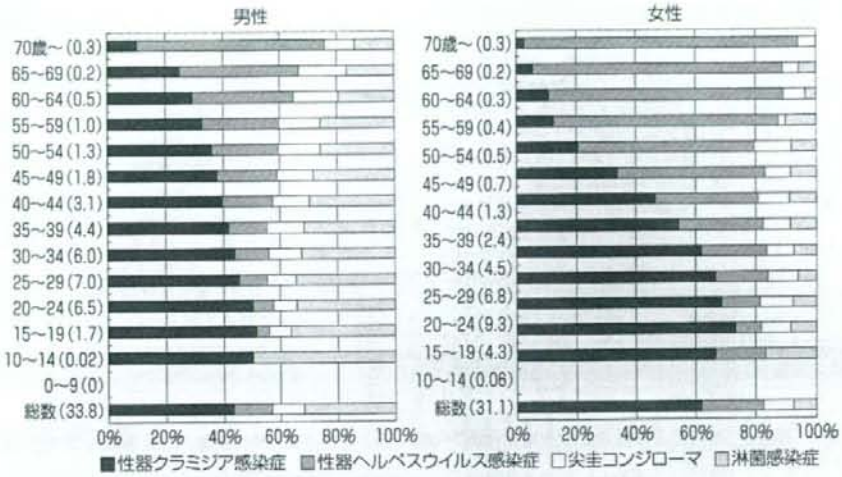


図5　年齢群別に見た定点把握4性感染症の割合(2006年)
(感染症発生動向調査　2008年1月16日現在)

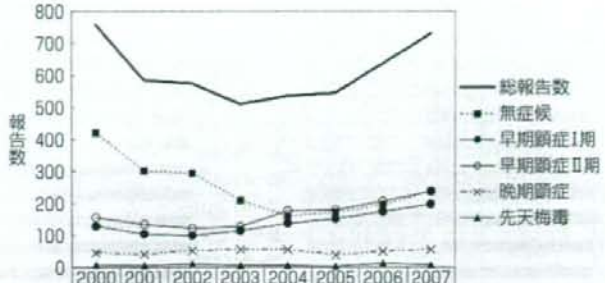
この点に関しては，届出基準の周知徹底が不十分な点や，定点医療機関におけるカルテの記載において，初発，再発の区別が明確に記載されていない場合も多いことなどが理由として考えられている[1]．

以上をまとめてみると，定点調査を見る限りでは，わが国における性感染症は特にクラミジア感染症，淋菌感染症において減少傾向にあり，特にこの減少は若年世代を中心にその傾向が強い．この理由については，これまで若者を中心として行ってきた性感染症対策が功を奏してきたとする考え方もあるが，後述の，全数調査が行われている梅毒において，特に女性患者において若年齢化が見られている状況を考えると，性器クラミジア感染症，淋菌感染症における定点調査が，若年齢層の罹患状況を必ずしも的確に把握していない可能性があり，決して楽観視することはできない状況と思われる．

事実，2006年にわれわれが行った4モデル県における性感染症の全数調査では，発生動向調査(定点調査)と全数調査の患者報告数の比較において，特に10歳代後半の世代において乖離が見られることが明らかとなっており，若い世代の性感染症患者は，定点に指定されている医療機関には受診していない可能性があることが示唆されている[2]．このことは，定点調査を検証するための何らかの追加的なサーベイランスを行って実態を比較することが重要であり，定点調査の精度についても総合的に評価していく必要性があることを示すものであろう．

2．梅毒の動向

2000年以降，2007年までの梅毒の年次推移について**図6**に示した．2000年以降，2003年までは緩やかな減少が見られていたが，2004年には増加に転じ，2007年の時点でも増加傾向が見られている．特に2006年，2007年はそれぞれ前年に比べ，約100例の増加が見られている．これを病型別に見ると先天梅毒，晩期顕症梅毒は横ばいになっているが，早期顕症Ⅰ，Ⅱ期，無症候梅毒ともにここ数年間において増加傾向が見られている．さらに，2006年の報告数を男女別，年齢別病気別に見てみると，男性では，早期顕症は15～19歳の年齢群から始まり，30歳代にピークがあるが，女性では早期顕症梅毒は，15～19歳がピークとなっており，年齢が高くなるにつれて報告数が減っていることが明らかになってい



	2000	2001	2002	2003	2004	2005	2006	2007
総報告数	759	585	575	509	533	543	637	731
無症候	420	301	293	209	160	172	195	236
早期顕症I期	129	104	99	114	136	151	175	196
早期顕症II期	157	134	121	127	177	180	205	237
晩期顕症	45	40	53	54	54	37	50	55
先天梅毒	8	6	9	5	6	3	12	7

図6　病期別梅毒患者報告数の年次推移(2000～2007年)(感染症発生動向調査　2008年2月3日現在)

る[1]．このことは，梅毒において，感染者の若年齢化が進行していることを示唆するものであり，今後の注意深い観察が重要であることを示すものである．

無症候の性感染症の実態と薬剤耐性淋菌の蔓延

前項で示した性感染症の定点調査による患者数は，いずれも何らかの症状があって医療機関を受診した患者数であり，性感染症には多くの無症候感染者が存在することを忘れてはならない．厚生労働省の班研究によるわれわれの調査では，例えば，性器クラミジア感染症の無症候感染者は，高校生の女子において，13%程度に存在し，男子においても7%前後に陽性者が存在する[3]．また，咽頭の淋菌感染はほとんどが無症候感染であるが，男子の淋菌性尿道炎患者の約10%，また女子の淋菌性子宮頸管炎患者でも50%程度に咽頭にも淋菌を有していることが明らかになっている[4]．

また，わが国では，薬剤耐性淋菌の蔓延が大きな問題になっており，淋菌感染症を確実に除菌し得る抗菌薬はきわめて少ない状況になっている．性感染症学会の診断・治療のガイドラインで淋菌感染症に推奨されている治療薬は，現在ではセフトリアキソン(CTRX)，セフォジジム(CDZM)，スペクチノマイシン(SPCM)の3薬のみで，いずれも注射薬である[5]．ただ，この中で咽頭の淋菌を1回の投与で確実に除菌できるのはセフトリアキソンだけであり，セフォジジム，スペクチノマイシンでは除菌率は低く，推奨されない[4]．少なくとも現段階では性器由来の淋菌を消失させるだけでなく，淋菌感染症の蔓延の原因になっている咽頭の淋菌感染にも有効なセフトリアキソンの投与を普及させることが重要である．

おわりに

わが国の性感染症の動向について，定点調査の成績を中心に述べ，トピックスとして，無症候の性感染症の実態と薬剤耐性淋菌の問題について述べた．

定点調査からは近年，淋菌感染症，性器クラミジア感染症とも減少傾向が見られる一方で，無症候の性感染症患者の実態調査では，特に若い世代において無症候の性器クラミジア感染者が予想を超えて存在することが重要である．このような現状を考えると，今後，若年層を中心とした予防対策を重点的に推進する必要があることは明らかである．そのためには，中学生の段階から性感染症予防のための教育を含む普及・啓発を行うこと，また，高校生の段階では，性感染症の早期発見・

早期治療に結び付けられるようなスクリーニングのシステムを構築することも重要であろう．さらにその蔓延を防ぐために，性感染症に対する適切な診断法・治療法の普及が急務であろう．

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映画の時間

ふみ子の海

●原作：市川信夫/監督：近藤明男/出演：鈴木理子，他/2007年/日本/105分/製作：CAL/配給：パンドラ，シネマ・ディスト/第31回山路ふみ子映画福祉賞受賞
●公式サイト：http://www.fumikonoumi.com
●自主上映主催・共催等の問合せはシネマ・ディスト（TEL 03-3505-1874）

篠田正浩監督による「はなれ瞽女おりん」という映画がありました．瞽女（ごぜ）とは三味線を弾きながら，民謡や俗曲を聞かせて地方を巡る盲目の女旅芸で，瞽女の組織から離れて生きる盲目の主人公を岩下志麻が好演した名作でした．

今回ご紹介する「ふみ子の海」の中でも，瞽女の集団が寄り添って生きる姿が垣間見られます．舞台は昭和初期の新潟．主人公のふみ子（鈴木理子）は幼くして視力を失います．先天的なものか，栄養不良によるものか，映画では原因は詳しく語られませんが，当時の公衆衛生水準，医療制度などを考えると，失明の背景には貧困もあるのでしょう．主人公の母親チヨ（藤谷美紀）は，ふみ子の視力の回復が望めない状況に絶望し，波の高い日本海で親子心中を図ります．そのとき，目の見えない主人公が「海ってきれいだね」と呟きます．チヨは我に返り，海から戻ります．題名の「ふみ子の海」はこのときの体験から名づけられたものでしょう．主人公ふみ子にはモデルがおり，一部は実話に基づいているとのことです．

主人公は盲学校への進学を望みますが，生活にゆとりはありません．当時の視力障害者が生きていくには，瞽女の親方に弟子入りして旅芸人の道を歩むか，あるいは江戸時代より盲人の職業とされていたマッサージ師に弟子入りするような進路しかなかったのでしょう．進学のための経済的援助を求められながら，それを断る本家の当主（中村敦夫）の判断も，当時の社会的状況からすれば，むしろ主人公の将来を考えてのことと思われます．

