fore, the data for the prevalence rates of *M. genitalium* are limited. Table 1 shows the prevalence of *M. genitalium* and *C. trachomatis* among male patients in Japan.[35–48] The study of *M. genitalium* in Japan began with Deguchi *et al.* at Gifu University. These researchers carried out many early studies and developed a new phylogeny-based real-time PCR test that amplifies a portion of the 16s *r*RNA gene, and can detect *M. genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplsma parvum*.[49,50] This test is supported by an examination company and commercially available. The detection rate of *M. genitalium* in patients with NGU was 10–15%, and although the rates in recent studies increased slightly to approximately 20%, it can be said that the detection rates of *M. genitalium* have not changed substantially. The detection rates of *M. genitalium* in European studies were higher than those in Japan;[7,10] it remains unclear why the prevalence of *M. genitalium* might have a regional difference.

Diagnosis for urethritis with *M. genitalium*

Male urethritis shows symptoms of discharge from the urethral meatus and urethral pain; in addition, it is important to detect increases in WBC counts in the urethral smear, urinary sediments or uncentrifuged urine. As the criterion for urethritis, counts of ≥5 polymorphonuclear leukocytes per high-power field (×1000) in the urethral smear by Gram stain are generally used.[35–40,45–47] Furthermore, counts of ≥5 WBC per high-power field (×400) in the urinary sediment of FVU[41,43–45] or counts of ≥10 WBC/μL in uncentrifuged FVU[44,48] are also used in clinical situations.

M. genitalium is closely related to symptomatic NGU; *M. genitalium* has been detected more frequently from specimens of men with symptomatic NGU than specimens of men without symptoms. Furthermore, *M. genitalium* is related to persistent or recurrent NGU after treatment of male urethritis by tetracyclines,[51,52] fluoroquinolones[37,53] or azithromycin.[54] The severities of discharge or the urethral pain by *M. genitalium*-infection were varied, and the clinical features of *M. genitalium*-related urethritis are indistinguishable from those of chlamydial NGU. The urethritis with *M. genitalium* cannot be diagnosed by symptoms only.

For detection of *M. genitalium*, the FVU or the urethral swab specimens are used for nucleic acid amplification tests as described in Table 1. Generally, FVU specimens are available as a painless method in Japan. In the Japanese literature, the amplicons for PCR to detect *M. genitalium* were portions of 16S *r*RNA or adhesion gene.[35–41,43–48]

Antimicrobial susceptibility of *M. genitalium*

Because it is still difficult to isolate of *M. genitalium* from clinical specimens, the antimicrobial susceptibility of *M. genitalium* is extremely limited. Earlier reports showed that *M. genitalium* strains were sensitive to tetracyclines, macrolides and fluoroquinolones; however, it has become clear that there are some differences among the responses to these types of antimicrobials. Table 2 shows the antimicrobial susceptibilities of 23 *M. genitalium* strains.[55] Among the 10 tested antimicrobials, macrolides showed the strongest activity against *M. genitalium*. However, the MIC of azithromycin and clarithromycin against one strain were ≥250 μg/mL and 128 μg/mL, respectively. This strain was evidently a macrolide-resistant strain, which signals a considerable problem, as this resistant strain has emerged and is spreading worldwide, as described later. Among the fluoroquinolones, there were large differences among the seven tested agents; sitafloxacin and moxifloxacin showed strong activity. The MIC of tetracyclines, such as minocycline, doxycycline and tetracycline, were not substantially lower; two strains had a higher MIC against tetracycline.

Clinical studies and antimicrobial resistance

Tetracyclines and macrolides

In earlier studies, the tetracyclines and the macrolides were tested, and their efficacies in the treatment of urethritis with *M. genitalium* were compared. In Gambini's report, the microbiological efficacies of doxycycline 200 mg/day for 7 days and azithromycin 1 g stat were 94.3% and 82.4%, respectively.[56] However, two more recent studies showed that the efficacy of doxycycline against *M. genitalium* was inferior to that of azithromycin.[57,58] The *M. genitalium* eradication rates for doxycycline 200 mg/day for 7 days at the urethra were found to be 45.2% and 30.8%, despite relatively good *in vitro* MIC of doxycycline in most *M. genitalium* strains.[55]

Clinical studies using azithromycin showed good results, with an eradication rate of *M. genitalium* of almost 100% before Bradshaw's report.[59] Bradshaw *et al.* carried out an open-label clinical study in Australia, and used azithromycin 1 g stat on 34 male patients with *M. genitalium*, among whom *M. genitalium* persisted in nine. Three patients were treated with additional azithromycin 1 g for three more attempts, but all of the trials failed. Finally, *M. genitalium* was treated by moxifloxacin 400 mg/day for 10 days. The urethral swab specimens of these patients were sent to Jensen's laboratory in Denmark and newer, high-level, macrolide-resistant *M. genitalium* strains were isolated.[15] Seven macrolide-resistant strains were isolated from Australia or Scandinavia. This macrolide-resistance was found to be related to genetic mutations on region V of the 23S *r*RNA gene, a similar type of mutation to that observed in macrolide-resistant *Mycoplasma pneumonia* strains.[60,61]

As aforementioned, small numbers of macrolide-resistant *M. genitalium* strains have actually been isolated and cultured. However, it became clear that mutations of the 23S

Table 1 Prevalence of *Mycoplasma genitalium* and *Chlamydia trachomatis* among male patients with urethritis in Japanese studies

Author	Study period	Patients Diagnosis for urethritis	Specimens Method for detecting *M. genitalium*	Prevalence % (*n*)		Others
				M. genitalium	*C. trachomatis*	
Deguchi[35]		114 with NGU ≥5 WBC/hpf (×1000) of urethral smear	Urethral swab, PCR for MgPa	14.9 (17)	–	
Maeda[36]	1995–1997	76 with NGU ≥5 WBC/hpf (×1000) of urethral smear	Urethral swab, PCR for MgPa	13.2 (10)	55.2 (42)	
Maeda[37]	1999–2000	72 with NGU ≥5 WBC/hpf (×1000) of urethral smear	FVU, PCR for 16S rRNA	17.1(13)	45.9 (34)	Clinical study by levofloxacin
Yoshida[38]	1999–2000	93 with NGU ≥5 WBC/hpf (×1000) of urethral smear	FVU, PCR-microtiter hybridization assay	15.1 (14)	50.5 (47)	
Maeda[39]	1999–2002	153 with NGU ≥5 WBC/hpf (×1000) of urethral smear or ≥15 WBC hpf of urinary sediments of FVU	FVU, PCR-microtiter hybridization assay	17.0 (26)	47.4 (73)	
Yokoi[40]	1999–2005	104 with post-GU ≥5 WBC/hpf (×1000) of urethral smear	FVU, PCR-microtiter hybridisation assay	9.6 (10)	49.0 (51)	*M. genitalium* was detected from 3.8% of GU
Takahashi[41]	2004	42 with NGU ≥5 WBC hpf of urinary sediments	FVU, PCR-microtiter hybridization assay	7.1 (3)	38.1 (16)	Clinical study by azithromycin
Shimada[42]	2006–2008	308 specimens from patients with urethritis	Stocked FVU	18.8 (58)	–	Retrospective study for stocked specimens
Takahashi[43]	2009–2010	87 with NGU ≥5 WBC hpf of urinary sediments	FVU, PCR-microtiter hybridization assay	4.6 (4)	21.8 (19)	Clinical study by levofloxacin
Hamasuna[44]	2008	135 with NGU ≥5 WBC hpf of urinary sediments or ≥10 WBC/1 μL of FVU	FVU, real-time PCR for adhesion gene, PCR for 16S rRNA gene	13.3 (18)	32.6 (44)	Clinical study by gatifloxacin
Hagiwara[45]	2004–2007	194 with NGU ≥5 WBC/hpf (×1000) of urethral smear or ≥15 WBC hpf of urinary sediments of FVU	FVU, PCR-microtiter hybridization assay	20.1 (39)	–	Clinical study by azithromycin
Shigehara[46]	2008–2010	176 with urethritis including GU ≥5 WBC/hpf of urethral swabs	Liquid-based samples, PCR for adhesion protein gene	18.2 (32)	25.6 (45)	Specimens were collected by rubbing the distal urethra
Kawaguchi[47]	2009–2010	121 with NGU ≥5 WBC/hpf of urethral swabs	Liquid-based urine samples, PCR for adhesion protein gene	15.7 (19)	19.0 (23)	
Ito[48]	2009–2011	89 with NGU ≥10 WBC/1 μL of FVU	FVU, PCR-microtiter hybridization assay	15.7 (14)	49.4 (44)	Clinical study using sitafloxacin

