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

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

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

RESULTS

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

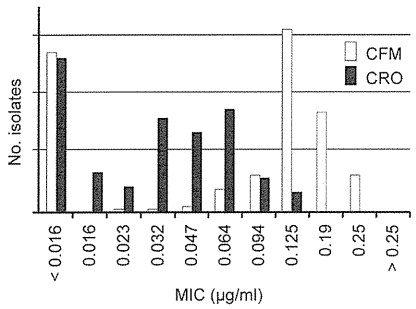

FIG 1 Cefixime (CFM) and ceftriaxone (CRO) MIC distribution of *Neisseria gonorrhoeae* isolated in Kyoto and Osaka, Japan, in 2010 to 2012 (n = 193).

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

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

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

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

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

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

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

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

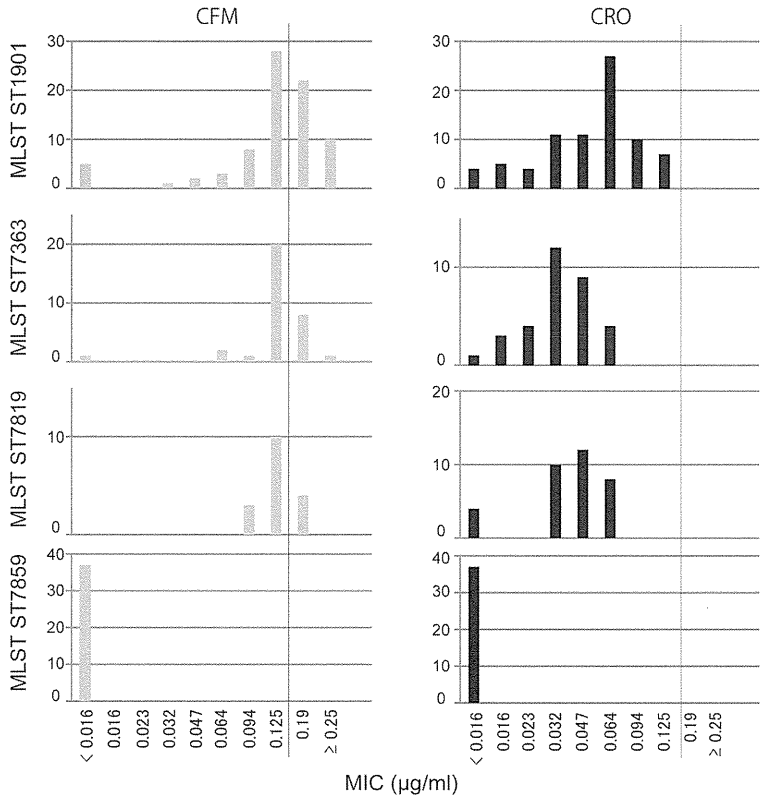

FIG 2 MIC of cefixime (CFM) and ceftriaxone (CRO) in *Neisseria gonorrhoeae* isolates from Kyoto and Osaka, Japan, in 2010 to 2012 belonging to the four major multilocus sequence typing (MLST) sequence types (STs), that is, ST1901 (n = 79), ST7363 (n = 33), ST7819 (n = 17), and ST7359 (n = 37).

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

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

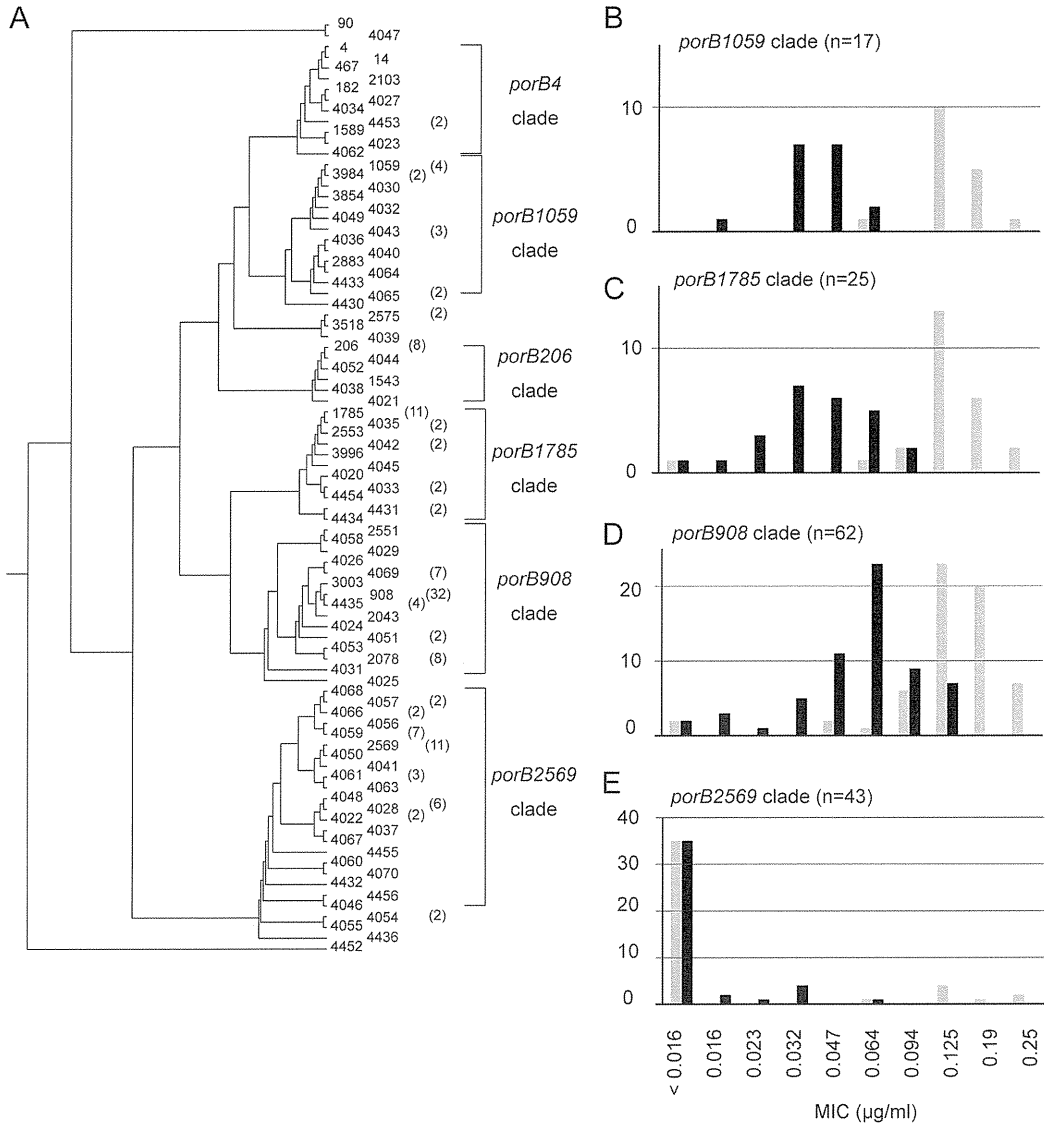

FIG 3 Sequence comparison of *porB* alleles (490 bp) from *N. gonorrhoeae* isolates (n = 193) from Kyoto and Osaka, Japan, and cefixime and ceftriaxone MIC profile of each *porB* group. (A) An internal region of the *porB* genes including loops 3, 4, and 5 (490 bp), which is used in NG-MAST, was utilized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

