期 | 134 | 121 | 127 | 179 | 180 | 205 | 234 | 283 | 251 | 236 | 289 |
| 晩期顕症 | 40 | 53 | 54 | 54 | 37 | 50 | 55 | 66 | 44 | 41 | 52 |
| 先天梅毒 | 6 | 9 | 5 | 7 | 3 | 12 | 7 | 9 | 5 | 1 | 6 |

**図4　病期別梅毒報告数の年次推移：2001～2011（男女合計）**

（感染症発生動向調査，2012年3月3日現在）

が再活性化することによるのではないかとされている[2]．

尖圭コンジローマは，男女とも4性感染症全体の約10%を占めている．2005年頃までは男女ともゆるやかな増加傾向がみられていたが，2006年以降は減少し，ここ数年はやや増加か横ばい状態となっていた．男性では，発症年齢に明らかなピークがみられないのは性器ヘルペスと同じ傾向であるが，2010年以降は20歳台前半，30歳台後半，40歳台など多くの年齢層で増加がみられている．一方，女性では20歳台前半がピークとなっており，多くの年齢層で横ばい傾向となっている．

## 梅毒の年次推移

全数把握性感染症の1つである梅毒は，男女合わせた合計数から年次推移をみると，2006年から2008年まで緩やかな増加がみられたが，2009年，2010年と減少し，2011年には再び増加していた[1]（**図4**）．梅毒は全数届け出が必要とされる疾患であるが，ここ8年間で最も報告数が多かった2008年においても男性617件，女性では214件で男女合わせても831件にとどまっており，現実とは乖離している印象がある．届出が行われていない患者，あるいは潜在的な患者が多数存在することが予想され，今後の届出制度の見直しが必要であろう．

なお，梅毒の感染経路をみると，男女の差が明らかになっている．女性では異性間の性的接触による感染が54%となっているのに対し，男性では，異性間の性的接触が38%，同性間の性的接触が約30%で同性間の性的接触による感染者が1/3を占めている[1]．最近の早期梅毒流行の原因として，MSM（男性同性愛者）における流行が問題

となっており，これらの患者ではHIVの感染率が高いことも重要である[3]．

わが国での性器クラミジア感染症や淋菌感染症の報告数は，すでに述べたように2003年頃から減少傾向にあったが，梅毒の届け出数は，逆にこの時期に増加傾向となっていることに注意をする必要がある．このことは，クラミジアや淋菌感染症と梅毒はそれぞれ異なる集団に発生していることを示していると思われる．すなわち，クラミジアや淋菌感染症の多くが主として異性間感染であるのと異なり，梅毒はMSMにおける発生率が高いことを示唆していると考えられる．

## 各性感染症における現状の問題点

### 性器クラミジア感染症

わが国における2009年頃までの性器クラミジアの減少傾向は，男女とも10歳台後半から20歳台までの若年世代で顕著にみられていることはすでに述べたとおりであるが，この減少の理由を証明する明確なデータは示されていないのが現状である．一方，近年，10歳台後半から20歳台の若年層では，性行為に関心がないあるいは嫌悪すると考える若者の比率が高くなっているともいわれており[4]，異性との性行為を求める若者が減少しているとする報告がある．こうした若者の性の変化は，最近よくいわれる若者のコミュニケーション不足と関連している可能性があり，時代背景として，ゲームやインターネットなどに熱中して異性との交流を求めない若者が増加していることと関連しているのかもしれない．もしそうであれば，近年の性器クラミジア感染症の減少は必ずしも性感染症に関する予防教育，あるいはその予防のための啓発活動が功を奏したとはいえないことになり，決して楽観視してはならないことを示唆していると思われる．

さらにこの定点サーベイランスによる報告数は，あくまでも有症状者を対象としたものであり，性感染症のスクリーニングによって発見された感染者，あるいは女性では約半数とされる無症候感染者はこのサーベイランスには含まれていないことに留意する必要がある．今後は無症候感染者を含めた性器クラミジア感染症のスクリーニングシステムを構築し，若者の性感染症を早期発見，早期治療に結びつけられる施策を講じることが必要であろう．