Table 2 Antimicrobial MIC distribution for 23 *Mycoplasm genitalium* strains

Antibacterial agent	MIC† (μg/mL)															
	≤0.002	0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	≥16	MIC50	MIC90
Azithromycin	22													1	0.001	0.002
Clarithromycin	9	9	4											1	0.004	0.008
Doxycycline						7	7	7	1		1				0.125	0.25
Minocycline					3	8	9	3							0.125	0.25
Tetracycline						3	11	5	2		1			1	0.125	0.5
Norfloxacin										2	1	2	3	15	32	64
Ciprofloxacin						1	1	1	3	1	3	10	3		4	8
Levofloxacin							1	5	1	11	5				1	2
Gatifloxacin					2	3	5	11	2						0.25	0.5
Moxifloxacin				1	5	11	5	1							0.06	0.125
Sitafloxacin			2	2	5	6	7	1							0.06	0.125

†MIC were measured using the broth-dilution method.

*r*RNA gene and the failure of azithromycin treatment regimens were closely related. Therefore, if mutations of the 23S *r*RNA gene are found in the *M. genitalium* genome obtained from the urine or urethral swab specimens, we can say that patients were infected by macrolide-resistant *M. genitalium*, although these genotypes were not detected in all specimens from patients who showed azithromycin treatment failure. Macrolide-resistance in *M. genitalium* seems to be spreading worldwide, and has been reported in Scandinavian countries,[15] France,[62] Australia,[59,63] New Zealand,[64] Greenland[65] and Japan.[66] In Australia, 27.2% of pretreatment specimens showed mutations related to macrolide-resistance.[63] In Greenland, these mutations were detected from all tested specimens of *M. genitalium*.[65] In Japan, Ito *et al.* showed that the same mutations were found in four urine specimens from seven patients with azithromycin treatment failure.[66] Importantly, it was discovered that the use of azithromycin, in particular at 1 g stat, had the potential to induce mutations on the 23S *r*RNA gene, as some reports indicated that the mutations on 23S *r*RNA were found in post-treatment specimens that had no mutations present in pretreatment.[15,67] In the recent randomized control trial of treatment of NGU by azithromycin or doxycycline regimen, the efficacy of azithromycin showed some decline from the 1990s.[68] However, azithromycin is still effective and can constitute a first-line treatment for NGU arising from both *M. genitalium* and *C. trachomatis*. In addition, azithromycin 2 g stat is acceptable for use in urethritis or cervicitis in Japan; therefore, evidence on the treatment of NGU will continue to build over the years.

Fluoroquinolones

The first clinical trial that used fluoroquinolone for urethritis with *M. genitalium* was carried out in Japan.[37] Levofloxacin 100 mg, three times/day for 7 days were tested, but the microbiological efficacy was just 36.4%. Furthermore, three other clinical studies were carried out in Japan that used regimens of levofloxacin,[43] gatifloxacin[44] or sitafloxacin[48] (Table 3). In Europe and Australia, two studies on regimens of moxifloxacin or ofloxacin were carried out.[59,69] We can extrapolate important information from these studies on the microbiological efficacies of fluoroquinolones and MIC.[44] The microbiological efficacies of levofloxacin, gatifloxacin, moxifloxacin and sitafloxacin were 33–60%, 83.3%, 100% and 100%, respectively, and the MIC90 of these agents were 2 μg/mL. 0.5 μg/mL, 0.125 μg/mL and 0.125 μg/mL, respectively. Considering the tissue level of fluoroquinolone, the optimal activity against *M. genitalium* might be at an MIC90 of 0.125 μg/mL. As the use of moxifloxacin is limited to only respiratory infections in Japan, sitafloxacin is the recommended fluoroquinolone for *M. genitalium* infection; however, further research on this agent is required.

Fluoroquinolone-resistance is known to exist in Gram-negative bacterial species and mutations in the gyrase genes, as *gyrA* and *parC* are known to be closely related to resistance. Deguchi *et al.* showed mutations on *gyrA* and *parC* genes from purified *M. genitalium* DNA obtained from urine specimens that had shown treatment failure with levofloxacin 100 mg, three times/day for 7 days.[70] They also detected mutations on the *gyrA*, *gyrB*, *parC* and *parE* genes of *M. genitalium* DNA from pretreated urine specimens.[42,71] In our past study on gatifloxacin, genetic mutations on *gyrA* or *parC* of *M. genitalium* were detected and linked to the efficacy of fluoroquinolone.[72] Of the 18 patients in the present study, *M. genitalium* remained in the specimens of three patients. Mutations on the *gyrA* or *parC* genes were found in the *M. genitalium* DNA of these three patients after treatment. No mutations on the *gyrA* gene of three *M. genitalium* DNA samples from pretreatment specimens were

Table 3 Clinical studies for treatment of urethritis with *Mycoplasma genitalium* by fluoroquinolones

Author Country	Year	Regimens for treatment	Microbiological efficacies† % (numbers of patients with treatment success/numbers of enrolled patients)	Comments
Maeda[37] Japan	2001	Levofloxacin 100 mg × 3/day, 7 days	36.4% (4/11)	
Bradshaw[59] Australia	2006	Second or third line treatment Moxifloxacin 400 mg × 1/day, 10 days	100% (9/9)	Moxifloxacin was used for patients with treatment failure by azithromycin regimens
Jernberg[69] Norway	2008	First line treatment		Ofloxacin or moxifloxacin was used for patients with treatment failure by azithromycin regimens
		Ofloxacin 200 mg × 2/day, 10 days	44.4% (4/9)	
		Moxifloxacin 400 mg/day, 7 days	100% (3/3)	
		Second or third line treatment		
		Ofloxacin 200 mg × 2/day, 10 days	58.3% (21/36)	
		Moxifloxacin 400 mg/day, 7 days	100% (24/24)	
Takahashi[43] Japan	2011	Levofloxacin 500 mg/day 7 days	60% (3/5)	
Hamasuna[44] Japan	2011	Gatifloxacin 200 mg × 2/day, 7 days	83.3% (15/18)	
Ito[48] Japan	2012	Sitafloxacin 100 mg × 2/day, 7 days	100% (11/11)	

†Microbiological efficacies showed the eradication rates of *Mycoplasma genitalium* after treatment by nucleic acid amplification tests.

found; thus, the mutations on the *gyrA* gene were thought to be selected for or induced by treatment. Regarding *parC*, mutations were found on *M. genitalium* DNA from two pretreatment specimens, but different mutations were found in the *M. genitalium* DNA from one of the post-treatment specimens. These data showed the presence of fluoroquinolone-resistant *M. genitalium* in Japan, but future studies are necessary to understand the mechanisms for the fluoroquinolone-resistance of *M. genitalium in vivo* and *in vitro*.

Treatment strategies for urethritis with *M. genitalium*

For NGU, macrolides and tetracycline are recommended in the guidelines of many countries.[2–5] However, tetracyclines are less effective against *M. genitalium*. Azithromycin regimens including 1 g stat or 2 g stat are now recommended, but if the azithromycin regimens fail, we must consider the use of fluoroquinolone, such as sitafloxacin, in Japan. The most important issues include the acceptance of *M. genitalium* examinations by the national insurance system in Japan and the individual treatment of *C. trachomatis* and *M. genitalium* in the not-too-distant future.

Conflict of interest

None declared.