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

DISCUSSION

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

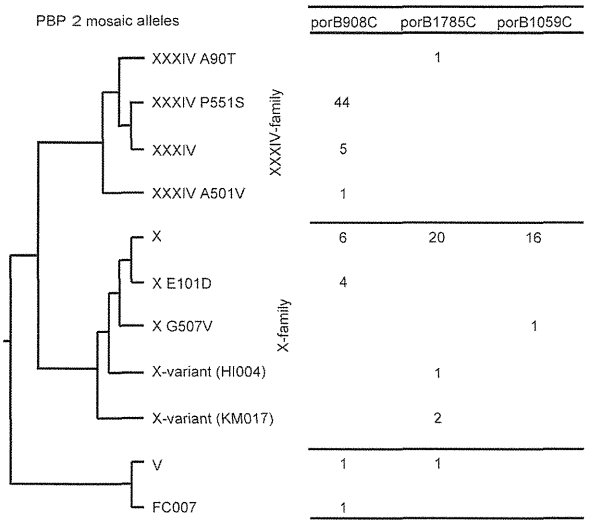

FIG 4 Phylogenetic tree of the amino acid sequences of penicillin binding protein 2 encoded by different *penA* mosaic alleles. The *penA* genes from a total of 104 *N. gonorrhoeae* isolates possessing alleles belonging to the *porB908*, *porB1785* and *porB1059* clades (C) were sequenced, and a phylogenetic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

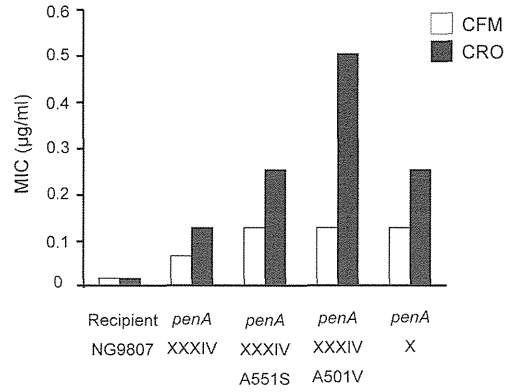

FIG 5 Capacity for increased MIC conferred by the mosaic PBP 2 alleles harbored by *penA* X and *penA* XXXIV, including amino acid substituted mutants identified in the present study. Each allele was introduced into the cefixime- and ceftriaxone-susceptible strain NG9807, and MIC values of each transformant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

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

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

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

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

rhoeae strains, it is difficult to strictly link *porB* alleles and accordingly NG-MAST STs to antimicrobial susceptibility phenotypes. Interestingly, seven of the eight isolates in the *porB2569* group that showed higher MICs of cefixime belonged to MLST ST7363 and MLST ST1901, which are the two major STs in which cefixime resistance has emerged. This might suggest that these MLST ST7363 and MLST ST1901 isolates have horizontally acquired their *porB2569* group allele from an authentic MLST ST7359 strain, which is the predominant MLST type among isolates within the *porB2569* group.

The first described isolate of NG-MAST ST1407 from San Francisco, CA, displayed a decreased susceptibility to cefixime, and this isolate had a mosaic type PBP 2 encoded by the *penA* mosaic allele SF-A, subsequently named *penA* allele XXXIV (23). The high-level ceftriaxone-resistant NG-MAST ST1407 isolates identified in France (14) and Spain (25) both had an identical mutated *penA* XXXIV allele (*penA* allele CI [14, 23, 25]) that encodes a PBP 2 XXXIV sequence with leucine replaced by proline at amino acid position 501 (L501P) (14). Accordingly, these high-level resistant strains have presumably evolved by a single nucleotide polymorphism in the *penA* XXXIV allele resulting in an A501P alteration in PBP 2 in an NG-MAST ST1407 (MLST 1901) strain. In the present study, although many NG-MAST ST1407 *N. gonorrhoeae* isolates with *penA* XXXIV allele or derivates of this allele were identified, no *penA* allele CI (14, 23, 25) was found. However, some other single-amino-acid-substituted alleles of *penA* XXXIV, i.e., *penA* XXXIV with P551S or A501V, were identified. The P551S and A501V amino acid substitutions have previously been associated with decreased susceptibility to ceftriaxone (10, 37, 38). In the present study, it was shown by transformation experiments that these substitutions in a *penA* XXXIV allele result in a 2- to 4-fold increase in the MICs of cefixime and ceftriaxone.

In conclusion, our intensified surveillance in 2010 to 2012 in the Kyoto and Osaka prefectures, Japan, did not identify any dissemination of the high-level ceftriaxone-resistant *N. gonorrhoeae* strain H041, suggesting that H041 caused only a sporadic case and has not further spread. Furthermore, no other ceftriaxone-resistant strain was identified. The antimicrobial resistance surveillance in this region has continued and should now be expanded nationally.

However, it is cause for great concern that *N. gonorrhoeae* strains with the *penA* XXXIV allele and its derivatives are also shown to be spreading in Japan, as in many other countries (10, 14). The *penA* mosaic XXXIV allele appears also to be evolving, resulting in further-enhanced MICs of ESCs, and it might be only a matter of time before an additional high-level ceftriaxone-resistant strain emerges. If this strain is not detected in a timely fashion and has a retained biological fitness, it will spread internationally and result in a large public health problem. Consequently, it is crucial not only to enhance the phenotypic antimicrobial susceptibility surveillance of *N. gonorrhoeae* but also to perform molecular typing and detection to monitor the spread and evolution of successful *N. gonorrhoeae* clones with decreased susceptibility and resistance to ceftriaxone worldwide.

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Note

Characterization of azithromycin-resistant *Neisseria gonorrhoeae* isolated in Tokyo in 2005–2011

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ABSTRACT

A total of 122 *Neisseria gonorrhoeae* isolated in the Tokyo metropolitan area in 2005–2011 were collected and analyzed by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) and for their susceptibility to azithromycin and ceftriaxone. All 122 strains were susceptible to ceftriaxone, but 8 strains were azithromycin-resistant, defined as an azithromycin MIC ≥ 1 μg/ml. The 8 azithromycin-resistant strains were in 6 NG-MAST types, 3 strains in NG-MAST type 1407 and each of the other 5 strains in a different NG-MAST type. NG-MAST type 1407 strains are multidrug-resistant and are disseminated worldwide.

Timely revision of treatment guidelines has been essential for control of *Neisseria gonorrhoeae* infections because of the rapid emergence and spread of drug resistant *N. gonorrhoeae* strains. Therefore, continuous surveillance and antimicrobial susceptibility testing of *N. gonorrhoeae* has been required.

Since the withdrawal of cefixime from the treatment guidelines for *N. gonorrhoeae* in Japan in 2006, the recommended drugs have been limited to three injectable antibiotics: ceftriaxone, spectinomycin, and cefodizime. The first report of a ceftriaxone-resistant *N. gonorrhoeae* strain was an isolate in 2009 from Kyoto, Japan. Spread of ceftriaxone-resistant *N. gonorrhoeae* and development of other treatment options then became a concern. Oral azithromycin (2 g) became available for treatment of *N. gonorrhoeae* infections in Japan in 2009. However, emergence of azithromycin-resistant *N. gonorrhoeae* in Japan would be a significant clinical problem because it would limit the utility of the only currently available oral drug treatment for *N. gonorrhoeae* infections in Japan. Indeed, azithromycin hyper-resistant *N. gonorrhoeae* isolates have been reported in several other countries [1–8].

From this background, we performed retrospective analyses of the clinical *N. gonorrhoeae* strains isolated and stocked in the Byoutai-Seiri Laboratory in 2005–2011. These 122 *N. gonorrhoeae* strains (8 strains isolated in 2005, 24 in 2006, 29 in 2007, 12 in 2008, 9 in 2009, 20 in 2010, and 20 in 2011) were analyzed by multiantigen sequence typing (NG-MAST), as described by Martin et al. [9], and for the minimum inhibitory concentrations (MIC) of penicillin G, cefixime, ceftriaxone, ciprofloxacin, spectinomycin, and azithromycin by the agar dilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI; http://www.clsi.org), with utilization of ATCC49226 strain as the quality control of MIC values.