母チヨが病に倒れ，ふみ子は高田市内のいわゆる「あんま屋」に弟子入りします．その女主人（高橋恵子）はふみ子を厳しくしつけます．折しもヘレン・ケラー女史が来日し，ふみ子は女史に会いに行こうとします…．

本作品は逆境に置かれた主人公ふみ子を中心に，彼女の頑張る姿と，彼女を取り巻く人々を暖かく描くハートウォーミングな映画ですが，それとともに，瞽女集団や「あんま屋」をはじめとして，昭和初期の視覚障害者の置かれた状況を実感できるのも見所のひとつです．主人公が進学を希望する高田盲学校も，2006年にその役割を終えて閉校となったようですが，高田周辺に残る歴史的建造物をロケーション場所に選ぶことによって，現実感溢れる画面を創造しています．

「ふみ子の海」はすでにロードショーを終了していますが，自主上映の機会もあるようです．視覚障害者教育のみならず，障害者の教育や福祉を考えるうえで，参考になることも多く，広く公衆衛生に従事する方々にご覧いただきたい映画です．

（桜山豊夫）

J Infect Chemother (2008) 14:195–203
DOI 10.1007/s10156-008-0610-7

ORIGINAL ARTICLE

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Analysis of amino acid sequences of penicillin-binding protein 2 in clinical isolates of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime and ceftriaxone

Received: January 4, 2008 / Accepted: March 4, 2008

Abstract *Neisseria gonorrhoeae* strains with reduced susceptibility to cefixime and ceftriaxone, with minimum inhibitory concentrations (MICs) of cefixime of 0.125–0.25 μg/ml and ceftriaxone of 0.031–0.125 μg/ml, were isolated from male urethritis patients in Tokyo, Japan, in 2006. The amino acid sequences of PenA, penicillin-binding protein 2, in these strains were of two types: PenA mosaic and nonmosaic strains. In the PenA mosaic strain, some regions in the transpeptidase-encoding domain in PenA were similar to those of *Neisseria perflava/sicca*, *Neisseria cinerea*, *Neisseria flavescens*, *Neisseria polysaccharea*, and *Neisseria meningitidis*. In the PenA nonmosaic strain, there was a mutation of Ala-501 to Val in PenA. In addition, we performed homology modeling of PenA wild-type and mosaic strains and compared them. The results of the modeling studies suggested that reduced susceptibility to cephems such as cefixime and ceftriaxone is due to a conformational alteration of the β-lactam-binding pocket. These results also indicated that the mosaic structures and the above point mutation in PenA make a major contribution to the reduced susceptibility to cephem antibiotics.

Key words Penicillin-binding protein 2 · PenA · *Neisseria gonorrhoeae* · Cefixime · Ceftriaxone

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Introduction

Gonococcal infections have existed as sexually transmitted diseases since early times and have never been regarded as intractable. In the late 20th century, the significant developments of antimicrobial agents facilitated the treatment and care of gonococcal infection disease.

Penicillins and tetracyclines are used for the treatment of gonococcal urethritis worldwide. After the emergence and worldwide spread of penicillin- and tetracycline-resistant *Neisseria gonorrhoeae* strains, fluoroquinolones were recommended as the primary therapeutic agent for uncomplicated gonorrhea in many countries.[1] Fluoroquinolones have been used extensively for the treatment of gonococcal urethritis due to their high degree of efficacy against the disease. However, intense selection pressure resulting from the continual exposure of *N. gonorrhoeae* to fluoroquinolones induced the emergence of quinolone-resistant strains with altered GyrA and ParC proteins.[2–6] In 2006, the Centers of Disease Control for prevention (CDC) updated the *Sexually Transmitted Diseases Treatment Guidelines, 2006* regarding the treatment of infections caused by *N. gonorrhoeae*.[7] In these guidelines, the CDC no longer recommends the use of fluoroquinolones for the treatment of gonococcal infections and associated conditions such as pelvic inflammatory disease (PID). In addition, intravenous ceftriaxone, which is a highly potent antimicrobial agent against *N. gonorrhoeae*, is now recommended as a first-line agent in the treatment of gonococcal infections worldwide.

In Japan, the numbers of gonococcal infections, including those resistant to antimicrobial therapy, have gradually increased since the mid-1990s.[8] Cefixime, an oral cephem, had been used previously, but recently an increased emergence and spread of gonococci resistant to oral cephems have also been reported.[9,10] Therefore, the use of cefixime was no longer recommended because the number of ineffective cases was increasing when cefixime was used as a therapeutic agent.[11] Nowadays, ceftriaxone and cefodizime, parenteral cephems, and spectinomycin, an aminoglycoside,

are widely used instead of fluoroquinolones and oral cephems for the treatment of gonococcal infections.[11] However, these strains also acquired reduced susceptibility to cefodizime or ceftriaxone, parenteral cephems.

N. gonorrhoeae has three penicillin-binding proteins (PBPs), denoted PBPs 1, 2, and 3. PBPs 1 and 2 of *N. gonorrhoeae* are the major targets of β-lactam antibiotics. PBP 2, encoded by the *penA* gene, has an approximately 10-fold higher affinity for penicillin G than PBP 1.[12] In previous reports, the insertion of Asp-345 into the *penA* gene has been proved to make a major contribution to reducing the affinity of gonococcal PBP 2 to penicillin G.[13] Other reports showed that C-terminal amino acid residues of the PenA transpeptidase domain were also altered in penicillin-resistant *N. gonorrhoeae*.[14–16] In our recent study, the strains with reduced susceptibility to oral cephems have mosaic structures in the transpeptidase-encoding domain in PenA, which is similar to those of *Neisseria perflava/sicca*, *Neisseria cinerea*, *Neisseria flavescens*, *Neisseria polysaccharea*, and *Neisseria meningitidis*.[17] These results suggest that the pathogens might have evolved by gene transformation between commensally resistant *Neisseria* spp. and the original susceptible gonococci owing to the existence of widespread commercial oral sex.[17]

Other genetic factors have been reported as enhancements of the efflux pump by mutations in *mtrR*, which is an *mtrCDE* transcriptional regulator, and that *penB* loci was due to β-lactam resistance.[18,19] A single substitution in *ponA* (the *ponA1* allele), which encodes an altered PBP1 with reduced affinity for penicillin, was reported to contribute to high-level penicillin resistance in *N. gonorrhoeae*.[20] The *pilQ* (previously named *penC*) gene mutants were reported to increase resistance to penicillin if *penA*, *penB*, and *mtrR* resistance determinants were present.[20–22]

This study was conducted to investigate the susceptibility to various antimicrobial agents of clinical isolates of *N. gonorrhoeae* isolated in Japan in 2006, and to clarify the mechanism of reduced susceptibility to cefixime and ceftriaxone in *N. gonorrhoeae*.

Materials and methods

Bacterial strains

The *N. gonorrhoeae* strains used in this study were clinical strains isolated from male urethritis patients at Jikei University School of Medicine and related hospitals in 2006. The specimens were directly streaked onto modified Thayer–Martin selective agar (Becton, Dickinson, Tokyo, Japan) in the hospitals. The plates were placed in a Bio-Bag environmental chamber (type C; Becton, Dickinson) and immediately transported to the laboratory, where they were incubated at 35°C for 20 h in a 5% CO_2 atmosphere. The organisms were identified by Gram staining, oxidase tests, and catalase tests. The identities of isolates cultured on Chocolate II agar (Becton, Dickinson) were further confirmed with a Gonochek-II kit (EY Laboratories, San Mateo, CA, USA). *N. gonorrhoeae* isolates were maintained at –80°C in modified skim milk until antimicrobial susceptibility testing.[23] The isolates were tested for β-lactamase production by a β-check (Nippon Bio-Supp. Center, Tokyo, Japan). In the antibiotic susceptibility test, 47 strains isolated in 2006 were used. In the PenA sequences of *N. gonorrhoeae*, 17 strains with reduced susceptibility to cefixime (minimum inhibitory concentrations (MICs) 0.125–0.25 μg/ml for strains isolated in 2006), 2 β-lactamase-producing strains, and 3 cefixime-susceptible strains (MICs 0.004–0.008 μg/ml) were used. In the comparison of PenA sequences of *N. gonorrhoeae*, LM306 (penicillin-susceptible strain; GenBank accession no. M320921),[16] NG-3 (reduced susceptibility to cefixime strain isolated in 2000; GenBank accession no. AB071984),[17] 6 isolates showing reduced susceptibility to cefixime and ceftriaxone in 2006 (NG-109, -110, -118, -120, -121, and -122), one cefixime-susceptible isolate (NG-127), and one β-lactamase-producing strain (NG-128) were used. In the comparison of PenA sequences of *N. gonorrhoeae* and other *Neisseria* species, those of *N. meningitidis* MC58 (GenBank accession no. NC-003112), *N. polysaccharea* NCTC11858 (GenBank accession no. X59626), *N. cinerea* NCTC10294 (GenBank accession no. X59540), *N. perflava/sicca* 1654/1659 (GenBank accession no. X76422), and *N. flavescens* NCTC8263 (GenBank accession no. M26645) were compared.