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4. 尿路・性器の感染症

性感染症

065 クラミジア感染症

濵砂良一*

1 概念・病因

クラミジア感染症は *Chlamydia trachomatis* による感染症である。*C. trachomatis* は偏性細胞内寄生体であり，特殊な生活環を持つ。細胞外では，感染性を持つ基本小体（elementary body：EB）という球体の形態をとる（核とリボゾームを充満した細胞質を有する）。EB が宿主細胞に感染すると，網様体（reticular body：RB）という形に変わる。RB は核，細胞質の区別のない構造で，二分裂を繰り返しながら増殖し，宿主の細部質内に封入体を形成する。感染後，20～24 時間後に成熟すると，EB に変わり細胞を破って細胞外に出る。

C. trachomatis は性感染症（sexually transmitted infection：STI）から最も高い頻度で分離される病原体である。性器クラミジア感染症として，男性では主に尿道炎と精巣上体炎を，女性では子宮頸管炎と骨盤内炎症性疾患（pelvic inflammatory diseases：PID）の病態を示す。男性尿道炎患者の 30～40%より分離され，女性では STI の 60～70%を性器クラミジア感染症が占める。また，淋菌感染症の約 20%に *C. trachomatis* が合併感染する[1,2]。

淋菌感染症同様，性器以外の *C. trachomatis* 感染例が増加している。オーラルセックスにより，男女の咽頭から *C. trachomatis* が検出される[3]。慢性扁桃炎や咽頭炎で治療に反応しないものから分離されることもある。またアナルセックスにより直腸炎を起こす。*C. trachomatis* 感染妊婦より新生児が産道感染すると，新生児クラミジア肺炎や新生児結膜炎が起こる。感染母体から生まれた新生児のクラミジア肺炎の発症率は 3～20%といわれる。このほか，わが国では稀であるが，鼠径リンパ肉芽腫症を引き起こすことがある。外陰部の丘疹が潰瘍化し，さらに鼠径部リンパ節が有痛性に大きく腫大し，自壊し排膿する。性器クラミジア感染症の分泌物による眼の汚染により，成人型封入体結膜炎を発症することがある。結膜の充血，眼脂，眼瞼腫脹を主訴とし，眼瞼結膜に濾胞を形成する。

C. trachomatis は，性的活動期にある男女から高い頻度で検出されることが知られている。Imai ら[4]の検討では，症状を有しない性交経験のある女子学生の 9.5%，男子学生の 8.7%より *C. trachomatis* が検出された。

2 診断のポイント

男性の尿道炎のうち，*C. trachomatis* が検出されるものをクラミジア性尿道炎と呼ぶ。感染機会後，1～3 週間で排膿（尿道分泌物），尿道痛といった尿道炎症状を呈する。淋菌性尿道炎と比較すると，発症は比較的緩徐で，症状は軽微なものが多い。尿道分泌物は漿液性で，少量から中等量である。尿道不快感や，尿道掻痒感から，無症候に近い症例も多い。クラミジア性尿道炎の 5%程度に精巣上体炎を合併する。性的活動期の年齢の精巣上体炎では，まずクラミジア性を疑う。ほかの細菌による精巣上体炎と比較すると，陰嚢内容の腫脹は軽度で，発熱の程度も低いことが多い。

女性では感染機会後，1～3 週間で子宮頸管炎を起こす。主たる症状は帯下の増加，不正出血などであるが，半数以上の症例では自覚症状を感じな

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表 1　わが国で使用できるクラミジア検出法

製品名（販売会社）	検出法	検体〔最少検出感度〕
アプティマ™ Combo2 クラミジア/ゴノレア（富士レビオ）	遺伝子増幅法 Transcription mediated amplification（TMA）法	男性：初尿*1，尿道分泌物（尿道スワブ） 女性：子宮頸管スワブ 男女：咽頭スワブ 〔1 IFU/アッセイ〕
BD プローブテック ET クラミジア・トラコマティス ナイセリア・ゴノレア（日本ベクトン・ディッキンソン）	遺伝子増幅法 Strand displacement amplification（SDA）法	男性：初尿*1，尿道分泌物（尿道スワブ） 女性：子宮頸管スワブ 男女：咽頭スワブ 〔1 IFU/アッセイ〕
アキュージーン® m-CT/NG（アボット・ジャパン）	遺伝子増幅法 Real-time PCR 法	男性：初尿*1，尿道分泌物（尿道スワブ） 女性：子宮頸管スワブ 〔1 IFU/アッセイ〕
淋菌，クラミジア・トラコマティス/リアルタイム PCR（ロッシュ・ダイアグノスティックス）	遺伝子増幅法 Real-time PCR 法	男性：初尿*1 女性：子宮頸管スワブ 男女：咽頭検体（うがい液）*2 〔1 IFU/アッセイ〕
イディア PCE クラミジア®（協和メディックス）	酵素抗体法	男性：初尿*1，尿道分泌物（尿道スワブ） 女性：子宮頸管スワブ 〔20 IFU/アッセイ〕
クリアビュークラミジア® アーリアメディカル ラピッドエスピー®クラミジア（DS ファーマバイオメディカル）	免疫クロマトグラフィー法	男性：初尿*1 女性：子宮頸管スワブ 〔1,000EB/ml（クリアビュー）〕 〔80 EB/アッセイ（ラピッドエスピー）〕
ヒタザイム®クラミジア（日立化成）	抗体測定（IgA，IgG）	血液

*1排尿後，1時間以上を経過した初尿を採取。
*2滅菌生理食塩水を 15～20 ml 口に含み，10～20 秒間うがいをしたものを検体とする。

いといわれている。*C. trachomatis* は子宮頸管から子宮付属器，腹腔内へ侵入する。子宮付属器炎（卵管炎，卵巣炎），PID を起こし，発熱，下腹部痛，腹部疝痛などの症状を呈する。肝周囲炎を起こすこともある。卵管炎の後遺症として，卵管内腔の狭小化より，卵管性不妊の原因となる。

クラミジア感染症の診断は *C. trachomatis* の検出による。*C. trachomatis* の分離，培養は極めて困難であるため，検出には核酸増幅法，酵素抗体法や免疫クロマトグラフィー法を用いる[1]。また，PID などで腹腔内に感染があるが，子宮頸管から *C. trachomatis* が検出されない場合，抗体検査法も用いられる。わが国では 4 種類の核酸増幅法が使用できる（表 1）が，その診断までに数日間かかる。このため，受診日に診断可能な酵素抗体法や免疫クロマトグラフィー法が用いられることがあるが，核酸増幅法と比較して，その感度は劣る。男性尿道炎では初尿，子宮頸管ではスワブ，また咽頭検体を検査する。

3 治　療

C. trachomatis 感染症に対する治療薬は，マクロライド系，テトラサイクリン系抗菌薬が第一選択薬となる[1,2,5]（表 2）。マクロライドの中でも azithromycin 1 g および 2 g 徐放製剤は単回での治療が可能である。また，ニューキノロン系抗菌薬も症例により選択可能である。テトラサイクリン，ニューキノロンでは 7 日間の投与が必要となる。セックスパートナーの治療，患児の両親の治療も積極的に行う。*C. trachomatis* の上記，マクロライド，テトラサイクリン，ニューキノロンに対する耐性株の報告はあるが，世界的にその蔓延は認められない。

4 ここがポイント

わが国の保険システム上，原則としてクラミジ

表 2　性器クラミジア感染症に対する推奨治療薬

・アジスロマイシン（ジスロマック®）1 g　経口　単回投与
・アジスロマイシン（ジスロマック SR®）2 g　徐放製剤　経口　単回投与
・クラリスロマイシン（クラリス®，クラリシッド®）1 回 200 mg　2 回/日　経口　7 日間
・ミノサイクリン（ミノマイ®）1 回 100 mg　2 回/日　経口　7 日間
・ドキシサイクリン（ビブラマイシン®）1 回 100 mg　2 回/日　経口　7 日間
・レボフロキサシン（クラビット®）1 回 500 mg　1 回/日　経口　7 日間
・トスフロキサシン（オゼックス®，トスキサシン®）1 回 150 mg　2 回/日　経口　7 日間
・シタフロキサシン（グレースビット®）1 回 100 mg　2 回/日　経口　7 日間

（三鴨廣繁，他：日性感染症会誌 **22**（1 Suppl.）：60-64，2011[1]より引用）

ア検査の結果を確認したうえで，治療を開始する。パートナーの治療は積極的に行うべきだが，プライバシーの問題などを考慮する必要がある。

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うがい液による*Chlamydia trachomatis*と*Neisseria gonorrhoeae*の口腔内性感染スクリーニングにおける核酸増幅検査法2種の比較

Performance of two nucleic acid amplification tests for oral infection screening of *C. trachomatis* and *N. gonorrhoeae* by gargle test