The 122 *N. gonorrhoeae* strains were in 82 NG-MAST types. The largest number of strains were NG-MAST type 2958 ($n = 16$), followed by NG-MAST type 1407 ($n = 14$) and NG-MAST type 247 ($n = 3$). Only 1 or 2 strains were in each of the other 79 NG-MAST types. The high prevalence of NG-MAST type 1407 and 2958 strains in Japan was also reported previously [10,11]. We then determined the MIC of azithromycin, ceftriaxone, and other 4 drugs described above, of the 122 *N. gonorrhoeae* strains. In this study, an antibiotic MIC ≥ 1 μg/ml was defined as azithromycin-resistant following the EUCAST Clinical Breakpoint Table v. 3.1 2013-02-11 (http://www.eucast.org/antimicrobial-susceptibility-testing/breakpoints) and MIC ≥ 0.5 μg/ml as ceftriaxone-

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Table 1
Properties of the 8 azithromycin-resistant *N. gonorrhoeae* strains in this study.

Strain number	Isolation date	NG-MAST ST	*porB* allele	*tbpB* allele	MIC (μg/ml)						Nucleotide at position 2611 in 23S rRNA gene[a]			
					PCG[b]	CFIX[b]	CTRX[b]	CPFX[b]	SPCM[b]	AZM[b]	Allele 1	Allele 2	Alle1e 3	Allele 4
50	Jun 2007	278	4	27	1	0.06	0.06	4	16	1	C	C	C	C
57	Jul 2007	7023	4222	21	0.5	0.25	0.125	8	16	1	C	C	C	C
112	May 2010	5687	2481	10	0.25	0.25	0.03	16	32	1	C	C	C	C
115	Jun 2010	6762	4	1307	0.5	0.25	0.03	4	16	8	T	T	T	T
139	Jan 2011	1407	908	110	2.0	0.125	0.06	32	32	16	T	T	T	T
142	Feb 2011	1407	908	110	2.0	0.125	0.06	16	16	16	T	T	T	T
148	Aug 2011	7048	4240	1365	1.0	0.06	0.03	8	16	1	C	C	C	C
149	Aug 2011	1407	908	110	4.0	0.25	0.06	16	16	1	C	C	C	C

[a] There were no mutations in of the four alleles of the 23S rRNA gene in these 8 strains except at position 2611 (*E. coli* numbering) of the three strains with an azithromycin MIC >4 μg/ml.
[b] PCG, penicillin G; CFIX, cefixime; CTRX, ceftriaxone; CPFX, ciprofloxacin; SPCM, spectinomycin; AZM, azithromycin.

resistant following the Clinical and Laboratory Standards Institute (CLSI; http://www.clsi.org), respectively, because CLSI has not set the breakpoint of azithromycin yet. All 122 *N. gonorrhoeae* strains were found to be susceptible to ceftriaxone, but 8 strains were azithromycin-resistant (Table 1). Five of these 8 azithromycin-resistant strains had an MIC = 1 μg/ml and the other 3 strains had an MIC >4 μg/ml (Table 1). For these 8 azithromycin-resistant strains, 3 strains were NG-MAST type 1407 (strains 139, 142, and 149) and there was 1 strain in NG-MAST type 278, 5687, 6762, 7023, and 7048 (Table 1). Although more *N. gonorrhoeae* strains in this study were NG-MAST type 2958 than any of the other 81 NG-MAST types, none of the 8 azithromycin-resistant strains was NG-MAST type 2958.

We noticed that, of the 14 NG-MAST type 1407 strains in this study, the first 11 strains, isolated from 2006 to 2010, were all azithromycin-sensitive (MIC <1 μg/ml). In contrast, the 3 NG-MAST type 1407 strains isolated in 2011 were all azithromycin-resistant. Each of the other 5 azitromycin-resistant strains in this study, isolated from 2007 to 2011, was a different NG-MAST type (Table 1).

The azithromycin-resistant *N. gonorrhoeae* strains detected in this study were different NG-MAST types than the azithromycin-resistant strains isolated in other countries [1–8]. Therefore, we constructed a phylogenetic tree of the *porB* gene sequences of *N. gonorrhoeae* strains from Japan and other countries to investigate the genetic relationships of these strains (Fig.1). No *porB* sequence in the *N. gonorrhoeae* strains from other countries was identical to any of the *porB* sequences of the strains isolated in the Tokyo metropolitan area, except for the *por4* allele that is common in azithromycin-resistant strains from England and Scotland (NG-MAST types 5, 225 and 738) and was found in two of the azithromycin-resistant strains from Tokyo (NG-MAST types 278 and 6762) in this study. The *por908* allele in the NG-MAST type 1407 strains isolated in Japan had only a one nucleotide difference from the *por2237* allele found in an NG-MAST type 3709 strain isolated in the United States [8] and was closely related to the *por971* allele found in an NG-MAST type 1503 strain isolated in Scotland (Fig.1) [4]. Moreover, these NG-MAST type 3709 [8] and type 1503 strains [4] have a *tbpB110* allele that is also in NG-MAST type 1407 strains (Table 1). Therefore, the *por* allele may have diverged in globally disseminated NG-MAST type 1407 strains and some of these NG-MAST type 1407 and related strains subsequently acquired azithromycin resistance.

A point mutation in the *N. gonorrhoeae* 23S rRNA gene has been shown to be responsible for azithromycin resistance [12]. Therefore, we sequenced the four 23S rRNA loci in the 8 azithromycin-resistant *N. gonorrhoeae* strains in this study (Table 1), referring 23S rRNA sequence of strain 20869 in reference 12. The three strains with an azithromycin MIC >4.0 μg/ml (strains 115, 139, and 142) had a C2611T transition (*E. coli* numbering) mutation in all four loci. But, these three strains did not have the A2059G mutation (*E. coli* numbering) found in azithromycin hyper-resistant strains (MIC ≥ 256 μg/ml) [13]. However, the other five *N. gonorrhoeae* strains in this study, with an azithromycin MIC = 1.0 μg/ml, had neither C2611T nor A2059G mutation in any of the four 23S rRNA gene loci.

In this study, we showed that up to the 3 of the 8 azithromycin-resistant *N. gonorrhoeae* strains detected in these analyses were NG-MAST type 1407. This is a public health concern because NG-MAST type 1407 strains have been reported to have reduced susceptibility to cefixime and ciprofloxacin, in several previous studies [11,12]. Moreover, of these three NG-MAST type 1407 strains, two (strains 139 and 142) had an azithromycin MIC as high as 16 μg/ml, the highest value detected in this study.

As an orally administered drug, azithromycin is an attractive treatment choice for *N. gonorrhoeae*. However, it has to be used cautiously because the results of this study indicated that azithromycin-resistant *N. gonorrhoeae* strains have spread, especially as of 2011, with significant clonality in NG-MAST type 1407 strains.

Although azithromycin therapy alone is not recommended in the Japanese Society for Sexually Transmitted Infections guideline, 2 g oral azithromycin is sometimes prescribed for *N. gonorrhoeae* treatment. A recent treatment guideline from the United States CDC recommends dual use of ceftriaxone/azithromycin or ceftriaxone/doxycycline because of their reported efficacy in the treatment of *N. gonorrhoeae* infections [14]. A regimen of azithromycin 2 g orally in a single dose is also proposed as an alternative if ceftriaxone cannot be given because of severe allergy. In this case, the CDC recommended that the patient should return 1 week after treatment for a test-of-cure.

