wide. Unfortunately, it was not possible to definitively verify that H041 caused a treatment failure because a posttreatment isolate was not available; however, treatment failure seems likely (see Discussion). Furthermore, H041 belongs to multilocus sequence typing (MLST) sequence type (ST) ST7363 and is closely related to the successful gonococcal cefixime-resistant subclones of ST7363 (23), which are prevalent in Japan (24) and now are also being transmitted in Europe. Accordingly, H041 may be a subclone of the MLST ST7363 cefixime-resistant strains that has acquired additional resistance determinant(s), resulting in high-level ceftriaxone resistance. Historically, gonococcal AMR has mostly emerged in the World Health Organization (WHO) Western Pacific Region (WPR) and, in particular, in Japan. This resistance has spread rapidly, via sex tourists, long-distance truck drivers, and forced migration in the WHO WPR, to the Pacific Rim countries, including the United States, Southeast and Central Asia, Europe, and globally (36). The spread of ceftriaxone-resistant gonococcal strains worldwide will probably follow the same pattern. Consequently, it is crucial to examine in detail, including elucidation of the mechanisms causing the ceftriaxone resistance, the first high-level ceftriaxone-resistant gonococcal strain worldwide, recently isolated in Japan (H041) (23).

The most common mechanism in gonococci for decreased ESC susceptibility is alteration of the *penA* gene, i.e., the acquisition of a *penA* mosaic allele or alterations of amino acid A501 in the encoded penicillin-binding protein 2 (PBP2) (1, 3, 11, 14–18, 21, 23, 24, 27, 28, 32, 33, 36, 38, 40, 41, 44, 50). Mutations in the promoter and/or coding sequence of the repressor gene *mtrR* cause an overexpression of the MtrCDE efflux pump system, which further decreases ESC susceptibility (3, 11, 12, 16–18, 30, 36, 38, 43, 48, 50). Furthermore, specific *porB1b* mutations that alter amino acid G101 and A102 in the PorB1b porin (the *penB* resistance determinant) result in additional decreases in ESC susceptibility (3, 11, 16–18, 25, 26, 30, 36, 38, 50). Nevertheless, based on the relatively few studies of gonococcal isolates with decreased ESC susceptibility, polymorphisms in *ponA* (encoding PBP1) and *pilQ* (encoding the pore-forming secretin PilQ protein in the type IV pili), which both can be involved in high-level penicillin resistance, do not seem to substantially enhance the MICs of ESCs (11, 29, 45, 49, 50). At least one unknown resistance determinant exists (11, 18, 38, 50).

The aims of this study were to perform a detailed characterization, phenotypic and genetic, of the first identified high-level ceftriaxone-resistant *N. gonorrhoeae* strain (H041) worldwide in order to confirm this finding, to thoroughly examine its antimicrobial resistance, and to elucidate the ESC resistance mechanisms.

MATERIALS AND METHODS

***Neisseria gonorrhoeae* strains.** The high-level ceftriaxone-resistant strain H041 (23) and gonococcal strains ($n = 9$) selected for transformation assays to verify the resistance mechanisms, i.e., five clinical strains and four of the eight 2008 WHO *N. gonorrhoeae* reference strains (41), were included in this study. For gonococcal species verification of H041, morphology on selective culture medium, catalase and oxidase tests, microscopy after Gram staining, and seven species confirmatory tests (sugar utilization, HN-20 Rapid system identification [ID] test [Nissui, Tokyo, Japan], PhadeBact GC monoclonal test [Bactus AB, Solna, Sweden], PhadeBact GC monoclonal serovar test [Bactus AB, Solna, Sweden], MicroTrak *N. gonorrhoeae* culture confirmation test [Trinity Biotech, Wicklow, Ireland], *porA* pseudogene PCR [13], and dual-target PCR [*porA* and *opa*] [10]) were used. All strains were grown on GC culture medium as previously described (42).

Antimicrobial susceptibility testing. Ceftriaxone MIC determination was performed using the Etest method (AB bioMérieux, Solna, Sweden) according to the manufacturer's instructions. The ceftriaxone MIC of H041 was also confirmed using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) standards (8). Finally, H041 was examined for its MICs of 29 additional antimicrobials (using the Etest method) and tested with the calibrated dichotomous sensitivity (CDS) disc diffusion method (5, 35), which is used in resistance surveillance of *N. gonorrhoeae* in many countries in the WHO WPR (six antimicrobials) (Table 1). β-Lactamase production was tested using nitrocefin discs. The 2008 WHO *N. gonorrhoeae* reference strains (41) were used for quality controls in all antimicrobial susceptibility testing.

Genetic characterization. DNA was isolated in a NorDiag Bullet instrument (NorDiag ASA Company, Oslo, Norway) using a Bugs'n Beads STI-*fast* kit (NorDiag ASA Company, Oslo, Norway) according to the manufacturer's instructions. For molecular epidemiological examination, strains were genotyped by means of MLST (24), *porB* gene sequencing, and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) as described previously (39). PCR and sequencing of resistance determinants, i.e., the *penA*, *mtrR*, *porB1b*, *ponA*, and *pilQ* genes, were performed as described elsewhere (18, 41, 45).

Sequence alignments and phylogenetic analysis. Multiple-sequence alignments (nucleotide and amino acid sequences) were performed using BioEdit Sequence Alignment Editor software (version 7.0.9.0). For examination of the evolutionary relationships of H041 with other *penA* mosaic strains displaying decreased ESC susceptibility and circulating worldwide, a phylogenetic analysis using the full-length *porB* sequences in H041 and previously reported *penA* mosaic strains (11) was performed with TREECON (version 1.3b) as previously described (39).

Transformation assays. To confirm that the unique *penA* allele in H041 ($penA_{H041}$) caused the high-level resistance to ceftriaxone, the full-length $penA_{H041}$ was PCR amplified and transformed into nine recipient strains as previously described (24). These nine recipient strains displayed different molecular epidemiological sequence types, ceftriaxone MICs, and composition of ESC resistance mechanisms, such as *penA* alleles, the *mtrR* promoter, and *penB* sequences (Table 2). Briefly, the recipients were suspended in GC broth (1×10^8 cells/100 μl) and incubated with 0.2 μg of the $penA_{H041}$ PCR product (after purification using a 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 with a 4-fold higher ceftriaxone concentration than the MIC of the respective recipient. After incubation, the colonies obtained were subcultured on an antimicrobial-free GC agar plate for single-clone isolation. For confirmation, the transformation assay was performed three times for each recipient.

Nucleotide sequence accession numbers. The GenBank/EMBL/DDBJ accession numbers for the two new *penA* alleles reported in this paper are AB546858 and AB608050.

RESULTS

Phenotypic characterization of the high-level ceftriaxone-resistant *N. gonorrhoeae* strain H041. All conventional bacteriological diagnostic tests and the seven species confirmatory assays verified that H041 was a gonococcal strain, which was assigned to serovar Bpyust.

The results of the antimicrobial susceptibility testing using the Etest method (30 antimicrobials) and CDS disc diffusion method (six antimicrobials) are summarized in Table 1. Briefly, H041 was resistant to various antimicrobials, including all β-lactam antimicrobials (with possible exceptions of carbapenems, at least ertapenem and meropenem, and piperacillin-tazobactam, for which no breakpoints are available), fluoroquinolones, macrolides, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, and nitrofurantoin. The MICs of all the cephalosporins, including the recommended first-line ESCs, were very high (e.g., 2 to 4 μg/ml of ceftriaxone and 8 μg/ml of cefixime). H041 did not produce any β-lacta-

TABLE 1. MIC using the Etest method and zone sizes with the calibrated dichotomous sensitivity disc diffusion method of *Neisseria gonorrhoeae* H041 to various antimicrobials

Antimicrobial	Class	MIC Etest result in μg/ml (agar dilution result), interpretation[a,b]	CDS (mm)[c]
Penicillin G	β-Lactams, penicillins	4, R[a,b]	0
Ampicillin		2	ND
Amdinocillin		>256	ND
Piperacillin-tazobactam		0.25	ND
Aztreonam	β-Lactam, monobactam	128	ND
Cefuroxime	β-Lactams, cephalosporins	16, R[a]	ND
Cefpodoxime		16, R[a]	3
Ceftazidime		16, R[a]	ND
Cefotaxime		8, R[a,b]	ND
Cefixime		8 (8), R[a,b]	ND
Ceftriaxone		4 (2), R[a,b]	1
Cefepime		16, R[a]	ND
Ertapenem	β-Lactams, carbapenems	0.064	ND
Meropenem		0.125	ND
Imipenem		2	ND
Ciprofloxacin	Fluoroquinolones	>32, R[a,b]	1
Levofloxacin		>32	ND
Moxifloxacin		6	ND
Azithromycin	Macrolides	1, R[b]	ND
Erythromycin		2	ND
Gentamicin	Aminoglycosides	4	ND
Kanamycin		16	ND
Tobramycin		6	ND
Spectinomycin	Aminocyclitol	16, S[a,b]	9
Tetracycline	Tetracycline	4, R[b]	5
Tigecycline	Glycylcycline	0.5	ND
Trimetoprim-sulfamethoxazole	Folic acid antagonists	1	ND
Chloramphenicol	—	4	ND
Nitrofurantoin	—	4	ND
Rifampin	—	0.25	ND

[a] Antimicrobial susceptibility testing was performed using the Etest method (AB bioMérieux, Solna, Sweden) on all antimicrobials according to the instructions from the manufacturer (results were rounded up to whole MIC dilutions). Furthermore, agar dilution was additionally performed for ceftriaxone and cefixime (in parentheses) according to the method described by the Clinical Laboratory and Standards Institute (CLSI) (8). Where available, interpretative criteria (S, susceptible; I, intermediate; R, resistant) from the Clinical and Laboratory Standards Institute (CLSI) (8) were used.

[b] Interpretative criteria (S, I, R) from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Available online at: http://eucast.org/ (Accessed 30 April, 2011).

[c] The calibrated dichotomous sensitivity (CDS) disc diffusion method (5, 35) is used for antimicrobial resistance testing in many countries in the World Health Organization (WHO) Western Pacific Region. ND, not determined.

mase. The strain was, however, susceptible to spectinomycin and rifampin. Furthermore, the MICs of aminoglycosides and tigecycline were also relatively low (no breakpoints are available for these antimicrobials). The CDS disc diffusion method also identified H041 as resistant to ceftriaxone, cefpodoxime, penicillin G, tetracycline, and ciprofloxacin but susceptible to spectinomycin (Table 1).

