TABLE 1. Description of *Neisseria gonorrhoeae* isolates from Kanagawa Prefecture and Tokyo with reduced susceptibility to cefixime, 1998 to 2005

Isolate	Yr of isolation	Hospital	Sex[a]	Specimen[b]	Cefixime MIC (μg/ml)	MLST ST	PBP 2 type
NG9806	1998	Kanagawa, H1	M	U	0.25	7363	X
NG9811	1998	Kanagawa, H1	F	VD	0.25	7363	X
NG9812	1998	Kanagawa, H1	F	VD	0.25	7363	X
NG9911	1999	Kanagawa, H2	M	UD	0.25	7363	X
NG9912	1999	Kanagawa, H3	M	U	0.25	7363	X
NG9913	1999	Kanagawa, H3	M	U	0.25	7363	X
NG9914	1999	Tokyo, H9	M	U	0.25	7363	X
NG0002	2000	Kanagawa, H4	F	VD	0.25	1901	X
NG0003	2000	Kanagawa, H2	M	UD	0.25	7363	X
NG0008	2000	Kanagawa, H2	M	UD	0.25	7363	X
NG0109	2001	Kanagawa, H6	M	UD	0.25	7363	X
NG0110	2001	Kanagawa, H5	M	UD	0.25	1596	X
NG0111	2001	Tokyo, H9	M	U	0.25	1590	XXXI
NG0201	2002	Kanagawa, H7	M	UD	0.25	1596	X
NG0204	2002	Kanagawa, H7	M	UD	0.25	7363	X
NG0205	2002	Kanagawa, H2	M	UD	0.25	7363	X
NG0206	2002	Kanagawa, H5	F	VD	0.25	7363	X
NG0207	2002	Kanagawa, H5	M	UD	0.25	7363	X
NG0303	2003	Kanagawa, H3	M	—	0.25	7363	X
NG0304	2003	Tokyo, H9	M	U	1.0	7363	XXX
NG0311	2003	Tokyo, H9	M	U	0.5	7363	XXX
NG0312	2003	Tokyo, H9	M	U	0.5	7358	XXVI
NG0401	2004	Kanagawa, H7	M	UD	0.5	7363	X
NG0404	2004	Kanagawa, H8	M	U	0.5	7363	X
NG0410	2004	Tokyo, H9	M	U	0.5	7363	X
NG0503	2005	Kanagawa, H3	M	U	0.25	1901	XXXII
NG0508	2005	Kanagawa, H5	M	—	0.25	1596	X
NG0509	2005	Kanagawa, H3	M	UD	0.25	7363	X
NG0511	2005	Kanagawa, H3	F	VD	0.25	7363	X
NG0512	2005	Kanagawa, H3	M	UD	0.25	1588	X
NG0513	2005	Kanagawa, H3	F	VD	0.5	1901	XXXII
NG0514	2005	Kanagawa, H8	M	U	0.25	7363	X
NG0202[c]	2002	Kanagawa, H7	M	UD	<0.008	1901	V
NG0402[c]	2004	Kanagawa, H5	M	U	0.031	1901	V
NGON03-079[c]	2003	Tokyo, H10	M	UD	0.5	1901	X
NGON03-092[c]	2003	Tokyo, H10	M	UD	0.25	1901	X
NGON03-115[c]	2003	Tokyo, H10	F	U	0.5	1901	X
NGON07-002[c]	2007	Tokyo, H10	M	U	0.25	1901	X

[a] M, male; F, female.
[b] U, urine; VD, vaginal discharge; UD, urethral discharge; —, no information.
[c] ST1901 isolates used for analysis of *penA*-flanking region.

was amplified and sequenced by using primers penA_F and penA_R (Table 2). 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. Purification of the PCR products was done with an ExoSAP IT kit (GE Healthcare). Sequencing was carried out with the appropriate sequencing primers and an ABI BigDye Terminator cycle sequencing kit (version 3.1; Applied Biosystems), followed by purification of the termination products. Both strands of the products were sequenced by use of an ABI 3130 xl sequencer. The translated amino acid sequences were compared with known PBP 2 amino acid sequences. Newly identified types were designated XXX to XXXII, as described by Ito et al. (10) and Whiley et al. (27).

A neighbor-joining tree with 33 PBP 2 amino acid sequences was generated by using the MEGA program (version 4) (22, 26). The reliability of the inferred relatedness was evaluated by the use of bootstrap tests (1,000 replicates) (7).

Amplification of the *penA*-flanking DNA was done by using primer set penA_3'F and dcaA_R and primer set penA_5'R and mraW_F (Table 2). The PCR mixtures were incubated for 2 min at 96°C, followed by 30 cycles of 10 s at 96°C, 10 s at 63°C, and 2 min at 72°C. The primers listed in Table 2 were used to sequence each PCR product.

MLST. PCR amplification and sequencing of the seven *N. gonorrhoeae* housekeeping genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC*, and *pgm*) were undertaken by using a previously described protocol (12). All nucleotide sequences were determined directly from the purified PCR products. After end trimming of the data obtained and editing by using Sequencher software (gene codes), the allele numbers of the STs were assigned by querying the *Neisseria* MLST database (http://pubmlst.org. /neisseria/) (13).

Pulsed-field gel electrophoresis (PFGE). Agarose plugs into which DNA was embedded were prepared as described previously (10), and the samples were digested with SpeI. The SpeI-digested genomic DNA was analyzed on a 1% agarose gel with 0.5× Tris-boric acid-EDTA buffer at 14°C by using a CHEF Mapper apparatus (Bio-Rad). The run time was 19.5 h at 6 V/cm, and the initial and final switch times were 0.5 and 35 s, respectively. The gel was stained with ethidium bromide.

***In vitro* genetic exchange of the *penA* allele during cocultivation.** An *in vitro* interstrain genetic exchange experiment was performed with strain NG0003 (cefixime MIC, 0.25 μg/ml; ciprofloxacin MIC, 0.031 μg/ml) and strain NG0202 (cefixime MIC, 0.004 μg/ml; ciprofloxacin MIC, 8 μg/ml). NG0202 was selected from 58 isolates susceptible to cefixime (cefixime MIC ≤ 0.125 μg/ml) and on the basis of the ciprofloxacin MICs. The strains were grown on GC agar plates with 5% CO_2 for 16 h and then suspended in GC broth. After adjustment of the optical density at 600 nm (OD_{600}) of the culture to 0.02 with GC broth, suspensions of strains NG0003 and NG0202 (500 μl each) were mixed and statically incubated for 16 h. One hundred microliters of sample was placed onto GC agar plates containing both cefixime (0.031 μg/ml) and ciprofloxacin (2 μg/ml) (Cef+Cip GC agar plate) in duplicate. Neither NG0003 nor NG0202 is able to form colonies on this medium. The plates were incubated for 20 h at 37°C with 5% CO_2, and the number of colonies on each plate was determined. The viable

TABLE 2. Oligonucleotide primers used in this study

Primer	Sequence (5′–3′)	Primer use
penA_F	CGGGCAATACCTTTATGG TGGAAC	Amplification of *penA*[a]
penA_R	ACAACGGCGGCGGGGAT ATAAC	
penA_SF1	CAAAGATAGAAGCAG CCTG	Sequencing of the *penA* region
penA_SF2	GATATTGACGGCAAA GGTC	
penA_SF3	CTTTGGATGTGCGCGGC	
penA_SR1	GCCGTCGGTATATTCGC	
penA_SR2	CCAAAGGGGTTAACTTGC	
penA_SR3	TTCTCAACAAACCTGCAG	
penA_SR4	CTTTGCCGTTTTGCGGGG	
penA_5′R	GCCATCAGGACGAAGCT AATCC	Amplification of the region upstream of *penA*[b]
mraW_F	GTGAGTGGAGCAGAAAG TTACCG	
mraW_S1	CCGTTACTGGTCATCG	Sequencing of the PCR product from penA_5′R and mraW_F
mraW_S2	TATCGGACCGGCAGTC	
mraW_S3	CCTCGTGCAAATCCTG	
mraW_S4	GGCGGTCAGAGAAGC	
penA_3′F	GCGGCAGCCTGAACATC TTGG	Amplification of the region downstream of *penA*[b]
dcaA_R	GGACACATCGGTAGCG GCTG	
murE_S1	TTCAAGATCGGAAA AACG	Sequencing of PCR product from penA_3′F and dcaA_R
murE_S2	TTGGCACAAAGCAAGG	
murE_S3	TGCGCGGTTTCTTCC	
murE_S4	TCGGACGGTTCAACG	
murE_S5	GCAGGCTTTGTTAACTC	
dcaA_S1	TCAATATCTTAACCG TATC	
dcaA_S2	GCGTATCGGGCAATGG	
dcaA_S3	CGGGAAGATTGCCGAC	
dcaA_S4	GGGGTATTTGCTGACG	
dcaA_S5	AGCTTGGCGAAGCAGG	
dcaA_S6	CGGTTTGATGCATGTCG	

[a] Amplification conditions were 96°C for 2 min and 30 cycles of 96°C for 10 s, 65°C for 10 s, and 72°C for 2 min.
[b] Amplification conditions were 96°C for 2 min and 30 cycles of 96°C for 10 s, 63°C for 10 s, and 72°C for 2 min.

counts of NG0202 and NG0003 were determined on GC agar plates containing either cefixime (0.031 μg/ml) or ciprofloxacin (2 μg/ml). The experiment was repeated three times. The transformation frequency was estimated on the basis of the number of viable recipient NG0202 colonies that grew on the Cef+Cip agar plates compared to the number of NG0202 colonies that grew on Cip agar plates. The MICs of clones (n = 12) resistant to both cefixime and ciprofloxacin were determined; and MLST typing, PFGE, and sequence analysis of the *penA*-flanking region of the clones were performed.

Nucleotide sequence accession numbers. The nucleotide sequences revealed in this study have been deposited in the DDBJ sequence library and assigned accession numbers AB511942 for *penA*-XXX, AB511943 for *penA*-XXXI, AB511944 for *penA*-XXXII, and AB511945 and AB511946 for the *penA*-flanking regions.

RESULTS

***penA* sequence variation.** To examine the possibility of the expansion of a single clone with reduced susceptibility to cefixime, we sequenced the *penA* alleles of the Cef^{Rs} isolates in our collection. Among 32 Cef^{Rs} isolates obtained from 1998 to 2005, five PBP 2 types were revealed, including three newly identified types. PBP 2-X was the predominant type (26/32, 81.3%), which is consistent with the findings presented in previous reports (10, 27). PBP XXVI, originally designated mosaic 4 (25), was also found. Newly identified types PBP 2-XXX and PBP 2-XXXI had replacements of Ala by Val at positions 502 and 533, respectively, compared with the sequence of PBP 2-X. PBP 2-XXXII was identical to PBP 2-X from positions 1 to 548, but its C-terminal portion was identical to that of the PBP 2-I allele from a strain that is susceptible to cefixime, strain LM306, suggesting the creation of a new mosaic structure.

Using phylogenetic analysis, we demonstrated that the amino acids sequences of the PBP 2 alleles among the Cef^{Rs} strains varied; however, the variation was restricted to a cluster, which was distinct from the other cluster formed by the PBP 2 types of Cef-susceptible isolates (Fig. 1), suggesting that *penA* of the Cef^{Rs} isolates evolved from a single origin through a point mutation or the replacement of a short segment, such as that in PBP 2-XXXII.