Susceptibility testing and antimicrobials

Bacteria were precultured at 35°C under a 5% CO_2 atmosphere on Chocolate II agar (Becton, Dickinson) for 20 h. MICs were determined by an agar dilution method according to the approved guidelines of the Clinical Laboratory Standards Institute,[24] with a GC agar base (Becton, Dickinson) containing 1% Iso VitaleX (Becton, Dickinson) and serial two-fold dilutions of antimicrobial agents. Plates were inoculated with 10^4 colony-forming units (CFUs) per spot, and were incubated for 20 h at 35°C in a 5% CO_2 atmosphere. MICs were defined as the lowest concentration of an antimicrobial agent that inhibited growth. The following reference antimicrobial agents were used: penicillin G (Banyu Pharmaceutical, Tokyo, Japan), clavulanic acid/amoxicillin (GlaxoSmithKline, Tokyo, Japan), ceftriaxone (Nippon Roche, Tokyo, Japan), cefteram (Toyama Chemical, Tokyo, Japan), cefodizime (Kyorin Pharmaceutical, Tokyo, Japan), cefixime (Astellas Pharma, Tokyo, Japan), azithromycin (Pfizer Japan, Tokyo, Japan), spectinomycin (Sigma Aldrich Japan, Tokyo, Japan), tetracycline (Sigma Aldrich Japan), levofloxacin (Daiichi Sankyo, Tokyo, Japan), and aztreonam (Eizai, Tokyo, Japan).

Nucleotide sequence of *N. gonorrhoeae penA* gene

Bacteria were grown at 37°C under a 5% CO_2 atmosphere on modified Thayer–Martin agar for 23 h. They were then suspended in 100 μl distilled water, heated at 100°C for 5 min, and then centrifuged at 15000 × *g* for 10 min. The

full-length gene was amplified by polymerase chain reaction (PCR) from the supernatant with oligonucleotides primer F1 and R1 (Table 1) and Ex *Taq* polymerase (Takara Bio, Otsu, Japan). PCR was performed as follows: 5 min of denaturation at 94°C, 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 0.5 min, and extension at 72°C for 2 min, concluding with a final extension at 72°C for 5 min. Sequencing was outsourced to the Dragon Genomics Center (Yokkaichi, Japan) using oligonucleotides F1, F2, F3, F4, R1, R2, R3, and R4 (Table 1). Oligonucleotides F1 to F4 and R1 to R4 were used for sequencing the forward and reverse sequences, respectively.

Homology modeling of PenA

Homology modeling of *Neisseria gonorrhoeae* PenA of wild-type strain LM306 and mosaic mutant strain NG-109 was performed using the Prime 1.5 homology modeling program.[25] The crystal structure of *Streptococcus pneumoniae* PBP2x protein (PDB No. 1RP5) was chosen as the template for modeling.[26] Then 600ps molecular dynamics (MD) simulations of the modeling structures were performed using the AMBER 8.0 package[27] with a TIP3P water model.[28] The model structures obtained after MD simulations were minimized using the AMBER 8.0 package.

Results

Antimicrobial susceptibility and β-lactamase production

The MICs of various antimicrobial agents and β-lactamase production were determined for 47 clinical isolates in 2006. Table 2 shows the MICs at which 50% of isolates are inhibited (MIC_{50}s) and the MIC_{90}s of the various antimicrobial agents for the clinical isolates in 2001 and 2006. The MIC_{50}s of ceftriaxone, cefodizime, cefixime, and cefteram, and the MIC_{90}s of ceftriaxone, cefodizime, and cefteram for the isolates recovered in 2006 were 2–4-fold higher than those for the isolates recovered in 2001. Seventeen of the 47 strains (36.2%) isolated in 2006 showed a reduction of susceptibility to cefixime (MICs 0.125–0.25 μg/ml), and also exhibited a reduction of susceptibility to ceftriaxone (MICs 0.031–0.125 μg/ml) (Table 2). These 17 strains also showed reduced susceptibilities to penicillins and other β-lactams such as cefodizime and aztreonam. Some strains showed resistance to both levofloxacin and tetracycline. In addition, β-lactamase production was detected in 2 of 47 clinical isolates in 2006.

Table 1. Oligonucleotides used in this study

Oligonucleotide	Sequence
Primer F1	5′-TCGGGCAATACCTTTATGGTGGAACAT-3′
Primer F2	5′-GAACGCCTGTCCGAGCTTGTC-3′
Primer F3	5′-ACAAGGCGGTCGAATACCATC-3′
Primer F4	5′-TATACCGCACTGACGCACGAC-3′
Primer R1	5′-ACAACGGCGGCGGGGATATAACT-3′
Primer R2	5′-AACGCCCGTTGACGAACTTGC-3′
Primer R3	5′-CATCGCGCACGGGAGACGGTC-3′
Primer R4	5′-GCGAAAGTTCCAAACCTTCCT-3′

Table 2. Susceptibilities of clinical isolates of *N. gonorrhoeae* from male urethritis patients in 2001 and 2006

Antimicrobial agents[a]	2001 ($n = 24$)[b]			2006 ($n = 47$)		
	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
PCG	1	2	0.063–2	1 (2)	4 (4)	0.063–64 (1–4)
CVA/AMPC	ND	ND	ND	0.5 (1)	1 (1)	0.063–2 (0.25–2)
CTRX	0.016	0.031	≤0.001–0.063	0.031 (0.063)	0.063 (0.125)	0.002–0.125 (0.031–0.125)
CDZM	0.016	0.063	0.002–0.125	0.063 (0.063)	0.125 (0.125)	0.002–0.125 (0.031–0.125)
CFIX	0.016	0.25	0.004–0.25	0.063 (0.125)	0.125 (0.25)	0.004–0.25 (0.125–0.25)
CFTM	0.063	0.25	0.002–0.25	0.125 (0.5)	0.5 (1)	0.004–1 (0.125–1)
AZM	ND	ND	ND	0.25 (0.25)	0.5 (0.25)	0.008–1 (0.031–0.5)
SPCM	>8	>8	4–>8	16 (16)	16 (16)	4–16 (4–16)
TC	ND	ND	ND	1 (1)	2 (4)	0.063–16 (0.5–16)
LVFX	4	>8	0.008–>8	4 (4)	8 (16)	0.004–16 (0.5–16)
AZT	0.5	4	0.063–>8	0.5 (4)	4 (8)	0.031–8 (0.25–8)

MIC, minimum inhibitory concentration; ND, not determined
Parenthetical values are MICs of 17 strains with reduced susceptibility to cefixime and ceftriaxone isolated in 2006
[a]Antimicrobial agents: PCG, penicillin G; CVA/AMPC, clavulanic acid/amoxicillin; CTRX, ceftriaxone; CDZM, cefodizime; CFIX, cefixime; CFTM, cefteram; AZM, azithromycin; SPCM, spectinomycin; TC, tetracycline; LVFX, levofloxacin; AZT, aztreonam
[b]MIC data from Ameyama[17]

Amino acid sequences of PenA in strains with reduced susceptibility to cefixime and ceftriaxone

Of the isolates in 2006, the PenA sequences of 17 strains with reduced susceptibility to cefixime and ceftriaxone (cefixime MICs 0.125–0.25 μg/ml, ceftriaxone MICs 0.031–0.125 μg/ml), 2 β-lactamase-producing strains, and 3 cefixime-susceptible strains (cefixime MICs 0.004–0.008 μg/ml) were determined. For a detailed analysis, 8 isolates in 2006 were selected from the 22 strains described above. These 8 strains were selected on the basis of three points: their susceptibility to cefixime and ceftriaxone, their β-lactamase-producing activity, and the amino acid sequence. Of these 8 strains, 5 strains (NG-109, -110, -118, -121, and -122) were PenA mosaic strains, NG-120 was a PenA nonmosaic strain, NG-127 was a cefixime-susceptible strain, and NG-128 was a β-lactamase-producing strain. Figure 1 shows the full-length sequences of PenA of LM306 (wild-type strain),[16] NG-3 (isolate in 2000),[17] and NG-109, -110, -118, -120, -121, -122, -127, and -128 (isolates in 2006). The MICs of the above 8 strains isolated in 2006, and the NG-3 and ATCC19424 against various antimicrobial agents are shown in Table 3. Figure 2 shows the full-length sequences of PenA of 3 clinical strains (NG-3, -109, and -120) and those of various *Neisseria* species, including *N. meningitidis* MC58, *N. polysaccharea* NCTC11858, *N. cinerea* NCTC10294, *N. perflava/sicca* 1654/1659, and *N. flavescens* NCTC8263.