Genetic characterization and elucidation of the mechanisms causing the high-level ceftriaxone resistance in *N. gonorrhoeae* strain H041. The molecular epidemiological characterization assigned H041 as MLST ST7363 and as the not-previously described NG-MAST sequence type ST4220 (www.ng-mast.net). A phylogenetic analysis using *porB* sequences showed that H041 is closely related to other *penA* mosaic strains with decreased ESC susceptibility that have been shown to circulate worldwide (Fig. 1).

The sequencing of ESC resistance determinants showed that H041 possessed a unique *penA* mosaic allele ($penA_{H041}$) and the previously described *mtrR*, *penB*, and *ponA1* (L421P) resistance determinants. No new *pilQ* mutations were found. Thus, the only new resistance determinant, which consequently was suspected to cause the high ESC MICs, was $penA_{H041}$ (GenBank accession number AB546858). $penA_{H041}$ was highly similar (i.e., 97.6% nucleotide identity and only 12 PBP2 amino acid differences that clustered in two regions) to the previously described *penA* mosaic allele X that has been correlated with cefixime treatment failures in Japan. Of these 12 amino acids, five were unique compared with any gonococcal PBP2 sequence previously described, but one of these (I486) has been found in *Neisseria meningitidis* and *Neisseria flavescens* (Fig. 2). Accordingly, $penA_{H041}$ contained only four PBP2 amino acid residues that have not been previously reported in any *Neisseria* species; compared to *penA* mosaic X, these consisted of A311V, T316P, A328T, and T484S (Fig. 2).

Transformation assays confirm that the unique $penA_{H041}$ caused the high-level resistance to ceftriaxone and other extended-spectrum cephalosporins. Based on their different genotypes, ceftriaxone MICs, and composition of ESC resistance determinants, nine strains were selected as recipients of $penA_{H041}$ in transformation assays (Table 2).

Upon transformation with $penA_{H041}$, the ceftriaxone MICs of the recipients increased to 0.125 to 8 μg/ml, i.e., by 16- to 500-fold. Accordingly, the ceftriaxone MICs of all recipients, with the exception of NG9901 (0.125 μg/ml), increased above

TABLE 2. *Neisseria gonorrhoeae* strains with different ceftriaxone MICs and containing divergent genetic ceftriaxone resistance mechanisms that were used as recipients in transformation of the full-length *penA* gene from H041

Strain	MLST[a]	NG-MAST[b]	Ceftriaxone MIC (μg/ml)[c]	*penA*[d] (allele)	*mtrR*[e]	*penB*[f]	*ponA*[g]
NG9901	ST7363	ST240	<0.002	*penA* XXXVI (mosaic[h])	WT	WT	WT
WHO F	NEW	ST3303	<0.002	*penA* XV (WT)	WT	WT	WT
NG9903	ST7359	ST4058	0.004	*penA* II (A345a)	WT	WT	WT
NG9807	ST7363	ST4093	0.016	*penA* II (A345a)	A-del	Yes	L421P
WHO M	ST7367	ST3304	0.016	*penA* II (A345a)	A-del	Yes	L421P
WHO K	ST7363	ST1424	0.064	*penA* X (mosaic)	A-del	Yes	L421P
NG0003	ST7363	ST4068	0.125	*penA* X (mosaic)	A-del	Yes	L421P
35/02	ST7363	ST326	0.125	*penA* XXVIII (mosaic)	A-del	Yes	L421P
WHO L	ST1590	ST1422	0.125	*penA* VII (A501V)	A-del	Yes	L421P

[a] MLST, multilocus sequencing typing (24).
[b] NG-MAST, *Neisseria gonorrhoeae* multiantigen sequence typing (39).
[c] Etest results were rounded up to whole MIC steps. MICs of <0.002 μg/ml were calculated as 0.001 μg/ml in the MIC ratios in Fig. 3.
[d] The *penA* mosaic allele encodes a mosaic penicillin binding protein 2 (PBP2) that causes a decreased susceptibility to extended-spectrum cephalosporins. Mosaic X has been found in cefixime-resistant *N. gonorrhoeae* isolates in Japan (18, 36, 50).
[e] A-del indicates a characteristic single nucleotide (A) deletion in the inverted repeat of the promoter region of *mtrR* that causes an overexpression of the MtrCDE efflux pump that results in a further decrease in susceptibility to ESCs (18, 36, 50).
[f] "Yes" indicates the presence of the alterations of amino acids 120 and 121 in porin PorB (*penB* alteration) that cause a decreased intake of ESCs and, accordingly, a further decrease in susceptibility to ESCs (18, 36, 50). WT, wild type.
[g] The alteration of amino acid 421 in PBP1 (encoded by *penA*) causes a decreased susceptibility to penicillins (18, 29, 50).
[h] *penA* mosaic allele that has not been previously described and whose sequence has been deposited in GenBank with accession number AB608050.

the resistance breakpoint (>0.25 μg/ml) (8) independent of other resistance determinants. Remarkably, WHO F, which has wild-type alleles of all ESC and penicillin resistance determinants, displayed a ceftriaxone MIC of 0.5 μg/ml after transformation (500-fold MIC increase) (Fig. 3).

All single-clone transformants (derived from all recipient strains) showed *mtrR*, *penB*, and *ponA* sequences identical to those in the recipients. All the single-clone transformants also contained the $penA_{H041}$ allele. In most transformants, the transformed $penA_{H041}$ sequence was identical to the sequence in H041. However, in a few transformants, such as those derived from the WHO F and WHO M strains, some point mutations differed from the $penA_{H041}$ sequence. These were considered to represent spontaneous mutations, mutations in junctions for recombination and/or belonging to the *penA* allele of the recipient. The majority of these mutations were nonsynonymous, and none was located in any segment of the mosaic *penA* allele affecting the ceftriaxone MICs. Consequently, the transformation experiments confirmed that $penA_{H041}$ was the cause of the high-level ceftriaxone resistance.

DISCUSSION

The present study describes the detailed phenotypic and genetic confirmation and characterization, including elucidation of the resistance mechanisms, of the first identified *N. gonorrhoeae* strain (H041) displaying high-level resistance to ceftriaxone worldwide. H041 was isolated from a female commercial sex worker in Japan (23), and the ceftriaxone MIC of H041 was 4- to 8-fold higher than any previously observed. Ceftriaxone is also the last remaining option for empirical first-line treatment of gonorrhea. Accordingly, *N. gonorrhoeae* has now shown its ability to develop resistance to ceftriaxone also and, although the biological fitness of ceftriaxone resistance in *N. gonorrhoeae* remains unknown, the gonococcus may become a true superbug that initiates a future era of untreatable gonorrhea.

Although a posttreatment isolate was unavailable (only one specimen positive with SDA [ProbeTec ET; Becton-Dickinson], sampled 2 weeks after treatment) to definitively verify treatment failure using 1 g ceftriaxone intravenously (23), it seems likely that this was the first gonorrhea clinical failure caused solely by high resistance of the bacteria to ceftriaxone, based on the posttreatment positive-SDA sample (all residual gonococcal DNA is expected to be eliminated before 2 weeks posttreatment) (2), the very high ceftriaxone MIC, and all available data regarding pharmacodynamic parameters for ESCs. Thus, according to Monte Carlo simulations, the 1 g ceftriaxone intravenously that was used for treatment (in full concordance with treatment recommendations for urogenital and pharyngeal gonorrhea in the Japanese treatment guidelines) results in median times of free ceftriaxone above the MIC ($fT_{>MIC}$) of only 6.0 h (0 to 20.3 h) and 0 h (0 to 5.6 h) for the detected MICs of 2 μg/ml (agar dilution method) and 4 μg/ml (Etest method), respectively (6). Accordingly, using 1 g ceftriaxone for treatment, the ceftriaxone MIC of H041 will make the strain escape eradication in most (if not all) patients. Furthermore, this was a case of pharyngeal gonorrhea, which is substantially harder to treat than urogenital gonorrhea (3, 36), and the infection probably resolved spontaneously within 3 months. Nevertheless, despite the fact that a clinical history was recorded, re-reinfection cannot be completely excluded, especially as the patient was a commercial sex worker.

The resistance determinants causing the high ESC MICs in H041 were also elucidated. The unique $penA_{H041}$ mosaic allele was found to be responsible; upon transformation of $penA_{H041}$ into recipients with different ESC MICs and resistance mechanisms, their ceftriaxone MICs increased to 0.125 to 8 μg/ml, i.e., by 16-fold to 500-fold. Nevertheless, additional resistance determinants, especially *mtrR* and *penB* (and "factor X," i.e., the still unidentified determinant), were needed to reach the same level of ceftriaxone MIC as H041, a synergy that was previously reported (18, 36, 50). Factor X was not transformable using the H041 genome (data not shown), which has also