In *N. gonorrhoeae*, the emergence of the initial drug-resistant strains and spread of these strains has been very rapid, partly because they have the high natural transformation ability and intra-species transfer of resistance gene may occur [15]. Therefore, there should be active surveillance to detect the emergence and spread of antibioitc-resistant *N. gonorrhoeae* strains, especially when a new drug is introduced for treatment of *N. gonorrhoeae* infections.

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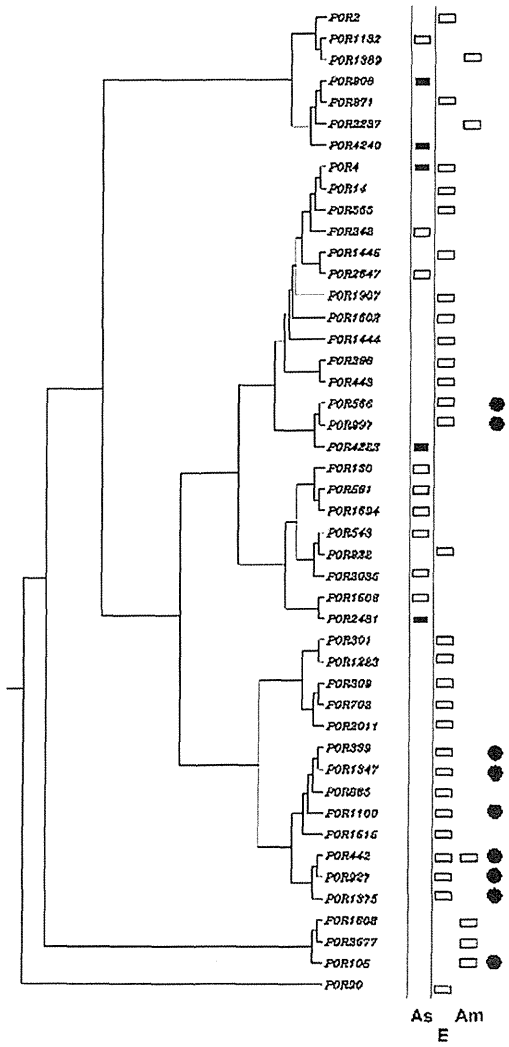

Fig. 1. Phylogenetic tree constructed from sequences of *porB* alleles from *N. gonorrhoeae* strains from Japan (filled boxes in column As) (data from this study), China (open boxes in column As) [6], Europe (open boxes in column E) [1,4,5], and United States and Argentina (open boxes in column Am) [2,3,7,8]. A 490 bp internal region of the *porB* gene, including loops 3, 4 and 5, which was used in NG-MAST, was used to reconstruct the phylogenetic tree using the MEGA 4 program. The numbers are the *porB* allele numbers assigned in the NG-MAST database. The *porB* alleles reported to be in azithromycin hyper-resistant strains (MIC ≥ 256 μg/ml) are marked with circles.

Conflict of interest

None of the authors has any conflicts of interest to declare.

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◆特集・痛みの性状からわかる耳鼻咽喉科疾患

Ⅱ．各　論
3．STIと咽喉頭の痛み

余田敬子*

Abstract　梅毒の第1期病変である硬結や潰瘍は無痛性で，第2期病変の粘膜斑や口角炎は，軽い痛みまたは違和感を訴える．HSVは初感染時に口腔咽頭粘膜に特徴的なアフタ・びらん・白苔を伴う咽頭炎や扁桃炎を発症し，激しい痛みと高熱がみられる．HIVは，感染から2～4週間頃の急性初期感染期に，インフルエンザまたは伝染性単核球症様の非特異的な咽頭痛を訴える．HIV感染の中期以降にあらわれる口腔咽頭病変には，カンジダ症，再発性アフタ性口内炎，多形性紅斑，舌癬，非特異的口腔潰瘍，カポジ肉腫，非ホジキンリンパ腫，扁平上皮癌等があり，それぞれ病変に伴う痛みや，違和感を訴える．淋菌とクラミジアは感染者数の多い性感染症で，どちらも咽頭に感染すると大多数は無症候性に感染する一方で，ごく一部に非特異的な咽頭炎，扁桃炎，上咽頭炎による痛みを訴える．HPVは中咽頭癌との関連性が指摘されているが，HPV感染そのものは無症候性である．

Key words　性感染症(sexually transmitted infection)，梅毒(syphilis)，単純ヘルペスウイルス感染症(herpes simplex virus infection)，ヒト免疫不全ウイルス感染症(human immunodeficiency virus infection)，淋菌感染症(gonococcal infection)，クラミジア感染症(chlamydial infection)，ヒトパピローマウイルス感染症(human papillomavirus infection)

はじめに

性感染症は従来よりSTD(sexually transmitted diseases)の略が普及しているが，多くの性感染症において無症候性感染者がキャリアとなって感染拡大に大きく関与していることが指摘されるようになり，性感染症をsexually transmitted diseasesではなく，sexually transmitted infection；STIと呼ぶようになっている．STIの原因微生物のうち，咽喉頭疾患に関連するものとして，梅毒トレポネーマ，単純ヘルペスウイルス(herpes simplex virus；HSV)，ヒト免疫不全ウイルス(human immunodeficiency virus；HIV)，淋菌，クラミジアトラコマティス(*Chlamydia trachomatis*；以下，クラミジア)，ヒトパピローマウイルス(human papillomavirus；HPV)が挙げられる．

これらの咽喉頭に関連する性感染症には，その特徴的な臨床像から診断しやすいものと，特徴に乏しく他の疾患との鑑別が難しいものとがある．本稿ではHSV感染症，梅毒，淋菌およびクラミジア感染症，HPV感染症について，咽喉頭の痛みの性状からみた臨床像，診断，治療について概説する．

梅　毒

梅毒はスピロヘータの一種である梅毒トレポネーマ(*Treponema pallidum subspecies pallidum*；以下，Tp)を病原体とする慢性感染症[1]で，体のあらゆる部分または全身の皮膚や粘膜，時に臓器に病変を生じる．胎児が経胎盤的に感染する先天性梅毒と，経胎盤感染以外の感染経路で梅毒

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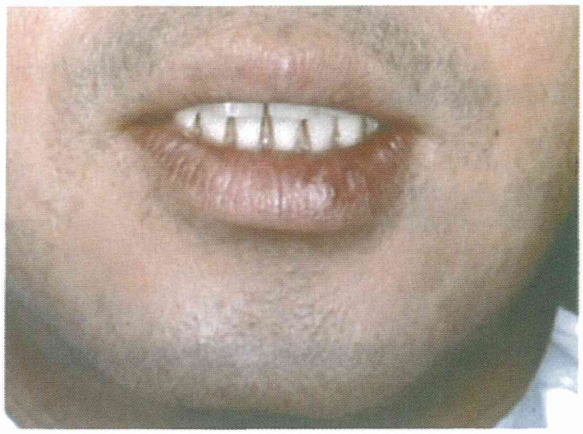

図 1. 口唇の初期硬結(41 歳，男性)文献 2 より転載
暗赤色で，無痛性の硬い腫瘤を下口唇左側に触れる(文献 2 より転載)