As a result, strains of NG-109, -110, -118, -121, and -122 have PenA mosaic structures which are similar to those of *N. meningitides*, *N. polysaccharea*, *N. cinerea*, *N. perflava/sicca*, and *N. flavescens* (Figs. 1 and 2). These PenA mosaic structures were mainly observed in the transpeptidase domain of PenA. PenA mosaic strains (NG-109, -110, -118, -121, and -122) had almost total concordant alignment. The MICs of cefixime and ceftriaxone for the NG-109 strain were the highest (cefixime MIC 0.25 μg/ml, ceftriaxone MIC 0.125 μg/ml) among the strains isolated in 2006. The PenA sequence of the NG-109 strain corresponds to that of the NG-3 strain isolated in 2000 (cefixime MIC 0.5 μg/ml, ceftriaxone MIC 0.063 μg/ml)[17] except for the replacement of Gly-83 with Val.

The PenA sequence of NG-120, -127, and -128 had an insertion of extra aspartate (Asp-345), and 4 PenA mosaic strains (NG-3, -109, -110, and -118) had another insertion of an extra asparagine (Asn-573). In PenA nonmosaic strain NG-120, the amino acid sequence of PenA was identical to that of NG-127 except for the replacement of Ala-501 with Val and Pro-551 with Ser. The mutation of Ala-501 to Val was detected in PenA nonmosaic strain NG-120, but not in various *Neisseria* spp. In addition, β-lactamase-producing strain NG-128 did not show a PenA mosaic mutation.

The active-site serine residue (Ser–X–X–Lys) of PenA as well as the Ser–X–Asn and the Lys–Thr–Gly motifs were conserved in clinical isolates in 2006 and various *Neisseria* spp.[29] (Figs. 1 and 2).

Overview of structures and mapping of mutations

The *penA* monomer consists of two domains: a PBP dimerization domain (residues 71–221) and a PBP transpeptidase domain (residues 263–557).[16] Figure 3 shows the stereo view of the modeled structures of *N. gonorrhoeae* PenA of the penicillin-susceptible strain LM306 and the mosaic strain isolated in 2006 (NG-109). The segment colored yellow indicates β-lactam binding site Ser-310. In Fig. 3A, the segments of Ala-501 to Val and Pro-551 to Ser mutations of nonmosaic strain NG-120 are depicted in green and light blue. This mutation of Ala-501 to Val was located near the β-lactam-binding region, but Pro-551 to Ser was not. In PenA mosaic strain NG-109, mosaic structures from other *Neisseria* spp. are depicted in red in Fig. 3B. These mutations were located in loop regions. Compared to wild-type PenA, NG-109 mosaic PenA shows substantial changes throughout the entire structure of PenA, including the PBP dimerization/transpeptidase domain linker region. In addition, the conformation of the β-lactam-binding pocket, the active site area, also changed its conformation.

Table 3. MICs of various antibiotics for *N. gonorrhoeae* ATCC19424 and clinical isolated strains

Antimicrobial agents	MIC (μg/ml)									
	ATCC19424[a]	NG-3[a]	NG-109	NG-118	NG-110	NG-120	NG-121	NG-122	NG-127	NG-128
PCG	0.004	2	4	2	2	1	1	1	0.063	64
CVA/AMPC	ND	ND	1	1	2	0.5	1	0.5	0.063	1
CTRX	0.00025	0.063	0.125	0.063	0.063	0.063	0.063	0.031	0.004	0.008
CDZM	0.00025	0.125	0.125	0.063	0.125	0.125	0.031	0.063	0.008	0.004
CFIX	0.001	0.5	0.25	0.25	0.125	0.125	0.125	0.125	0.008	0.016
CFTM	0.004	0.5	1	0.5	1	0.25	0.25	0.25	0.016	0.031
AZM	ND	ND	0.25	0.125	0.25	0.031	0.25	0.125	0.016	0.25
SPCM	2	4	16	16	16	8	16	16	4	16
TC	ND	ND	1	1	4	1	1	1	0.125	16
LVFX	<0.004	8	16	4	4	4	8	4	0.008	2
AZT	0.008	8	4	8	4	0.5	4	4	0.125	0.063
β-lactamase[b]	–	–	–	–	–	–	–	–	–	+
PenA type[c]	NM	M	M	M	M	NM	M	M	NM	NM

ND, not determined
[a]MIC data from Ameyama[17]
[b]β-lactamase: –, negative; +, positive
[c]PenA type: NM, nonmosaic; M, mosaic

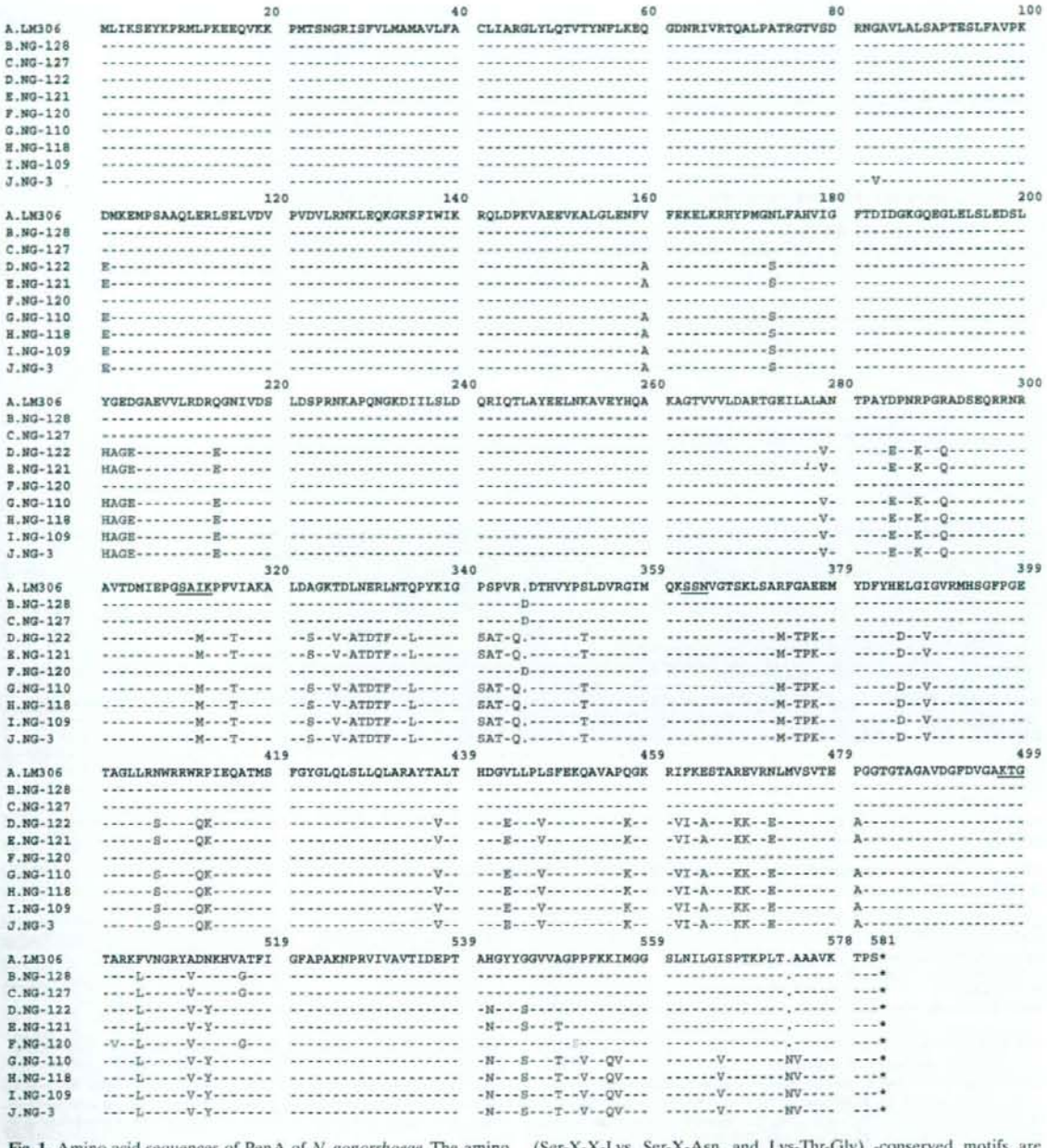

Fig. 1. Amino acid sequences of PenA of *N. gonorrhoeae*. The amino acid sequences of PenA of (**A**) the penicillin-susceptible strain LM306, (**B**) the β-lactamase-producing strain NG-128 isolated in 2006, (**C**) the cefixime-susceptible strain NG-127, (**D**)–(**I**) strains with reduced susceptibility to cefixime and ceftriaxone isolated in 2006, and (**J**) the mosaic strain with reduced susceptibility to cefixime isolated in 2000 (strain NG-3) are shown. The insertion of an extra aspartate (Asp-345) is shown in NG-120, -127, and -128, but not in other strains. The insertion of an extra asparagine (Asn-573) is shown in NG-3, -109, -110, and -118, but not in other strains. The replacement of alanine with valine (Ala-501 to Val) is shown in only NG-120. Active sites of serine residue (Ser-X-X-Lys, Ser-X-Asn, and Lys-Thr-Gly) -conserved motifs are indicated by *underlining*. The amino acid residues colored in *red* indicate mutations from other *Neisseria* spp., *blue* indicates specific mutations which are not in other *Neisseria* spp., *purple* is a previously reported mutation of Asp-345 with reduced affinity of gonococcal PBP2 to penicillin,[13] *green* is a specific mutation Ala-501 to Val of nonmosaic strain NG-120, and *light blue* is specific mutation Pro-551 to Ser of nonmosaic strain NG-120. *Dashes* indicate amino acid residues identical to those of LM306, *periods* are blanks, and *asterisks* are stop codons