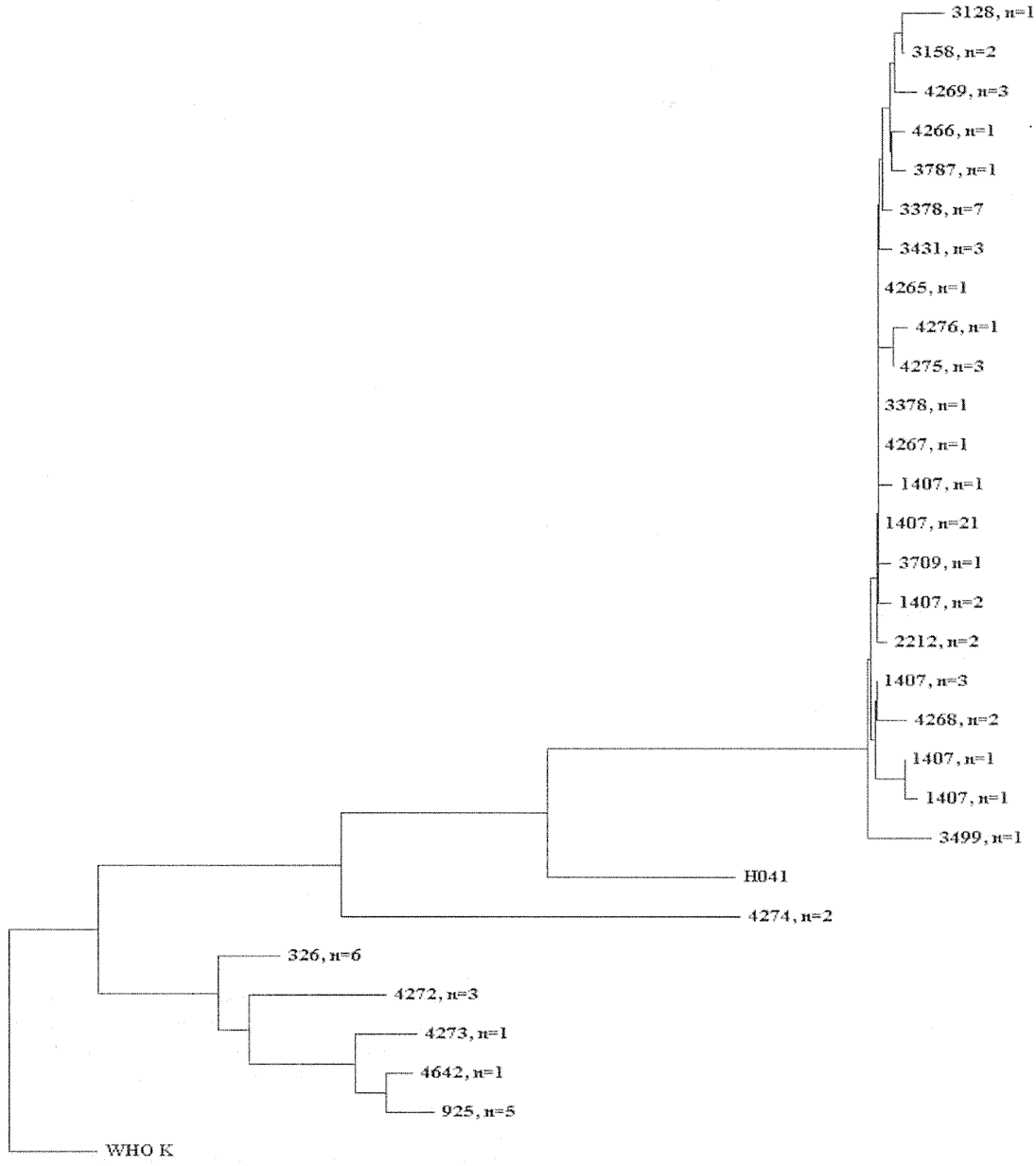

FIG. 1. Phylogenetic tree describing the evolutionary relationships of full-length *porB* gene sequences of the high-level ceftriaxone-resistant *Neisseria gonorrhoeae* strain H041 compared with those of previously published *N. gonorrhoeae penA* mosaic isolates (11). The 2008 WHO K reference strain (41), containing a *penA* mosaic allele X and cultured in Japan in 2001, was used to root the tree. The *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) sequence type (ST) and number of isolates are indicated.

previously been described using other gonococcal genomes (18, 50). $penA_{H041}$ is highly similar to the previously described *penA* mosaic allele X (causing ceftriaxone MICs of only 0.064 to 0.125) (Table 2), which has been correlated with cefixime treatment failures in Japan, having only 12 PBP2 amino acid differences clustering in two regions. Of these 12 amino acids, only four have not been previously reported in any *Neisseria* species; compared with *penA* mosaic X, these consist of A311V, T316P, A328T (in region A), and T484S (in region B) (Fig. 2). It was also confirmed that transformation of only the $penA_{H041}$ region A into the recipients caused, for most, as high a ceftriaxone MIC as transforming the full-length $penA_{H041}$ (data not shown). Although further confirmatory studies are needed, it is highly probable that A311V and T316S are the alterations causing the high ceftriaxone resistance, i.e., due to the proximity to the β-lactam active site in PBP2. Despite this fact, the MICs of some β-lactam antimicrobials, such as penicillins (especially piperacillin-tazobactam) and carbapenems (particularly ertapenem and meropenem), were surprisingly low. $penA_{H041}$ could also easily be transformed to other gono-

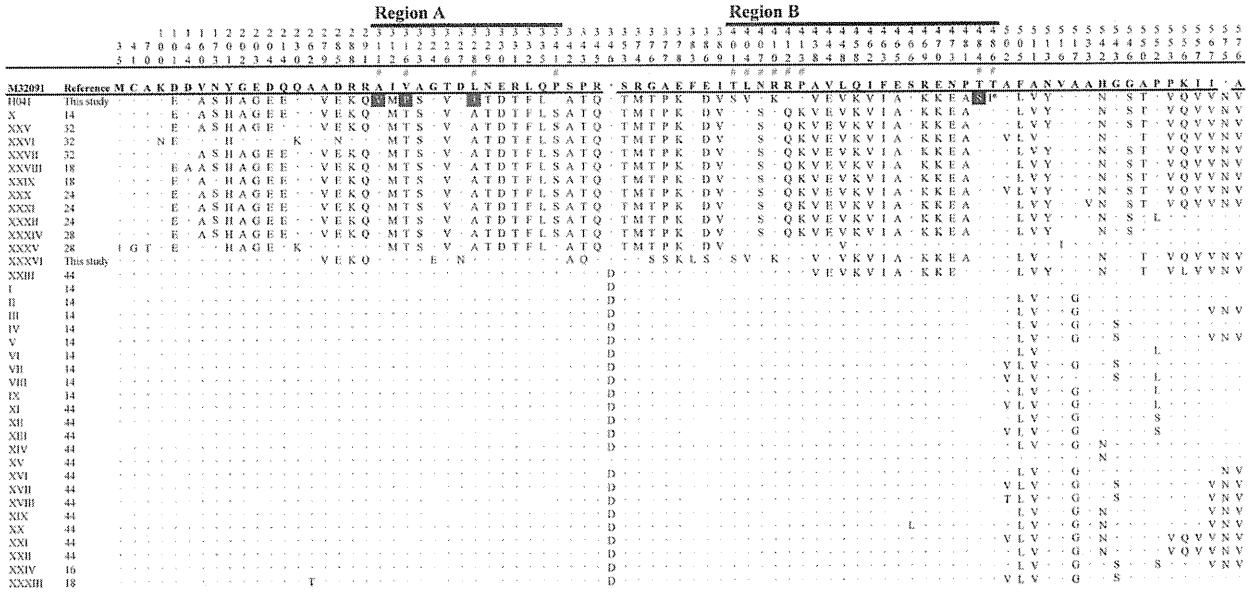

FIG. 2. A schematic figure describing all reported penicillin-binding protein 2 (PBP2) amino acid sequences in *Neisseria gonorrhoeae*, which are aligned to the wild-type PBP2 sequence M32091. All amino acid alterations in the different PBP2 sequences are illustrated with a single capital letter. The amino acids in PBP2 of H041 differing from PBP2 mosaic X (n = 12) are indicated (#). The four amino acid residues in the highly ceftriaxone-resistant *N. gonorrhoeae* strain H041 not previously observed in any *Neisseria* species and which explained the ceftriaxone resistance are shown by white letters on a blue ground. The amino acid residue marked with an asterisk has previously been found in *N. meningitidis* (unpublished) and *N. flavescens* (GenBank accession number M26645).

coccal strains in cocultivation experiments (data not shown) performed as previously described (24), which shows that this ceftriaxone resistance can rapidly spread within the *N. gonorrhoeae* population.

H041 seems to represent a subclone of the previously described MLST ST7363 cefixime-resistant *N. gonorrhoeae* circulating in Japan (23, 24). This clone has caused treatment failures using oral ESCs, has successfully spread worldwide, and now seems to have evolved further and developed resistance to ceftriaxone as well. The fear is that this ceftriaxone-resistant subclone will now spread in Japan, to WHO WPR countries, to Pacific Rim countries, and globally, which has been the scenario for emergence and worldwide spread of most gonococcal AMR. Based on previous experience (e.g., for fluoroquinolones), AMR can be widely disseminated internationally only 1 or 2 decades after the first emergence of AMR in WHO WPR (34, 36). The finding of this single high-level ceftriaxone-resistant gonococcal strain is important, especially because it was identified in a female commercial sex worker belonging to a high-risk, frequently transmitting population and because no national gonococcal antimicrobial resistance surveillance programs (including no sentinel sites for identification of gonorrhea treatment failures) are active in Japan. Accordingly, the strain should have excellent opportunities for a rapid spread. An enhanced but still limited gonococcal antimicrobial resistance surveillance in Kyoto, Japan, was initiated after the finding of H041; however, any secondary spread of H041 (or additional treatment failures) has yet not been identified. Despite the suboptimal Japanese surveillance systems, this fact may indicate that H041 has a lower biological fitness that results in limited further spread. Accordingly, the biological fitness of H041, compared to that of its wild type lacking $penA_{H041}$ that causes the ceftriaxone resistance, would be valuable to examine in a well-designed study, i.e., investigating quantitatively the fitness *in vitro* (different culture media, solid and liquid based) and also in an appropriate animal model, i.e., *in vivo*.

Nevertheless, *N. gonorrhoeae* has now shown its ability to develop resistance to ceftriaxone also, in which case gonorrhea may become untreatable in certain circumstances; although the biological fitness of H041 remains unknown, a serious public health problem seems to be approaching. To at least limit the spread of ESC (cefixime and ceftriaxone) resistance, timely and decisive multidisciplinary and multicomponent public health actions are essential not only in Japan but also globally. A recent expert review described WHO initiatives and approaches to AMR containment and how to meet public health challenges of untreatable gonorrhea (36). Nevertheless, to succeed with any AMR containment, enhanced gonorrhea control activities are needed to reduce the burden of infection (36). Furthermore, it is crucial to explore options, in industrialized settings as well as in less-resourced settings, for future treatment of ESC-resistant gonorrhea. This includes exploration of optimized dose regimens of presently used antimicrobials, new antimicrobials (or rediscovery of old drugs, such as gentamicin, ertapenem, and perhaps, piperacillin-tazobactam in emergent situations of ESC-resistant *N. gonorrhoeae*) or other substances, and combination therapy (6, 7, 19, 20, 22, 31, 36, 37; M. Unemo and J. Tapsall, unpublished data).