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Key words : gargle, C. trachomatis, N. gonorrhoeae

目 的

医療機関を受診しにくい若年者に対し、自己採取可能なうがい液で口腔内性感染症スクリーニングができれば、早期発見や受診勧奨につながると考え、複数の核酸増幅検査を試行した。

対象と方法

平成24、25年度に保健所の夜間HIV抗体検査受検者のうち若年者240人に、生理食塩水10 mlで10～20秒うがいした液によりtranscription-mediated amplification（TMA）とstrand displacement amplification（SDA）の2種類の核酸増幅検査で同時に*Chlamydia trachomatis*と*Neisseria gonorrhoeae*の検出を試みた。いずれかの陽性例は参考にreal-time polymerase chain reaction（RT-PCR）を加えて行った。また、性感染症の知識や予防、口腔性交の有無等に関する性行動アンケートの回答から単純集計を行った。

なお、本調査は日本性感染症学会倫理委員会で承認された。

成 績

性・年齢構成は、男性114例（49%）、女性126例（51%）、10歳代13例（5%）、20歳代193例（81%）、30歳以上34例（14%）、計240例であった。うがい液による*C. trachomatis*（CT）陽性はTMA 3件、SDA 3件で、*N. gonorrhoeae*（NG）陽性はTMA 7件、SDA 15件であった（**Table 1**）。CTのTMAとSDAによる検査一致率は99.2%、NGのTMAとSDAによる検査一致率は95.8%であった（**Table 2**）。参考に行ったRT-PCRは18例のうち、陽性はCT 2件、NG 7件で、RT-PCRとの陽性一致率はCTがTMA 94.4%、SDA 94.4%、NGはTMA 94.4%、SDA 55.6%であった。

アンケートでは口腔性交の経験は男女ともペニスと口の接触が約90%、膣と口の接触は約70%で、いずれも

1）神戸市保健福祉局：Health and Welfare Bureau of Kobe city
2）兵庫県予防医学協会：Hyogo Health Service Association
3）神戸大学医学部附属病院感染制御部：Department of Infection Control and Prevention, Kobe University Hospital
2014（平成26）年5月8日受付、同6月27日掲載決定
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Table 1 Positive cases of CT and NG by gargle test

		CT：gargle		NG：gargle	
		TMA	SDA	TMA	SDA
Female	126	3	2	2	5
Male	114	0	1	5	10
Total	240	3	3	7	15

CT, *Chlamydia trachomatis* ; NG, *Neisseria gonorrhoeae* ; SDA, strand displacement amplification ; TMA, transcription-mediated amplification.

Table 2 Concordance of TMA vs. SDA for detecting CT/NG

CT	SDA+	SDA−	Total	Concordance	
TMA+	2	1	3	Positive	66.7% (2/3)
TMA−	1	236	237	Negative	99.6% (236/237)
Total	3	237	240	Total	99.2% (238/240)

NG	SDA+	SDA−	Total	Concordance	
TMA+	6	1	7	Positive	85.7% (6/7)
TMA−	9	224	233	Negative	96.1% (224/233)
Total	15	225	240	Total	95.8% (230/240)

CT, *Chlamydia trachomatis* ; NG, *Neisseria gonorrhoeae* ; SDA, strand displacement amplification ; TMA, transcription-mediated amplification.

膣性交を併用していた。性感染症予防にコンドームが必要と答えたのは95%である一方で、実行している予防行動は口腔性交を含め必ずコンドームを使うと答えたのは5%未満であった。

考　察

自主的にHIV抗体検査を受けるような若年者は口腔性交を高率に行っていたが、うがい液によるCT、NGの検出は低率でそれぞれの病原体でTMA、SDAの一致率が異なった。口腔内のCT、NGいずれも培養で検出しにくいためRT-PCRを参考に行ったが、SDAでNGの陽性一致率が低かったのは口腔内常在*Neisseria*属との交差反応が考えられた。性感染症クリニック受診者の調査から咽頭のCT検出は3～10%、NG検出は14～16%と報告されている[1,2]が、多くは口腔の自覚症状がなく医療機関を受診しない。特に若年者は性器と口腔の接触を、無防備な性行為として日常化しており、その一部で口腔内感染が生じていると推察する。うがい液も咽頭ぬぐい液同様、有用である[3-5]と評価されており、感染伝播を防ぐには、保健所等で自ら採取しやすいうがい液でCTおよびNGスクリーニングを行い、医療機関受診を促したい。しかし本調査では、陽性率が低く核酸増幅法の検査キット間で感度にバラツキがあり、真の陽性の判断が困難であったことから、検査一致率の低い結果の解釈は慎重にすべきである。今後、これらの原因を明らかにするため、罹患率の高い対象と陽性率や背景を比較し、スクリーニングに適切な検査方法についてさらに検討したい。

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Antimicrobial Agents and Chemotherapy

Antimicrobial Resistance and Molecular Typing of Neisseria gonorrhoeae Isolates in Kyoto and Osaka, Japan, 2010 to 2012: Intensified Surveillance after Identification of the First Strain (H041) with High-Level Ceftriaxone Resistance

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In 2009, the first high-level ceftriaxone-resistant *Neisseria gonorrhoeae* strain (H041) was isolated in Kyoto, Japan. The present study describes an intensified surveillance (antimicrobial resistance and molecular typing) of *Neisseria gonorrhoeae* isolates in Kyoto and its neighboring prefecture Osaka, Japan, in 2010 to 2012, which was initiated after the identification of H041. From April 2010 to March 2012, 193 *N. gonorrhoeae* isolates were collected and the MICs (μg/ml) to six antimicrobials, including ceftriaxone, were determined. All isolates showed susceptibility to ceftriaxone and cefixime (MIC values, <0.5 μg/ml), and spectinomycin. The rates of resistance (intermediate susceptibility) to azithromycin, penicillin G, and ciprofloxacin were 3.6% (19.7%), 24.4% (71.0%), and 78.2% (0.5%), respectively. Multilocus sequence typing (MLST) showed that 40.9%, 19.2%, and 17.1% of isolates belonged to ST1901, ST7359, and ST7363, respectively. Furthermore, *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) revealed that 12 (63%) of the 19 isolates with decreased susceptibility to ceftriaxone (MIC > 0.064 μg/ml) were of ST1407. NG-MAST ST1407 was also the most prevalent ST (16.1%; 31 of 193 isolates). In those NG-MAST ST1407 strains, several mosaic type *penA* alleles were found, including SF-A type (penicillin binding protein 2 allele XXXIV) and its derivatives. These were confirmed using transformation of the *penA* mosaic alleles as critical determinants for enhanced cefixime and ceftriaxone MICs. The intensified surveillance in Kyoto and Osaka, Japan, did not identify any dissemination of the high-level ceftriaxone-resistant *N. gonorrhoeae* strain H041, suggesting that H041 might have caused only a sporadic case and has not spread further.

Neisseria gonorrhoeae infections are major public health concerns worldwide. In 2008, the World Health Organization (WHO) estimated 106 million gonorrhea cases among adults globally, which places the infection as the most prevalent bacterial sexually transmitted infection (STI) (with a global incidence similar to that of genital chlamydial infections) (1). Resistance in *N. gonorrhoeae* to previously recommended first-line antimicrobials for treatment of gonorrhea is also prevalent worldwide. During the recent decade, the susceptibility to the extended-spectrum cephalosporins (ESCs) cefixime (oral) and ceftriaxone (parenteral), which currently are the first-line antimicrobials in most countries, has decreased rapidly worldwide (2–12). Clinical treatment failures with cefixime have been verified in many countries (13–19). In regard to ceftriaxone, which is the last remaining option for empirical first-line antimicrobial monotherapy in most countries, a few cases of failure in treating pharyngeal gonorrhea despite relatively low ceftriaxone MICs of the gonococcal strains have been confirmed in Australia (20), Sweden (21), and Slovenia (22). However, these cases likely reflected the fact that pharyngeal gonorrhea commonly is harder to treat than urogenital gonorrhea and not treatment failure due to the slightly increased ceftriaxone MICs of the gonococcal strains. Nevertheless, it is most perturbing that recently the first extensively drug-resistant (XDR) gonococcal strain H041 (23, 24) in Kyoto, Japan, followed by an XDR strain in France (14) and Spain (25), which all have been confirmed to have a high-level resistance to ceftriaxone, were described. If these strains start to spread globally, gonorrhea will become untreatable in certain circumstances and especially in settings where dual antimicrobial therapy is not feasible and/or affordable. In response to this developing situation, the WHO (26, 27), the European Centre for Disease Prevention and Control (ECDC), and the Centers for Disease Control and Prevention (CDC) in the United States have published global and region-specific response plans for their respective regions, i.e., the European Union/European Economic Area (EU/EEA) countries (28) and the United States (29). One main component of these action/response plans is to enhance the surveillance of antimicrobial resistance and treatment failures. Nevertheless, for future treatment of gonorrhea it is imperative to develop new treatment options.