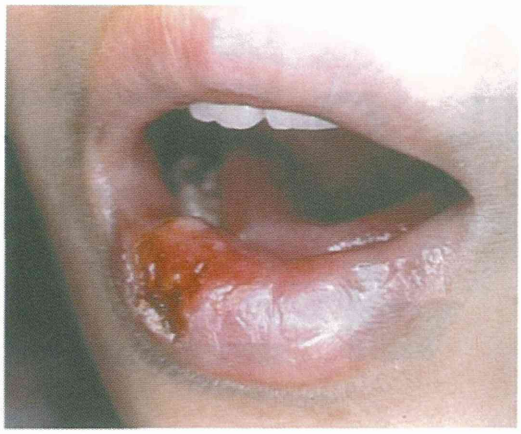

図 2. 口唇の硬性下疳(16 歳，女性)
初期硬結が潰瘍化したもの．無痛性．潰瘍面のスワブの鏡検にて，ラセン状のトレポネーマが検出される(文献 2 より転載)

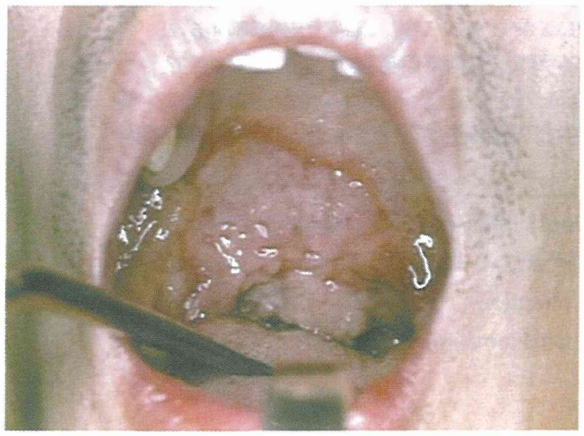

図 3. 梅毒 2 期の咽頭粘膜斑(43 歳，男性)
口蓋垂から口蓋粘膜に拡大した粘膜斑．粘膜斑は扁平で若干の隆起があり，周囲は薄い赤色の紅暈で囲まれ青みがかった白灰色で「乳白斑」とも呼ばれる(文献 2 より転載)

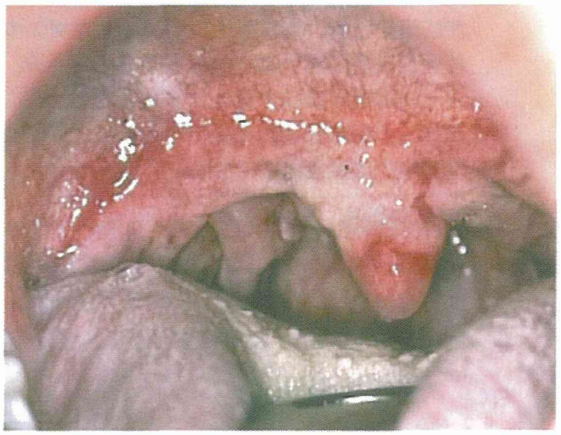

図 4. 梅毒 2 期の咽頭粘膜斑(27 歳，女性)
粘膜斑が口峡部に沿って弧状に拡大融合して，蝶が羽を広げたような“butterfly appearance”を呈している(文献 3 より転載)

に感染する後天梅毒に分けられ，後天梅毒には血清梅毒検査は陽性であるが臨床症状のない無症候梅毒と，皮膚や粘膜に病変がみられる顕症梅毒とがある．顕症梅毒は Tp に感染してからの時期(第 1～4 期)によってあらわれる病変，疾患が異なる．

耳鼻咽喉科領域では，第 1 期の初期硬結，硬性下疳，第 2 期の粘膜斑，口角炎が口腔，咽頭にみられる場合がある[1)～4)]．

1．痛みの性状

1）初期硬結・硬性下疳

感染後 3ヶ月頃まで(第 1 期)にみられる病変で，Tp が侵入した部位にしこりが生じ(初期硬結，図 1)，数日後には硬結の中央に潰瘍ができる(硬性下疳，図 2)．初期硬結・硬性下疳は痛みがないのが特徴で，アズキ大から指頭大の大きさで暗赤色を呈し軟骨のように硬く触れる．耳鼻咽喉科領域では口唇，舌，扁桃[1)2)]に 1 個，時に 2～3 個現れる．患側頸部に軟骨様に硬く触れるリンパ節腫脹を伴い，これも無痛性[4)]である．

2）口角炎・粘膜斑

感染後 12 週目頃(第 2 期)には，口腔・咽頭の粘膜斑(乳白斑ともいう)と口角炎がみられる．咽頭の粘膜斑(図 3)は，扁平で若干の隆起があり，青みがかった白または灰色を呈して周囲は薄い赤色

表 1. 梅毒血清反応　定性検査の結果の解釈

STS	TPHA 抗原法	結果の解釈
−	−	非梅毒 稀に感染初期#
+	−	生物学的偽陽性(BFP)* 稀に感染初期#
+	+	梅毒(早期から晩期) 梅毒治癒後の抗体保有者
−	+	梅毒治癒後の抗体保有者

(文献 1 より転載)

第 1 期の梅毒感染初期が疑われる場合は，2〜4 週後に再検査が必要となる

＊生物学的偽陽性(BFP)梅毒に感染していなくても，ウイルス・細菌などによる感染症，膠原病，妊娠，担癌状態，老齢，静注薬物乱用者などで STS が陽性を示す場合をいう

表 2. 梅毒血清反応　定性検査(用手法)の結果の解釈

検査法		抗体価(血清希釈倍数)									
STS	RPR 法	1°	2	4	8	16	32	64	128	256	512
	ガラス板法	1°	2	4	8	16	32	64	128	256	512
Tp 抗原	TPHA		80		320	1,280		5,120	20,480		81,920
	FTA-ABS		20			定性法のみ					
抗体価の読み方					低い←	中等度		→高い			

(文献 1 より転載)

○印は定性検査の血清希釈倍数

感染初期には STS 群抗体価が TPHA 法の抗体価に先行して陽性となる

の紅暈で囲まれる．乳白斑が拡大・融合すると軟口蓋に特徴的な“butterfly appearance”を呈する(図 4)．梅毒性口角炎は口角に白斑を伴う所見で，カンジダ性口角炎に似ているが梅毒の白斑は擦過にて剝離されない．粘膜斑，口角炎ともに，病変部の比較的軽い痛みや違和感を訴える[4]．自覚症状がなく，病変に気づいて受診する場合もある．

2．検査・診断

Tp は分離培養ができないため，直接検出する直接法または梅毒血清反応によって診断する．

臨床所見からカンジダ症との鑑別を要する場合は，病変部から採取したスワブを鏡検とともに真菌培養へ提出する．

1） 直接法

硬性下疳や粘膜斑などの口腔咽頭の梅毒病変には Tp が多く存在するため直接法での検出が有用である．硬性下疳や粘膜斑の表面を擦って採取した漿液をスライドグラスに塗抹，染色し観察する．ただし，Tp と口腔内常在性トレポネーマとの鑑別は困難，必ず梅毒血清反応の結果とあわせて診断する．また，抗菌薬がいったん投与されると病変部の Tp が減少し検出率が低下するため，直接法は必ず抗菌薬投与前に行う．

2） 梅毒血清反応

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)と，Tp 抗原法がある．STS にはガラス板法や RPR(rapid plasma reagin)があり，抗原法には TPHA (treponema pallidum heamagglutination assay) と FTA-ABS (fluorescent treponemal antibody absorption test) 法がある．はじめに STS の 2 法と TPHA の定性検査を行い(表 1)，陽性の場合に STS および TPHA の定量検査で確定診断する(表 2)．梅毒血清反応は血行性感染が始まる第 2 期以降の診断に有用である．血清梅毒反応陰性でも，問診や臨床所見から第 1 期が疑われる場合は，2〜4 週後に再検査を行う．

これまで用手法で行われていた STS，TPHA の定量検査は，近年高感度の自動定量測定が開発され，各医療施設に導入されつつある．自動定量測定と従来の用手法による定量検査の数値との相関性は自動測定キットのメーカーにより異なるの

で注意する.