In conclusion, the first high-level ceftriaxone-resistant *N. gonorrhoeae* strain has now been characterized in detail, including an elucidation of its resistance mechanisms. Accordingly, *N. gonorrhoeae* has now shown its ability to develop

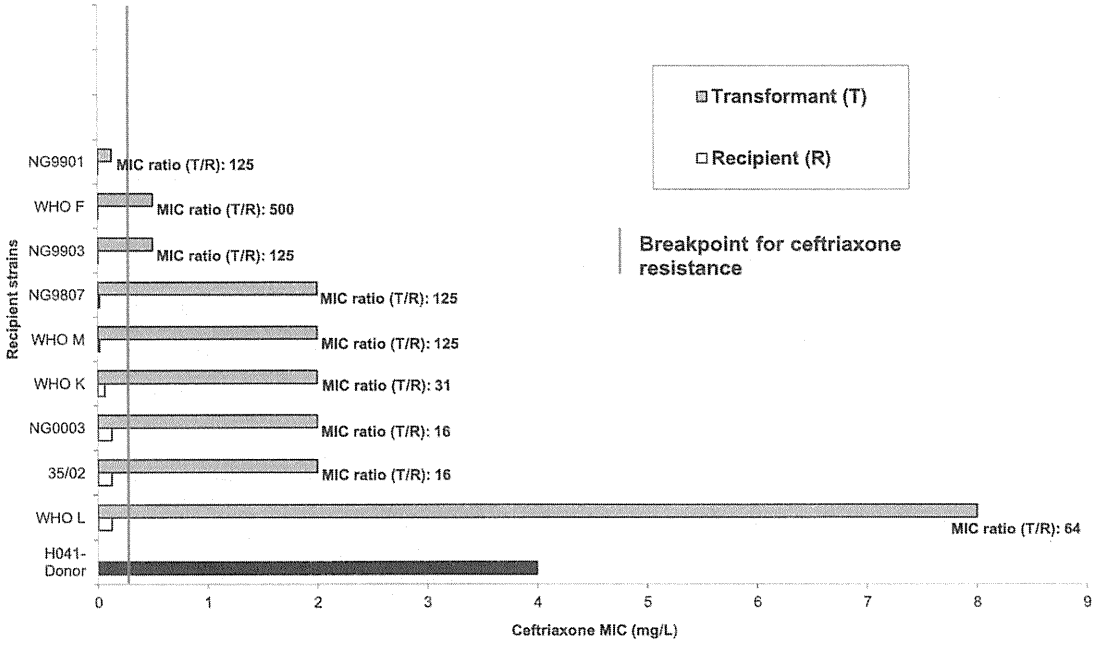

FIG. 3. Transformation of the full-length *penA* allele ($penA_{H041}$) from the high-level ceftriaxone-resistant *Neisseria gonorrhoeae* strain H041 (Donor) into *N. gonorrhoeae* strains (Recipients) with different ceftriaxone MICs and genetic resistance determinants affecting the susceptibility to ceftriaxone. The ceftriaxone MICs using the Etest method (shown as mean results of three repeated experiments) of the donor strain, recipient strains (R), and transformants (T) and the MIC ratio (T/R) are given. The breakpoint for ceftriaxone resistance is according to reference 8.

ceftriaxone resistance also and, although the biological fitness of ceftriaxone resistance in *N. gonorrhoeae* remains unknown, the gonococcus may soon become a true superbug that initiates a future era of untreatable gonorrhea. To at least slow the spread of ESC (cefixime and ceftriaxone) resistance, a reduction in global gonorrhea burden by enhanced disease prevention and control activities is crucial. As well, the implementation of much wider strategies for general AMR control, better understanding of the mechanisms and global monitoring of the emergence and spread of AMR, and global and national public health response plans (including sustainable clinical, microbiological, and epidemiological components) are needed. Any such plan alone will most probably not be able to prevent the emergence, establishment, and spread of ceftriaxone resistance; nevertheless, these plans will be valuable to delay and limit a global spread of ESC resistance (cefixime and ceftriaxone). Ultimately, a major focus important for public health globally is the timely development of effective new drugs (for single or combined use) for the treatment of gonorrhea.

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Molecular Analyses of TEM Genes and Their Corresponding Penicillinase-Producing *Neisseria gonorrhoeae* Isolates in Bangkok, Thailand

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***Neisseria gonorrhoeae* is a major public health problem globally, especially because the bacterium has developed resistance to most antimicrobials introduced for first-line treatment of gonorrhea. In the present study, 96 *N. gonorrhoeae* isolates with high-level resistance to penicillin from 121 clinical isolates in Thailand were examined to investigate changes related to their plasmid-mediated penicillin resistance and their molecular epidemiological relationships. A β-lactamase (TEM) gene variant, $bla_{TEM-135}$, that may be a precursor in the transitional stage of a traditional bla_{TEM-1} gene into an extended-spectrum β-lactamase (ESBL), possibly causing high resistance to all extended-spectrum cephalosporins in *N. gonorrhoeae*, was identified. Clonal analysis using multilocus sequence typing (MLST) and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) revealed the existence of a sexual network among patients from Japan and Thailand. Molecular analysis of the $bla_{TEM-135}$ gene showed that the emergence of this allele might not be a rare genetic event and that the allele has evolved in different plasmid backgrounds, which results possibly indicate that it is selected due to antimicrobial pressure. The presence of the $bla_{TEM-135}$ allele in the penicillinase-producing *N. gonorrhoeae* population may call for monitoring for the possible emergence of ESBL-producing *N. gonorrhoeae* in the future. This study identified a bla_{TEM} variant ($bla_{TEM-135}$) that is a possible intermediate precursor for an ESBL, which warrants international awareness.**

Neisseria gonorrhoeae is the causative agent of gonorrhea, which is the second most prevalent bacterial sexually transmitted infection globally. During recent decades, *N. gonorrhoeae* has rapidly developed resistance to most classes of antimicrobials used for treatment of gonorrhea (4, 6, 17, 18, 20). Penicillinase-producing *N. gonorrhoeae* (PPNG), with plasmid-mediated high-level resistance to penicillin, was first reported in 1976 (1, 14) and has since been disseminated worldwide (2). The first gonococcal strain with high-level clinical resistance to ceftriaxone, which is the last remaining option for first-line gonorrhea treatment, was recently found in Japan and completely characterized (9, 11). However, the resistance to ceftriaxone was chromosomally mediated, and no extended-spectrum β-lactamase (ESBL) has yet been identified in *N. gonorrhoeae*. If an ESBL did emerge in *N. gonorrhoeae* and spread internationally, gonorrhea would become an extremely serious public health problem.

PPNG strains are rare in Japan, but these strains have remained highly prevalent in several other countries in Asia (19) and worldwide (20). Penicillin is still also used as the first-line drug in, e.g., some Pacific island countries and the northern part of Australia, because of maintained efficacy in the settings and its low cost.

Although the β-lactamase (TEM) gene of authentic PPNG is the bla_{TEM-1} allele, a recently isolated PPNG in Thailand possessed the $bla_{TEM-135}$ allele, which differs from the bla_{TEM-1} allele with one single nucleotide polymorphism (SNP) at position 539, resulting in a single amino acid substitution, M182T (16). However, the prevalence and characteristics of TEM-135 strains worldwide are unknown and seem critical to study, especially in countries where PPNG strains are highly prevalent. Furthermore, the knowledge regarding the genetic relationships of PPNG strains, their TEM genes, and plasmids carrying β-lactamase is highly limited.

Therefore, in the present study, PPNG isolates cultured from 2005 to 2007 in Thailand, which has a relatively high prevalence of PPNG, were investigated. To detect $bla_{TEM-135}$ in the PPNG strains, a simple and rapid mismatch amplification mutation assay (MAMA) PCR method (3) was developed and successfully used. To reveal the population structure of the PPNG isolates, molecular epidemiological typing by means of multilocus sequence typing (MLST) (5), *porB* gene sequencing, and *N. gonorrhoeae* multiantigen sequence typing NG-MAST (7) were used to compare the detected TEM-135 strains with the TEM-1 strains.

MATERIALS AND METHODS

Bacterial isolates. *N. gonorrhoeae* isolates were collected from Siriraj Hospital, Bangkok, Thailand. Among 121 isolates collected during 2005 to 2007, based on resistance to penicillin and a positive nitrocefin test, a total of 96 PPNG isolates were detected and analyzed (see the supplemental material). These isolates were systematically collected in a previous research project (16).

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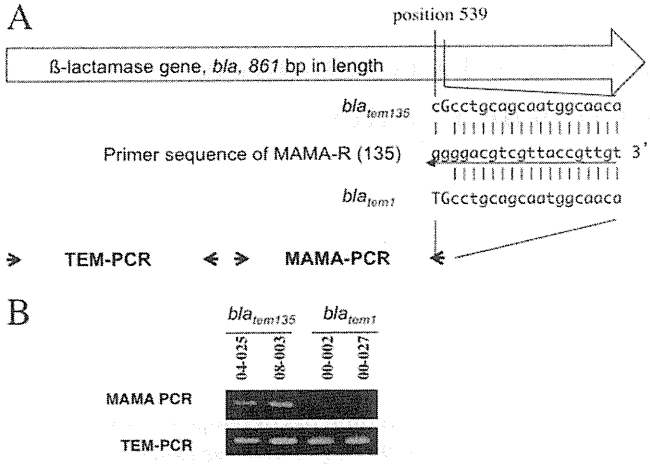

FIG 1 MAMA-PCR for $bla_{TEM-135}$ detection. (A) The TEM PCR primer set (TEM-F and TEM-R), which can amplify a 231-bp amplicon from bla_{TEM-1} and $bla_{TEM-135}$, and the MAMA-PCR primer set, specific for $bla_{TEM-135}$ (MAMA-F and MAMA-R), are shown schematically with arrows. The sequence of primer MAMA-R (middle) and the corresponding regions from bla_{tem135} (top) and bla_{tem1} (bottom) are also shown. (B) The PCR results for the Japanese penicillinase-producing *N. gonorrhoeae* (PPNG) TEM-135 and PPNG TEM-1 isolates, which were used as controls in all PCRs, are presented.