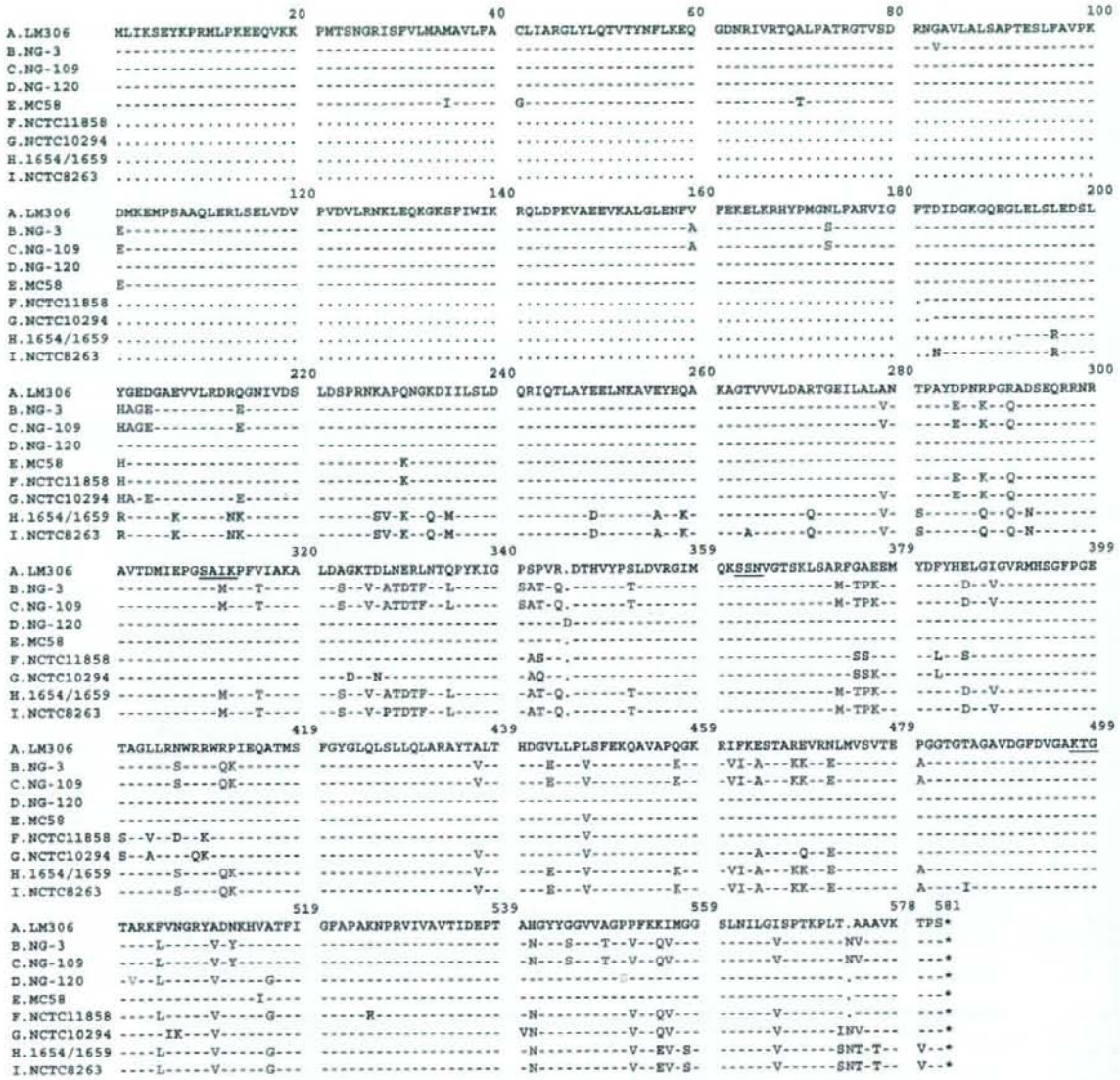

Fig. 2. Amino acid sequences of PenA of *N. gonorrhoeae* and other *Neisseria* spp. Amino acid sequences of PenA of (**A**) the penicillin-susceptible strain LM306, (**B**) the mosaic strain with reduced susceptibility to cefixime isolated in 2000 (strain NG-3), (**C**) the mosaic strain isolated in 2006 (strain NG-109), (**D**) the nonmosaic strain isolated in 2006 (strain NG-120), (**E**) *N. meningitidis* MC58, (**F**) *N. polysaccharea* NCTC11858, (**G**) *N. cinerea* NCTC10294, (**H**) *N. perflava/sicca* 1654/1659, and (**I**) *N. flavescens* NCTC8263 are shown. The active sites of serine residue (Ser–X–X–Lys, Ser–X–Asn, and Lys–Thr–Gly)-conserved motifs are indicated by *underlining*. The colored amino acid residues are indicated as in Fig. 1. *Dashes* indicate amino acid residues identical to those of LM306, *periods* are blanks, and *asterisks* are stop codons

Discussion

N. gonorrhoeae is a major pathogen isolated from patients with sexually transmitted diseases. *N. gonorrhoeae* strains with reduced susceptibility to cephems evolved by the acquisition of β-lactamases target modifications (alteration of PBPs) and alterations of the outer membrane transport or the enhancement of MtrCDE efflux pumps.[19,30–32] It has been reported that in Japan, *N. gonorrhoeae* with reduced susceptibility to cefixime has been isolated from areas unrelated to the urethra, such as the pharynx.[33] It is known that the source of infection is the diversity of commercial sex, such as oral sex.

In 2001, *N. gonorrhoeae* strains with reduced susceptibility to cephems or fluoroquinolones were isolated in

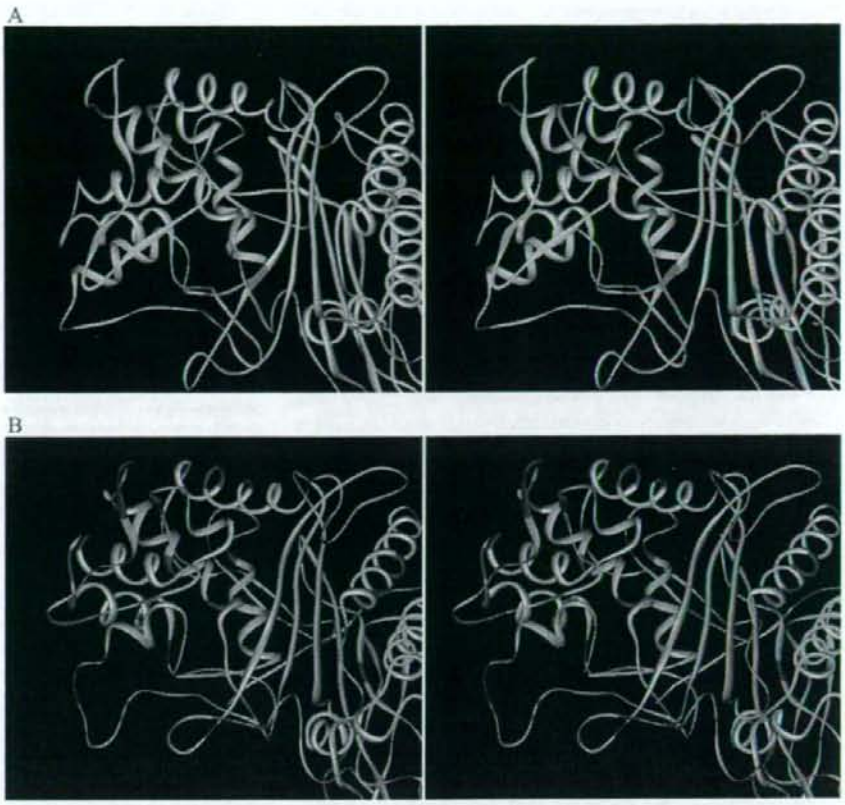

Fig. 3. The stereo view of modeled structures of *N. gonorrhoeae* PenA of (**A**) the penicillin-susceptible strain LM306 and (**B**) the mosaic strain isolated in 2006 (NG-109). A *yellow*-colored segment indicates β-lactam binding site Ser-310. In **A**, the segment colored *green* is an amino acid residue of Ala-501, and that colored *light blue* is an amino acid residue of Pro-551. In **B**, mutations are colored as in Fig. 1

Fukuoka, Japan.[22,34] It was reported that these strains had multidrug resistance against such drugs as penicillin G, tetracycline, azithromycin, and ciprofloxacin. In the last few years, various guidelines for the appropriate use of antimicrobial agents against gonococcal infections have been established worldwide.[7,11] Therefore in this study, in order to survey the tendency of such resistance, we compared the results of susceptibility testing between strains isolated in 2001 and 2006. In isolates of 2006, MIC_{50}s and MIC_{90}s against cephems were increased by two- to four-fold, but not against quinolone, aminoglycoside, and monobactam (Table 2). These showed that the resistant level of *N. gonorrhoeae* strains with reduced susceptibility to cephems was slightly elevated.