In Japan, a sentinel surveillance system for sexually transmitted infections, including gonorrhea, was launched in 1999. This system includes approximately 1,000 sentinel sites that monthly report their gonorrhea cases. The number of reported gonorrhea cases peaked in 2002 (n = 21,921; 23.9 cases/site) but has since declined to 10,247 (10.6 cases/site) in 2011. Unfortunately, this

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TABLE 1 Antibiotic susceptibility of *Neisseria gonorrhoeae* isolates from Kyoto and Osaka, Japan, 2010 to 2012 (n = 193)

Antimicrobial	Breakpoints[a] (μg/ml)	No. (%) of isolates showing:		
		Susceptibility	Intermediate susceptibility	Resistance
Ceftriaxone	S ≤ 0.25/R > 0.25	193 (100)		0
Cefixime	S ≤ 0.25/R > 0.25	193 (100)[b]		0
Penicillin G	S ≤ 0.06/R > 1	9 (4.7)	137 (71.0)	47 (24,4)
Ciprofloxacin	S ≤ 0.06/R > 0.5	41 (21.2)	1 (0.5)	151 (78.2)
Azithromycin	S ≤ 0.25/R > 0.5	148 (76.7)	38 (19.7)	7 (3.6)
Spectinomycin	S ≤ 64/R > 64	193 (100)		0

[a] Antibiotic susceptibility and resistance criteria according to the Clinical and Laboratory Institute (CLSI; www.clsi.org), with the exception of azithromycin (not stated by the CLSI), for which the breakpoints from the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) were applied. S, susceptible; R, resistant.

[b] All isolates were categorized as susceptible to cefixime according to the CLSI breakpoints; however, 48 (24.9%) of the isolates were resistant to cefixime according to the European EUCAST breakpoints (MIC > 0.12 μg/ml).

sentinel surveillance system does not include any requirements to perform antimicrobial susceptibility testing on the identified gonococcal isolates. Nevertheless, after the identification of the first high-level ceftriaxone-resistant XDR strain H041 (23, 24) in Kyoto, Japan, surveillance of antimicrobial resistance in *N. gonorrhoeae* was initiated in April 2010 in Kyoto and in April 2011 in the neighboring prefecture Osaka.

The present study describes the antimicrobial resistance and molecular typing of *Neisseria gonorrhoeae* isolates in Kyoto and Osaka, Japan, in 2010 to 2012, a study initiated after the identification of the first high-level ceftriaxone-resistant XDR *N. gonorrhoeae* strain H041 in Kyoto (23, 24).

MATERIALS AND METHODS

Study settings and *N. gonorrhoeae* isolates. Five outpatient clinics, three in Kyoto and two in Osaka, Japan, provided mainly consecutive urethral discharge samples to the National Institute of Infectious Diseases (NIID), Tokyo, for isolation of *N. gonorrhoeae*. The specimens were transported using Seed swab γ no. 2 (Eiken Chemical, Tokyo, Japan) to NIID, where the samples were inoculated on selective BBL Modified Thayer Martin (MTM II) agar plates (Becton, Dickinson and Company, Sparks, MD). After 18 to 24 h of incubation, suspected colonies on the MTM II agar plates were verified as *N. gonorrhoeae* by identification of Gram-negative diplococci in microscopy, by the rapid oxidase reaction, and with the biochemical test ID Test HN-20 Rapid (Nissui Pharmaceutical, Tokyo, Japan). If culture was negative after 18 to 24 h of incubation, the agar plates were incubated for an additional 18 to 24 h before being classified as negative for *N. gonorrhoeae*. From April 2010 to March 2012, in total 154 males and 39 females were culture positive for *N. gonorrhoeae*, and accordingly, 193 *N. gonorrhoeae* isolates (one per patient) were obtained (53 from Kyoto and 140 from Osaka).

Antimicrobial susceptibility testing. The MICs (μg/ml) of ceftriaxone, cefixime, penicillin G, ciprofloxacin, azithromycin, and spectinomycin were determined using the Etest method (AB bioMérieux, Solna, Sweden) according to the manufacturer's instructions. MIC breakpoints used for determination of susceptibility, intermediate susceptibility, and resistance (Table 1) were in accordance with the Clinical and Laboratory Standards Institute (CLSI; www.clsi.org), with the exception of azithromycin, for which CLSI does not state any breakpoints. For azithromycin, the breakpoints stated by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) were used. The 2008 WHO *N. gonorrhoeae* reference strains (30) were used for quality control in all antimicrobial susceptibility testing.

DNA extraction. The bacterial isolates were suspended in Tris-EDTA (TE) buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and boiled for 10 min. After centrifugation to remove the cell debris, the supernatant was promptly used as the template DNA for the PCRs.

Molecular epidemiological characterization. Molecular epidemiological characterization by means of multilocus sequence typing (MLST) and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) was performed as described previously (31, 32). The diversity index for MLST and NG-MAST was calculated as described earlier (33). Neighbor-joining trees based on partial *porB* gene sequences (490 bp) were generated by using MEGA 4. Similarity of alleles was evaluated by individual pairwise alignment against the representative alleles in each clade (*porB4*, *porB1059*, *porB206*, *porB1785*, *porB908*, and *porB2569*) to determine the numbers of base pair differences. Alleles of *porB* showing ≥99% similarity (<5-bp difference) were grouped.

***penA* sequencing.** The *penA* gene was PCR amplified and sequenced by using the previously described primers penA_F and penA_R (34). Briefly, the PCR mixtures were incubated for 2 min at 96°C, followed by 30 cycles of 10 s at 96°C, 10 s at 65°C, and 2 min at 72°C. The PCR products were subsequently purified with an ExoSAP IT kit (GE Healthcare Limited, Buckinghamshire, United Kingdom). Both DNA strands of the PCR products were sequenced with an ABI BigDye Terminator cycle sequencing kit (version 3.1) on an ABI 3130 xl sequencer, in accordance with the instructions from the manufacturer (Applied Biosystems, Foster City, CA).

Transformation assays. To assess the capacity of a unique *penA* allele to result in increased MICs of cefixime and ceftriaxone, the full-length *penA* allele was PCR amplified and transformed into a recipient strain as previously described (34). The recipient gonococcal strain NG9807 was of MLST ST7363 and NG-MAST ST4093 and had a ceftriaxone as well as cefixime MIC of 0.016 μg/ml (23). Briefly, the recipient was suspended in GC broth containing 1.5% (wt/vol) proteose peptone 3 (Becton, Dickinson and Company, Sparks, MD), 0.4% (wt/vol) K_2HPO_4, 0.1% (wt/vol) KH_2PO_4, 0.5% (wt/vol) NaCl, and 1% (vol/vol) isovitalex (1×10^8 cells/100 μl) and incubated with 0.2 μg of PCR product of the specific *penA* allele (after purification using the High Pure PCR Product Purification kit [Roche Diagnostics GmbH, Mannheim, Germany]) for 4 h. Aliquots of 10 μl and 100 μl were inoculated on GC agar containing cefixime (0.064 μg/ml). After incubation for 18 to 24 h, the obtained colonies were subcultured on an antimicrobial-free GC agar plate for single-clone isolation. For confirmation, the full-length *penA* allele was sequenced in all transformants.

Nucleotide sequence accession numbers. The nucleotide sequences of *penA* determined in this study have been deposited in the DDBJ sequence library and assigned the accession numbers AB819103 to AB819115.