DNA isolation. To obtain genomic DNA, isolates were suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and boiled for 10 min. After removing cell debris by centrifugation, the supernatant was used directly as template DNA in the PCR.

PCR identification of bla_{TEM} gene. The MAMA-PCR to detect sequence polymorphism between the bla_{TEM-1} and $bla_{TEM-135}$ alleles focused on nucleotide position 539 of the bla_{TEM} gene (Fig. 1A). A conserved forward primer (MAMA-F, 5′-GCATCTTACGGATGGCATGAC-3′) and a $bla_{TEM-135}$ allele-specific polymorphism detection primer (MAMA-R, 5′-TGTTGCCATTGCTGCAGGGG-3′) were designed (Table 1). The $bla_{TEM-135}$ allele-specific primer carries a specific nucleotide, G (bold and underlined), at the 3′ end. Furthermore, to enhance the 3′ end mismatch effect, an additional nucleotide alteration of G, rather than C (bold), at the second nucleotide from the 3′ end of the primer was introduced. Thus, the $bla_{TEM-135}$ allele-specific primer contained two mismatched bases at the 3′ end relative to the sequence of bla_{TEM-1} (Fig. 1A). In brief, the 10-μl-volume PCR master mix contained diluted template DNA, 0.8 μl of 2.5 mM deoxynucleoside triphosphate (dNTP) mixture (final concentration, 200 μM each), 0.25 μl each of 10 μM MAMA-F and MAMA-R primers (final concentration, 250 nM each), and 0.25 units of the DNA polymerase Takara Ex *Taq* (Takara Bio Co., Kyoto, Japan). The parameters of the PCRs were as follows: incubation for 2 min at 96°C followed by 25 cycles of 10 s at 96°C, 10 s at 56°C, and 30 s at 72°C and then final extension for 2 min at 72°C. The previously described *N. gonorrhoeae* strains NGON 00-002 and NGON 00-027 (containing bla_{TEM-1}) and NGON 04-025 and NGON 08-003 (containing $bla_{TEM-135}$) (10) were used as controls in all PCRs (Fig. 1). The universal TEM PCR was done as described above except that the PCR master mix contained TEM-F and TEM-R primers (Table 1). To confirm TEM alleles, we sequenced PCR-amplified products of the whole bla_{TEM} coding region using the primer set bla-F and bla-R as described previously (10).

Molecular epidemiological characterization. Molecular epidemiological characterization by means of MLST (5), *porB* gene sequencing, and NG-MAST was performed as described previously (7). The type of plasmid carrying the β-lactamase (TEM) gene was determined by a multiplex PCR method developed by Palmer et al. (12). Neighbor-joining trees with *por* and *tbpB* nucleotide sequences were generated by using MEGA4.

Drawing of minimum spanning tree. Based on the MLST data, a minimum spanning tree was generated by using BioNumerics (version 5.1; Applied Math), using the categorical coefficient of similarity and the priority rule of the highest number of single-locus variants as parameters. No hypothetical sequence or reported sequences other than those identified in the present study were included in the calculation.

RESULTS AND DISCUSSION

Development and use of the MAMA-PCR for detection of $bla_{TEM-135}$. To differentiate the $bla_{TEM-135}$ allele from the bla_{TEM-1} allele in PPNG strains, by detection of the SNP at position 539, a MAMA-PCR (detecting only $bla_{TEM-135}$) was successfully developed and was used together with a TEM PCR (detecting both $bla_{TEM-135}$ and bla_{TEM-1}) (Fig. 1).

Nine of the 96 PPNG isolates from Thailand were positive in both the MAMA-PCR and TEM PCR, suggesting that these isolates possessed the $bla_{TEM-135}$ allele. Sequencing analysis of the full-length PCR products from the *bla* gene confirmed that these nine isolates (9.4%) indeed contained the $bla_{TEM-135}$ allele, and the remaining 87 isolates (90.6%) possessed bla_{TEM-1}.

Genetic relationships of PPNG TEM-1 and PPNG TEM-135 isolates. In order to examine the genetic relationships of PPNG isolates containing TEM-1 and TEM-135, MLST was carried out. Twenty-three MLST STs were identified among the 96 PPNG isolates, 17 STs among the TEM-1 isolates and 6 among the TEM-135 isolates. Among the 17 MLST STs identified among the TEM-1 isolates, ST1588 was the most prevalent (55 out of the 87 TEM-1 isolates, 63.2%) (Table 2). A minimum spanning tree analysis showed that most of the other STs in TEM-1 isolates were closely related to ST1588, with few exceptions (Fig. 2). Accordingly, 83 out of the 87 (95.4%) TEM-1 isolates belonged to a large cluster comprising 15 STs and centered around ST1588 (cluster A) (Fig. 2 and Table 2). The remaining four TEM-1 isolates were assigned ST8782 ($n = 2$) and ST8775 ($n = 2$), which formed an additional smaller cluster (cluster B) (Fig. 2 and Table 2).

Six different MLST STs were found in the nine TEM-135 isolates (Fig. 2 and Table 2). ST8778 was the most common ($n = 4$, 44.4%), and the other five STs were singletons. All these TEM-135 isolates, with the exception of the singleton ST7822 (isolate Thai_026) that was placed in the TEM-1 cluster A, belonged to the same separate cluster (cluster C) (Fig. 2 and Table 2). Taken together, Thailand PPNG TEM-1 and PPNG TEM-135 strains seem to belong to distinct clonal groupings with different genetic backgrounds, and also, TEM-135 strains have emerged from multiple independent origins.

Plasmid typing. Plasmid typing has been used as another classification method for PPNG surveillance. We also performed plasmid typing and investigated relationships with the results of MLST and the specific alleles of the *bla* genes bla_{TEM-1} and $bla_{TEM-135}$.

As shown in Table 2, the Africa-type β-lactamase plasmid was the predominant type (79 of 96 isolates, 82.3%) in the isolates

TABLE 1 Primers used in the MAMA-PCR for detection of $bla_{TEM-135}$ and the TEM-PCR for detection of both bla_{TEM-1} and $bla_{TEM-135}$

Primer	Primer sequence (5′ to 3′)	Position
MAMA-F	GCATCTTACGGATGGCATGAC	327-347
MAMA-R[a]	TGTTGCCATTGCTGCAGGGG	558-539
TEM-F	GTCGCCCTTATTCCCTTTTTTG	22-43
TEM-R	TAGTGTATGCGGCGACCGAG	284-268

[a] Binds only $bla_{TEM-135}$.

TABLE 2 MLST sequence type and plasmid type of PPNG isolates cultured in Thailand in 2005 to 2007[a]

No. of isolates	MLST		No. of isolates with plasmid type[c]:		
	ST	Cluster[b]	Africa	Asia	Toronto/Rio
55	1588	A	53	1	1
5	8780	A	5		
4	1903	A	4		
4	8774	A	4		
2	1584	A	2		
2	1921	A	2		
2	8779	A	2		
1	7827	A	1		
1	8145	A	1		
1	8776	A	1		
1	8783	A	1		
1	8777	A	1		
2	7823	A		2	
1	1600	A		1	
1	7822	A		1 (1)[d]	
1	8781	A		1	
2	8775	B		2	
2	8782	B	2		
4	8778	C			4 (4)
1	1582	C			1 (1)
1	8136	C			1 (1)
1	8143	C			1 (1)
1	8784	C			1 (1)
96		TOTAL	79	8 (1)	9 (8)

[a] MLST, multilocus sequencing typing (5); PPNG, penicillinase-producing *Neisseria gonorrhoeae*.
[b] Clusters were defined by the minimum spanning tree in Fig. 2.
[c] Plasmid typing was determined by a multiplex PCR (12).
[d] The number of PPNG TEM-135 isolates is shown in parentheses.

analyzed in the present study. Asia- and Toronto/Rio-type β-lactamase plasmids were found in only eight and nine isolates, respectively. Recently, a new type of the β-lactamase plasmid (Johannesburg plasmid) was reported by Muller et al. (8). If the Johannesburg-type plasmid had existed in our isolates, it would have generated a 450-bp amplicon with the BL1 and BL3 primers in our multiplex PCR system. However, we did not find any isolates containing this plasmid. Notable, all TEM-135 isolates, except Thai_026 (MLST ST7822) which had an Asia-type plasmid, carried the Toronto/Rio-type plasmid. As described above, Thai_026 (MLST ST7822) was the only isolate that belonged to cluster A formed by the TEM-1 isolates. Thus, the plasmid typing supported separation of this isolate from the other TEM-135 isolates, which further supports the hypothesis that TEM-135 strains have emerged from multiple independent origins. There was no TEM-135 isolate with the Africa-type plasmid. On the other hand, although all three plasmid types were found among the TEM-1 isolates, the Africa-type plasmid was the most abundant among the TEM-1 isolates (79 out of 87 isolates, 90.8%). Thus, this plasmid typing, again, implied a genetic difference of TEM-135 and TEM-1 strains. The most abundant MLST ST in TEM-1 isolates, ST1588, was strongly related to the Africa-type plasmid (53 out of 55 isolates, 96.4%). The remaining two MLST ST1588 isolates carried Toronto/Rio- and Asia-type plasmids, respectively. In total, the Africa-type plasmid was also abundant in other MLSTs, although both of the MLST ST8775 isolates had the Asia-type plasmid. Other isolates with the Asia-type plasmid were limited to MLST ST8781, MLST ST7822 (single TEM-135 isolate), MLST ST7823, and MLST ST1600 (Table 2). Interestingly, three of these MLST ST (except MLST ST1600) were linked and formed a stem in the left part of the minimum spanning tree (Fig. 2).

NG-MAST analysis. To thoroughly evaluate the genetic diversity and relatedness of the TEM-135 isolates, all PPNG isolates were also analyzed using a substantially more discriminative typing method, NG-MAST (7). The 96 PPNG isolates were divided into 58 NG-MAST STs. Each NG-MAST ST is shown in the supplemental material.