Our recent studies have indicated that the strains with reduced susceptibility to oral cephems had mosaic structures in PenA which were similar to those of other *Neisseria* spp.[17] In this investigation of the relations between mutations of PenA and the MICs of cephems, the PenA sequences of wild-type strain LM306[16] and various *N. gonorrhoeae* strains isolated in 2001 and 2006 were determined and compared with the other *Neisseria* spp. (Figs. 1 and 2). We performed homology modeling of *N. gonorrhoeae* PenA of wild-type strain LM306 and PenA mosaic strain NG-109 to research the impact of the mosaic mutation of PenA.

It was reported that the most significant change for reduced susceptibility against antimicrobial agents was an amino acid insertion of Asp-345 into PenA, which was shown to make a major contribution to reducing the affinity of gonococcal PBP2 to penicillin by 4–5-fold in 1990.[13] In isolates from 2006, this insertion was found in PenA nonmosaic strain NG-120, cefixime-susceptible strain NG-127, and β-lactamase-producing strain NG-128. It is suggested that this mutation is a generality in the clinical isolates of cefixime-susceptible strains and PenA nonmosaic strains.

From a comparison of these three strains, in the PenA nonmosaic strain NG-120, two additional differences, Ala-501 to Val and Pro-551 to Ser, were found. It was reported that the mutation of Ala-501 to Val was associated with reduced susceptibility to cefixime and other oral cephems.[35,36] The mutation of Ala-501 to Val was located downstream of the conserved Lys-497–Thr–Gly motif. Modeling studies of PenA suggest that this mutation of Ala-501 to Val is located near the binding site, although a mutation of Pro-551 to Ser is distant (Fig. 3A). This result indicated that the mutation of Ala-501 to Val may contribute mainly to the reduced susceptibilities to penicillins and other β-lactam antimicrobial agents in PenA nonmosaic strains. In addition, it was considered that these were not horizontal transfers but

specific mutations, because other *Neisseria* spp. did not have this mutation. Interestingly, a PenA nonmosaic strain, NG-120, tended to be more sensitive to aztreonam, a monobactam, unlike other PenA mosaic strains. It remains possible that these strains have other features, but it was difficult to explain such phenomena in this study.

In PenA mosaic strains with reduced susceptibility to cefixime and ceftriaxone isolated in 2006, the PenA amino sequences of NG-109, -110, -118, -121, and -122 were quite similar to the PenA of *N. perflava/sicca* and *N. cinerea* as well as *N. flavescens* and *N. meningitidis* (Fig. 2). In other *Neisseria* spp., it was reported that one of the donors conferring *penA* to *N. meningitidis* was identified as a naturally penicillin-resistant species, *N. flavescens*.[37] These gene transformations between commensally resistant *Neisseria* spp. and the original susceptible gonococci might have been caused by widespread commercial oral sex, and have enhanced the spread of reduced susceptibility to cephems.[17] An *N. gonorrhoeae* PenA with mosaic structures that confers reduced susceptibility to cefixime might have been constructed by various partial PenA from other *Neisseria* spp. (Fig. 3A,B). In this modeling study of mosaic PenA in NG-109, it is suggested that the PenA 3D conformation of the mosaic strain exchanged with other *Neisseria* spp. reduced the affinity to PenA of cephem antibiotics, including cefixime or ceftriaxone. Moreover, each domain in PenA of NG-109 had other alterations of amino acid residues which were not found in other *Neisseria* spp., suggesting that these alterations were induced to maintain the activity of PenA, since they were not found in other *Neisseria* spp.

In conclusion, we consider that the cause of reduced susceptibility to cephems is a conformational alteration of the β-lactam-binding pocket in PenA. A homology modeling simulation of PenA suggests that PenA mosaic sequences and the mutation of Ala-501 to Val near the β-lactam binding site Ser-310 of the PenA nonmosaic strain caused reduced susceptibility to penicillins or other cephem antibiotics. *N. gonorrhoeae* PenA with mosaic structures might have emerged by the transduction of regions from PenA of other *Neisseria* spp.

The reduction of susceptibility to antimicrobial agents has obviously progressed. Continual surveillance and monitoring of susceptibility to antimicrobial agents and genetic analysis are needed not only to reveal the detailed mechanism of resistance in *N. gonorrhoeae*, but also to select new therapeutic agents.

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J Infect Chemother (2006) 12:207–209
DOI 10.1007/s10156-006-0447-x

NOTE

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Enhancement of antimicrobial activities of cefteram or clavulanic acid/amoxicillin against cefixime-resistant *Neisseria gonorrhoeae* in the presence of clarithromycin or azithromycin

Received: March 27, 2006 / Accepted: April 24, 2006

Abstract We investigated the enhancement of the antimicrobial activities of β-lactams against cefixime (CFIX)-resistant *Neisseria gonorrhoeae* in the presence of macrolides. Ten strains of CFIX-resistant *N. gonorrhoeae*, isolated between 2000 and 2003 from male patients with urethritis at Jikei University Affiliated Hospital and its related clinics in the Tokyo metropolitan area, were tested. The fractional inhibitory concentrations of clavulanic acid/amoxicillin (CVA/AMPC), CFIX, or cefteram (CFTM), in the presence of clarithromycin (CAM) or azithromycin (AZM), against these strains were determined. Synergism, partial synergism, or additivity was recognized between CVA/AMPC or CFTM and macrolides against nine strains. Additivity and partial synergism between CFTM and macrolides against nine and ten strains, respectively, were also recognized. On the other hand, antagonism between CFIX and macrolides was recognized. These results indicate that combination antimicrobial chemotherapy, using CFTM or CVA/AMPC with macrolides, is a possible alternative treatment for CFIX-resistant *N. gonorrhoeae* infections.

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Key words *Neisseria gonorrhoeae* · Drug resistance · β-Lactam · Macrolide · Synergy

Since the 1990s, the spread of fluoroquinolone-resistant *Neisseria gonorrhoeae* has been an important issue in Japan. In response to this, cephems such as cefixime (CFIX), cefodizime, and ceftriaxone were recommended as alternative treatment options for *N. gonorrhoeae* infections in Japanese patients in 2002.[1] However, CFIX-resistant *N. gonorrhoeae* strains have recently[2–4] appeared. Therefore, the rechecking of patients treated with CFIX[5] for *N. gonorrhoeae* infections is essential. In addition, the spread of cephem-resistant *N. gonorrhoeae* is a source of worries in the future. In view of these findings, we investigated the combined effects of β-lactams and macrolides so as to have a new strategy for CFIX-resistant *N. gonorrhoeae* infections.

Ten strains of CFIX-resistant *N. gonorrhoeae*, isolated between 2000 and 2003 from male patients with urethritis at Jikei University Affiliated Hospital and its related clinics in the Tokyo metropolitan area, were tested. The minimum inhibitory concentration (MIC) of CFIX was 0.25 μmg/ml or more against all these strains. The MICs of levofloxacin (LVFX) ranged from 0.25 to 0.5 μg/ml against two strains, and from 4 to 16 μg/ml against eight strains. Al strains showed intermediate or high resistance to CFIX and fluoroquinolones, according to the breakpoint of ciprofloxacin defined by the Clinical and Laboratory Standards Institute (CLSI) M100-S15.[6] The antimicrobial agents we tested were three β-lactams, i.e., clavulanic acid/amoxicillin (CVA/AMPC), CFIX, and cefteram (CFTM); and two macrolides, i.e., clarithromycin (CAM) and azithromycin (AZM). The fractional inhibitory concentration (FIC) index of each β-lactam and each macrolide was determined by the checkerboard method,[7] using plate dilution with modified GC agar medium (Becton Dickinson, Sparks, MD, USA) according to the CLSI.[6] The initial inoculum was 10^4 cfu/ml. Synergism, partial synergism, additivity, and antagonism between each β-lactam

Table 1. Antimicrobial susceptibilities of ten *Neisseria gonorrhoeae* strains to five antibiotics

Strain no.	MIC (μg/ml)				
	CAM	AZM	CVA/AMPC	CFIX	CFTM
TB1464	1	0.25	2	0.25	0.5
TB14673	1	0.25	2	0.25	0.5
TB19928	1	0.25	2	0.25	1
TB19933	1	0.12	2	0.5	1
TB19939	0.5	0.12	2	0.25	0.5
TB19936	0.5	0.12	2	0.5	1
TB19950	1	0.25	2	0.25	1
TB14675	1	0.25	2	0.5	1
NSD00116	1	0.25	1	0.25	1
NSD001981	1	0.12	2	0.25	1

CAM, clarithromycin; AZM, azithromycin; CVA/AMPC, clavulanic acid/amoxicillin; CFIX, cefixime; CFTM, cefteram

Table 2. FIC indexes of β-lactam-macrolide combinations for ten *Neisseria gonorrhoeae* strains

Combination	FIC index		No. of strains		
	Mean	Range	Synergistic or partially synergistic	Additive	Antagonistic
CVA/AMPC and CAM	0.819	≦0.501–2.000	7	2	1
CVA/AMPC and AZM	0.705	≦0.504–2.000	8	1	1
CFIX and CAM	1.775	0.750–2.000	1	1	8
CFIX and AZM	1.177	0.508–2.000	3	4	3
CFTM and CAM	0.913	0.625–2.000	6	3	1
CFTM and AZM	0.710	0.531–1.000	8	2	0

FIC, fractional inhibitory concentration; CAM, clarithromycin; AZM, azithromycin; CVA/AMPC, clavulanic acid/amoxicillin; CFIX, cefixime; CFTM, cefteram

and each macrolide were defined when the FIC index was 0.5 or less, more than 0.5 and less than 1, 1 or more, respectively.