RESULTS

Antimicrobial susceptibility testing. The results of the antimicrobial susceptibility testing of the *N. gonorrhoeae* isolates (n = 193) are summarized in Table 1. All 193 examined isolates were susceptible to ceftriaxone, cefixime, and spectinomycin. The rates of resistance (intermediate susceptibility) to azithromycin, peni-

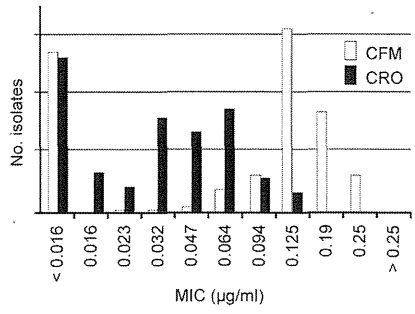

FIG 1 Cefixime (CFM) and ceftriaxone (CRO) MIC distribution of *Neisseria gonorrhoeae* isolated in Kyoto and Osaka, Japan, in 2010 to 2012 (n = 193).

cillin G, and ciprofloxacin were 3.6% (19.7%), 24.4% (71.0%), and 78.2% (0.5%), respectively. However, although there were no isolates resistant to cefixime and ceftriaxone according to the CLSI breakpoints (MIC, >0.25 μg/ml), 48 (24.9%) isolates were resistant to cefixime according to the European breakpoints (EUCAST; MIC, >0.125 μg/ml) and the MIC_{90} value for ceftriaxone was 0.094 μg/ml (Fig. 1).

Multilocus sequence typing investigation and relationship between MLST and cephalosporin resistance. In Japan, the initial emerged *N. gonorrhoeae* strains with intermediate susceptibility and resistance to cefixime possessed a *penA* mosaic allele X, which encodes a penicillin binding protein 2 (PBP 2) X. These strains belonged mainly to two major MLST sequence types (ST), particularly ST7363 but also ST1901 (34). These STs and their *penA* alleles have subsequently further evolved. The first strain with high-level resistance to ceftriaxone identified in Kyoto, Japan (H041; 23, 24), was of ST7363. The additional high-level ceftriaxone-resistant strains isolated in France (14) and Spain (25) were of ST1901. In the present study, a total of 29 MLST STs were found among the 193 isolates from Kyoto and Osaka. ST1901 was the most common MLST ST (n = 79, 40.9%), followed by ST7359 (n = 37, 19.2%), ST7363 (n = 33, 17.1%), and ST7819 (n = 17, 8.8%) (Table 2). The diversity index for MLST of the 193 isolates was 0.76.

Among those four most prevalent MLST STs, isolates of ST1901, ST7363, and ST7819 showed relatively high MIC values for cefixime with similar MIC distribution patterns (Fig. 2). In regard to ceftriaxone, isolates of ST1901 showed slightly higher MICs than did the ST7363 and ST7819 isolates. In contrast, all ST7359 isolates were highly susceptible to both cefixime and ceftriaxone (Fig. 2).

***N. gonorrhoeae* multiantigen sequence typing analysis and relationship between NG-MAST and MLST.** NG-MAST has a substantially higher discriminatory power than MLST, and NG-MAST has been applied for molecular epidemiologic investigations for gonococci isolated worldwide (10, 35). In recent years, NG-MAST ST1407 has been a prevalent ST in many countries, and this ST has also accounted for a substantial proportion of the decreased susceptibility and resistance to ESCs in those countries (10). Furthermore, the high-level ceftriaxone-resistant XDR isolates cultured in France (14) and in Spain (25), which might represent one and the same strain, were of NG-MAST ST1407. In the present study, a total of 95 NG-MAST STs were identified among the 193 isolates from Kyoto and Osaka (Table 2). ST1407 (n = 31, 16.1% of all isolates) was the most prevalent NG-MAST ST, followed by ST4186 (n = 10, 5.2%), ST3505 (n = 8, 4.1%), and ST6780 (n = 7, 3.6%) (Table 2). The diversity index for NG-MAST of the 193 isolates was 0.96.

N. gonorrhoeae isolates assigned to the four most common MLST STs, ST1901, ST7359, ST7363, and ST7819, were subdivided into 30 (intra-MLST ST diversity index, 0.83), 18 (diversity index, 0.89), 23 (diversity index, 0.94), and 7 (diversity index, 0.71) NG-MAST STs, respectively. These results clearly illustrate the substantially higher discriminatory power of NG-MAST compared to MLST. However, the number of different *tbpB* alleles within these MLST STs was relatively small, that is, MLST ST1901, ST7363, ST7359, and ST7819 possessed only six, two (*tbpB10* and *tbpB3*), one (*tbpB241*), and one (*tbpB27*) different *tbpB* alleles, respectively. Accordingly, the high discriminatory power of NG-MAST was highly dependent on the diversified *porB* gene (see below), and the different level of genetic heterogeneity within the main MLST STs might reflect how long these STs have been spreading and thus evolving. As aforementioned, ST1407 (n = 31) was the most prevalent NG-MAST ST, and all except one (belonging to MLST ST10241) of these isolates belonged to MLST ST1901 (30/79 [38.0%] of all ST1901 isolates). The MIC_{50}s (ranges) of cefixime and ceftriaxone for the NG-MAST ST1407 isolates were 0.19 μg/ml (0.064 to 0.25 μg/ml) and 0.064 μg/ml (0.016 to 0.125 μg/ml), respectively. Furthermore, 12 (63%) of the 19 isolates with decreased susceptibility to ceftriaxone (MIC, >0.064 μg/ml) were of ST1407.

***porB* and *penA* sequencing.** To further examine the genetic relationships of the 193 *N. gonorrhoeae* isolates from Kyoto and Osaka, a phylogenetic analysis of the *porB* sequences (490 bp) used in the NG-MAST was performed.

TABLE 2 MLST and NG-MAST sequence types of *N. gonorrhoeae* isolates from Kyoto and Osaka, Japan, 2010 to 2012

ST	No. (%) of isolates
MLST ST	
1901	79 (40.9)
7359	37 (19.2)
7363	33 (17.1)
7819	17 (8.8)
7358	3 (1.6)
1579	2 (1.0)
1584	2 (1.0)
7371	2 (1.0)
7827	2 (1.0)
10241	2 (1.0)
Others (19 STs)	24 (12.4)
NG-MAST ST	
1407	31 (16.1)
4186	10 (5.2)
3505	8 (4.1)
6780	7 (3.6)
2958	6 (3.1)
6771	6 (3.1)
4019	5 (2.6)
6767	5 (2.6)
3431	4 (2.1)
4015	4 (2.1)
7381	4 (2.1)
6765	4 (2.1)
6754	3 (2.1)
6769	3 (2.1)
Others (81 STs)	93 (48.2)

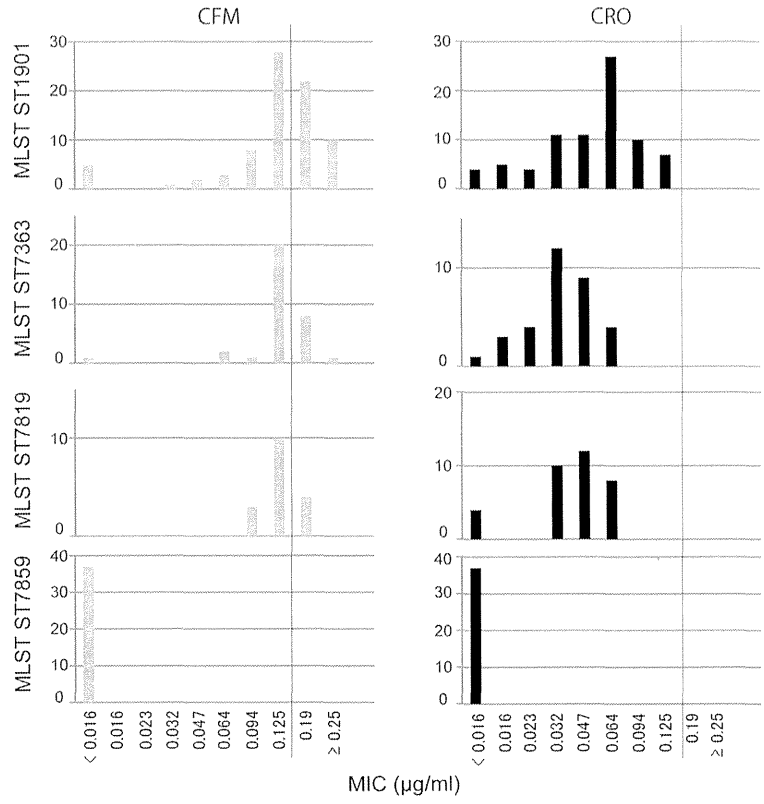

FIG 2 MIC of cefixime (CFM) and ceftriaxone (CRO) in *Neisseria gonorrhoeae* isolates from Kyoto and Osaka, Japan, in 2010 to 2012 belonging to the four major multilocus sequence typing (MLST) sequence types (STs), that is, ST1901 (n = 79), ST7363 (n = 33), ST7819 (n = 17), and ST7359 (n = 37).