Among the four TEM-135 isolates assigned to MLST ST8788, three belonged to NG-MAST ST5134 (*porB3109* and *tbpB98*; Fig. 3A), indicating clonal dissemination. Also, four of the additional TEM-135 isolates contained highly similar *porB* alleles (Thai_098, Thai_045, Thai_003, and Thai_032) (Fig. 3B). This similarity and, accordingly, the clustering were further supported by analyzing the *tbpB* alleles of all the TEM-135 isolates (Fig. 3C). Accordingly, the NG-MAST supported the conclusion that seven of the nine TEM-135 isolates had originated from a common ancestor. Both the remaining TEM-135 isolates (Thai_026 and Thai_073) were genetically separated from this cluster by the NG-MAST (Fig. 3). This was also in full concordance with the results of the MLST and plasmid typing (Table 2 and Fig. 2).

Comparison of the Thai isolates with previously characterized Japanese PPNG isolates. Using several molecular typing methods, we tried to identify any spread of PPNG between Thailand and Japan and found the possible spread of only one TEM-1

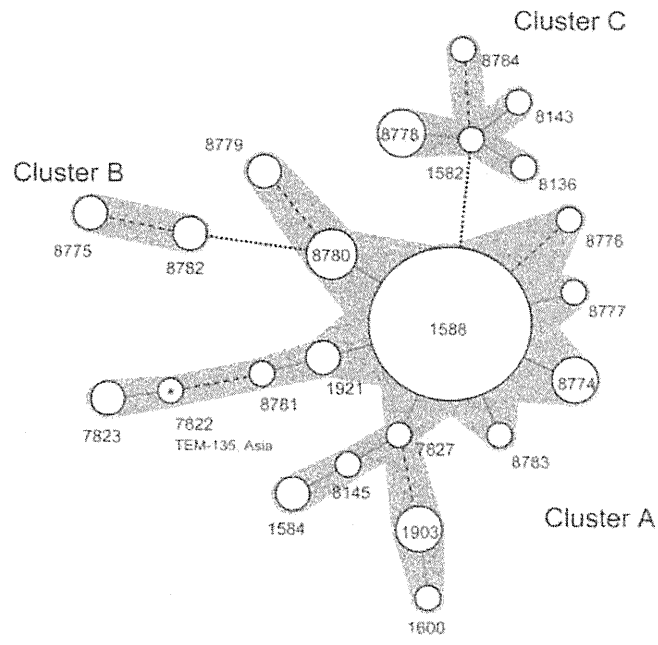

FIG 2 Minimum spanning tree analysis of multilocus sequence typing (MLST) STs observed in penicillinase-producing *N. gonorrhoeae* (PPNG) isolates cultured in Thailand in 2005 to 2007. Numbers beside the circles denote ST. Solid lines, dashed lines, and dotted lines show the interrelationship of "single-locus variant," "double-locus variant," and "triple-locus variant," respectively. The types directly or indirectly connected through single- or double-locus-variant relationships were judged to form one cluster. Each cluster is shaded gray. Sizes of circles reflect the numbers of isolates belonging to each type (for details, see text and tables). The only PPNG TEM-135 isolate belonging to cluster A (ST7822) is marked with an asterisk, and its plasmid type is given.

A

Strain	MLST	*por*	*tbpB*	NG-MAST
Thai_022, _078, _127	8778	3109	98	5134
Thai_003	8778	2809	1084	5138
Thai_045	8143	1830	1088	5132
Thai_098	8784	832	98	1292
Thai_032	1582	53	21	147
Thai_026	7822	3110	186	5135
Thai_073	8136	90	58	211

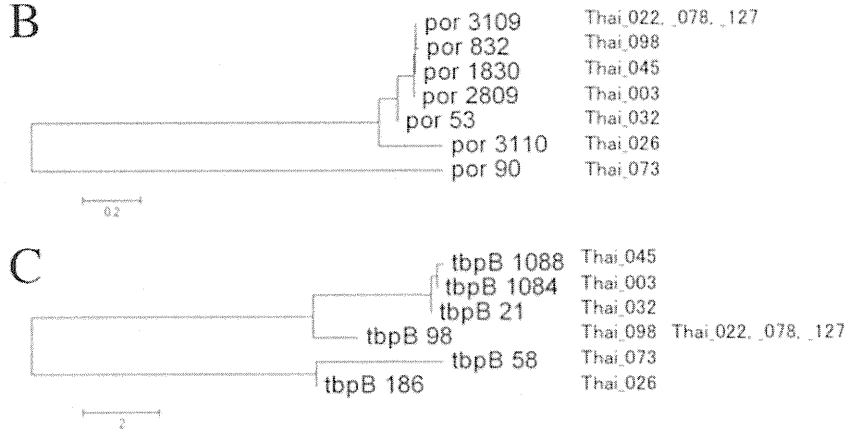

FIG 3 Molecular characterization of penicillinase-producing *N. gonorrhoeae* PPNG TEM-135 isolates. (A) Sequence types revealed by multilocus sequence typing (MLST) and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) are shown, along with *por* and *tbpB* alleles. (B and C) Neighbor-joining clustering showing similarity of *por* alleles (B) and *tbpB* alleles (C) from PPNG TEM-135 isolates.

clone. Accordingly, the previously characterized Japanese isolates NGON 08-041 and NGON 08-046 (10) and Thai_036 and Thai_093 were all, in the present study, assigned to MLST ST1584 and NG-MAST ST1478 and carried the Africa-type plasmid with bla_{TEM-1}. Despite some similarities in the MLST STs supporting, e.g., a cluster of isolates with the TEM-135 Toronto/Rio-type plasmid, no clear evidence to support international spread of any TEM-135 strains was found.

It is well-known that the β-lactamase plasmid can also easily be transferred between different *N. gonorrhoeae* strains. As the number of analyzed isolates in the present study was relatively low and they were cultured in restricted regions, Thailand (Bangkok) and Japan (Tokyo), more extensive international studies are crucial to reveal the origin and the evolutionary pathway of the TEM-135 strains, as well as the possible existence of PPNG with other TEM alleles.

Possible motive force of emergence of TEM-135. Still, the reasons and mechanisms for the emergence and dissemination of PPNG TEM-135 strains are unknown. The $bla_{TEM-135}$ allele was first found in *Salmonella enterica* serovar Typhimurium (13), and there are no major differences in the MICs of any β-lactam antimicrobials between $bla_{TEM-135}$ and bla_{TEM-1} allele-possessing isolates. The $bla_{TEM-135}$ allele has now been found in two different types of β-lactamase plasmid in PPNG, which are known to originally carry the bla_{TEM-1} allele. This fact indicates that $bla_{TEM-135}$ emerged independently in *N. gonorrhoeae* and was not acquired due to, for example, a transformational event. However, due to the similar MICs of β-lactam antimicrobials in PPNG TEM-1 and TEM-135 isolates, other factor(s) than β-lactam antimicrobial selective pressure must be the selective force in the emergence of $bla_{TEM-135}$. One possibility might be a pressure by other antibiotic(s) than penicillins. If so, we could expect some different patterns of resistance or rate of resistance to nonpenicillin antibiotics between TEM-1 and TEM-135 isolates. However, we did not observe any significant difference in those, at least when comparing susceptibility and resistance to ceftriaxone, ciprofloxacin, and tetracycline (data not shown). Another possibility is that this selective force may be an enhanced stability of the β-lactamase enzyme, which the TEM-135-specific amino acid substitution (M182T) is considered to establish (15, 21). Usually, this amino acid substitution is found in extended-spectrum TEM-type β-lactamase, as the second substitution. Since an amino acid substitution close to the active site of β-lactamase, which results in an increased MIC of cephalosporins, tends to decrease the stability of the enzyme, the M182T substitution may play a role as a stabilizer. In this context, the M182T in $bla_{TEM-135}$ in PPNG might be a prerequisite to allow the subsequent substitutions, which could extend the antimicrobial resistance spectrum of the enzyme, like several TEM-type β-lactamases found in other bacteria, e.g., TEM-20 carriers.

Necessity of monitoring TEM-135 PPNG. In conclusion, an emergence of ESBL-producing *N. gonorrhoeae* would be highly threatening to public health, because this would also be resistant to ceftriaxone, which is the first-line and last remaining option for treatment of *N. gonorrhoeae* infection in many countries world-

wide. Recently, the first *N. gonorrhoeae* strain with chromosomally mediated high-level resistance to ceftriaxone was isolated in Japan (9, 11). Although this strain was not PPNG, i.e., it had a *penA*-dependent resistance mechanism, this calls for a substantially strengthened monitoring of ceftriaxone-resistant *N. gonorrhoeae* infection and gonorrhea treatment failures, including consideration of possible emergence of ESBL-producing *N. gonorrhoeae* isolates.

In Thailand, about 10% of PPNG had TEM-135, a possible direct precursor of an ESBL. However, the prevalence and characteristics of TEM-135 strains and possible strains containing other TEM variants worldwide is unknown. This seems crucial to investigate in larger, international studies, including studies of recent geographically, phenotypically, and genetically diverse PPNG.