梅毒患者ではHIV感染を合併する率が高いため，梅毒血清反応陽性者の場合は必ずHIV検査を追加する.

3．治　療

Tpはほとんどの抗菌薬に感受性がある．診断に先行したむやみな抗菌薬の投与は病変のみが消失して見逃され，潜伏梅毒に移行させてしまうおそれがあるため注意する.

最も推奨される抗菌薬はペニシリンで，経口合成剤AMPC，ABPCなどを1回500 mg，またはDEBCPCG(ベンジルペニシリンベンンザチン；バイシリンG®)1回400万単位を1日3回投与する．ペニシリンアレルギーの場合は塩酸ミノサイクリンを1回100 mg，1日2回投与する.

治療期間は，第1期では2〜4週間，第2期では4〜8週間投与を継続する．抗体価が高い症例や，感染時期が不明な場合には投与期間を延長する.

治療開始にあたり，内服開始直後の2〜12時間後に，悪寒戦慄・発熱・倦怠感・咽頭痛・筋肉痛・頭痛・頻脈などの症状が一過性に現れ，ほぼ8時間以内に消失する．この現象はJarish-Herxheimaer反応と呼ばれ，第1期で50%，第2期では75%現れる，Tpが多量に死滅し菌体のリポ多糖類が放出されて生じるエンドトキシン反応で，駆梅療法を中止する必要はない．投薬開始時にこの現象を説明し，副作用と誤って薬の服用を中断しないように患者を指導しておくことは重要で，解熱薬も頓用で予め処方しておいてもよい.

治療後，体内のTpが消失するとSTS抗体価は下がりはじめるので，STS定量値は治療効果判定に用いられる．一方，TPHA定量値は治療後に必ずしも低値にならず，治療効果を反映しない．病期に応じた十分な投薬を行った後，臨床症状の持続や再発がないことと，STS抗体価を定期的に追跡して定量値が8倍以下に低下するまで確認する必要がある．治療後半年過ぎてもSTS定量値が16倍以上示す例は，治療が不十分または再感染例が疑われるため，検査と治療を追加する.

HSV性咽頭・扁桃炎

HSVには，1型(HSV-1)と2型(HSV-2)がある．HSV-1，HSV-2ともに，初感染の90%以上は不顕性感染し，潜伏感染に移行する．残りの約10%に，歯肉口内炎，咽頭・扁桃炎，性器ヘルペスの発症がみられる．主にHSV-1は口唇・顔面・眼に，2型は性器に病変を生じるが，HSV-1による性器ヘルペスや，HSV-2による口唇ヘルペスの報告もある.

HSV性咽頭・扁桃炎は，10歳代後半〜30歳代前半の青壮年期にHSVに初感染した場合にみられることが多く，HSV-1，HSV-2，どちらも原因となる[5)]

1．痛みの性状

著明な咽頭痛を訴え，咽頭痛・嚥下痛のため摂食障害をきたす患者が多い．38〜40℃の弛張熱と，上頸部リンパ節の高度腫脹を伴う．口蓋扁桃・舌扁桃・咽頭後壁のリンパ濾胞に白苔をともなう発赤腫脹がみられる(図5)．陰窩性扁桃炎と異なり，HSV性扁桃炎の口蓋扁桃の白苔は必ずしも陰窩に一致しない．白苔の周囲の口蓋扁桃(図5)，舌扁桃，口腔粘膜，口唇(図6)にヘルペス特有のアフタがみられる.

口腔・咽頭の帯状疱疹と鑑別を要するが，帯状疱疹は正中を越えず一側性のことが多い．性器ヘルペスや皮膚のヘルペス疹を併発する場合もある.

2．検査・診断

HSV性咽頭・扁桃炎の診断には，抗原検査(モノクローナル抗体による蛍光抗体法)が簡便で，迅速に結果が得られる．特異性が高く型判定も可能でありきわめて有用である．綿棒で擦過採取したアフタや潰瘍病変の細胞をスライドグラスに塗抹し，抗HSV-1および抗HSV-2モノクローナル抗体を用いて，ウイルス感染細胞を同定する.

抗原検査ができない施設においては，他に保険適応である血清抗体検査による診断には時間がかかるため，臨床症状，問診，視診から総合的に診

断し，抗ウイルス薬の投与を判断することが求められる．しかし，症例経験のある医師であれば，その特徴的な所見から，臨床診断は難しくない．HSV 性咽頭扁桃炎であれば，抗ウイルス薬の投与を開始して3日目頃から症状が急速に改善し始める．

移植後患者など重篤化の恐れがある患者で，治療上他の疾患との鑑別診断が必須となる場合は，下記の方法で咽頭の病変から採取した擦過細胞や生検組織から HSV 感染の証拠を検索する．

1）ウイルス分離培養：ゴールドスタンダード，特異性が高く，型判定が可能．

2）核酸増幅法（PCR 法，LAMP 法）：特異性が高く型判定が可能．

3）Tzanck 試験：外来で迅速簡便に検査が可能，感染細胞に特徴的な full 型または Cowdry 型の核内封入体，またはウイルス巨細胞を確認する．HSV と VZV の判別は不可．

4）血清抗体検査：単回検査では臨床的意義が低い．急性期と回復期のペア血清で有意の抗体上昇によって診断することが可能である．HSV 初感染後は生涯持続感染するので，血清抗体価が陽性であることだけでは診断的意味はない．HSV は同一個体において HSV-1 初感染，HSV-2 初感染，潜伏持続感染，再発といった様々な病態があり，また HSV-1 と HSV-2，また HSV と水痘帯状疱疹ウイルス（varicella zoster virus：VZV）間で交叉反応が存在するため，血清抗体価から HSV-1, HSV-2, VZV を判別できない場合もある．

3．治　療

バラシクロビル1回 500 mg，1日2回を経口で5日間，またはアシクロビル1回 200 mg，1日5回を経口で5日間，のどちらかで治療する．経口摂取困難な重症例では，アシクロビル注 5 mg/kg/回，1日3回8時間毎，7日間投与する．腎機能障害合併例では，表3[6]に従い，クレアチニンクリアランス値（CCr）により投与量を決定する．