Multilocus sequence typing of Cef^{Rs} isolates. In order to examine whether the whole genomes of the Cef^{Rs} strains were clonal, we applied an MLST strategy. Thirty-two Cef^{Rs} isolates were divided into six different STs (Table 3), including three singleton STs. The predominant ST was newly assigned ST7363 (n = 23, 71.9%). ST1901 (n = 3) and ST1596 (n = 3) were the second most dominant STs among the Cef^{Rs} isolates.

ST7363 and ST1588 differed from ST1596 only in the *pdhC* locus and the *fumC* locus, respectively, suggesting that ST7363, ST1588, and ST1596 are closely related to each other (Table 3). The MLST sequence type might alter during passages *in*

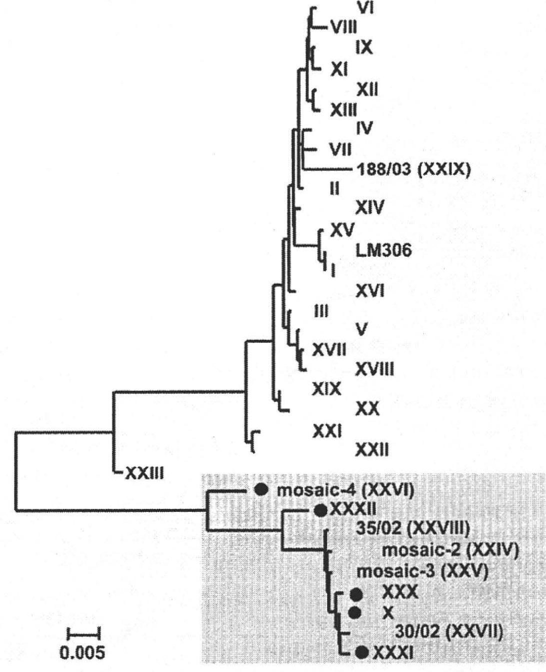


FIG. 1. Relationships of 33 PBP 2 types. A neighbor-joining tree was constructed from the PBP 2 amino acid sequences. The tree contains the LM306, PBP 2, and the PBP 2 types reported by Ito et al. (10), Whiley et al. (27), Takahata et al. (25), and Lindberg et al. (14) and in this study. The PBP 2 types that resulted in the reduced susceptibility of *N. gonorrhoeae* to cefixime are shaded in gray. Black dots indicate the PBP 2 types found in this study.

TABLE 3. MLST types and *penA* alleles of isolates with reduced susceptibility to cefixime

ST	No. of isolates	Allele at locus[a]:							No. of isolates with *penA* allele:				
		abcZ	*adk*	*aroE*	*fumC*	*gdh*	*pdhC*	*pgm*	X	XXVI	XXX	XXXI	XXXII
7363	23	59	39	67	78	148	153	65	21	0	2	0	0
1596	3	59	39	67	78	148	**71**	65	3	0	0	0	0
1588	1	59	39	67	**158**	148	**71**	65	1	0	0	0	0
1590	1	**126**	39	67	78	**149**	153	65	0	0	0	1	0
7358	1	**109**	39	67	78	**149**	153	**133**	0	1	0	0	0
1901	3	**109**	39	**170**	**111**	148	153	65	1	0	0	0	2

[a] Boldface data indicate alleles different from that of ST7363.

vivo and *in vitro* due to a point mutation or an interstrain recombinational event. However, the other three STs, ST1901, ST1590, and ST7358, showed at least two differences from the other STs of the Cef^{Rs} isolates. It is unlikely that this was because of allele exchange in all these isolates, indicating that the concept of the expansion of a single clone of Cef^{Rs} could not completely explain the spread of Cef^{Rs}.

Correlation of *penA* allele type with MLST typing. If Cef^{Rs} isolates emerged as different STs through independently generated mosaic structures of the *penA* allele, we would expect isolates with unique *penA* alleles in each ST. As shown in Table 3, the *penA-X* of the dominant PBP, PBP 2-X, was widely distributed in four different STs, ST7363, ST1596, ST1588, and ST1901, while unique *penA* alleles of PBP 2-XXX and PBP 2-XXXII, which were found in more than two isolates, were detected only in ST7358 and ST1901, respectively. From the results, we speculate that one of the possible reasons for this is that in some Cef^{Rs} isolates the transfer of the *penA-X* allele occurs between different *N. gonorrhoeae* strains.

***In vitro* transfer of *penA* gene.** To explore the possibility that the *penA-X* allele spread between different *N. gonorrhoeae* strains, we tested whether *penA-X* could be transferred by the *in vitro* cocultivation of Cef^{Rs} isolate (NG0003, ST7363) and a cefixime-susceptible (Cef^{S}) strain (strain NG0202, ST1901). NG0003 is susceptible to ciprofloxacin, and NG0202 is resistant to ciprofloxacin.

When a portion (0.1 ml) of a 16-h static culture of strain NG0003 or strain NG0202 (0.71×10^8 and 1.01×10^8 CFU/ml, respectively) was plated on a Cef+Cip GC agar plate, no colonies appeared, indicating that no spontaneous antibiotic resistance mutations occurred (Table 4). When a mixture of NG0003 and NG0202 was plated after cocultivation for 16 h, we obtained colonies resistant to both drugs (4.33×10^3 CFU/ml) on Cef+Cip GC agar plates (Table 4). We randomly selected 12 colonies from these mutants. All clones were ST1901, and the PFGE profiles of all the resistant clones were identical to the PFGE profile of NG0202 (Fig. 2), suggesting that NG0202 received *penA-X* from NG0003 and became resistant to cefixime.

The transformation frequency was estimated to be 2.1×10^{-4} (Table 4). When DNase (200 μg/ml) was present in the cocultivated mixture, no colonies resistant to both drugs were obtained, suggesting that the transfer was dependent on naked DNA released from the donor strain in the broth.

Sequence comparison of *penA* alleles. To confirm the transfer of the *penA-X* allele, we determined the nucleotide sequence of the *penA* allele (1,752 bp) in the double-resistant clones derived from NG0202, which originally possessed a *penA-V* allele. Sequence diversity between the *penA-X* and the *penA-V* alleles was identified at a total of 221 polymorphic sites after nucleotide position 294 of the *penA* gene, and the overall sequence identity was 87.3%. Eight of 12 clones had the same *penA-X* allele as NG0003. The clones with the other *penA* alleles, clones Tf-3, Tf-13, Tf-14, and Tf-15, had alleles highly similar to the alleles in *penA-X* (99.6 to 99.9%), and all se-

TABLE 4. *In vitro* transfer of reduced susceptibility to cefixime

Strain[a]	No. of CFU on plates with:		
	Cefixime[b]	Ciprofloxacin[c]	Cefixime and ciprofloxacin[d]
NG0202 (ST1901)	<10	0.71×10^8	<10
NG0003 (ST7363)	1.01×10^8	<10	<10
NG0202 + NG0003	1.03×10^8	0.2×10^8	4.33×10^3

[a] Strain NG0202, strain NG0003, and a suspension of equal numbers of cells of both strains (OD_{600}, 0.02) were incubated for 16 h.
[b] Containing 0.031 μg/ml of cefixime.
[c] Containing 2 μg/ml of ciprofloxacin.
[d] Containing 0.031 μg/ml of cefixime and 2 μg/ml of ciprofloxacin.

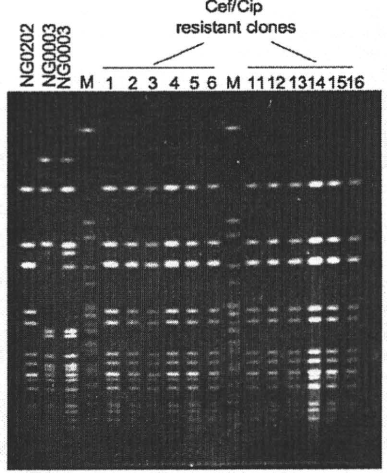


FIG. 2. PFGE patterns of clones obtained by *in vitro penA-X* transfer. NG0202 (Cef^{S} of ST1901) and NG0003 (Cef^{Rs} of ST7363) were cocultivated overnight, and then colonies that were resistant to both cefixime (Cef) and ciprofloxacin (Cip) were identified by using GC agar plates containing 0.031 μg/ml of cefixime and 2 μg/ml of ciprofloxacin. SpeI-digested genomic DNA from 12 of the clones obtained was analyzed by PFGE. Lanes M, size marker consisting of SpeI-digested *Salmonella enterica* serovar Braendecup strain H9812 genomic DNA.

FIG. 3. Comparison of sequences from nucleotides 241 to 720 among *penA-X*, *penA-V*, and minor variants of the transformants. Dashes indicate the same nucleotide as *penA-X* of strain NG0003 (shown on the first line). Shaded boxes in *penA* Tf-3 and NG0202 (positions 241 to 417) indicate regions where the sequences between them are identical. The underlined region indicates the possible junction site of Tf-3.

quence divergences were found between nucleotide positions 294 and 669 of *penA* (Fig. 3). We concluded that all clones analyzed acquired *penA-X* or its derivatives and that these were responsible for the reduced susceptibility to cefixime in the clones. We also showed that *penA-X* allelic diversity was generated in the Tf-3, Tf-13, Tf-14, and Tf-15 clones.

Junction site of recombination of *penA-X*. The 5′ portion of *penA* (positions 1 to 417) in clone Tf-3 was identical to that of *penA-V* in strain NG0202, while the 3′ portion after nucleotide position 456 was identical to that of *penA*-X (Fig. 3), implying that a recombination junction site was located between positions 417 and 456 in *penA* of Tf-3. To determine the junction sites of the other clones, we first sequenced the *penA*-flanking regions (6,299 bp) in strains NG0003 and NG0202 (Fig. 4A). The overall nucleotide sequence identity of the region between NG0003 and NG0202 was 95.7%, significantly less than the identity of the concatenated seven loci of ST7363 and ST1901 determined by MLST analysis (3 bp different in 3,284 bp; 99.9%). As shown in Fig. 4A, the sequence divergence accumulated in the *penA* locus and also in the 5′ part of *murE*. Only three polymorphic sites were identified in the *dcaA* gene (1,647 bp), at about nucleotide position 5500, outside the highly variable region (Fig. 4A and 4B).

As shown in Fig. 4A and B, since the upstream region (positions 1 to 1590) was highly conserved and there were no polymorphic sites between strains NG0003 and NG0202, we could not determine the left junction site, other than that of Tf-3. As for the right junction site, we detected a possible junction site within the highly variable region in the *penA*-flanking region of Tf-15 (Fig. 4B). Although we could not determine the right junction site for *penA* recombination other than that in Tf-15, our analysis of the other 11 clones showed that the nucleotide sequence of *dcaA* was identical to that of NG0003, indicating that *penA-X* was replaced along with *murE* and *dcaA*.