ACKNOWLEDGMENTS

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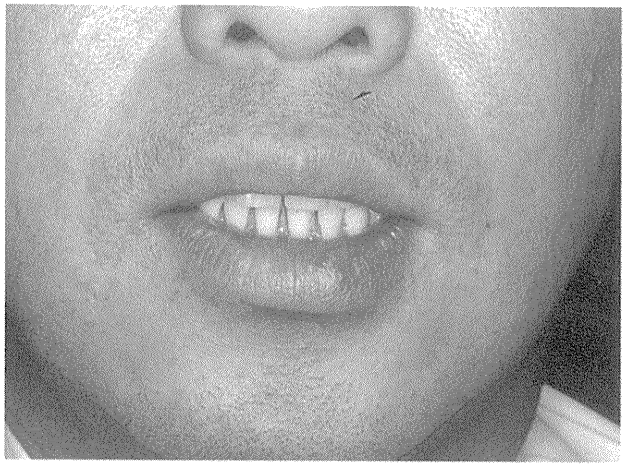

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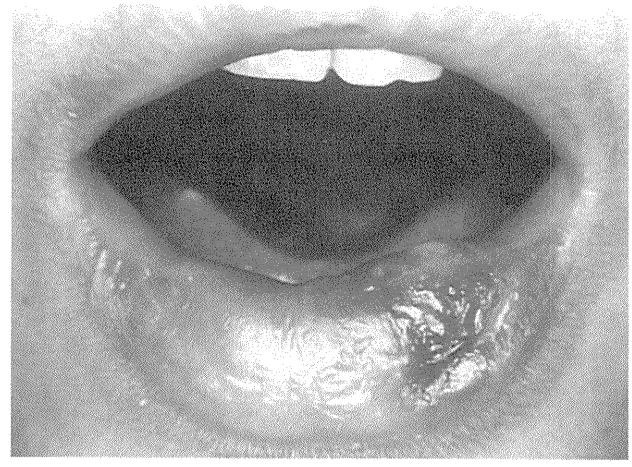

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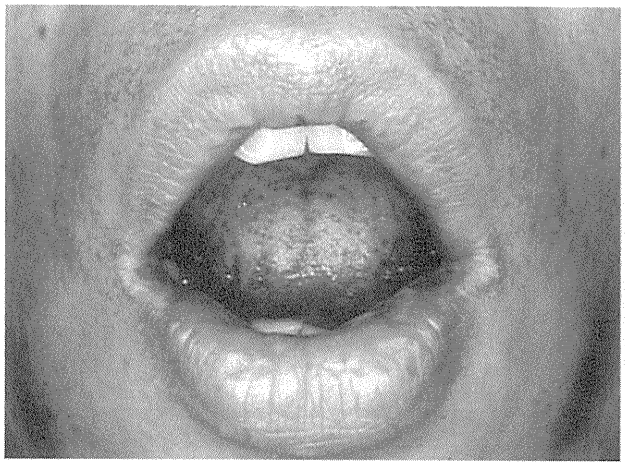

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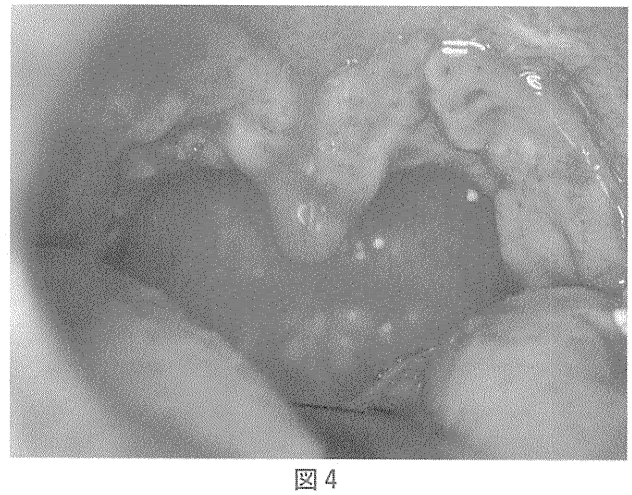

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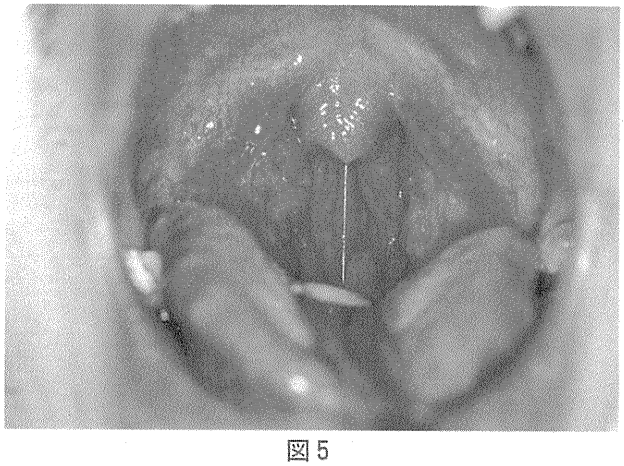

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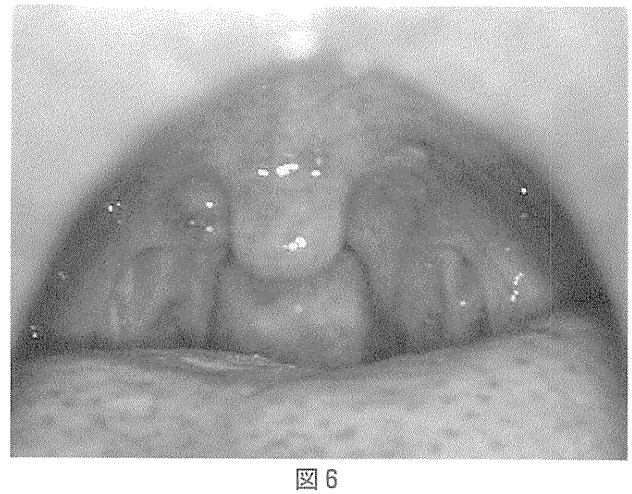

図6

図1　梅毒第1期　下口唇の初期硬結
図2　梅毒第1期　下口唇の硬性下疳
図3　梅毒第2期　梅毒性口角炎
図4　梅毒第2期　咽頭粘膜斑：butterfly appearance を呈した粘膜斑
図5　淋菌性急性咽頭扁桃炎：扁桃表面の白色調のびらんを中心に咽頭全体の発赤を認める
図6　淋菌陽性の CSW の咽頭所見：咽頭に発赤などの異常は見られず、自覚症状もない無症候性感染。

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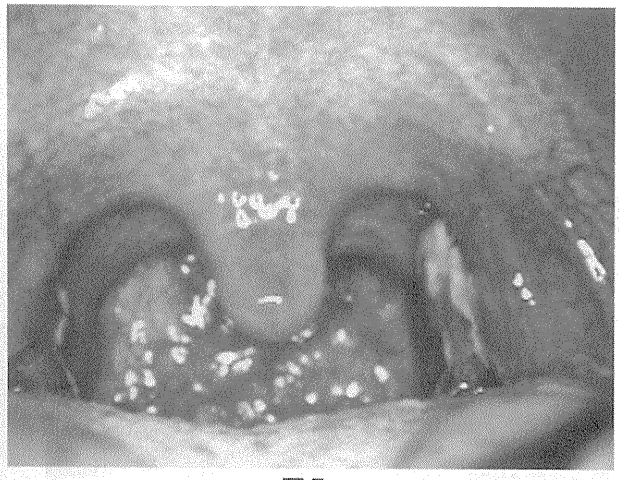

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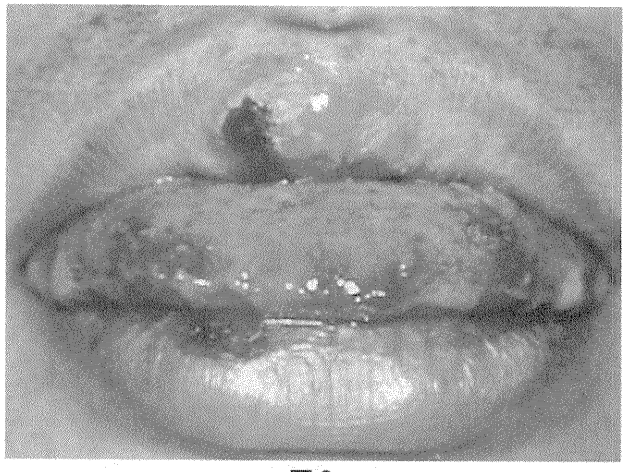

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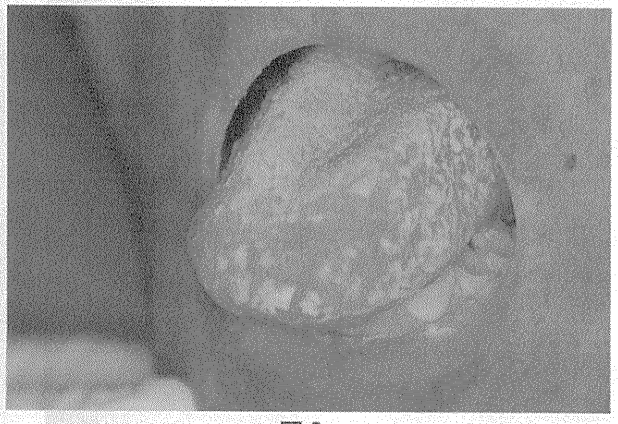

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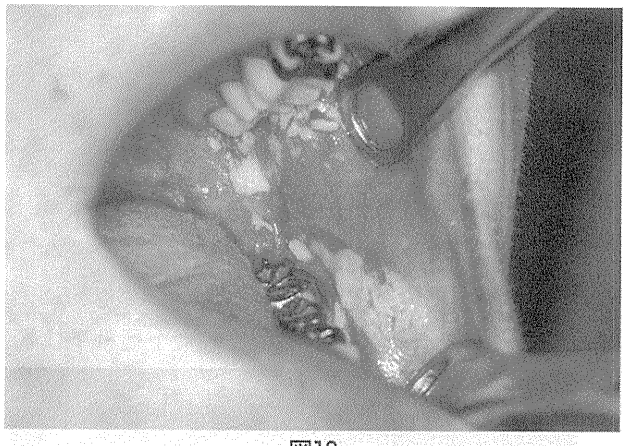

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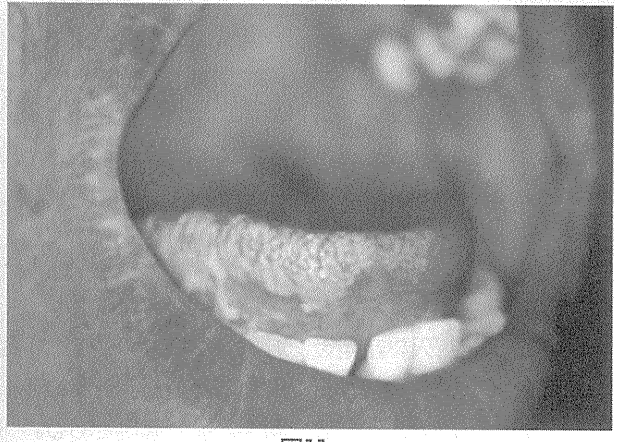