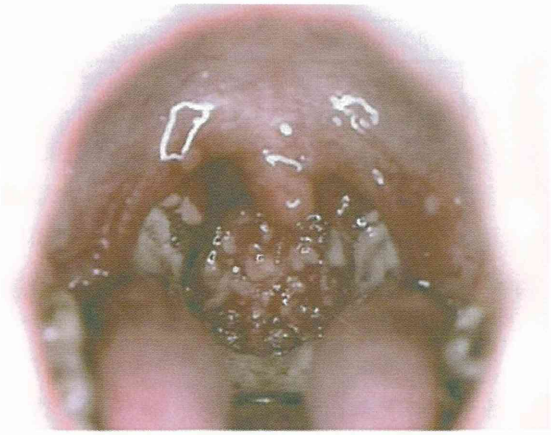

図 5. HSV 咽頭扁桃炎（20 歳，女性）
口蓋扁桃陰窩の白苔，咽頭後壁リンパ濾胞の白苔を伴う発赤腫脹を認める（文献6より転載）

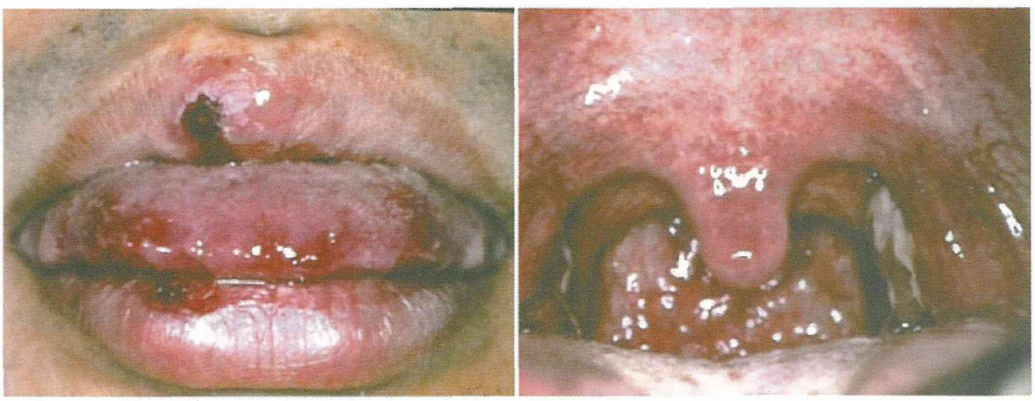

図 6. HSV 性扁桃炎（25 歳，男性）
血清抗体価および扁桃組織生検により HSV-1 初感染による扁桃炎と診断された．偽膜性扁桃炎にアフタを伴う歯肉口内炎の併発がみられる

表 3. 腎機能障害患者における HSV 感染症のアシクロビルの用量

クレアチニンクリアランス	アシクロビル錠（1回 200 mg）	バラシクロビル錠（1回 500 mg）	アシクロビル注射用
25 ml＜/min	1日5回		12時間ごと 5 mg/kg
10～25 ml＜/min	1日5回		24時間ごと 5 mg/kg
＜10 ml＜/min	1日2回		24時間ごと 2.5 mg/kg
30＜～ml＜/min		1日2回	
15～35 ml＜/min		1日2回	
＜15 ml＜/min		1日1回	
血清クレアチニン値からクレアチニンクリアランスを推定する計算式 男性　体重(kg)×(140－年齢)/{72×血清クレアチニン値(mg/dl)} 女性　男性の計算式×0.85			

（文献 6 より引用）

表 4. 急性 HIV-1 感染（ARS）の症状・所見とその頻度

臨床所見	発症頻度
発熱	＞80～90%
倦怠感	＞70～90%
皮疹	＞40～80%
頭痛	32～70%
リンパ節腫脹	40～70%
咽頭炎	50～70%
筋肉痛，関節痛	50～70%
嘔気，嘔吐，下痢	30～60%
寝汗	50%
無菌性髄膜炎	24%
口腔潰瘍	10～20%
陰部潰瘍	5～15%
血小板減少	45%
白血球減少	40%
肝酵素値の上昇	21%

（文献 7 より引用）

HSV 感染では，可及的早期に抗ヘルペスウイルス薬で治療することが重要で，十分量の抗ウイルス薬治療を早期から開始することで，潜伏感染するウイルス量を減らし，その後の再発回数を抑制できることが指摘されている．抗ヘルペスウイルス薬のアシクロビル，バラシクロビルは，HSV-1，HSV-2，VZV 感染細胞にのみ作用するため，重篤な副作用はほとんど生じない．臨床的に HSV 性咽頭・扁桃炎が強く疑われたら，迷わず抗ヘルペスウイルス薬による治療を開始する．抗ウイルス薬投与を開始した後，5 日目になっても軽快しない，あるいはむしろ悪化する場合には，細菌の二次感染の併発または他の疾患を考えた治療に切り替える．

HIV 感染症

HIV には 1 型（HIV-1）と 2 型（HIV-2）がある．世界流行（pandemic）の主体は HIV-1（HIV-2 感染者は主に西アフリカ地域に限局）で，我が国においても同様である．

HIV-1 に感染すると 2～4 週間で，血中の HIV ウイルス量は急速にピークに達し，急性初期感染期の急性レトロウイルス症候群（acute retroviral syndrome；ARS）の症状が出現する．感染から 6～8 週後，血中に抗体が産生されはじめ，ピークに達していたウイルス量が減少し，一定のレベルの定常状態となり，無症候期に入る．無症候期を過ぎ，CD4 リンパ球数が減少し始めるとエイズを発症する．

1．痛みの性状

HIV-1 に感染して 2～4 週間目頃にみられる ARS は，自覚症状のない無症候性から無菌性髄膜炎に至る重症まで，その程度は様々で，2～3 週間以内に自然に消退する．主な症状として，発熱，倦怠感，筋肉痛，関節痛，咽頭痛，皮疹，リンパ節腫脹，下痢，頭痛などがある（表 4）[7]．ARS 症状の大部分が HIV-1 感染特有のものではなく，咽頭痛も一般的な急性咽頭炎，インフルエンザ，または伝染性単核症様の痛みや症状を訴える場合が多い．ほかに，全身リンパ節腫張，皮疹，口腔（頬粘膜，歯肉，口蓋）あるいは性器（肛門，陰茎，膣）に円形もしくは卵形の潰瘍がみられる場合は，特

表 5. HIV 感染に関連する口腔病変

感染症	真菌感染，細菌感染，ウイルス感染
新生物	カポジ肉腫，非ホジキンリンパ腫，扁平上皮癌
炎症性	再発性アフタ性口内炎，多形性紅斑，苔癬
原因不明	唾液腺疾患，非特異的口腔潰瘍，メラニン色素の過度の沈着

（文献 8 より引用）

に ARS が示唆される．

HIV-1 感染者の無症候期以降でも，初発症状の 40％が耳鼻咽喉科領域，特に口腔咽頭に病変（表 5）[8]が生じ，それらが診断の契機となる場合が多いとされる．最も多いのがカンジダ症で，約半数を占める．他，再発性アフタ性口内炎，多形性紅斑，苔癬，非特異的口腔潰瘍，カポジ肉腫，非ホジキンリンパ腫，扁平上皮癌等があり，それぞれの病変に伴う痛みや，違和感を訴える．

2．検査・診断

第一に血清 HIV 抗体のスクリーニング検査（酵素抗体法：ELISA，粒子凝集法：PA，免疫クロマトグラフィー：IC 等）を行う．スクリーニング検査陽性の場合には，①抗体確認検査（Western blot 法，蛍光抗体法：IFA），②HIV-RNA 定量検査（RT-PCR 法）の，①②いずれかが陽性の場合に HIV 感染症と診断する．感染から 2ヶ月間ほどは血清 HIV 抗体が検査では検出できない（ウインドウ期）ため，ARS では血清 HIV 抗体のスクリーニング検査で陰性となる場合がある．臨床経過から ARS が疑われ，抗体検査が陰性の場合は HIV-RNA 定量検査で確認する[9]．

3．治　療

AZT（azidothymidine）を代表とする逆転写酵素阻害薬（reverse transcriptase inhibitor；RTI），プロテアーゼ阻害薬（protease inhibitor；PI）（あるいは非ヌクレオシド系逆転写酵素阻害薬），インテグラーゼ阻害薬，CCR5 阻害薬の組み合わせによる抗 HIV 療法（antiretroviral therapy；ART）を行う．この治療法の導入により，AIDS で死亡する例は激減している．