Sequencing analysis of *murE-dcaA* region of ST1901 clinical isolates with PBP 2-X allele. To investigate the horizontal transfer of *penA*, we analyzed a *murE-dcaA* region of additional an ST1901 Cef^{s} isolate (n = 1) and ST1901 Cef^{Rs} isolates (n = 5) (Table 1). As shown in Fig. 4C, the *penA-murE-dcaA* region of the ST1901 Cef^{s} clinical isolate (NG0402) was identical to that of NG0202. The sequence of the *penA-murE-dcaA* region of the ST1901 Cef^{Rs} strains NG0002, NGON03-079, NGON03-092, and NGON03-115 was identical to that of NG0003 and most of the clones (type I) obtained in the *in vitro* experiment. NGON07-002 had a *murE* sequence identical to that of NG0003, but the polymorphism sites in *dcaA* of NGON07-002 were the same as those of NG0202, implying that the recombination junction of NGON07-002 was within the region from positions 4100 to 5500. The results suggested that similar DNA transfer and recombination events involving *penA-X* might occur *in vivo*.

DISCUSSION

Reduced susceptibility to cefixime has been associated with the mosaic-type *penA-X* allele encoding PBP 2-X or its derivatives with minor differences (10, 25, 27). However, the genetic relatedness between Cef^{Rs} *N. gonorrhoeae* isolates has not been completely elucidated. In the study described here, we applied MLST analysis to reveal the clonality of the Cef^{Rs} *N. gonorrhoeae* isolates in our collection and showed that Cef^{Rs} *N. gonorrhoeae* isolates belong to six different MLST types. One possible explanation for the wide distribution of Cef^{Rs} *N. gonorrhoeae* is the introduction of *penA* from other species to these STs by interspecies recombination (3, 10). We found that the minor types of PBP 2, PBP 2-XXX and PBP 2-XXXII, were seen only in ST7363 and ST1901 strains, respectively. Although we should analyze more Cef^{Rs} isolates, this may imply that the independent

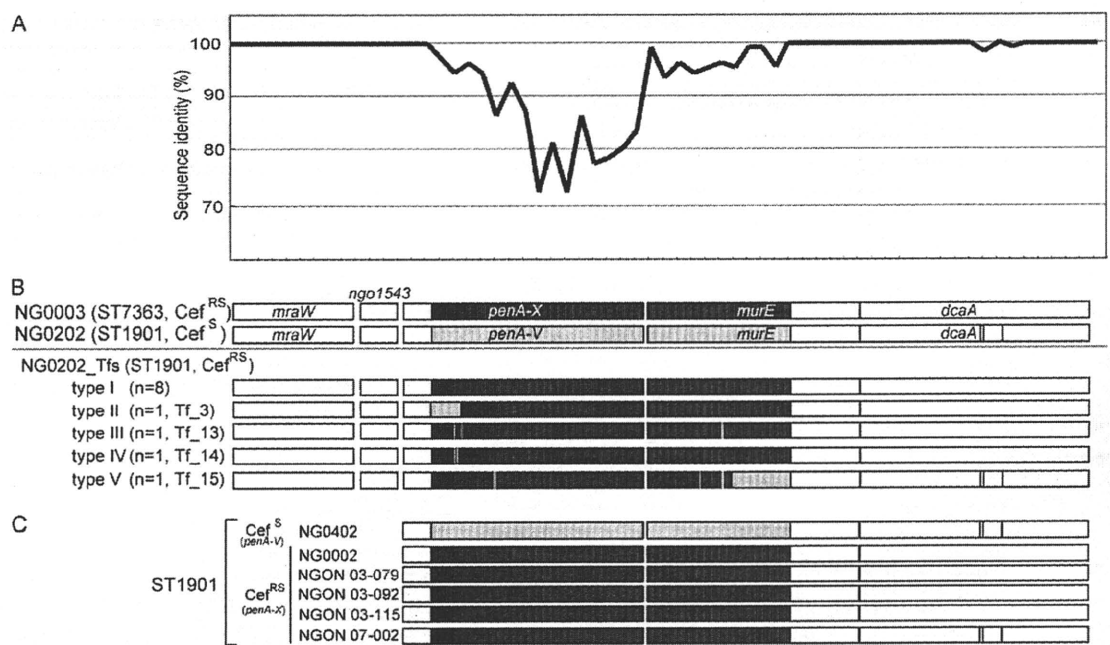


FIG. 4. Sequence diversity in *penA*-flanking regions (6,299 bp) among strain NG0003, strain NG0202, and the transformants generated by *in vitro* cocultivation. (A) Sequence identity of each 100 bp between NG0003 and NG0202. (B) Boxes indicate the five open reading frames in this region, *mraW*, *NGO1543*, *penA*, *murE*, and *dcaA*. Gray boxes, the highly variable region; dark gray boxes, sequences that are identical to the sequence of the *penA-X*-flanking region of NG0003; bright gray boxes, sequences identical to the sequence of the *penA-V*-flanking region of NG0202; fine vertical lines (white and black), polymorphic sites that match the nucleotide bases of NG0202. (C) Sequence diversity in the *penA-murE-dcaA* regions of additional clinical isolates of ST1901.

introduction of a DNA segment from a putative common ancestor has occurred, as proposed previously (24).

However, the putative original *penA-X* allele was the predominant allele among Cef^{Rs} strains in this study as well as in other studies (10, 27). All nine types of Cef^{Rs}-associated PBP 2 seem to be derived from the putative original *penA-X* (Fig. 1). Therefore, another possible explanation for the wide distribution of Cef^{Rs} is that a putative original Cef^{Rs} clone may emerge in a given lineage and clonally expand worldwide (14, 27). This is also suggested by our finding that ST7363 with the *penA-X* allele is predominant. During the spread of the Cef^{Rs} clone, mutations may be introduced, resulting in the emergence of new variants of *penA-X*. Another possibility is that the observed predominance may reflect fitness. If the *penA-X* allele has an advantage in cell growth over other alleles, the result is the elimination of the other alleles, although there is no evidence for such a difference.

The horizontal transfer of the *penA-X* allele shown in the present study can explain the clonality of Cef^{Rs}-associated PBP 2 even in isolates of different STs. We demonstrated the *in vitro* transfer of the *penA-X* allele from Cef^{Rs} ST7363 to Cef^{S} ST1901. Our sequence analysis of the *penA*-flanking region in the clones that acquired *penA-X* (8 of 12) showed that *penA* and the downstream open reading frames for *murE* and *dcaA* were replaced. Furthermore, the sequences from the Cef^{Rs} clinical isolates of ST1901 were also identical to those of the clones generated *in vitro*, supporting the possibility of the *in vivo* spread of the *penA*-flanking DNA segment. To our knowledge, this is the first case that suggests the interstrain transfer of a chromosomally encoded antibiotic resistance-conferring gene in *N. gonorrhoeae* by natural transformation in nature.

In addition to the horizontal transfer of *penA*, we observed the generation of *penA* allele diversity by the introduction of point mutations and the formation of a mosaic structure between a donor and a recipient *in vitro*. The *penA* alleles of one-third (4 of 12) of the transformants analyzed had minor differences from those of both the donor and the recipient. This is inconsistent with an observation mentioned by Spratt et al. (24). They could not detect any sequence variation during experimental transformation by using a PCR-amplified *N. meningitidis penA* gene. This discrepancy may be due to differences in the experimental procedures used, for example, a coculture assay versus transformation by use of a PCR product. However, we should examine more details about the natural transformation system, including the repair process, in *N. gonorrhoeae*. Nonetheless, the dynamic change observed in the allele during transformation may explain the diversity of the *penA* allele-derived Cef^{Rs} clinical isolate. Determination of the mutation rate for the *penA* allele during *in vitro* passages and analysis of more Cef^{Rs} isolates from various geographical areas will help improve our understanding of the diversity of the *penA* allele.

N. gonorrhoeae is a highly recombinogenic pathogen. DNA transformation contributes to the interspecies acquisition of chromosomally encoded antibiotic resistance (10, 23). DNA uptake in *Neisseria* is directly affected by piliation of the cells and the 10-bp-specific DNA uptake sequence (1, 9). After the DNA is internalized, it can be efficiently recombined with a homologous sequence on the recipient chromosome. As the efficiency of homologous recombination is correlated with se-

quence homology, intraspecies genetic exchange may be more efficient than interspecies exchange (8). If so, once *N. gonorrhoeae* acquires a genetic element from another bacterium that provides an advantage for *N. gonorrhoeae* survival *in vivo*, the acquired element would easily be spread among *N. gonorrhoeae* strains under selective pressure.

MLST is used for phylogenetic analysis for many other bacteria because the nucleotide sequence variation of housekeeping genes is likely to accumulate slowly and to be selectively neutral (4, 6, 16). However, the phylogeny of highly recombinogenic bacteria such as *Neisseria* species are difficult to study due to the exchange of DNA segments by natural transformation, resulting in the formation of nonclonal populations (21). Therefore, Cef^{Rs} isolates also might exchange the allele(s) utilized in MLST analysis by a recombinational event. As the allele profiles of ST7363, ST1588, and ST1596 were very similar to each other, these STs might be expected to be genetically related (the ST1596 complex). If we can assume that the housekeeping genes are exchangeable between strains, Cef^{Rs} isolates belonging to ST1596 complex might emerge by allele exchange, despite *penA* allele transfer. Other than the ST1596 complex, ST1901, which is one of the STs found in Cef^{Rs} isolates with the *penA-X* allele, has three loci, *abcZ*, *fumC*, and *aroE*, different from those in ST7363 (Table 3). These loci are scattered on the *N. gonorrhoeae* chromosome (5). Because even the loci closest to each other, *abcZ* and *fumC*, are 140 kb apart on the *N. gonorrhoeae* chromosome (5), evolution from ST7363 to ST1901 (or the other direction) would require three independent genetic events. However, we cannot suggest that this scenario is completely exclusive, since *N. gonorrhoeae* has a high likelihood of acquiring DNA from other cells.

As *N. gonorrhoeae* is an obligate human pathogen, there is neither transmission to other animals nor an environmental reservoir. Genetic exchange between two different strains must take place when one strain meets another strain within an individual host. Recently, two independent groups showed evidence for *N. gonorrhoeae* mixed infections (15, 17). The spread of an antibiotic resistance gene demonstrated in this study could also occur during a mixed infection, probably in highly sexually active persons. It should be noted that the frequency of *penA* allele transfer was relatively high (approximately 2 cells per 10^4 recipients). As expected previously and also as demonstrated in this study, the high natural competence of *N. gonorrhoeae* plays an important role in the transfer of a mosaic *penA* allele among different types of *N. gonorrhoeae* strains. As a result, the prevalence of the allele would be increasing in the population, although it remains unclear whether the other determinants are spread like the *penA* allele. If it is assumed that the spread occurs frequently, we need to reinforce surveillance for asymptomatic mixed gonococcal infections to prevent the spread of resistance-conferring genes.

ACKNOWLEDGMENTS

We thank H. Takahashi and H. Izumiya for helpful discussions. We thank H. Matsuoka for technical assistance.

This work was partly supported by grants-in-aid from the Ministry of Health, Labor, and Welfare of Japan (grants H21-Shinkou-Ippan-001 and H21-Shinkou-Ippan-012).