The phylogenetic analysis identified six clades, in which common representative alleles for each clade were *porB4*, *porB1059*, *porB206*, *porB1785*, *porB908* (the *porB* allele in NG-MAST ST1407), and *porB2569*, respectively (Fig. 3A). The MIC distributions for cefixime and ceftriaxone varied substantially for those different *porB* clades. Interestingly, the isolates in the *porB908* clade (including all 31 NG-MAST ST1407 isolates but also 31 additional isolates of other STs [n = 15]) had clearly the highest MICs of cefixime and particularly ceftriaxone; in fact, this clade contained all isolates showing an MIC of 0.125 μg/ml for ceftriaxone (Fig. 3D). NG-MAST ST1407 isolates have been previously described to possess a *penA* mosaic allele XXXIV encoding a PBP 2 mosaic XXXIV, which results in elevated MICs of ESCs (10, 14). Accordingly, in the present study the *penA* gene in all isolates from the *porB908* clade (n = 62) and, for comparison, isolates from the *porB1059* clade (n = 17) and the *porB1785* clade (n = 25), which also had substantially elevated MICs of cefixime particularly (Fig. 3B and C), were sequenced. In total, 11 *penA* alleles, including nine mosaic alleles, were revealed among those 104 isolates (Fig. 4). Of the nine *penA* mosaic alleles, five encoded PBP 2 mosaic X or closely related sequence variants (found in 50 of the isolates), which were distributed in all three examined *porB* clades. In contrast, all isolates possessing a *penA* mosaic allele XXXIV or closely related sequence variants (in 51 isolates) were in the *porB908* clade, with the exception of one isolate from the *porB1785* clade (Fig. 4), indicating that this *penA* mosaic allele might have emerged in the *porB908* clade (including the 31 NG-MAST ST1407 isolates). Nevertheless, only a few (n = 5) of the isolates in the *porB908* clade possessed the authentic *penA* mosaic allele XXXIV, and most contained PBP 2 mosaic XXXIV with an additional P551S substitution (n = 44) (Fig. 4). Furthermore, all isolates showing an MIC of 0.125 μg/ml of ceftriaxone possessed this PBP 2 mosaic XXXIV with an additional P551S alteration, with the exception of one isolate with PBP 2 mosaic XXXIV containing an additional A501V substitution. As a matter of great concern, these results indicate that the authentic *penA* mosaic XXXIV allele is evolving, resulting in further enhanced MICs of ESCs.

Transformation of specific *penA* mosaic alleles. To assess the capacity of specific *penA* mosaic alleles to result in increased MICs of ceftriaxone and cefixime, the full-length *penA* mosaic alleles X, XXXIV, XXXIV-P551S, and XXXIV-A501V were separately transformed into the ESC-susceptible strain NG9807 (ceftriaxone MIC, 0.016 μg/ml; cefixime MIC, 0.016 μg/ml). As shown in Fig. 5, the transformants with *penA* XXXIV variants (P551S and A501V) showed ceftriaxone MICs of 0.125 μg/ml (and cefixime MICs of 0.25 μg/ml and 0.5 μg/ml, respectively), which were clearly higher than those of transformants with *penA* XXXIV (ceftriaxone MIC, 0.064 μg/ml; cefixime MIC, 0.125 μg/ml). The authentic *penA* mosaic allele X transformant showed a ceftriaxone MIC of 0.125 μg/ml and a cefixime MIC of 0.25 μg/ml. Accord-

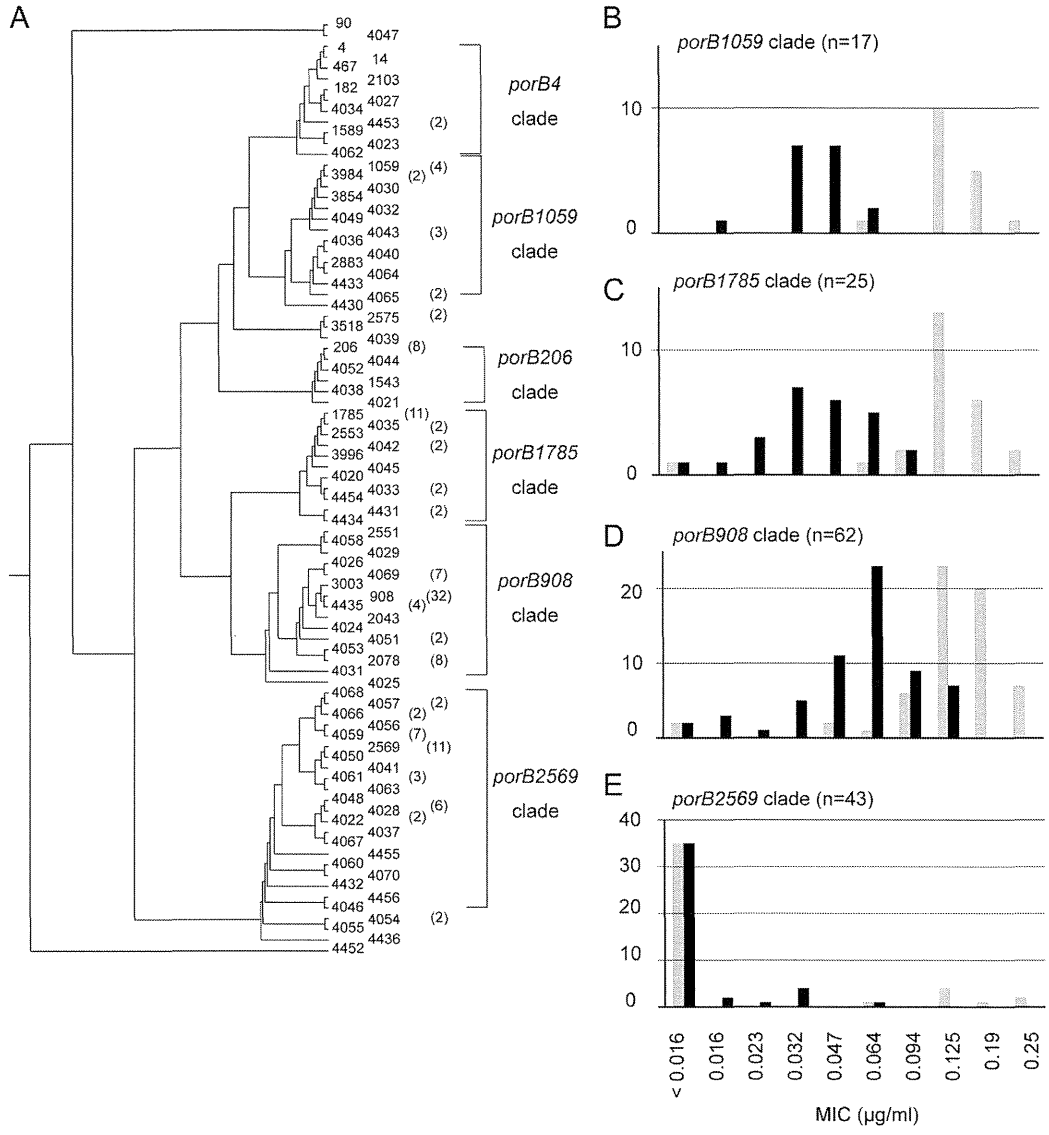

FIG 3 Sequence comparison of *porB* alleles (490 bp) from *N. gonorrhoeae* isolates (n = 193) from Kyoto and Osaka, Japan, and cefixime and ceftriaxone MIC profile of each *porB* group. (A) An internal region of the *porB* genes including loops 3, 4, and 5 (490 bp), which is used in NG-MAST, was utilized to create a phylogenetic tree with the MEGA 4 program. The numbers indicate *porB* allele numbers assigned in the NG-MAST database. The number of isolates with the same *porB* allele is indicated in parentheses. (B to E) Similar *porB* alleles are grouped as *porB1059*, *porB1785*, *porB908*, and *porB2569* clades, and the MICs to cefixime (gray bars) and ceftriaxone (black bars) are shown in panels B to E, respectively.

ingly, in regard to ceftriaxone, *penA* alleles XXXIV-P551S and XXXIV-A501V showed impact on MIC identical to that of the *penA* X allele, and the MIC was raised twice as much as that seen with the *penA* XXXIV allele. Interestingly, the *penA* allele XXXIV-A501V could increase the MIC of cefixime up to 0.5 μg/ml, which is above the resistance breakpoint (Fig. 5).