淋菌感染症，クラミジア感染症

我が国で，性感染症の患者報告数の第 1 位がクラミジア，第 2 位が淋菌感染症である．

淋菌感染症はナイセリア属の細菌 *Neisseria gonorrhoeae* を病原体とし，尿道炎，子宮頸管炎，結膜炎の原因となる．尿道炎と結膜炎は，強い痛みを伴い膿性分泌物も多く，顕著な炎症所見がみられる．子宮頸管炎では自覚症状がない感染者が多く，男性の尿道炎でも再感染では症状・所見が現れにくい．

Chlamydia trachomatis を病原体とするクラミジア感染症は．尿道炎，子宮頸管炎，結膜炎の原因となる．尿道炎も結膜炎も淋菌に比べて病状が軽い．

淋菌とクラミジア，ともに感染しても無症状で他覚的所見もみられない無症候性感染者が存在することが，患者数の多い原因の一つとなっている．性器感染も無症候性のことが多く，また無症候性感染であっても未治療でいると男女とも不妊の原因となる．性器感染者の 10～30％に，咽頭感染の合併がある．

1．痛みの性状

大多数は無症状で咽頭発赤や扁桃腫脹など他覚的所見がみられない無症候性感染である[10][11]．淋菌もクラミジアも感染者のごく一部に，咽頭炎や扁桃炎の発症がみられる．

1）淋菌の口腔・咽頭感染

ごく一部の感染者において，口内炎，咽頭炎，扁桃炎を発症し[12]，咽頭痛や乾燥感，灼熱感を訴える場合がある．それぞれ淋菌感染症に特有の自覚症状や他覚的所見はなく，臨床所見から他の感染症と判別することは困難である．

(1) 淋菌性口内炎

口腔粘膜が易出血性の黄白色の偽膜を伴って浮腫状に腫脹を呈し，口腔内の乾燥感，灼熱感を訴える．

(2) 淋菌性咽頭炎

咽頭炎では，びまん性紅斑と浮腫を呈し，扁桃

と口蓋垂に斑状の発赤と浮腫がみられるウイルス感染症に似た咽頭所見を呈し，咽頭痛を訴える．

(3) 淋菌性扁桃炎

陰窩性扁桃炎もともなう溶連菌感染症に似た咽頭所見を呈し，咽頭痛を訴える．

2）クラミジアの口腔・咽頭感染

症例数は少ないが，上咽頭炎，咽頭炎，扁桃炎を発症する場合がある．淋菌感染症と同様に，それぞれクラミジア感染症に特有の自覚症状や他覚的所見はなく，臨床所見から他の感染症と判別することは難しい．

(1) クラミジア性上咽頭炎

上咽頭炎では高率に滲出性中耳炎や頸部リンパ節腫脹を伴い，耳閉感，難聴，咽頭痛，鼻汁を訴える．内視鏡で上咽頭の発赤腫脹やアデノイド様の腫瘤が観察される[13]．*C. trachomatis* の眼内感染症である成人型封入体結膜炎の約半数の上咽頭炎を併発する．

(2) クラミジア性咽頭炎，扁桃炎

咽頭炎，扁桃炎は，*C. trachomatis* よりも，呼吸気感染症の原因となる *Chlamydia pneumonia* による場合が多い．一般的な咽頭炎や扁桃炎の所見を呈し，咽頭痛を訴える．

2．検査・診断

臨床所見からの淋菌とクラミジアの判別は困難で，一部には淋菌とクラミジアの混合感染例も存在するため，診断に際しては淋菌とクラミジアを同時に検査することが推奨される．咽頭感染では，感度の高い核酸増幅法での検出が最も有用である．核酸増幅法には，① SDA (Strand Displacement Amplification：鎖置換増幅法)法(BD プローブテック ET CT/GC®：日本ベクトン・ディッキンソン)，② TMA (Transcription-Mediated Amplification：転写介在増幅法)法(アプティマコンボ 2®：富士レビオ)，③ Real Time PCR 法(コバス 4800 システム CT/NG®：ロシュ・ダイアグノスティックス)の 3 種類がある．①② は尿道用または子宮頸管用検査キットを用いて咽頭または上咽頭からスワブを採取，③ は尿検査キットを用いて咽頭うがい液を採取(生食 15～20 m*l* で，10～20 秒間上を向いてガラガラとうがいをさせる)して提出する．①～③ いずれも，1 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である．

3．治　療

1）淋菌の口腔・咽頭感染

近年，淋菌の抗菌薬多剤耐性化が深刻な問題となっており，不適切な抗菌薬投与は極力避けなければならない．組織移行性の違いから性器感染に有効でも，咽頭感染では無効の抗菌薬が多く，日本性感染症学会でも性感染症 診断・治療ガイドラインを 2 年毎に改正して注意を促している[14]．このガイドラインでは，咽頭感染の治療として，セフトリアキソン CTRX(ロセフィン®)静注 1 g 単回投与を推奨ランク A，セフォジジム CDZM (ケニセフ®，ノイセフ®)静注 1 または 2 g を 1～2 回/日，1～3 日間投与を推奨ランク B としている．しかし，2009 年に世界初のセフトリアキソンに高度耐性を示す株が日本国内から報告されていること，筆者も CTRX 静注 1 g 単回投与が無効であった例を複数経験していることから，本稿ではあえて CTRX 2 g×1 回/日，1～3 日間投与を推奨する．

2）クラミジアの口腔・咽頭感染

クラミジアの咽頭感染は性器と同じレジメで，アジスロマイシン(ジスロマック®)1,000 mg 単回投与，またはクラリスロマイシン(クラリス®・クラリシッド®)200 mg×2　7 日間投与する．

HPV 感染症

HPV は，ヒトの皮膚上皮に感染して，種々の腫瘍性病変を生じる[15]．HPV はゲノム DNA の塩基配列の相違によって，現在 150 以上の遺伝子型が分類されている．性感染症で性器クラミジア感染症，淋菌感染症，性器ヘルペスに続いて 4 番目に多い尖圭コンジローマは，主に HPV6 または HPV11 が感染して生じるウイルス性疣贅である．

一方，HPV は子宮頸癌や前癌状態を結発する

高リスク型HPVとして，15種類以上の遺伝子型が同定されている．特にHPVに関連した若年性子宮頸癌患者が増加をうけ，HPVワクチンの実用化が我が国でも始まっている．

また，HPVは子宮頸癌以外の癌，特に中咽頭癌とHPV16の関連性が注目されている[16]．

1．痛みの性状

HPV感染や種々の腫瘍性病変そのものは痛みを伴わない．HPVに関連する中咽頭癌患者において，その多くは頸部リンパ節腫脹や腫瘍の増大による症状が診断の契機となる．

2．検査・診断

HPV感染は，腫瘍性病変の組織からのHPVの検出による．病理学的所見，*in situ* hybridization (ISH)で病変部細胞からのHPV-DNAの証明，PCR法またはLAMP法によるHPV遺伝子型を決定する．

3．予　防

病変のみられない咽頭からも，PCR法で検索すると高率にHPVが検出される[17]．若い女性の子宮頸部HPV感染者の約9割は感染から2〜3年以内に自然治癒する[18]ことから，子宮頸癌と同様に，咽頭のHPVも自己の免疫力で，その多くは排除されるものと考えられる．

感染そのものへの治療法は確立していない．予防として初交前のHPVワクチン接種の効果が期待されているが，我が国では女性のみへの適応となっている．

おわりに

HIV，淋菌，クラミジアのように，特徴的な所見がない性感染症においては，医師側が疑ってその病原体を狙った検査をしないと診断に至らない．性感染症は，古今東西，普遍的な感染症であり，耳鼻咽喉科外来で診療する患者のなかに紛れている可能性は十分考えられる．日常診療の鑑別診断に性感染症も加えて対処するべきと考える．

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