REFERENCES

1. **Aas, F. E., M. Wolfgang, S. Frye, S. Dunham, C. Lovold, and M. Koomey.** 2002. Competence for natural transformation in *Neisseria gonorrhoeae*: components of DNA binding and uptake linked to type IV pilus expression. Mol. Microbiol. **46:**749–760.
2. **Akasaka, S., T. Muratani, Y. Yamada, H. Inatomi, K. Takahashi, and T. Matsumoto.** 2001. Emergence of cephem- and aztreonam-high-resistant *Neisseria gonorrhoeae* that dose not produce beta-lactamase. J. Infect. Chemother. **7:**49–50.
3. **Ameyama, S., S. Onodera, M. Takahata, S. Minami, N. Maki, K. Endo, H. Goto, H. Suzuki, and Y. Oishi.** 2002. Mosaic-like structure of penicillin-binding protein 2 gene (*penA*) in clinical isolates of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime. Antimicrob. Agents Chemother. **46:**3744–3749.
4. **Bennett, J., K. A. Jolley, P. F. Sparling, N. J. Saunders, C. A. Hart, I. M. Feavers, and M. C. J. Maiden.** 2007. Species status of *Neisseria gonorrhoeae*: evolutionary and epidemiological inferences from multilocus sequence typing. BMC Biol. **5:**35.
5. **Chung, G. T., J. S. Yoo, H. B. Oh, Y. S. Lee, S. H. Cha, S. J. Kim, and C. K. Yoo.** 2008. The complete genome sequence of *Neisseria gonorrhoeae* NCCP11945. J. Bacteriol. **190:**6035–6036.
6. **Enright, M. C., and B. G. Spratt.** 1999. Multilocus sequence typing. Trends Microbiol. **7:**482–487.
7. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.
8. **Fraser, C., W. P. Hanage, and B. G. Spratt.** 2007. Recombination and the nature of bacterial speciation. Science **315:**476–480.
9. **Goodman, S. D., and J. J. Scocca.** 1988. Identification and arrangement of the DNA sequence recognized in specific transformation of *Neisseria gonorrhoeae*. Proc. Natl. Acad. Sci. U. S. A. **85:**6982–6986.
10. **Ito, M., T. Deguchi, K. Mizutani, M. Yasuda, S. Yokoi, S. Ito, Y. Takahashi, S. Ishihara, Y. Kawamura, and T. Ezaki.** 2005. Emergence and spread of *Neisseria gonorrhoeae* clinical isolates harbouring mosaic-like structure of penicillin-binding protein 2 in central Japan. Antimicrob. Agents Chemother. **49:**137–143.
11. **Janda, W. M., and C. A. Gaydos.** 2007. *Neisseria*, p. 601–620. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
12. **Jolley, K. A.** 2001. Multi-locus sequence typing, p. 173–186. *In* A. J. Pollard and M. C. Maiden (ed.) Meningococcal diseases: methods and protocols. Humana Press, Totowa, NJ.
13. **Jolley, K. A., M. S. Chan, and M. C. Maiden.** 2004. mlstdbNet—distributed multi-locus sequence typing (MLST) database. BMC Bioinform. **5:**86.
14. **Lindberg, R., H. Fredlund, R. Nicholas, and M. Unemo.** 2007. *Neisseria gonorrhoeae* isolates with reduced susceptibility to cefixime and ceftriaxone: association with genetic polymorphisms in *penA*, *mtrR*, *porB1b*, and *ponA*. Antimicrob. Agents Chemother. **51:**2117–2122.
15. **Lynn, F., M. M. Hobbs, J. M. Zenilman, F. M. T. F. Behets, K. Van Damme, A. Rasamindrakotroka, and M. C. Bash.** 2005. Genetic typing of the porin of *Neisseria gonorrhoeae* from clinical noncultured samples for strain characterization and identification of mixed gonococcal infections. J. Clin. Microbiol. **43:**368–375.
16. **Maiden, M. C. J., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A. **95:**3140–3145.
17. **Martin, I. M. C., and C. A. Ison.** 2003. Detection of mixed infection of *Neisseria gonorrhoeae*. Sex. Transm. Infect. **79:**56–58.
18. **Muratani, T., S. Akasaka, T. Kobayashi, Y. Yamada, H. Inatomi, K. Takahashi, and T. Matsumoto.** 2001. Outbreak of cefzopran (penicillin, oral cephems, and aztreonam)-resistant *Neisseria gonorrhoeae* in Japan. Antimicrob. Agents Chemother. **45:**3603–3606.
19. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial susceptibility testing, 9th informational supplement M7-A4 (M100-S9). National Committee for Clinical Laboratory Standards, Wayne, PA.
20. **Ochiai, S., H. Ishiko, M. Yasuda, and T. Deguchi.** 2008. Rapid detection of the mosaic structure of the *Neisseria gonorrhoeae penA* gene, which is associated with decreased susceptibilities to oral cephalosporins. J. Clin. Microbiol. **46:**1804–1810.
21. **O'Rourke, M., and B. G. Spratt.** 1994. Further evidence for the non-clonal population structure of *Neisseria gonorrhoeae*: extensive genetic diversity within isolates of the same electrophoretic type. Microbiology **140:**1285–1290.
22. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
23. **Spratt, B. G.** 1988. Hybrid penicillin-binding proteins in penicillin-resistant gonococci. Nature **332:**173–176.
24. **Spratt, B. G., L. D. Bowler, Q.-Y. Zhang, J. Zhou, and J. M. Smith.** 1992. Role of interspecies transfer of chromosomal genes in the evolution of penicillin resistance in pathogenic and commensal *Neisseria* species. J. Mol. Evol. **34:**115–125.

25. **Takahata, S., N. Senju, Y. Osaki, T. Yoshida, and T. Iida.** 2006. Amino acid substitutions in mosaic penicillin-binding protein 2 associated with reduced susceptibility to cefixime in clinical isolates of *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **50:**3638–3645.
26. **Tamura, K., J. Dudley, M. Nei, and S. Kumar.** 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:**1596–1599.
27. **Whiley, D. M., E. A. Limnios, S. Ray, T. P. Sloots, and J. W. Tapsall.** 2007. Diversity of *penA* alterations and subtypes in *Neisseria gonorrhoeae* strains from Sydney, Australia, that are less susceptible to ceftriaxone. Antimicrob. Agents Chemother. **51:**3111–3116.
28. **Yamai, S., Y. Obara, T. Nikkawa, Y. Shimoda, and Y. Miyamoto.** 1979. Preservation of *Neisseria gonorrhoeae* by the gelatin-disc method. Br. J. Vener. Dis. **55:**90–93.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2010, p. 3021–3023
0066-4804/10/$12.00 doi:10.1128/AAC.00245-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.
Vol. 54, No. 7

Identification of TEM-135 β-Lactamase in Penicillinase-Producing *Neisseria gonorrhoeae* Strains in Japan[▽]

Makoto Ohnishi,[1]* Emi Ono,[2] Ken Shimuta,[1] Haruo Watanabe,[1] and Noboru Okamura[2]

Department of Bacteriology I, National Institute of Infectious Diseases,[1] and Department of Moleculo-Genetic Science, Graduate School of Health Care Science, Tokyo Medical and Dental University,[2] Tokyo, Japan

Received 19 February 2010/Returned for modification 21 March 2010/Accepted 17 April 2010

Ten penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains isolated from 2000 to 2008 were characterized by multilocus sequence typing, multiantigen sequence typing, and plasmid typing. Sequence analysis showed that 8 strains contained a TEM-1 β-lactamase gene. However, two other genetically distinct PPNG strains, isolated in 2004 and 2008, each contained a TEM-135 β-lactamase on different plasmids, a Toronto/Rio type R plasmid and an Asia type R plasmid, suggesting independent origins of these PPNG strains.

Antibiotic-resistant *Neisseria gonorrhoeae* is a major public health concern (15). An essential element in gonococcal-infection control is the availability of effective antimicrobial therapy. However, *N. gonorrhoeae* has developed resistance to multiple classes of antimicrobials. In Japan, the prevalence of fluoroquinolone-resistant *N. gonorrhoeae* strains is over 80% (12), and *N. gonorrhoeae* strains with reduced sensitivity and with resistance to cefixime (CFM) have emerged and spread nationwide (5, 7). In contrast to the high prevalence of *N. gonorrhoeae* strains with chromosomal β-lactam resistance genes, the prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG) strains with a β-lactamase gene carried on a plasmid is relatively low in Japan. However, the prevalence of PPNG strains in other countries in Asia is high (16). To study the epidemiology of *N. gonorrhoeae*, nucleotide sequence-based typing methods, like multilocus sequence typing (MLST) and multiantigen sequence typing (MAST), are useful tools, since the analyses yield highly reproducible and easy-to-compare data from different laboratories.

Among the 719 *N. gonorrhoeae* strains isolated from January 2000 to December 2008 in the Nakano Sogo Hospital in Japan, 10 strains (1.4%) were found to be penicillinase-producing *N. gonorrhoeae* (PPNG) by the nitrocefin test (data not shown). The MICs of penicillin (PEN), cefixime (CFM), and ceftriaxone (CRO) were determined by the agar dilution method (6), suggesting that the strains were highly resistant to penicillin but not to cephalosporins (Table 1). This low prevalence was consistent with other reports (14, 16).

MLST and MAST (3, 4) were used to characterize these PPNG strains. As shown in Table 1, both MLST and MAST divided the 10 PPNG strains into 7 types, with 4 (NGON 00-002, NGON 00-027, NGON 04-025, and NGON 08-003) of the PPNG strains having unique sequence types (ST) by both MLST and MAST. However, three pairs of strains (NGON 05-042 and NGON 06-041, NGON 08-041 and NGON08-046, and NGON 08-043 and NGON 08-044) had identical sequence types by MLST and by MAST (Table 1). Although we have no information linking the patients from whom each pair of strains was isolated, transmission of PPNG strains might be considered in these cases.

Plasmids of the PPNG strains carrying the β-lactamase gene (*bla*) have been typed based on plasmid size, since deletion mutants have been reported previously (9). To investigate plasmid diversity in the PPNG strains in this study, plasmid DNAs were purified using QIAprep Spin miniprep kits (Qiagen). To estimate β-lactamase plasmid size, we amplified the complete DNA of each plasmid by long PCR using LA *Taq* polymerase (TaKaRa) and primers bla-IR, 5′-TCGTGGTGTCACGCTC GTCG, and bla-IF, 5′-CTGCAGCAATGGCAACAACGTTG, which anneal to nucleotides 7426 to 7404 and 1 to 23, respectively, of the 7,426-bp pJD4 plasmid (Fig. 1A) (9). The PCR products were incubated for 2 min at 96°C followed by 30 cycles of 10 s at 96°C, 10 s at 63°C, and 8 min at 72°C. As shown in Fig. 1B, analysis of the amplified plasmid DNAs in a 1% agarose gel showed three plasmid sizes: 5.2, 5.6, and 7.4 kb. By use of a multiplex PCR method for plasmid typing (10), the 5.2-, 5.6-, and 7.4-kb plasmids were identified as Toronto/Rio, Africa, and Asia type R plasmids, respectively (Fig. 1A and C).

Although the molecular sizes of *N. gonorrhoeae* R plasmids are diverse, plasmids carrying β-lactamases are genetically related and carry a TEM-1 type *bla* gene, bla_{TEM-1} (12). To confirm the conservation of bla_{TEM-1}, the *bla* genes of the 10 PPNG isolates were analyzed by DNA sequencing (8). The primers used for amplification and sequencing were bla-F, 5′-CGCTCATGAGACAATAACCCTGG, and bla-R, 5′-GGGTCTGACGCTCAGTGGAACG. The PCR products were incubated for 2 min at 96°C followed by 30 cycles of 10 s at 96°C, 10 s at 60°C, and 1 min at 72°C. Nucleotide sequencing was carried out as described previously (8). As shown in Table 1, two distinct bla_{TEM} alleles were found: 8 PPNG strains contained bla_{TEM-1}, and the other 2 strains (NGON 04-025 and NGON 08-003) contained $bla_{TEM-135}$, a TEM allele originally identified in *Salmonella enterica* serovar Typhimurium (11). These alleles, bla_{TEM-1} and $bla_{TEM-135}$, had one base difference, which resulted in a single amino acid

* Corresponding author. Mailing address: Department of Bacteriology I, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku, Tokyo 162-8640, Japan. Phone: 81-3-5285-1111. Fax: 81-3-5285-1163. E-mail: ohnishi7@nih.go.jp.
▽ Published ahead of print on 26 April 2010.