### 淋菌感染症

わが国での淋菌感染症の動向に影響を与えている要因として，咽頭の淋菌感染者の存在と薬剤耐性淋菌の増加の問題を見逃すことはできない．

性器の淋菌感染症患者の10～30％に咽頭からも淋菌が検出されることが知られており[5]，この多くは炎症症状が自覚されないか，あるいは咽頭の違和感などの軽い症状しか呈さない．しかも咽頭に存在する淋菌を除菌できる抗菌薬はきわめて限定されているため，治療を行って性器の淋菌が消えても咽頭の淋菌は存続し，感染が蔓延する温床となっていると考えられる．さらに薬剤耐性淋菌については近年，キノロン耐性淋菌や経口セフェム耐性淋菌が増加し，現在わが国のガイドラインで淋菌感染症治療薬として推奨されているのは，いずれも注射薬で，セフトリアキソン，セフォジジム，スペクチノ

マイシンの3剤のみとなっていることが問題である[5]．さらに重要な問題は，このなかで唯一咽頭の淋菌も確実に除菌可能であったセフトリアキソンに対し，高度耐性を示す淋菌がわが国で報告されたことである[6]．もし仮にこのセフトリアキソン高度耐性淋菌が蔓延したとしたら，現状では単独でこの淋菌を完全に除菌しうる抗菌薬は存在しないことになり，大きな社会問題になる．今後，このセフトリアキソン高度耐性淋菌の推移には十分な注意を払う必要がある．

### 性器ヘルペス

HSVは初感染後に知覚神経節に潜伏感染し，再発を繰り返す場合が多い．現在，性器ヘルペスの治療には，アシクロビル，バラシクロビルなどの抗ヘルペスウイルス薬が使用され，一定の治療効果をあげているが，これらの抗ウイルス薬によっても潜伏感染しているHSVを排除することはできない．さらに，性器ヘルペスの再発は，肛門，臀部，大腿部などにも起こりうることが知られており，コンドームの使用だけでは完全に防止することができないことが問題である．理想としてはワクチン戦略が最も有効な予防法であるが，現状ではいまだ十分な予防効果が期待できるワクチンの開発までには達していない．

### 尖圭コンジローマ

わが国では2011年9月に，ヒトパピローマウイルス（HPV）16, 18型に加え，尖圭コンジローマの原因となる6型，11型のHPVを含む4価ワクチンが使用可能になった．その有効性については，オーストラリアでこの4価のワクチンを女性に接種したことによって男性の尖圭コンジローマも減少傾向になったとの報告がある[7]．現時点ではこのワクチンの適応はわが国では女性に限られているが，子宮頸がんとともに，尖圭コンジローマに対してもワクチンによって予防が可能であることについては，広く情報提供を行うことが必要である．

## 「性感染症に関する特定感染症予防指針」の改正について

「性感染症に関する特定感染症予防指針」は，2012年1月19日に改正された予防指針が告示されたが，ここでは主な改正事項について紹介する．

まず前文で，性感染症は，性器，口腔などを介した性的接触で感染することが追記された．また，性的接触を介して感染する可能性があり，連携して対策をとる感染症の例示として後天性免疫不全症候群のほかにB型肝炎が追加された．

「第一　原因の究明」のなかの「発生動向の調査の活用」では，国は定点把握の性感染症の発生動向が実態を反映したものとなるよう，指定届出機関の指定の基準（定点選定法）をより具体的に示すことが追記された．

「第二　発生の予防及び蔓延の防止」では，性感染症の予防方法として予防接種が追記された．また，コンドームについては，その効果とともに，コンドームだけでは防ぐことができない性感染症があることや，その正しい使い方などの具体的な情報の普及啓

発に努めるべきとされた．また，性器クラミジア感染症および淋菌感染症の病原体検査に，尿を検体とするものを含むことが追記され，対象者の実情に応じた対策として，若年層に対する情報提供では適切な媒体を用いることなどが追記された．さらに，尖圭コンジローマについては，子宮頸がんとともにワクチンによっても予防が有効であることから，ワクチンの効果などについての情報提供を行うことが重要と追記された．

