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Various *penA* mutations together with *mtrR*, *porB* and *ponA* mutations in *Neisseria gonorrhoeae* isolates with reduced susceptibility to cefixime or ceftriaxone

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Objectives: To examine mutations within the *penA*, *mtrR*, *porB*, *ponA* and *pilQ* genes of *Neisseria gonorrhoeae* to determine their contribution to cephalosporin resistance.

Methods: A total of 46 *N. gonorrhoeae* isolates with reduced susceptibility to cefixime or ceftriaxone (MICs $\geq$ 0.12 mg/L) and two susceptible isolates were selected. The full sequence of *penA* and partial sequences previously reported as hot mutation sites of the other genes were analysed. Genotyping by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) was also performed.

Results: A mosaic penicillin-binding protein 2 (PBP 2) was found in a single isolate that exhibited the highest cefixime MIC (0.5 mg/L). The majority of the isolates with reduced susceptibility to cephalosporins contained non-mosaic PBP 2 sequences, of which PBP 2 pattern XIII was most common (28/46). All isolates with reduced susceptibility to cephalosporins also had *mtrR* and *porB* mutations. Two susceptible isolates had the PBP 2 pattern XIV and an incomplete MtrR protein, which was a new mutation. Isolates with identical PBP 2 patterns comprised multiple NG-MAST sequence types.

Conclusions: Reduced susceptibility of *N. gonorrhoeae* to ceftriaxone and cefixime was associated with diverse *penA* mutations, particularly PBP 2 pattern XIII containing an Ala-501→Val substitution, together with *mtrR* and *porB* mutations. The existence of only one strain having the mosaic *penA* sequence indicated that ceftriaxone and cefixime resistance in Korea is mostly not associated with a mosaic *penA* sequence. Highly heterogeneous NG-MAST sequence types excluded the clonal expansion of a particular subtype.

Keywords: penicillin binding protein 2, genotyping, antibiotic resistance, cephalosporins

Introduction

Gonorrhea remains a major sexually transmitted disease (STD) in Korea, with 3115 reported cases in 2007 according to the STD Sentinel Surveillance System from the National Institute of Health, Korea (http://stat.cdc.go.kr). Effective treatment options for *Neisseria gonorrhoeae* infections have been significantly reduced over time by the emergence and spread of gonococci resistant to penicillins, tetracyclines, macrolides and quinolones.[1] Cephalosporins are now recommended as the mainstay of treatment.[2] The Korea Centers for Disease Control and Prevention (KCDC) has recommended a single dose of 125 mg of intramuscular ceftriaxone or a single dose of 400 mg of oral cefixime since 2006. However, the emergence of *N. gonorrhoeae* isolates with reduced susceptibility to cephalosporins *in vitro*,[3–13] along with occasional treatment failures with cefixime[10,14] and ceftibuten,[12] have been reported.

Sequential alterations of the *penA*, *mtrR*, *porB*, *ponA* and *pilQ* genes induce chromosomally mediated high-level resistance to penicillin.[15] A point mutation in the *pilQ* gene (*pilQ2*, previously named *penC*) was recently identified, but this mutation has not been observed in a clinical setting.[15,16] Similarly, an association has been proposed between mutations in *penA*, *mtrR*, *porB* and *ponA* and reduced susceptibility of *N. gonorrhoeae* strains to

broad-spectrum cephalosporins such as cefixime and ceftriaxone.[6,9,17] The *penA* mutations usually comprise insertion of a single aspartic acid codon between Arg345 and Asp346 (R345_D346insD), along with a five or six amino acid substitution downstream.[18,19] Recently, *penA* mosaic alleles different from the former non-mosaic *penA* mutations were identified,[4,6,8,9,11,13] with mosaic alleles particularly linked to reduced susceptibility to cefixime.[4,6,8,13] However, recent studies suggest that reduced susceptibility to ceftriaxone is not due to the presence of a mosaic *penA* allele.[11,20] Accordingly, the aims of this study were to examine mutations in the *penA*, *mtrR*, *porB*, *ponA* and *pilQ* genes of *N. gonorrhoeae* isolates with reduced susceptibility to cefixime or ceftriaxone and to determine their contribution to resistance.