DISCUSSION

In 2009, the first high-level ceftriaxone-resistant *N. gonorrhoeae* strain (H041) was isolated in Kyoto, Japan (23, 24). H041 was also the first extensively drug-resistant (6) *N. gonorrhoeae* strain, displaying resistance to most available antimicrobials (23). The present study describes an intensified surveillance (antimicrobial resistance and molecular typing) of *N. gonorrhoeae* isolates in Kyoto and its neighboring prefecture Osaka, Japan, in 2010 to 2012, which was initiated after the identification of H041 (23, 24). All *N. gonorrhoeae* isolates (n = 193), collected at five clinics, were susceptible to ceftriaxone and cefixime (MIC values, <0.5 μg/ml) and to spectinomycin. The rates of resistance (intermediate susceptibility) to azithromycin, penicillin G, and ciprofloxacin were

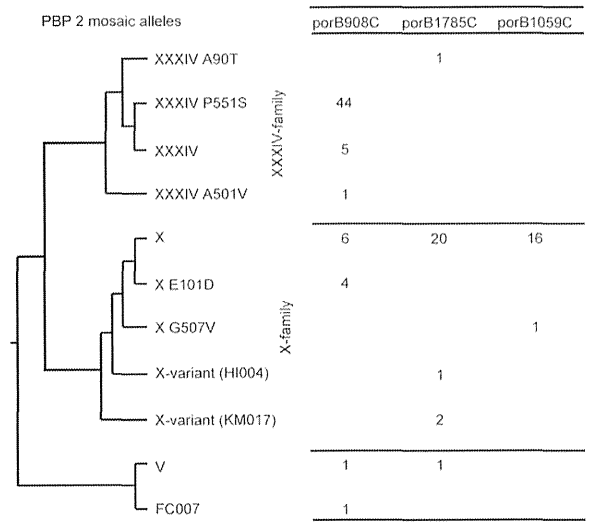

FIG 4 Phylogenetic tree of the amino acid sequences of penicillin binding protein 2 encoded by different *penA* mosaic alleles. The *penA* genes from a total of 104 *N. gonorrhoeae* isolates possessing alleles belonging to the *porB908*, *porB1785* and *porB1059* clades (C) were sequenced, and a phylogenetic tree based on the PBP 2 amino acid sequences was constructed by the MEGA 4 program. Two major *penA* mosaic allele groups of PBP 2 were revealed, the PBP 2 X family and the PBP 2 XXXIV family. Strains HI004 and KM017 had 3 and 11, respectively, amino acid substitutions in their PBP 2 X group alleles.

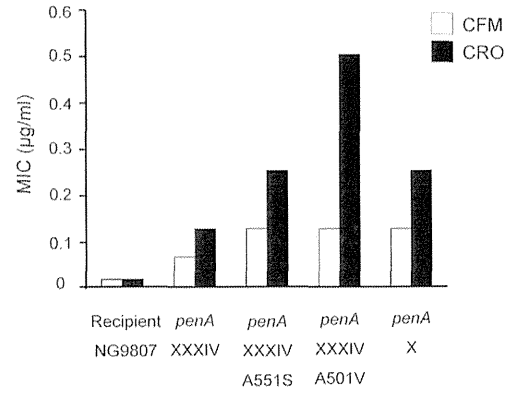

FIG 5 Capacity for increased MIC conferred by the mosaic PBP 2 alleles harbored by *penA* X and *penA* XXXIV, including amino acid substituted mutants identified in the present study. Each allele was introduced into the cefixime- and ceftriaxone-susceptible strain NG9807, and MIC values of each transformant are shown.

3.6% (19.7%), 24.4% (71.0%), and 78.2% (0.5%), respectively (Table 1). These data are basically consistent with a previous recent report from Japan, except for the azithromycin resistance (12). In a previous publication (12), only 0.4% (1 of 242 isolates) was reported to be resistant to azithromycin. Accordingly, the high-level ceftriaxone-resistant *N. gonorrhoeae* strain H041 might have caused only a sporadic event, and no further dissemination in the Kyoto or Osaka area could be found. The reasons for this are unknown; however, the high-level ceftriaxone resistance (i.e., the remodeled PBP 2) might affect the biological fitness of H041. Studies investigating the biological fitness of H041 in competitive experiments *in vitro* and *in vivo* (in a mouse model) are in progress. In the present study, no other ceftriaxone-resistant strain was identified either. However, only five clinics were included in the present surveillance and, due to this lack of complete surveillance, it cannot be entirely excluded that some ceftriaxone-resistant gonococcal strain was spreading in the region. The antimicrobial resistance surveillance in this region has continued, further strengthened in the area, and should now be expanded nationally.

All clinics included in this surveillance adhered to the treatment guideline from the Japanese Society for STI, which recommends using 1 g ceftriaxone intravenously or 2 g spectinomycin intramuscularly. However, in some cases also azithromycin (2 g orally) can be used. During the study period, no cases of suspected treatment failure using 1 g ceftriaxone were identified, which further supports the lack of ceftriaxone resistance in the region.

Recently, the antimicrobial resistance surveillance for *N. gonorrhoeae* has been enhanced worldwide (26–28), and some of those surveillance systems have also performed molecular typing of the *N. gonorrhoeae* isolates (36). As to molecular typing for *N. gonorrhoeae*, MLST (31) and particularly NG-MAST (32) have been most widely applied worldwide (35). MLST, which identifies allelic variations in seven housekeeping genes, describes the phylogenetic relationship between isolates. However, since the housekeeping genes are relatively conserved, the discrimination of MLST is rather low and the method is more suitable for long-term epidemiology, macroepidemiology (e.g., on a global scale), and evolutionary studies (35). In contrast, NG-MAST is more applicable to short-term epidemiology and microepidemiology because it is based on sequence variations of genes encoding two bacterial cell surface proteins, that is, PorB and TbpB (35). These antigens are exposed to the human immune response, and this selective pressure results in antigenic changes giving the *N. gonorrhoeae* strain an opportunity to escape from the human immune system. Although NG-MAST has a very high ability to discriminate *N. gonorrhoeae* isolates, several publications have reported a high prevalence of the NG-MAST ST1407, which appears to be disseminated basically worldwide and account for a high proportion of the decreased susceptibility and increased resistance to ESCs and multidrug resistance (10, 14, 18, 36). As found in the present study, the NG-MAST ST1407 (30 of those 31 isolates belonged to MLST ST1901) was also the most prevalent ST in Osaka and Kyoto, Japan, and as in other countries the ST1407 isolates had decreased susceptibility to ESCs and were resistant to fluoroquinolones (data not shown).

Thus, the discriminatory ability of NG-MAST is substantially higher than MLST (35; this study); however, the majority of this discrimination is due to the high variation of the *porB* allele and far fewer *tbpB* alleles have been described. The *porB908* allele (present in the NG-MAST ST1407) and its closely related alleles (*porB908* group) were widely distributed in the isolates examined in the present study; these isolates also in general had higher MICs of ESCs. The other major *porB* groups were the *porB1059* group, the *porB1785* group, and the *porB2569* group (Fig. 3). The *porB2569* group isolates (mostly belonging to MLST ST7359) were mainly fully susceptible to ceftriaxone and cefixime, although some isolates showed higher MICs, that is, MICs of 0.016 to 0.064 μg/ml of ceftriaxone and MICs of 0.064 to 0.25 μg/ml of cefixime. Because *porB* alleles might be horizontally transferred among *N. gonor-*