The MICs of CAM, AZM, CVA/AMPC, CFIX, and CFTM against the ten CFIX-resistant strains ranged from 0.5 to 1 μg/ml, 0.12 to 0.25 μg/ml, 1 to 2 μg/ml, 0.25 to 0.5 μg/ml, and 0.5 to 1 μg/ml, respectively (Table 1). Partial synergism against seven strains and additivity against two strains were recognized for the combination of CVA/AMPC and CAM. Partial synergism against eight strains and synergism against one strain were recognized for the combination of CVA/AMPC and AZM. In the combinations of CFTM with CAM or AZM, partial synergism against six or eight strains and additivity against three or two strains were recognized, respectively. On the other hand, antagonism against eight or three strains was recognized for the combinations of CFIX with CAM or AZM, respectively (Table 2).

The antimicrobial activities of CVA/AMPC and CFTM against eight strains were enhanced twofold to fourfold in the presence of 1/2 the MIC of CAM (0.25 or 0.5 μg/ml). However, the antimicrobial activities of CFIX against eight strains were not enhanced in the presence of these macrolides (Table 3). In addition, the antimicrobial activities of CVA/AMPC and CFTM were enhanced twofold to fourfold and fourfold to eightfold in the presence of 1/2 the MIC of AZM (0.06 or 0.12 μg/ml), respectively. On the other hand, the antimicromial activity of CFIX against nine strains was not enhanced in the presence of 1/2 the MICs of macrolides.

Table 3. Comparison of the MICs of β-lactams tested in combination with the MICs of β-lactams tested alone

Combination		MIC ratio[a]			
		1	1/2	1/4	≦1/8
CVA/AMPC		1	7	1	1
CFIX	1/2 MIC CAM	8	2	0	0
CFTM		1	3	5	1
CVA/AMPC		1	6	3	0
CFIX	1/2 MIC AZM	3	6	1	0
CFTM		0	2	4	4

[a] MIC ratio, MIC tested in combination with macrolide (1/2 MIC)/MIC tested alone

Numbers in Table body are numbers of strains

CAM, clarithromycin; AZM, azithromycin; CVA/AMPC, clavulanic acid/amoxicillin; CFIX, cefixime; CFTM, cefteram

From these results, in vitro combination effects were found between CVA/AMPC or CFTM, and CAM or AZM. Therefore, these combination therapies were possible alternative strategies to use against CFIX-resistant *N. gonorrhoeae* infections. The macrolides tested in this study are also effective against chlamydial infections, which are present in 20.8% of *N. gonorrhoeae* infections.[8] Therefore, these combination therapies should to be effective against

mixed infections with *N. gonorrhoeae* and *C. trachomatis*. Although clinical trials of these combination therapies will be performed in the future, we should pay attention to adverse effects, such as diarrhea.

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若年者における無症候性器クラミジア感染症の実態把握と蔓延防止システムについて

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日本性感染症学会誌
Vol.17,No.1

若年者における無症候性器クラミジア感染症の実態把握と蔓延防止システムについて

The actuality of Japanese youth asymptomatic *Genital Chlamydial infection* and a prevention system of STD control

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若年者は無症状の段階で無防備に性感染症を広げているのではないかと危惧される。性感染症予防システムを構築するため、性器クラミジアの病原体保有状況と性行動や検査・治療に関する要望を調べた。14～25歳までの803人(女性707人、男性96人)のうち、クラミジアトラコマティスPCR陽性率は、女性11%、男性9%であった。性感染症予防行動として、コンドームを常時使っているのは20%、性に関して相談したいのは、彼氏や彼女や友人、その次に医療従事者であった。若年者はプライバシーを守ってもらい保護者の保険証を使わなくてすむ気軽に受診できる医療機関を求めている。性感染症の蔓延防止のためには、病原体の早期発見と確実な治療によって、二次感染を防ぐことが重要である。今後の展望として、無症状の段階でのスクリーニング検査から相談、医療、パートナーへの感染予防を包括した、若年者への具体的支援システムが望まれる。

The spread of STDs among Japanese youth is disturbing because it increases insidiously without demonstrating obvious symptoms. For the purpose of establishing a prevention system of STD control in Japan, the authors investigated carriers of sexually transmitted pathogens and their sexual behavior, and their desire for medical treatment. Among 803 young subjects (707 females, 96 males), ranging from 14 to 25 years of age, *Chlamydia trachomatis* was found in 11% of females and 9% of males by PCR test. Concerning their sexual behavior, condoms were regularly used during sexual intercourse in approximately 20% of the subjects. They want to choose their friends and sexual partners to consult with medical staff concerning sexual matters. Young people demand accessible medical facilities, which conduct physical examinations while maintaining their privacy, without having to show the guardian's health insurance certificate. To decrease the spread of STDs in the community, it is important to prevent secondary infection by early etiological diagnosis of STDs and early treatment and care appropriately. In the near future, we hope the STD prevention system for asymptomatic youth including screening tests, medical consultation and treatment, will protect them from contracting infection from sexual partners.

Key words : Chlamydia trachomatis, Youth, Asymptomatic infection, STD prevention

はじめに

性行動開始年齢の低下によって若年者において性感染症が広がっていることが危惧される[1]。性感染症は症状に気づかなくても、病原体を持っていれば無防備な行為によってパートナーに感染を広げてしまう[2]。感染症法に基づく国の指針である、エイズ及び性感染症予防に関する特定感染症予防指針では、感染の可能性のある者、特に青少年、若年層への普及啓発を重要としており[3,4]、性感染症の蔓延を防止するためには、性行動開始の初期の段階で、性感染症（性器クラミジア感染症、淋菌感染症等）の病原体の保有状況を調べ、性行動のリスクを減らすための相談や指導とともに早期発見・早期治療につなげる必要がある。WHOが示す性感染症予防のための重要な公衆衛生的枠組みの中にも、「早期の症候性および無症候性感染の発見」が挙げられている。このため、若年者を対象に、無症候性器クラミジアの病原体保有者スクリーニングとあわせて、受検者における性行動や医療アクセスに関するアンケートを行った。若年者にとって身体的侵襲や精神的負担の少ないスクリーニング検査の

導入と円滑で適切な相談および医療へつなげる連携システムを試行し、その事業化に向けて提言する。

対象と方法

性行為を経験した若年者（14～25 歳：中高大学・専門学校生等）で性感染症の自覚症状がなく治療中でない者を対象に、調査研究に賛同を得た学校での授業や健康教育、自主グループ、医療機関での思春期相談やメーリングリスト、HIV 夜間検査時や各種啓発イベント等の機会に本調査参加を呼びかけた。H15 年度から H17 年度までの 3 年間で、群馬、横浜、神戸、岡山、北九州の地区を合わせて約 800 人が調査に参加した。

1）スクリーニング検査：*Chlamydia trachomatis* について 803 人から遺伝子増幅検査（PCR）によって病原体保有を調べた。検体採取方法は、女性（707 人）は専用キットを使って、膣分泌液を自己採取し郵送で、男性（96 人）は初尿を自己採取し当日、研究協力者へ提出した。また、協力を得られる参加者（女性 32 人）の咽頭ぬぐい検体から *C. trachomatis* の分離培養による検出を試みた。検体検査は全て三菱化学 BCL へ委託した。

2）アンケート調査：検体を提出した者のうち、アンケートに答えた 788 人（女性 695 人、男性 93 人）から、性感染症の知識、性行動、性感染症の検査や医療に関する要望をまとめた（回収率 98%）。

3）倫理面および個人情報保持の配慮：対象者に本調査の趣旨とともに、部分的な協力や参加の中止も可能であること、研究結果を公表する際には、特定の個人を同定できないよう報告することを書面で説明した。目的を理解し同意書を提出した参加者へ検体容器と質問紙を配布し、検体とアンケート用紙はそれぞれ無記名で提出してもらった。結果通知を希望する者には検査結果報告書を郵送あるいは手渡して結果を説明した。本研究：課題「若年者を対象とした無症候性感染者（性器クラミジア感染症および淋菌感染症）の実態調査と蔓延防止システムの構築」は、東京慈恵会医科大学倫理委員会で平成 15 年 10 月 6 日承認「受付番号 15-99（4124）、平成 17 年 4 月 11 日更新」。なお、受診の必要な場合には再検査や治療を勧め、パートナーとともに適切な医療へつなげるよう、希望に応じて随時、電話や E－メールなどで相談を受けた。