Materials and methods

N. gonorrhoeae isolates

N. gonorrhoeae isolated from patients attending STD clinics and public health centres during 2001–07 were studied. Of the 877 gonococci isolated, 208 were randomly selected for antimicrobial susceptibility testing and were stored at −70°C until further use.

A total of 48 isolates were selected for further investigation on the basis of their ceftriaxone or cefixime MICs: 46 had reduced susceptibility (MICs 0.12–0.5 mg/L); and 2 were susceptible (MICs ≤ 0.008 mg/L).

MIC determinations

MICs of penicillin G, cefixime and ceftriaxone were determined by the agar dilution method.[21] The medium used was GC agar base (BBL; Becton Dickinson, Cockeysville, MD, USA) supplemented with 1% haemoglobin and 1% IsoVitaleX (BBL). Penicillin G (Sigma Chemical Co., St Louis, MO, USA), cefixime (Dong A Pharmaceutical Co., Seoul, Korea) and ceftriaxone (Hanmi Pharmaceutical Co., Seoul, Korea) were used. Inocula of 10^4 cfu were applied using a Steers replicator (Craft Machine Inc., Woodline, PA, USA). Plates were incubated in a 5% CO_2 incubator at 35°C for 24 h after which the results were read. *N. gonorrhoeae* ATCC 49226 was used as a control strain.

Nucleotide sequencing of penA genes

Full-length *penA* nucleotide sequences from the *N. gonorrhoeae* strains were determined as reported previously.[13] The primers used in this study are listed in Table 1. PCR was carried out in a total volume of 20 μL, with 1 μL of heat-extracted template DNA, 10 pmol of each primer and PreMix (Bioneer, Cheongwon, Korea) containing 1 U of Taq DNA polymerase. A thermal cycler (Eppendorf, Hamburg, Germany) was used with the following conditions: 35 cycles of 94°C for 1 min; 50°C (*penA*-A1, B1), 56°C (*penA*-A2, B2) or 52°C (*penA*-A3, B3) for 1 min; and 72°C for 1 min. The PCR products were extracted using a DNA extraction kit (Qiagen, Hilden, Germany) and subjected to direct sequencing. All PCR products were sequenced twice with forward and reverse primers using an automatic sequencer (model 3730*xl*; Applied Biosystems, Weiterstadt, Germany). Multiple-sequence alignments of nucleotide and amino acids were performed using BioEdit (version 7.0.9.0). The amino acid sequence patterns of PBP 2 were classified according to those previously described by Ito *et al.*[13] (I–X) and Whiley *et al.*[11] (XI–XXIII).

Mutations in mtrR, porB, ponA and pilQ

The promoter and coding regions of *mtrR*, *porB*, *ponA* and *pilQ* were amplified using previously described *mtrR*,[5] *porB*[22] and *ponA*[5] primers and *pilQ* primers newly designed from the nucleotide sequence of *N. gonorrhoeae* FA 1090 (GenBank accession no. AE004969) (Table 1). The primers were intended to amplify the partial sequences previously reported as hot mutation sites. The parameters of the amplifications were as follows: 35 cycles of 94°C for 30 s; 50°C (*mtrR*), 46°C (*porB*), 56°C (*ponA*) or 52°C (*pilQ*) for 30 s; and 72°C for 1 min. PCR products were extracted and sequenced using the PCR primers mentioned above.

Table 1. Primers used for PCR amplification and sequencing of the *penA*, *mtrR*, *porB*, *ponA* and *pilQ* genes