TABLE 1. Penicillinase-producing *Neisseria gonorrhoeae* strains isolated in Tokyo from 2000 to 2008

Strain	Time of isolation	Sex of patient[a]	Age (yr) of patient	Specimen type[b]	MLST type	MAST type	Plasmid type	*bla* type	MIC (μg/ml)		
									PEN	CFM	CRO
NGON 00-002	January 2000	M	26	UD	ST-1590	ST-270	Africa	TEM-1	16	0.032	0.016
NGON 00-027	June 2000	M	42	U	ST-1921	ST-1817	Asia	TEM-1	>64	0.008	0.008
NGON 04-025	April 2004	M	27	U	ST-1597	ST-1549	Toronto/Rio	TEM-135	>64	0.004	≤0.004
NGON 05-042	August 2005	F	30	VD	ST-1588	ST-4012	Africa	TEM-1	64	0.25	0.064
NGON 06-041	October 2006	M	56	U	ST-1588	ST-4012	Africa	TEM-1	32	0.25	0.032
NGON 08-003	January 2008	F	31	VD	ST-7823	ST-4013	Asia	TEM-135	>64	0.032	0.032
NGON 08-041	September 2008	M	52	U	ST-1584	ST-1478	Africa	TEM-1	64	0.008	0.004
NGON 08-043	September 2008	M	31	U	ST-7823	ST-1288	Asia	TEM-1	>64	0.064	0.064
NGON 08-044	September 2008	F	25	VD	ST-7823	ST-1288	Asia	TEM-1	>64	0.064	0.064
NGON 08-046	October 2008	F	59	VD	ST-1584	ST-1478	Africa	TEM-1	64	0.25	0.032

[a] M, male; F, female.
[b] U, urine; VD, vaginal discharge; UD, urethral discharge.

substitution, M182T (residue numbering follows that of Ambler et al. [1]).

Interestingly, the two PPNG strains with $bla_{TEM-135}$ were genetically different: the sequence types of strain NGON 04-025 were MLST ST-1597 and MAST ST-1549, and those of strain NGON 08-003 were MLST ST-7823 and MAST ST-4013 (Table 1). The plasmids carried by strains NGON 04-025 and NGON 08-003 were also distinct: the plasmid for the former was a Toronto/Rio type, and that for the latter was an Asia type. Taken together, these findings suggest that $bla_{TEM-135}$ may have been introduced independently into these two *N. gonorrhoeae* strains or may have emerged by a point mutation in each. Recently, Srifeungfung et al. (13) reported that a PPNG strain isolated in Thailand contained a $bla_{TEM-135}$ allele. PPNG strains containing $bla_{TEM-135}$ might be widespread in Asian countries, although further study is needed to determine the prevalence.

The TEM type β-lactamase genes, which are widely distributed in Gram-negative bacteria, are diverse in sequence and in substrate spectrum. Some types of TEM β-lactamases can hydrolyze extended-spectrum cephalosporins with an oxyimino side chain, including ceftriaxone, which is still an effective antibiotic for *N. gonorrhoeae*. The diverse substrate spectra of TEM β-lactamases are due to mutations in the bla_{TEM} gene that alter the amino acid configuration around the β-lactamase active site. Since bacteria with $bla_{TEM-135}$ have a restricted β-lactamase substrate spectrum, as reported in a previous study (10) and also in this study (Table 1), the selective pressure for emergence of *N. gonorrhoeae* $bla_{TEM-135}$ is not known. It is noteworthy that there are other TEM β-lactamases with extended substrate spectra that may have arisen as a single point mutation in bla_{TEM-1} or $bla_{TEM-135}$, e.g., bla_{TEM-29} and bla_{TEM-20} (2). Since point mutations in bla_{TEM-1} and $bla_{TEM-135}$ could lead to emergence of *N. gonorrhoeae* β-lactamases with extended substrate spectra, the antibiotic resistance profiles of PPNG strains should be monitored, especially in areas of high PPNG prevalence.

Nucleotide sequence accession number. The sequence data for the $bla_{TEM-135}$ gene have been assigned DDBJ accession number AB551787.

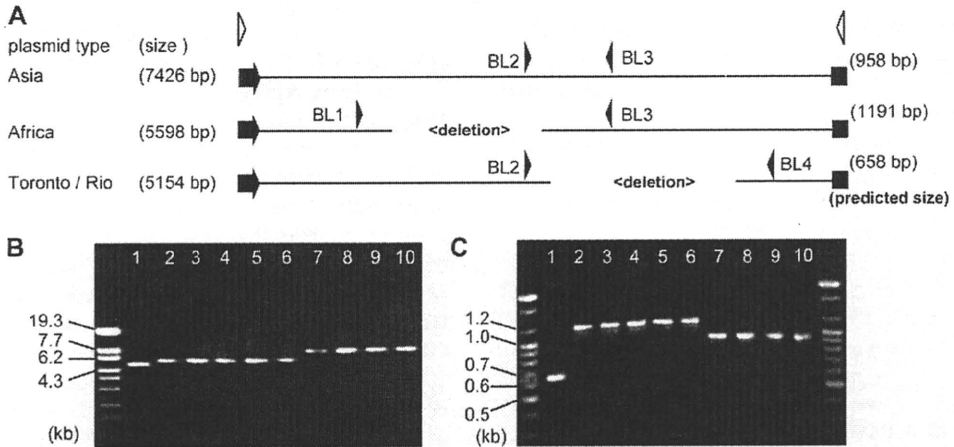


FIG. 1. Typing of plasmids carrying β-lactamases from *Neisseria gonorrhoeae* strains. (A) Schematics of Asia, Africa, and Toronto/Rio type plasmids. Each β-lactamase gene is shown by an arrowhead. The annealing sites of the primers used in this study for plasmid size determination (white arrowheads) and for plasmid type determination (black arrowheads) are shown. (B) Products of whole-plasmid PCR amplification, separated on a 1% agarose gel. (C) Products of multiplex PCR, separated on a 2% agarose gel. The size marker lanes contain StyI-digested lambda DNA (Toyobo) (B) or a 100-bp DNA ladder (Bioneer) (C). Lane 1, NGON 04-025; lane 2, NGON 00-002; lane 3, NGON 05-042; lane 4, NGON 06-041; lane 5, NGON 08-041; lane 6, NGON 08-046; lane 7, NGON 00-027; lane 8, NGON 08-003; lane 9, NGON 08-043; lane 10, NGON 08-044.

We thank Hiroko Matsuoka for technical assistance.

This work was partly supported by grants-in-aid from the Ministry of Health, Labor and Welfare of Japan (H21-Shinkou-Ippan-001 and H21-Shinkou-Ippan-012).

REFERENCES

1. **Ambler, R. P., A. F. W. Coulson, J.-M. Frère, J.-M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–272.
2. **Arlet, G., S. Goussard, P. Courvalin, and A. Philippon.** 1999. Sequences of the genes for the TEM-20, TEM-21, TEM-22, and TEM-29 extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **43:**969–971.
3. **Jolley, K. A.** 2001. Multi-locus sequence typing, p. 173–186. *In* A. J. Pollard and M. C. Maiden (ed.), Meningococcal disease: methods and protocols. Humana Press, Totowa, NJ.
4. **Martin, I. M., C. A. Ison, D. M. Aanensen, K. A. Fenton, and B. G. Spratt.** 2004. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J. Infect. Dis. **189:**1497–1505.
5. **Muratani, T., S. Akasaka, T. Kobayashi, Y. Yamada, H. Inatomi, K. Takahashi, and T. Matsumoto.** 2001. Outbreak of cefzopran (penicillin, oral cephems, and aztreonam)-resistant *Neisseria gonorrhoeae* in Japan. Antimicrob. Agents Chemother. **45:**3603–3606.
6. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial susceptibility testing, 9th informational supplement M7-A4 (M100-S9). National Committee for Clinical Laboratory Standards, Wayne, PA.
7. **Ochiai, S., H. Ishiko, M. Yasuda, and T. Deguchi.** 2008. Rapid detection of the mosaic structure of the *Neisseria gonorrhoeae penA* gene, which is associated with decreased susceptibilities to oral cephalosporins. J. Clin. Microbiol. **46:**1804–1810.
8. **Ohnishi, M., Y. Watanabe, E. Ono, C. Takahashi, H. Oya, T. Kuroki, K. Shimuta, N. Okazaki, S. Nakayama, and H. Watanabe.** 2010. Spread of a chromosomal cefixime-resistant *penA* gene among different *Neisseria gonorrhoeae* lineages. Antimicrob. Agents Chemother. **54:**1060–1067.
9. **Pagotto, F., A. T. Aman, L. K. Ng, K. H. Yeung, M. Brett, and J. A. Dillon.** 2000. Sequence analysis of the family of penicillinase-producing plasmids of *Neisseria gonorrhoeae*. Plasmid **43:**24–34.
10. **Palmer, H. M., J. P. Leeming, and A. Turner.** 2000. A multiplex polymerase chain reaction to differentiate β-lactamase plasmids of Neisseria gonorrhoeae. J. Antimicrob. Chemother. **45:**777–782.
11. **Pasquali, F., C. Kehrenberg, G. Manfreda, and S. Schwarz.** 2005. Physical linkage of Tn*3* and part of Tn*1721* in a tetracycline and ampicillin resistance plasmid from *Salmonella* Typhimurium. J. Antimicrob. Chemother. **55:**562–565.
12. **Sanchez-Pescador, R., M. S. Stempien, and M. S. Urdea.** 1988. Rapid chemiluminescent nucleic acid assays for detection of TEM-1 β-lactamase-mediated penicillin resistance in *Neisseria gonorrhoeae* and other bacteria. J. Clin. Microbiol. **26:**1934–1938.
13. **Srifeungfung, S., A. Roongpisuthipong, S. Asavapiriyanont, R. Lolekha, C. Trubyddharat, S. Lokpichart, P. Sungthong, and P. Tongtep.** 2009. Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in HIV-seropositive and gonococcal antimicrobial susceptibility: an update in Thailand. Jpn. J. Infect. Dis. **62:**467–470.
14. **Tanaka, M., H. Nakayama, T. Notomi, S. Irie, Y. Tsunoda, A. Okadome, T. Saika, and I. Kobayashi.** 2004. Antimicrobial resistance of *Neisseria gonorrhoeae* in Japan, 1993–2002: continuous increasing of ciprofloxacin-resistant isolates. Int. J. Antimicrob. Agents **24**(Suppl. 1)**:**S15–S22.
15. **Tapsall, J. W., F. Ndowa, D. A. Lewis, and M. Unemo.** 2009. Meeting the public health challenge of multidrug- and extensively drug-resistant *Neisseria gonorrhoeae*. Expert Rev. Anti Infect. Ther. **7:**821–834.
16. **WHO Western Pacific Gonococcal Antimicrobial Surveillance Programme.** 2008. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in the WHO Western Pacific Region, 2006. Commun. Dis. Intell. **32:**48–51.