「第三　医療の提供」に関して，学会などの関係団体は標準的な診断や治療の指針などについて積極的に情報提供し，普及を図ることが重要とされ，国および都道府県などは，学会などとの連携により，性感染症の専門家養成のための教育および研修機会の確保を図ることが重要とされた．また，若年層などが性感染症に関して，受診しやすい医療体制の整備などの環境づくりとともに，保健所などでの検査から受診・治療に結び付けられる体制づくりを推進することが重要であることも記述されている．

「第四　研究開発の推進」では，発生動向などに関する疫学研究の推進にあたって，病原体の分子疫学や薬剤耐性に関する研究を行うことが追記されたが，これはわが国での薬剤耐性淋菌の増加への対応について述べられたものである．さらに，社会面と医学面における性の行動様式などに関する研究として，感染リスクや感染の防止に関する意識・行動などを含むことも記載されている．

今回の改正では，口腔を介した性感染症の予防策や予防接種による予防など新たな感染対策も追記された．全体に性感染症の予防のための施策に重点をおいた改正であるといえるが，若年層が受診しやすい環境づくりや医療体制の整備を推進することの重要性についても明記され，今後の性感染症対策をより具体的に示した改正と思われる．

## おわりに

わが国の性感染症の動向について定点把握調査の結果を中心に述べ，2012年1月に改正された「性感染症に関する特定感染症予防指針」の改正の概要についても述べた．わが国の性感染症対策は，疾患ごとにその特徴を把握しながら具体的な対策を構築していくべきであると思われる．また，口腔を介した性的接触に伴う性感染症の情報提供，コンドームだけでは予防できない性感染症も存在すること，またワクチンによる性感染症予防に関する情報提供を進めることなどが重要であり，さらには若者が受診しやすい環境の整備をより積極的に行うことも重要な課題であろう．

### 文献


1) 岡部信彦，多田有希：性感染症発生動向調査から見たわが国の性感染症の動向．性感染症に関する予防，治療の体系化に関する研究班（研究代表者：小野寺昭一）平成23年度総括研究報告書，2011
2) 川名　尚：性器ヘルペスの現状と治療の問題点．化療の領域28：70-83，2012
3) 佐藤文哉，河野真二，加藤哲朗，他：HIV感染者の梅毒に関する検討．日性感染症会誌20：192-197，2009
4) 北村邦夫：「第5回男女の生活と意識に関する調査」結果報告．JASE現代性教育ジャーナル（7）：1-6，2011
5) 淋菌感染症，性感染症診断・治療ガイドライン2011．日性感染症会誌22(supple)：52-59，2011
6) 山元博貴，雑賀　威，保科眞二，他：淋菌感染症におけるセフトリアキソン（CTRX）耐性の1例．日性感染症学会誌21：98-102，2010
7) Donovan B, Franklin N, Guy R：Quadrivalent human papillomavirus vaccination and trends in genital warts in Australia：analysis of national sentinel surveillance data. Lancet Infect Dis 11：39-44, 2011

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REVIEW ARTICLE

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

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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

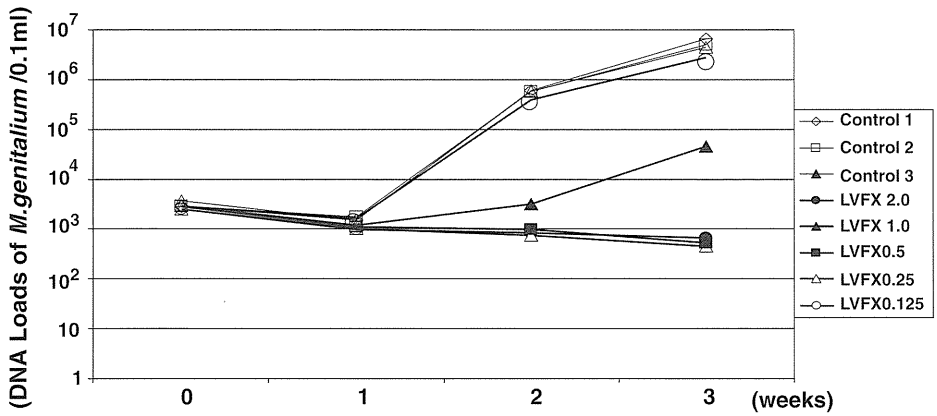
obtaining *M. genitalium* strains that can grow in axenic culture medium takes a long time.

"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 |