Primer	Nucleotide sequence (5′ to 3′)	Nucleotide positions	Reference
penA-A1	CGGGCAATACCTTTATGGTGGAAC	8 to 31	13
penA-B1	AACCTTCCTGACCTTTGCCGTC	655 to 676	13
penA-A2	AAAACGCCATTACCCGATGGG	597 to 617	13
penA-B2	TAATGCCGCGCACATCCAAAG	1157 to 1177	13
penA-A3	GCCGTAACCGATATGATCGA	1003 to 1022	13
penA-B3	CGTTGATACTCGGATTAAGACG	1844 to 1865	13
mtrR-F	GCCAATCAACAGGCATTCTTA	−210 to −191	5
mtrR-R	GTTGGAACAACGCGTCAAAC	190 to 171	5
porB-F	CCGGCCTGCTTAAATTTCTTA	−41 to −22	22
porB-R	TATTAGAATTTGTGGCGCAG	1030 to 1049	22
ponA-F	GAGAAAATGGGGGAGGACCG	1171 to 1190	5
ponA-R	GGCTGCCGCATTGCCTGAAC	1395 to 1376	5
pilQ-F	CGTTACGCCGAACATCACG	1833 to 1851	this study
pilQ-R	TGACCGAAACTGAACGGACTG	2358 to 2338	this study

Genotyping

N. gonorrhoeae multiantigen sequence typing (NG-MAST) was conducted by the sequencing of internal fragments of two highly polymorphic antigen-encoding loci, *por* and *tbpB*.[23] The sequence data were uploaded onto the NG-MAST website (www.ng-mast.net) to obtain the allele numbers and the sequence types.

Nucleotide sequence accession numbers

Nucleotide sequence data for the *penA* gene encoding PBP 2 with amino acid pattern XXIV, the *mtrR* gene encoding the incomplete MtrR protein and the *mtrR* gene encoding the MtrR protein with new amino acid substitutions (Ala-39→Thr, Leu-47→Pro) were submitted to the GenBank nucleotide database under accession numbers FJ465093, FJ465094 and FJ465095, respectively.

Results

Alterations of PBP 2 in clinical isolates

The complete nucleotide sequences of the *penA* genes from 48 clinical isolates were determined. Eight amino acid sequence patterns in PBP 2 were identified. These sequences were compared with those of penicillin- and cephalosporin-susceptible *N. gonorrhoeae* strain LM306 (GenBank accession no. M32091) (Figure 1). Overall, sequence pattern XIII was most common (28 strains), followed by pattern IV (9 strains) and pattern V (4 strains). Sequence pattern X, a previously described mosaic PBP 2 sequence,[13] was found in only one strain. Pattern XXIV was newly identified in this study, which was different from pattern V by a single amino acid at position 551 (Pro-551→Ser).