その他の感染症

STI

余田敬子*
Keiko YODA

● Key Words ●性感染症，STI ●

はじめに

性感染症（sexually transmitted infection：STI）は，性行為によって伝搬する感染症の総称で，多種多様な疾患が含まれる。現在では30種類以上の微生物が性行為によって伝搬する[1]ことが知られているが，性行動の多様化に伴い口腔咽頭を介して性感染症に感染する人が増えている。実際，口腔咽頭の性感染症患者の対応に苦慮している泌尿器科医，婦人科医は少なくない。性感染症関連組織の啓蒙活動やインターネットサイトによって口腔咽頭にも性感染症が感染するという認識が広がり，最近，性感染症検査を目的に自ら耳鼻咽喉科を受診する人も増えている。性感染症は普遍的な疾患であり，耳鼻咽喉科医にも性感染症に適切な対応できることが求められつつある。

本稿では，代表的性感染症の最近のトピックスや動向について紹介する。

Ⅰ．主な性感染症の臨床像，検査，治療

1．梅　毒

梅毒は以前からHIV感染者における陽性率が顕著に高いことが指摘されている。一般成人での梅毒陽性率1％に対し，HIV感染者では40～50％の陽性率と報告されている[2]。梅毒は一般に予後良好であるが，HIV感染者が梅毒に感染すると悪性梅毒[3]などの非典型疹がみられる例，病期の順序で症状が出現しない例，異常に早く進行して早期から神経梅毒を発症する例の報告がある。梅毒血清反応の定量値も，HIV感染者では異常な高値や低値を示したり，激しく変動したりする。HIV感染者では治癒が遷延する場合や，再感染する場合もあり長い経過観察が必要となる。

梅毒の診断に用いられる梅毒血清反応には，リン脂質のカルジオリピンを抗原とする脂質抗原試験（serologic tests for syphilis：STS）法と，梅毒トレポネーマ（TP）抗原法が用いられてきた。STS法とTPHA法の倍数希釈法による定量は用手法で手技が煩雑であるため，近年ラテックス凝集法や化学発光などのOD値を数値で測定する高感度の自動定量測定が導入され各社からキットが発売されている[4]。しかし，倍数希釈法と自動分析装置による検査法での各キット間の相関性は十分に検討されていないため自動分析法の結果の解釈は難しく，今のところ従来の倍数希釈法による定量検査が推奨されている[5]。

治療に用いる抗菌薬は梅毒に耐性のないペニシリンが今日まで第一選択として推奨されている。治療の完遂，簡便性，費用の面からペニシリン以外の抗菌薬も使用される海外からは近年アジスロマイシン耐性の梅毒が報告されており，今後も新たな薬剤耐性梅毒の出現の可能性が指摘されている[5]。

2．淋菌感染症・性器クラミジア感染症

淋菌，クラミジアともにオーラルセックスを介して咽頭にも感染し，その多くが無症候感染であることが指摘されている[6]。われわれが2005年～2009年の間行った性感染症クリニック受診者854人を対象におこなった前向き調査の結果（図1）[7]によると，咽頭における淋菌の陽性率は男性が16％，女性が14％，また咽頭におけるクラミジア

* 東京女子医科大学東医療センター耳鼻咽喉科
〔〒16-0011 東京都荒川区西尾久2-1-10〕

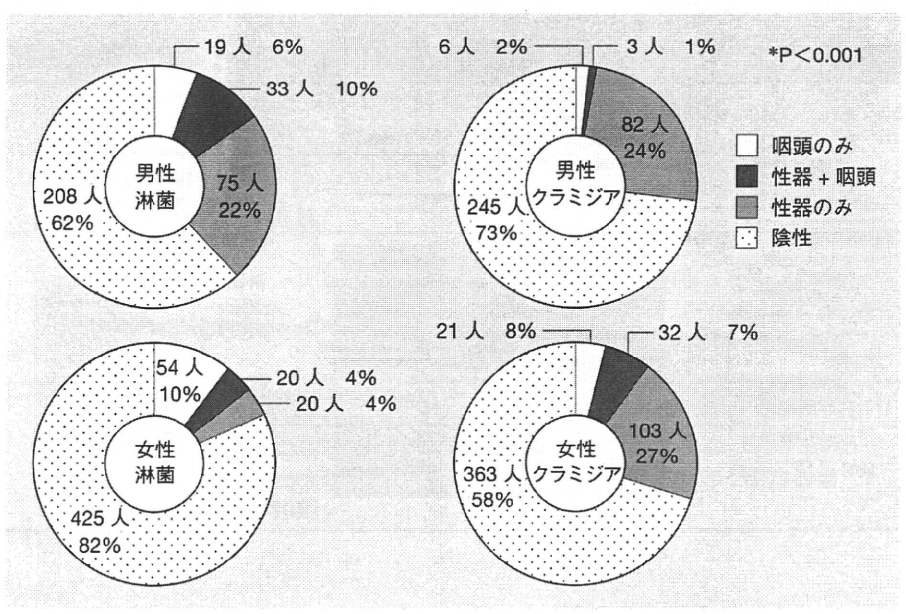


図 1　咽頭と性器の同時検査における陽性者数と割合（文献7より引用改変）

の陽性率は男性3%，女性10%という結果で，有意差はないものの女性の淋菌は咽頭の陽性率が性器の値を上回っていた。また，男性の淋菌，女性の淋菌，女性のクラミジアでは，咽頭のみ陽性の感染者は少なくなかった。また淋菌・クラミジアとも咽頭の陽性者のほとんどが無症候感染者であった。この調査結果は性風俗店従業者やその利用者における淋菌・クラミジアの感染状況を反映する結果であり，現時点ではこのデータが一般的な状況に該当するとはいえないが，今後これらの性感染症の咽頭感染者が風俗産業外でも増加することが懸念される。

2003～2008年に若年者1,585人を対象に行った無症候の性器クラミジア感染症のスクリーニング調査[8]の陽性率は，男性5%，女性6%であった。また，性器クラミジア陽性率は男子よりも女子が高く，特に20～25歳の陽性率5%に比べて14～19歳は14%と，10歳代女子で感染率が高いことも指摘されている。クラミジアの咽頭感染は男性より女性に有意に多く，特に15～19歳の女子の陽性率が高い傾向が以前から示されており，性風俗店従業女性だけでなく若年層での感染率が高いこと，また性行為の在り方が多様化している状況下では，口腔・咽頭の淋菌，クラミジア感染は今後さらに拡大することが懸念される。

淋菌およびクラミジア感染症の検査として，分離培養，酵素抗体法，核酸検出法，核酸増幅法がある[9]。淋菌では感染部位局所の菌量は，尿道，子宮頸管，直腸，咽頭の順に少なくなり，培養，遺伝子検査法ともに淋菌検出の正診率が低くなる[5]。菌量が少ない咽頭からの淋菌・クラミジアの検出には最も感度が高い核酸増幅法を用いることが推奨される。咽頭検体からの淋菌・クラミジアの核酸増幅検査（**図2**）には，現在PCR（polymerase chain reaction：ポリメラーゼ連鎖反応）法のアンプリコアSTD-1ナイセリアゴノレアおよびアンプリコアSTD-1クラミジアトラコマティス（ロシュ・ダイアグノスティックス，以下PCRと略す），SDA（strand displacement amplification：鎖置換増幅）法のBDプローブテックET CT/GC（日本ベクトン・ディッキンソン，以下SDAと略す）の2つと，平成21年10月1日よりTMA（transcription-mediated amplification：転写介在増幅）法のアプティマコンボ2（富士レビオ，以下TMAと略す）が新たに保険収載された。このうち，口腔咽頭の常在性ナイセリアとの交叉反応

表1 淋菌・クラミジアの咽頭検査キット

	採取容器	項目	提出先
アンプリコア 滅菌スワブ		クラミジア のみ	SRL BCL
プローブテック wet スワブ 黄色		淋菌 and/or クラミジア	三菱化学 メディエンス
コンボ2 wet スワブ 白色		淋菌 and クラミジア	SRL BCL

が生じるPCRの淋菌検査に限り咽頭検体の検査は適用外で，PCRは咽頭検体においてはクラミジア検査のみの適用となっている。

一方，SDAおよびTMAは咽頭の淋菌，クラミジア双方の検査が可能である。ただし，TMAは淋菌とクラミジア同時検査のみの適用で，淋菌，クラミジアどちらか一種のみの検査はできない。SDAは，同一検体から淋菌とクラミジアの同時検査も，どちらか一種のみの検査も可能となっている。どちらの検査も，咽頭スワブ（専用の子宮頸管または尿道検査キットを利用）またはうがい液（生理食塩水10 m*l*ほどを10秒以上うがい，専用の尿検査キットを利用）を採取して検査する。

治療として，薬剤耐性菌が増加している淋菌の咽頭感染にはセフトリアキソン（ロセフィン®）1 g単回静注のみ有効である[10]。クラミジアは性器と治療法は同じでアジスロマイシン（ジスロマック®）1000 mg単回投与などがある。

3．性器ヘルペス

性器ヘルペスは単純ヘルペスウイルス1型(herpes simplex virus type 1：HSV-1)，または単純ヘルペスウイルス2型(herpes simplex virus type 2：HSV-2）の感染によって性器に有痛性の水疱または潰瘍を生じる疾患である。主に性行為によって伝搬し，初感染初発，非初感染初発，再発の3型に分類される。

HSV-1・2に共通して，初感染の90％以上は不顕性感染であるが，顕性，不顕性を問わずHSV-1は主に三叉神経節，HSV-2は主に腰仙骨神経節に潜伏感染する傾向がある。初感染ではHSV-1・2とも体のいずれの部分にも感染して病変を生じるが，再発しやすい場所としてHSV-1は口唇・顔面・眼などの上半身に，HSV-2は性器・臀部などの下半身に分かれる。疲労，妊娠，怪我，熱性疾患，その他の原因によって腰仙骨神経節に潜伏したウイルスが再活性化されると，性器ヘルペスを生じる。HSV感染者は，症状がない無症候の状態であってもHSVを排泄し他者への感染源となっていることが問題となっている[11]。

HSV感染症の診断では，血清HSV抗体検査はHSVとVZV間，またHSV1型と2型間でも交叉反応が存在するため，血清抗体価からHSV感染を診断することは困難とされる。病変部からのウイルス分離，PCRなどによるウイルス核酸の検出，蛍光抗体法や免疫組織染色によるウイルス抗原の検出によって，病変部組織からウイルスを直接証明することによって診断されるが，これらの検査は臨床現場でHSV感染症を疑うすべての症例に行えるものではない。現在，特異度が高く約30分間と短時間で結果が出る新しい核酸増幅法のLAMP法[12]，インフルエンザウイルスの迅速診