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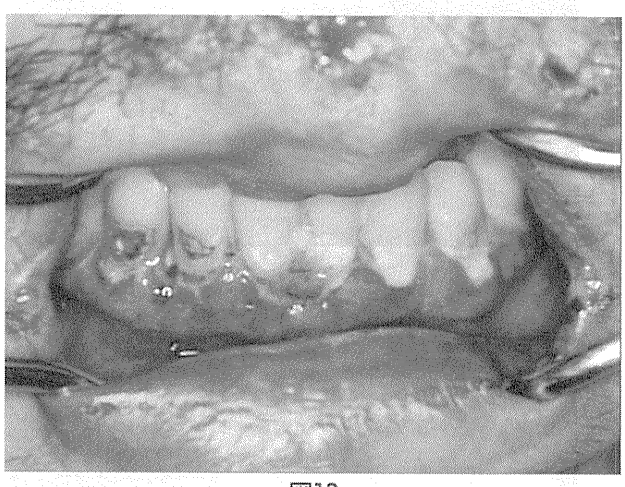
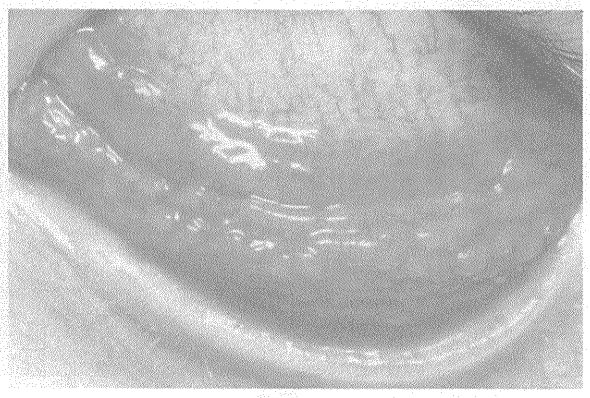


図12

図7　ヘルペス（HSV）性咽頭扁桃炎
図8　ヘルペス（HSV）性口唇舌炎：図7と同じ症例
図9　エイズーカンジダ症：舌

図10　エイズーカンジダ症：頬部粘膜
図11　エイズー口腔毛様白斑症
図12　HIV感染に伴う歯肉炎



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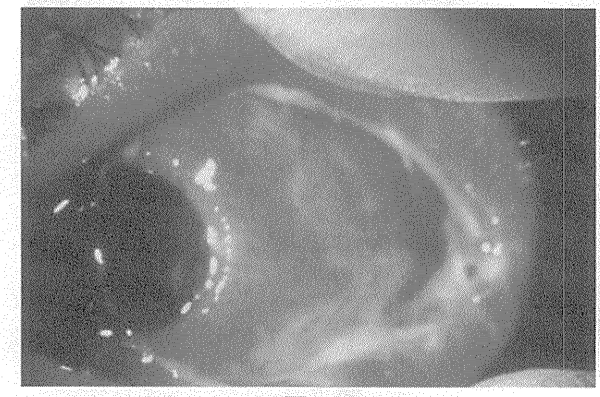

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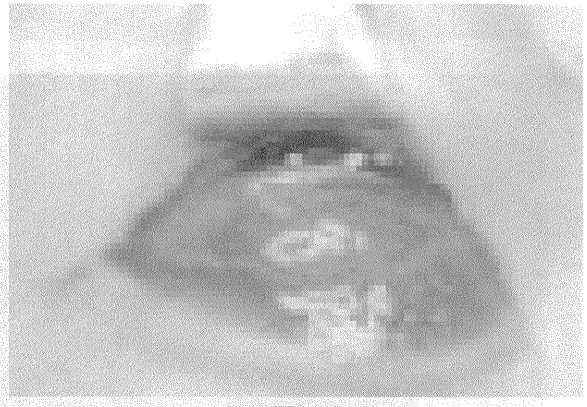

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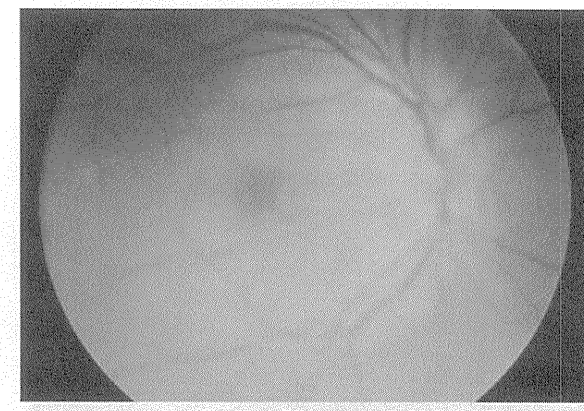

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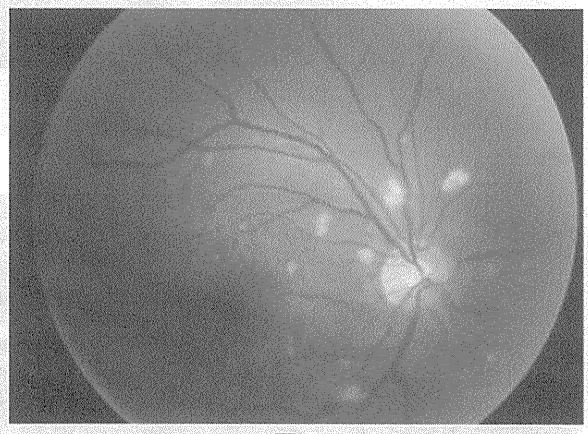

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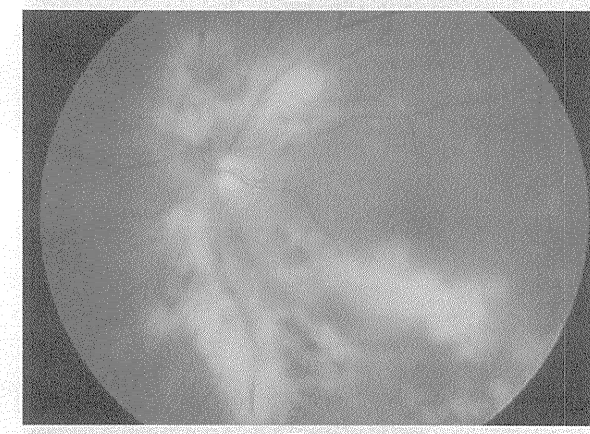

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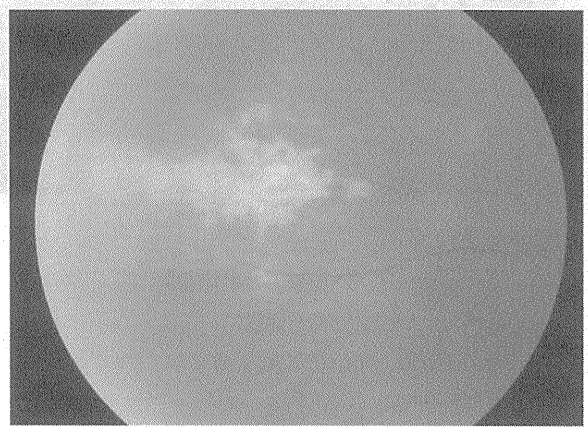

図7

図1　成人型封入体結膜炎：堤防状の濾胞形成を認める。
図2　淋菌性結膜炎：クリーム状の膿性眼脂を認める。
図3　新生児膿漏眼：結膜、眼瞼腫脹が著明である。
図4　梅毒性網脈絡膜炎：黄色の大小滲出斑を認める。
図5　HIV 網膜症に認める綿花様白斑
図6　AIDS 患者に発症した Fulminant/edematous lesion（激症 / 浮腫状病変）型の CMV 網膜炎。
図7　AIDS 患者に発症した Indolent/granular lesion（緩徐 / 顆粒状病変）型の CMV 網膜炎。

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2011（平成23）年6月

日本性感染症学会
前 理 事 長　守 殿 貞 夫
理 事 長　小野寺 昭 一
ガイドライン委員長　岸 本 寿 男

日本性感染症学会では、2002年にわが国初の性感染症（sexually transmitted infections：STI）に関するガイドラインとして「性感染症　診断・治療　ガイドライン」を発行しました。諸外国では、アメリカCDCや、オーストラリアなどのガイドラインがよく知られていますが、諸外国とわが国では疾患の分布や割合、重要性、医療制度の違いによる診断・治療の保険適応等が異なることから、わが国に適したガイドラインが必要であるという認識のもとに作成されたものです。2002年の初版以来、新しい検査法や治療薬剤の登場、また薬剤耐性菌の出現や、新たな知見等による臨床対応の変更に応えるため、2004年版、2006年版、2008年版と2年ごとに改訂が行われてきました。

今回も、当初は2010年版として発行の準備を進めていましたが、同年12月に福岡市で開催された第23回日本性感染症学会学術大会（田中正利会長）で、このガイドラインのコンセンサスミーティングを開催することとなり、会場では主要疾患の暫定版を配布し、報告・討論が行われました。その会場での討論内容や、その後寄せられた会員からのご意見を加味しました。さらに、折から改訂されたCDCガイドライン2010の内容を点検して、ここに3年ぶりに2011年版として発行することとなりました。

今回の2011年改訂版の基本的なコンセプトは、これまでの改訂とほぼ同様であり、2008年版をもとに、必要に応じて執筆者やコメンテーターの交代や追加、内容の見直し、加筆等を行って、よりup to dateなものを目指しました。主な改訂のポイントとしては、「クラミジア感染症」と「淋菌感染症」について、男性と女性の項目の書き分けをしました。「梅毒」については、血清検査におけるガラス板法の廃止に伴うコメントを記しています。また「非クラミジア性非淋菌性尿道炎」については、近年の原因病原体についての知見を盛り込みました。その他にも保険適応などの状況の変化への対応や、2008年度版以降に盛り込まれた治療薬の推奨度をグレード分けするという記載方法を、さらに臨床により即したものになるように努めました。また、性感染症の若年化が進んでいる現状を踏まえて、思春期への対応の必要性を鑑み、今回新たに3部として「思春期と性感染症」の総論的な記述を加えました。今後の改訂で各論の追加を検討する予定です。

なお、本ガイドラインでは、担当者・コメンテーターは現在の最良の治療方法を記載していますが、中には、保険が適応されないものもあります。本ガイドラインに記載することにより、これらの診断・治療が将来保険適応となるための推進力となればと思っています。

また、今回の改訂版の発行後には、諸外国への情報発信の重要性を考え、内容をコンパクトにまとめた英語版を作成しホームページに掲載することも予定しています。

最後になりましたが、今回の改訂にご尽力いただいた担当者ならびにコメンテーターの方々に深謝いたします。また、本ガイドラインの内容についてお気付きの点は是非事務局にご一報下さい。

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