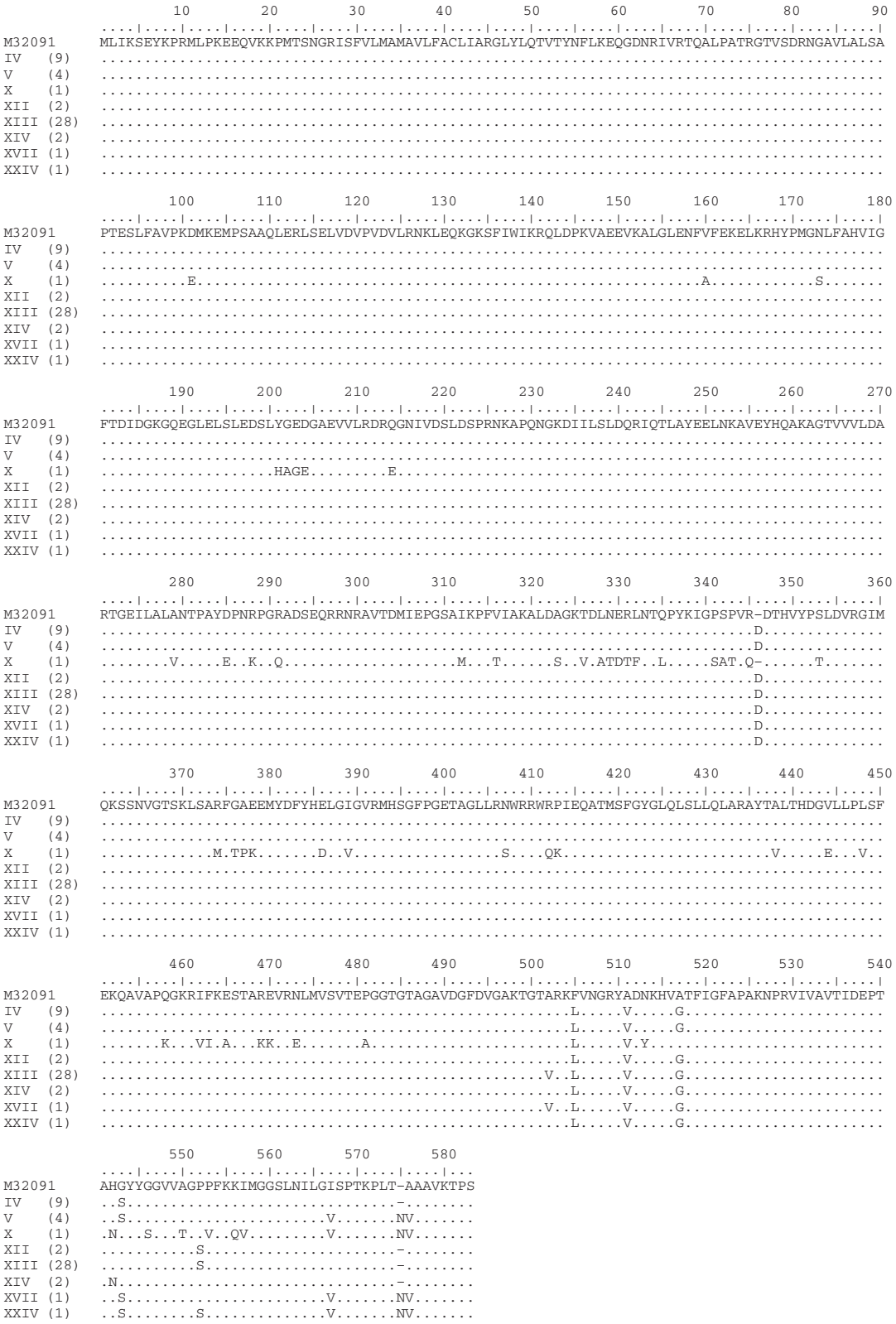

Figure 1. Amino acid sequences of PBP 2 from the 48 *N. gonorrhoeae* clinical isolates used in this study. The sequences are classified into different amino acid patterns (patterns I–XXIV) and are aligned with a GenBank sequence (accession number M32091). Sequence patterns I–X and XI–XXIII were previously described by Ito *et al.*[13] and Whiley *et al.*,[11] respectively, while pattern XXIV was identified in this study. The number of isolates displaying each pattern is indicated in parentheses.

Table 2. MICs of penicillin G, cefixime and ceftriaxone for the 48 *N. gonorrhoeae* clinical isolates with various patterns of alterations in PBP 2

Isolate (no. of isolates tested)				No. of isolates with MIC (mg/L)												
β-Lactamase	PBP 2 pattern	No. of NG-MAST STs	Antibiotic	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	32	64	≥128
Positive	all (11)		PEN												3	1	7
Negative	all (37)		PEN						1	2	6	21	6	1			
All	IV (9)	8	CFX				2	3	4								
			CRO					7	2								
	V (4)	4	CFX			1	1	1	1								
			CRO					3	1								
	X (1)	1	CFX							1							
			CRO						1								
	XII (2)	2	CFX				1	1									
			CRO				1	1									
	XIII (28)	19	CFX				3	22	3								
			CRO				16	11	1								
	XIV (2)	2	CFX	2													
			CRO	2													
	XVII (1)	1	CFX					1									
			CRO					1									
	XXIV (1)	1	CFX					1									
			CRO					1									

PEN, penicillin G; CFX, cefixime; CRO, ceftriaxone; NG, *Neisseria gonorrhoeae*; MAST, multiantigen sequence typing; STs, sequence types.

Association of alteration in PBP 2 with antimicrobial susceptibility

The distributions of the MICs of antimicrobial agents for the clinical isolates associated with the PBP 2 sequence patterns are shown in Table 2. The penicillin MIC for the isolate with the mosaic PBP 2 structure (pattern X) was the highest for the β-lactamase non-producing strains (Table 3). This isolate also had the highest ceftriaxone and cefixime MICs (0.25 and 0.5 mg/L). However, four other strains with non-mosaic *penA* mutations also had ceftriaxone MICs of 0.25 mg/L. The two susceptible isolates had PBP pattern XIV and displayed the lowest MICs of the antimicrobial agents.