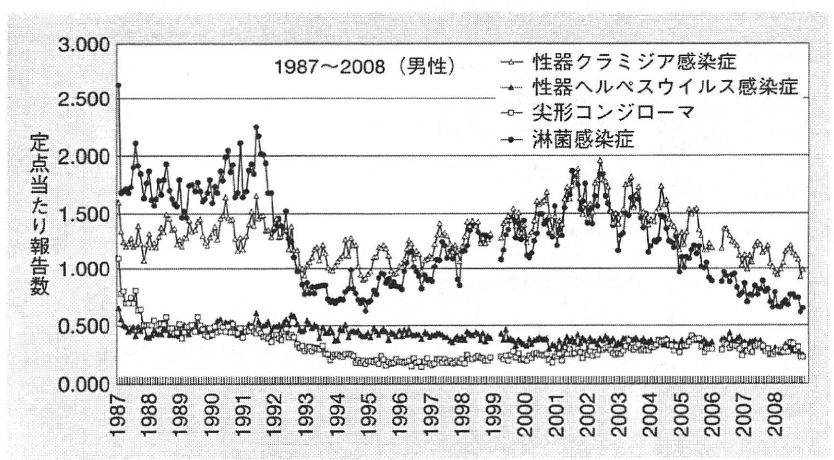


図 2　定点把握感染症の年次推移〔男性，感染症発生動向調査（2009 年 1 月 13 日現在）文献 16 より引用〕

断に標準的に使用されているイムノクロマト法を原理とする HSV 検出キット[13]の治験が現在進んでおり，近い将来臨床現場でより簡便で確実に HSV 感染症が診断できるようになることが期待されている。

4．尖圭コンジローマ・子宮頸癌

尖圭コンジローマは，ヒトパピローマウイルス（human papillomavirus：HPV）感染症により疣贅を形成する疾患で，一般に疼痛などの症状をともなわない。治療として，2007 年 12 月に保険適用となった外用の化学療法薬のイミキモイドクリームが使用できるようになり，臨床効果を上げている。

HPV は，現在 100 種類以上の遺伝子型に分類されており，その中で性器から検出される型は 40 種類以上ある。わが国での子宮頸癌からの HPV 検出頻度は高く，子宮頸癌の発症と HPV 感染との関係が注目されている。子宮頸癌はわが国では年間約 15,000 人が発症し，約 3,500 人が死亡している癌であり，近年，20 代 30 代の若年女性で増加傾向にある。性交渉により子宮頸部に感染した HPV の一部が数年から数十年かけて持続的に感染し，前癌状態をへて癌を発症する。子宮頸癌を生じる HPV の約 7 割が 16 型と 18 型であることから，HPV-16/18 の感染を予防する組換え沈降 2 価 HPV 様粒子ワクチン（サーバリックス®）が 2009 年 10 月に承認され，同年 12 月より市販されている，10 歳以上の女性を接種対象とし，0，1，6 カ月の 3 回接種する。

本ワクチンは予防効果，費用対効果ともに非常に高いとされる。接種費用は 3 回で 5 万円前後と高額のため，高い接種率を向上させるために，公費負担による無料接種を求める声が高い。また初交より先に接種されなければ予防効果が得られないため，接種年齢の設定も議論されている。

5．その他の性感染症

性感染症の 1 つである男性尿道炎は淋菌性尿道炎（GU）と非菌性尿道炎（NGU）に分けられ，NGU は男子尿道炎の約 70％を占めるとされる。そのうち *Chlamydia trachomatis* が検出されるのは 30～40％で，ほかは非クラミジア性 NGU とされる[14]。*Ureaplasma urealyticum*，*Mycoplasma genitalium*，*Staphylococcus saprophyticus*，*Bacteroides ureolytics*，*herpes simplex virus*，adenovirus などの各種病原体，さらに口腔内細菌による NGU の可能性が検討されている。その中で，*Mycoplasma genitalium* と *Ureaplasma urealyticum* は咽頭にも colonization しオーラルセックスにて男子尿道へ伝搬する可能性が示唆されている[15]が，これらの菌の検出は，一部の研究施設を

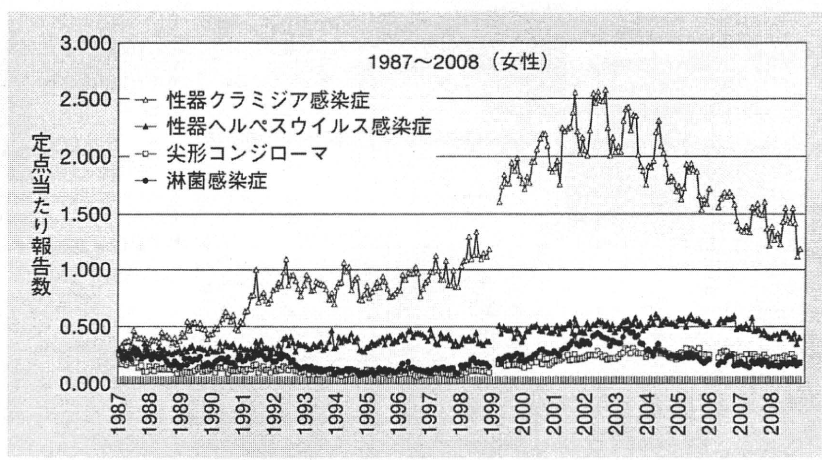


図 3　定点把握感染症の年次推移〔女性，感染症発生動向調査（2009年1月13日現在）文献16より引用〕

除き現時点では困難である。

II．わが国における性感染症の最近の動向

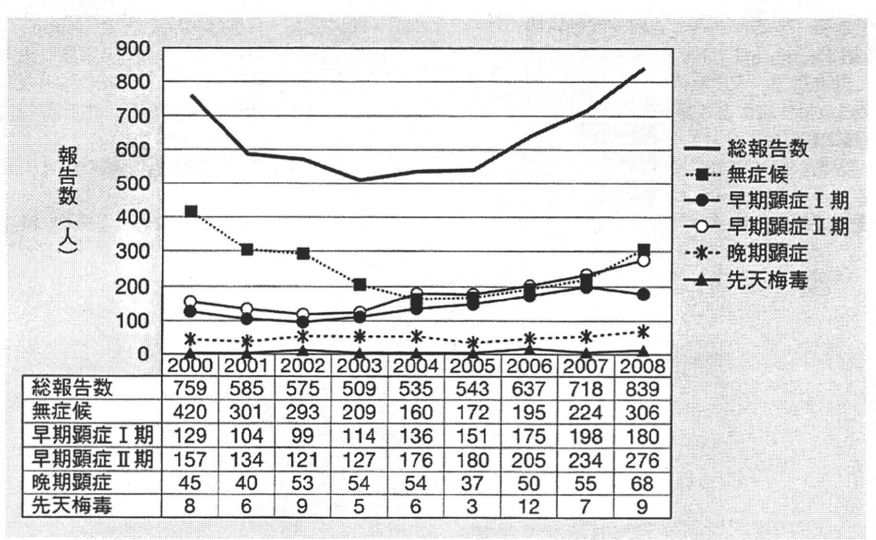
性感染症法に基づいて発生動向が調査されている性感染症は6疾患あり，梅毒，ヒト免疫不全ウイルス（human immunodeficiency virus：HIV）感染者および後天性免疫不全症候群（acquired immunodeficiency syndrome：AIDS）患者は全数届出，性器クラミジア感染症，性器ヘルペスウイルス感染症，尖圭コンジローマ，淋菌感染症は定点把握疾患として，各都道府県で性感染症定点として指定された医療機関から届け出が行われている。**図2，3**にわが国における性感染症の男女別発生件数の推移を示す[16]。

男性においては，淋菌感染症と性器クラミジア感染症が多く，同様の増減を示し推移している。いわゆるエイズショックで危険な性行動を避ける風潮が広まり1993年から減少していたが，1997年頃から再び増加に転じている。淋菌感染症ではその原因として，ニューキノロン耐性淋菌をはじめとする薬剤耐性淋菌の増加，性風俗店でオーラルセックスが日常的に行われるようになり，そこから感染する淋菌性尿道炎が増加したことが指摘されている。

女性においては，性器クラミジア感染症が最多で，第2位が性器ヘルペスウイルス感染症，尖圭コンジローマ，淋菌感染症はほぼ同数で推移している。1999年から2000年の間のグラフの途切れは，定点の見直しがおこなわれたことを示しており，それまで泌尿器科に偏っていた定点設定を産婦人科と泌尿器科がほぼ同数になるように調整が行われた。この後からみられる女性の性器クラミジア感染症の急増は，定点の見直しに加えPCR法などの感度の高いクラミジア診断法の普及によると推察されている。

淋菌感染症，性器クラミジア感染症も2002年をピークに男女とも患者数の減少がみられる。この2つの感染症の年次別・年齢別患者報告数から男女とも10歳代20歳代の若い世代で最も減少していることがわかっているが，その理由は明らかになっていない。性感染症予防キャンペーンや啓蒙活動の効果とみるか，定点報告にかからない感染者が増えているのか，今後検証が必要とされている[8]。

梅毒患者数は1980年代に年間2,000例以上の感染者の報告があったが，1990年以後減少し続け1997年には445例までになった。しかし，HIV無症候性キャリアの増加と連動して梅毒患者数は2004年以降漸次増加傾向に転じている（図4）。HIV/AIDSが20～40歳代の男性同性愛者に多いことを背景に，男性の梅毒患者は20～30歳代を中心に増加している。女性では20歳代前半が最も多



	2000	2001	2002	2003	2004	2005	2006	2007	2008
総報告数	759	585	575	509	535	543	637	718	839
無症候	420	301	293	209	160	172	195	224	306
早期顕症Ⅰ期	129	104	99	114	136	151	175	198	180
早期顕症Ⅱ期	157	134	121	127	176	180	205	234	276
晩期顕症	45	40	53	54	54	37	50	55	68
先天梅毒	8	6	9	5	6	3	12	7	9

図 4　病期別梅毒患者報告数の年次推移感染症発生動向調査（2009 年 3 月 5 日現在）

く，若年化の傾向がみられている。2006 年以降は年間約 100 例ずつ患者数が増加し，病期別では無症候梅毒や早期顕症梅毒Ⅱ期が増加している。早期顕症梅毒Ⅱ期では，性器や皮膚の梅毒病変がなく口腔・咽頭の梅毒病変が診断の契機になる場合が少なくない[17]ため，耳鼻咽喉科でも今後注意が必要である。

おわりに

性感染症が蔓延する大きな要因として，潜伏梅毒，淋菌およびクラミジアの性器および咽頭へ無症候感染，性器ヘルペスの無症候性排泄，前癌状態に至るまでの子宮頸部 HPV 感染など，感染しても無症状で他覚的所見もみられないこれらの無症候性感染者の存在が挙げられている。

女性の淋菌・クラミジア感染症は性器への持続感染を放置すると，不妊症や母子感染，子宮外妊娠の一因となるため，特に次世代を生み育てる若年女性に対する早期の診断治療は重要となる。無症候であるがゆえに早期受診につながりにくいという問題もあり，感染拡大の悪循環に歯止めをかけられていない。性感染症の実態を正しく理解し，日常診療の鑑別診断に加えるのみならず，患者やコメディカルをはじめ周囲の若い世代に性感染症の正確な情報を提供し，予防に努めるよう啓蒙できる耳鼻咽喉科医が増えることを切に願う。