調査結果

1）性器クラミジア PCR 陽性率

女性 707 人、男性 96 人を母数として、男女別（3 年間の全地区平均）では、女性 11%、男性 9%、咽頭ぬぐい検体による *C. trachomatis* 培養陽性が、女性 32 人中 1 人（3%）であった。地区別及び窓口別の PCR 陽性率は、学校を窓口にした場合（横浜・神戸・北九州：大学生が中心）をまとめて、女性 4.8%　男性 5.4%、医療機関を窓口とした場合（群馬・岡山：高校生が中心）は、それぞれ女性 18.5%　男性 11.9%（群馬）、女性 9.9%（岡山）であった。また、女性のみについて、年齢層別の平均では 14～19 歳が 14%、20～25 歳が 5%であった（Table 1）。

2）アンケート調査の概要

(1)　性感染症の知識

「知っている性感染症」と「性感染症の知識」について、HIV/AIDS の名前はほぼ全員が知っていたが、「何らかの性感染症にかかっていると HIV に感染しやすい」は 40%が知らないと回答した。

(2)　性行動

初交年齢について、最年少は女性 11 歳、男性 12 歳であった（女性 1 人はオーラルセックスのみ 8 歳で経験あり）。回答者の 60%は 15～16 歳で初交を経験していた。

Table 1　性器クラミジア感染の PCR 陽性率（%）

	female	male
Total participator	n=707 11.0%	n=96 9.0%
Participator by school	n=144 4.8%	n=54 5.4%
Participator by medical facility of gynecologist		
Gunma	n=442 18.5%	n=42 11.9%
Okayama	n=121 9.9%	—
Participator of separate generation (female)		
age	14－19	20－25
	14%	5 %
Pharyngeal smear (female)		
	n=32 1（3 %）	

過去1年間のパートナーの数は2〜5人が、40〜50%で最も多く、パートナー数が1人であったのは30%、パートナー数6人以上は20%、過去1年間はセックスの経験なしは5%であった。

性感染症の既往があったのは、女性20%、男性5〜8%（地区による差）であった。

(3) コンドームについて

初めてのセックスでコンドーム使用は約60%、2回目からのコンドーム使用では、「いつも使う」のは20%に留まり、「使うことが多い」30%、「使わないことが多い」40%であった。岡山地区では性感染症リスクに関して、高校生と大学生の年齢階級別に性行動の差を検定したところ、初めてのセックスと比べて、2回目以降のセックスで常時使用する割合が減っており、より若年者層で初交年齢が低く、コンドームを選択しないという有意差がみられた（Table 2）。

(4) 性感染症予防のための行動

「コンドームがないときはセックスしない」女性50%、男性30%、「パートナーは一人にする」女性70%、男性40%、「何もしない」女性20%、男性40%と、性感染症予防の意識は特に男性で低い傾向であった。「特定のパートナー1人とのセックス」という選択は女性で多く、「現在特定のパートナーがいる」は約60%であったが、その30%は「今までに特定のパートナー以外とのセックスあり」と回答しており、「特定のパートナー以外とのセックスはない」という残りの70%でも、過去の性交相手の人数は複数であった。

(5) 性に関して困ったときに相談したい人（Fig. 1）

多い順に「友人」約80%、「彼氏彼女（パートナー）」約60%、に次いで「医療関係者に相談したい」約40%、という回答であった。携帯電話やメール・インターネットによる相談は、実際の使用経験によって高まる可能性があり、岡山地区での調査では大学生より高校生の世代でIT利用の希望が多かった（Table 3）。その他意見では「顔と名前が知られないような場所で相談したい」があった。

Table 2 性感染症のリスクに関する行動（岡山地区：年齢階級別女性）

	Participator in Okayama of separate generation (female) generation	14-18	19-21	有意差
初交年齢	age of first sexual intercours	15.3±1.3	17.2±1.6	0.000*
コンドームがないときはセックスしない	no sexual intercours without condom	27.9%	50.0%	0.046*
初交時コンドーム使用	use condom at first intercourse	54.8%	66.7%	0.346
2回目から常時コンドーム使用	use condom regularly from two times	15.0%	28.6%	0.078
何もしない	do nothing	39.5%	21.4%	0.099

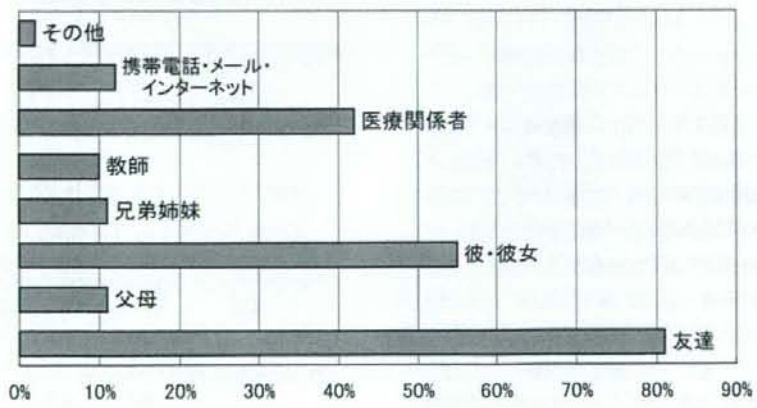

Fig. 1 性に関して相談したい人

Table 3　性に関して相談したい相手や要望（岡山地区：年齢階級別女性）

	Participator in Okayama of separate generation (female) generation	14-18	19-21	有意差
彼氏彼女に相談したい	consultation with sexually partners	36.4%	66.7%	0.006＊
携帯メールやインターネットで相談したい	use of cellular phone or internet services	47.7%	26.2%	0.047＊
自宅で検査したい	having screening test at home	18.2%	31.7%	0.209
親の保険証を使わずに済ませたい	without the guardian's health insurance certificate	68.2%	39.0%	0.009＊

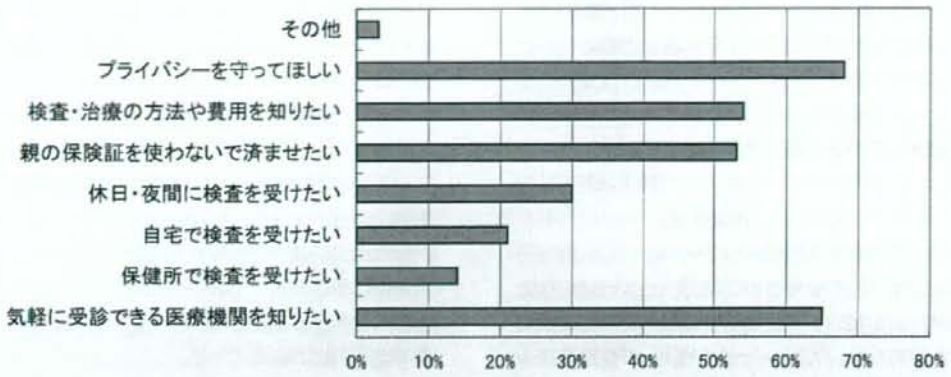

Fig. 2　検査や治療に関しての要望

(6)　検査・治療への要望（Fig. 2）

多い順に「プライバシーを守ってほしい」「気軽に受診できる医療機関を知りたい」「具体的な検査・治療方法やその費用について知りたい」「親の保険証を使わないで済ませたい」について、全体では半数以上の参加者が望んでいた。

その他の要望は自由記載より、「検査する人の対応が心を傷つけるものであってほしくない」「HIV 検査のように他の検査も無料化が進めばいい」「パソコンや携帯電話で、検査できる機関か治療などについて、調べられるサイトなどを多くの人が知られるようにしてもらいたい」という意見があった。

考　察

病原体が検出された割合は 20 代より 10 代で高かったが、その特徴として性行動が活発で初交年齢が低く、過去に複数のパートナーとセックスの経験があり、コンドームを常時使用していない傾向がみられた。また、参加者の一部では、パートナーの病原体陽性の判明から同時に複数の検査希望が増加し、その数人は知人どうしであるなど、若年者のカジュアルな性のネットワーク[5]がみられた。

自己採取スクリーニング検査について

本調査では、試行的に自己採取による膣分泌液から性器クラミジアの病原体を PCR 法で検出した。膣分泌液（自己採取）と子宮頸管粘液（婦人科医による採取）の陽性一致率は高く、検査精度にも大きな問題はないとされる[6,7]。従来行われている血清抗体によるスクリーニング検査では過去の感染の影響も受け、陽性率が高い。新たな感染を把握し、自分で採取可能な性器クラミジア感染症のスクリーニング検査として核酸増幅検査の導入が望まれる。

窓口別のクラミジア PCR 陽性率

医療機関を窓口とした場合に陽性率は高く、学校を窓口とした場合との差が大きかったことについて、性感染