Mutations in mtrR, porB, ponA and pilQ

Forty-four of the 46 *N. gonorrhoeae* isolates with reduced susceptibility to cephalosporins harboured a single nucleotide (A) deletion in the 13 bp inverted repeat located between the −10 and −35 sequences of the *mtrR* promoter (c.−57delA). The other two isolates contained missense mutations at codons 39 and 47 in the coding segments of *mtrR* that resulted in Ala-39→Thr, Leu-47→Pro amino acid substitutions. The two susceptible isolates contained a single nucleotide (G) deletion at position 81 of the *mtrR* coding sequence (c.81delG). This deletion induced a frame shift and produced a premature stop codon in *mtrR*, resulting in an incomplete MtrR protein consisting of 41 amino acids.

Non-synonymous substitutions at Gly-101 and Ala-102 of porin encoded by *porB* were found in 47 clinical isolates. Among the amino acid substitutions, Gly-101→Lys and Ala-102→Asp were the most common (42 strains), followed by Gly-101→Lys, Ala-102→Asn (2 strains) and Gly-101→Asn, Ala-102→Asp (2 strains). The *ponA1* allele (Leu-421→Pro in PBP 1) was detected in 44 of the 46 clinical isolates with reduced susceptibility and not in the two susceptible isolates. Finally, the *pilQ* mutation was not found in any of the clinical isolates tested.

Genotyping

A total of 38 NG-MAST sequence types were observed, of which 26 were new. The identical PBP 2 patterns were divided into multiple NG-MAST sequence types (Table 2). The 28 strains with PBP 2 pattern XIII, the most common PBP 2 alteration, resolved into 19 NG-MAST sequence types.

Discussion

In the present study, the reduced susceptibility of *N. gonorrhoeae* isolates to cefixime or ceftriaxone was associated with mutations in the *penA*, *mtrR*, *porB* and *ponA* genes (Table 3). We found the *penA* mosaic allele in a single clinical isolate, for the first time in Korea. This *penA* mosaic sequence was identical to the sequence (PBP 2 pattern X) identified in *N. gonorrhoeae* strains with reduced susceptibility to cefixime and ceftriaxone in Japan[4,8,9,13] and to ceftriaxone in Australia.[11] However, the mosaic allele was found in only one of the 46 isolates with reduced susceptibility to cephalosporins. This finding emphasizes the importance of non-mosaic *penA* alleles in mediating reduced susceptibility to broad-spectrum cephalosporins. Among non-mosaic *penA* alleles, PBP 2 pattern XIII was observed in a large proportion of the isolates with reduced susceptibility (28 out of 46). This

Table 3. Antibiogram, mutations of *penA*, *mtrR*, *porB* and *ponA* genes and genotypes of the 48 *N. gonorrhoeae* clinical isolates