文　献

1) 松本哲朗：性感染症（STI）治療のファーストステップ　性感染症とは？　概念と現状．臨床研修プラクティス **7**（2）：6-13，2010．
2) 大里和久，永尾朝江，犬角紀代美，他：HIV-1 感染者に見られる梅毒感染の動向．日性感染症会誌 **12**（1）：5-119，2001．
3) 赤股　要，白井　明，鳥居秀嗣：悪性梅毒の 1 例．皮膚科の臨床 **52**（2）：241-243，2010．
4) 本田まりこ：産婦人科検査マニュアル　感染症　梅毒．産科と婦人科 **77**（suppl）：34-38，2010．
5) 古賀哲也：重要な皮膚感染症　梅毒の症状，検査，治療（近年の傾向）．日本皮膚科学会誌 **19**（13）：2513-2514，2009．
6) 安田　満：特集／氾濫する性感染症（STI）を再考する　淋菌・クラミジアの咽頭感染．Urology View **7**（5）：87-92，2009．
7) 余田敬子，尾上泰彦，西田　超，他：淋菌およびクラミジアの咽頭および性器感染　性感染症クリニック受診者からみた現状．口咽科 **23**：207-212，2010．
8) 小野寺昭一：特集／氾濫する性感染症（STI）を再考する　わが国における性感染症の現状．Urology View **7**（5）：10-17，2009．
9) 余田敬子，新井寧子：咽頭からの淋菌およびクラミジア検出方法の検討．日耳鼻感染症研会誌 **26**：153-157，2008．
10) 日性感染症学会：性感染症 診断・治療ガイドライン 2008 疾患別診断と治療 淋菌感染症．日性感染症会誌 **19**（suppl）：49-56，2008．
11) 松尾光馬，尾上智彦，伊東秀記，他：ヘルペス感染症

その診断と治療 性器ヘルペスの無症候性排泄の現状と対策．MB Derma 147：5-12，2008．
12）東出誠司，保坂憲光，太田嘉則，他：新しい核酸抽出法を用いたLAMP法による単純ヘルペスウイルスの検出．日性感染症会誌 21（1）：120-127，2010．
13）堀場千尋，大黒 徹，白木公康，他：イムノクロマト法を原理とする単純ヘルペスウイルス抗原検出キットの基礎的性能評価．日性感染症会誌 21（1）：128-133，2010．
14）出口 隆，守殿貞夫，松本哲朗：非クラミジア性非菌性尿道炎．日性感染症会誌 19（suppl）：89-91，2008．
15）前田真一，清家健作，久保田恵章，他：オーラルセックスにより男子マイコプラズマ性尿道炎およびウレアプラズマ性尿道炎は引き起こされるか？ 日性感染症会誌 21（1）：115-119，2001．
16）国立感染症研究所感染症情報センタ：感染症発生動向調査．
17）余田敬子：口腔・咽頭梅毒．口咽科 14（3）：255-265，2002．

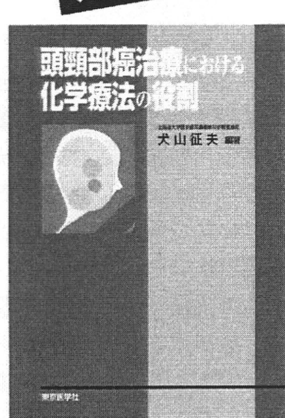
＊　＊　＊

頭頸部癌の化学療法の変遷と30有余年の治療成績を集大成した労作！

頭頸部癌治療における化学療法の役割

好評書

北海道大学医学部耳鼻咽喉科学教室教授
犬 山 征 夫 編著



主要目次

1. 癌化学療法の歴史
2. 動注化学療法
3. 単剤化学療法（1973～1978）
4. Cell kineticsに基づいた多剤併用療法
5. 頭頸部癌治療効果判定基準の作成
6. わが国における抗癌剤の第2，3相試験
7. Cisplatinを組み入れた多剤併用療法の開発とその臨床成績
8. Neo-adjuvant chemotherapy
9. 放射線治療と化学療法の同時併用
10. Adjuvant chemotherapy
11. 姑息的治療としての化学療法
12. 薬物有害反応（副作用）とその対策
13. 薬剤耐性克服およびSecond Line Chemotherapyの開発に関する基礎的・臨床的研究
14. 扁平上皮癌以外の組織型に対する化学療法
15. 頭頸部癌の化学予防に関する基礎的研究

B5判上製 480頁
カラー写真100点・図表500点
定価（12,000円＋税）
ISBN4-88563-106-8

東京医学社 〒101-0051 東京都千代田区神田神保町2-20-13Y's コーラルビル TEL 03-3265-3551 FAX 03-3265-2750

淋菌およびクラミジアの咽頭感染の現状

●

東京女子医科大学東医療センター
耳鼻咽喉科　准教授　余田　敬子

微研ジャーナル友　Vol.33　No.1（2010年1月号）別刷

学　術

淋菌およびクラミジアの咽頭感染の現状

東京女子医科大学東医療センター
耳鼻咽喉科　准教授　余田　敬子

1　はじめに

淋菌およびクラミジア感染症は、性感染症（sexually transmitted infections：STI）のなかで罹患者数が特に多い疾患である。淋菌感染症・クラミジア感染症とも、あらかじめ性感染症定点として指定された医療機関が毎月の患者数を管轄の保健所へ届け出る定点把握疾患として感染症法に定められ、定点報告数に基づく発生動向調査（サーベイランス）[1]、http://idsc.nih.go.jp/idwr/index.html として発表されている。2008年では、男性の性感染症の45%、女性の性感染症の63%を性器クラミジアが占め、また、男性の性感染症の30%を淋菌性尿道炎が占めていた（表1）。

これほど淋菌およびクラミジア感染症が蔓延している大きな要因として、どちらも罹患者の多くが無症候性で感染したことに本人が気づきにくいことが挙げられる。さらに、最近の日本の若者（とくに10代後半から20代前半の男女を示す）の性の自由化の流れに歯止めがかからない状況が、無症候性の性感染症の蔓延に拍車をかけている。複数の研究や性意識調査により、若者における貞節概念の希薄化、初性交年齢の低下、セックスパートナー数の増加は明らかで[2]、性感染症はもはや性風俗に関連した人がかかりやすい時代ではなく、若年世代を中心に誰もが感染しうる疾患になっている。

無症候性の性感染症には淋菌・クラミジア感染症の他に、HIV／エイズ（後天性免疫不全症候群 aquired immunodeficiency syndrome：AIDS）、ヒューマンパピローマウイルス（HPV）感染症、単純ヘルペス（herpes simplex virus：HSV）感染症がある。このなかで、淋菌とクラミジア感染症は無症候性であっても男女とも将来不妊に高率に結びつく疾患である。次の世代を産み育てるべき若者の中での淋菌・クラミジア感染症の蔓延は、重大な健康問題として中高年者のメタボリック症候群と同等に扱われてしかるべきである。しかし、この日本の性感染症の危機的状況が、厚生・教育行政、マスコミ、医療従事者、そして当事者である若者のなかで十分認識されていないのが現状である。性感染症は性器の病気と思い込み、オーラルセックスによって口腔咽頭にも性感染症が感染すること、口腔咽頭からパートナーへ性感染症をうつしてしまうことを知らない一般人が多い。性行動の多様化が急速に進み、オーラルセックスのみをサービスする性風俗店の人気が高い日本では、

表1　日本における性感染症定点報告数

	男　性		女　性	
第1位	性器クラミジア感染症	45%	性器クラミジア感染症	63%
第2位	淋菌感染症	30%	性器ヘルペスウイルス感染症	20%
第3位	性器ヘルペスウイルス感染症	12%	尖圭コンジローマ	10%
第4位	尖圭コンジローマ	12%	淋菌感染症	7%

厚生労働省・性感染症に関する特定感染症予防指針の推進に関する研究　2008年より

学 術

性感染症の咽頭感染の増加が懸念されている[3]。

本稿では、罹患者数が多い性感染症である淋菌およびクラミジアの咽頭感染症について、その背景、臨床像、診断について解説する。

2 淋菌感染症

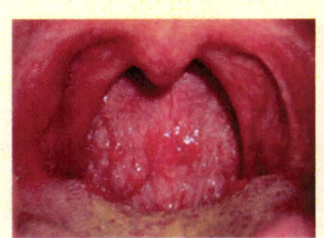
淋菌感染症はクラミジア感染症に次いで症例数が多い性感染症である。淋菌 *Neisseria gonorrhoeae* は環境変化に弱く外界ではすぐ死滅するが、1回の性行為による感染率は約30%[4]と感染リスクが高い病原体といえる。淋菌は、感染症として男性に尿道炎、女性に子宮頸管炎を生じる。淋菌性結膜炎は、新生児の経産道感染として生じることが多いが、成人においても性行為または感染している性器からの自家接種による重症の化膿性結膜炎が生じることがある。淋菌感染症は感染部位により症状の現れ方に差があり、尿道炎（排尿痛、膿性尿道分泌物）や結膜炎（多くは一側性で、著名な眼瞼と結膜の浮腫、大量の膿性浸出物がみられる）は著明な臨床症状がみられるが、女性の子宮頸管炎では分泌物を生じるものの感染女性の多くは自身の感染に気づかない場合が多い。また、男性の淋菌性尿道炎でも、再感染では症状・所見が現れにくい[5]。

感染経路として、日本人男性の淋菌性尿道炎の感染源の第1位はオーラルセックスをサービスする性産業従業女性の咽頭に存在する淋菌であることが指摘されており[6]、咽頭の淋菌感染が最多の感染源として問題となっている。咽頭の淋菌感染は偽膜形成を伴う淋菌性咽頭扁桃炎を生じることはごくまれで、感染者のほとんどは無症状で咽頭発赤や扁桃腫脹など他覚的所見が見られない（図1）[3,7]。耳鼻咽喉科医である著者も、これまでの経験から咽頭の診察によって淋菌の咽頭感染を診断することは困難と考えている。

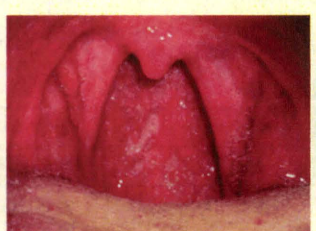
3 クラミジア感染症

性感染症としてのクラミジア感染症は *Chlamydia trachomatis* による。*C. trachomatis* は、血清型でA～L 3の18に分けられ、性器クラミジア感染症は主にD、E、F、G型による。伝染性角結膜炎（トラコーマ）の原因となるのはA、B、C型で性器感染するものと別の血清型の *C. trachomatis* であるが、新生児の経産道感染による角結膜炎はD、E、F、G型による場合が多い。このD、E、F、G型によるクラミジア性尿道炎とクラミジア性角結膜炎は淋菌性尿道炎と結膜炎に比べるとかなり病状が軽い。性器クラミジア感染では尿道炎と子宮頸管炎の5分の4が無症候で潜在していると推定され[2]、このことが性感染症としてのクラミジア感染症が最も罹患者数の多い大きな原因となっている。特に問題なのが、10代後半から20代前半の若年層でのクラミジア陽性率が高いことである。この背景にあるのは、冒頭に挙げたこの世代での性交経験率の上昇だけでなく、子宮頸管に円

図1 咽頭の淋菌およびクラミジア陽性者の局所所見



23歳 女性 風俗店従業
咽頭淋病 陽性



20歳 女性 風俗店従業
咽頭クラミジア 陽性

●感染者の多数は無症状。
●咽頭発赤や扁桃腫脹など他覚的所見が認められないことが多い。

宮本町中央診療所 尾上泰彦先生 撮影