		MIC (mg/L)			Mutations in				
Penicillinase	Isolate no.	PEN	CFX	CRO	*penA* (PBP 2)	*mtrR*	*porB*	*ponA*	NG-MAST ST
Positive	2001-12	>128	0.12	0.06	XIII	deletion of A[a]	G101K, A102D	WT	3946[b]
	2002-1	128	0.12	0.06	XIII	A39T, L47P	G101K, A102D	L421P	495
	2004-2	32	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	270
	2004-3	32	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	270
	2006-39	128	0.25	0.12	IV	deletion of A	G101K, A102D	L421P	3956[b]
	2006-43	>128	0.25	0.25	IV	deletion of A	G101K, A102D	L421P	3956[b]
	2007-17	128	0.12	0.12	IV	deletion of A	G101K, A102D	L421P	3485
	2007-32	64	0.06	0.12	IV	deletion of A	G101K, A102D	L421P	3965[b]
	2007-38	128	0.25	0.12	IV	deletion of A	G101K, A102D	L421P	3966[b]
	2007-53	128	0.12	0.12	IV	deletion of A	G101K, A102D	L421P	3965[b]
	2002-8	32	0.12	0.06	XII	A39T, L47P	G101K, A102D	L421P	3948[b]
Negative	2003-9	0.25	≤0.008	≤0.008	XIV	truncated MtrR	WT, WT	WT	1798
	2006-17	0.5	≤0.008	≤0.008	XIV	truncated MtrR	G101K, A102D	WT	3945[b]
	2001-2	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	1623
	2002-3	4	0.12	0.12	XIII	deletion of A	G101K, A102D	L421P	1623
	2002-5	4	0.12	0.12	XIII	deletion of A	G101K, A102N	L421P	3947[b]
	2002-15	4	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	1623
	2002-21	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	1623
	2003-8	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	3950[b]
	2003-25	2	0.12	0.12	XIII	deletion of A	G101K, A102D	L421P	3951[b]
	2004-6	2	0.12	0.06	XIII	deletion of A	G101K, A102N	L421P	3952[b]
	2004-28	1	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	3954[b]
	2006-6	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	972
	2006-12	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	3955[b]
	2006-19	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	3955[b]
	2006-20	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	3955[b]
	2006-21	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	1623
	2006-23	2	0.12	0.06	XIII	deletion of A	G101K, A102D	L421P	437
	2006-34	2	0.12	0.12	XIII	deletion of A	G101K, A102D	L421P	437
	2006-35	1	0.12	0.12	XIII	deletion of A	G101K, A102G	L421P	3976[b]
	2006-42	0.5	0.25	0.12	XIII	deletion of A	G101K, A102D	L421P	3957[b]
	2007-14	2	0.25	0.25	XIII	deletion of A	G101K, A102D	L421P	3962[b]
	2007-19	2	0.25	0.12	XIII	deletion of A	G101K, A102D	L421P	3963[b]
	2007-45	1	0.12	0.12	XIII	deletion of A	G101K, A102D	L421P	3967[b]
	2007-50	1	0.06	0.12	XIII	deletion of A	G101K, A102D	L421P	3967[b]
	2007-51	1	0.06	0.12	XIII	deletion of A	G101K, A102D	L421P	3968[b]
	2007-61	1	0.06	0.12	XIII	deletion of A	G101K, A102D	WT	3970[b]
	2007-9	4	0.12	0.12	IV	deletion of A	G101K, A102D	L421P	3961[b]
	2007-60	2	0.25	0.25	IV	deletion of A	G101N, A102D	L421P	3969[b]
	2007-64	4	0.06	0.12	IV	deletion of A	G101K, A102D	L421P	3971[b]
	2006-44	2	0.06	0.12	V	deletion of A	G101K, A102D	L421P	3959[b]
	2006-45	4	0.25	0.25	V	deletion of A	G101K, A102D	L421P	1868
	2007-6	2	0.12	0.12	V	deletion of A	G101K, A102D	L421P	3960[b]
	2007-65	2	0.03	0.12	V	deletion of A	G101K, A102D	L421P	3972[b]
	2004-16	8	0.5	0.25	X	deletion of A	G101K, A102D	L421P	3953[b]
	2006-38	2	0.06	0.12	XII	deletion of A	G101K, A102D	L421P	1614
	2007-30	2	0.12	0.12	XVII	deletion of A	G101N, A102D	L421P	3964[b]
	2002-37	2	0.12	0.12	XXIV	deletion of A	G101K, A102D	L421P	3949[b]

PEN, penicillin G; CFX, cefixime; CRO, ceftriaxone; WT, wild-type; NG, *Neisseria gonorrhoeae*; MAST, multiantigen sequence typing; ST, sequence type.
[a]Nucleotide (A) deletion within the 13 bp inverted repeat located between the −10 and −35 sequences of the *mtrR* promoter.
[b]New sequence types found in this study.

PBP 2 pattern contained a substitution at position 501 (Ala-501→Val), which was considered to cause reduced susceptibility to cephems in a homology modelling study.[24] PBP 2 pattern XVII, which also contained the Ala-501→Val substitution, was found in one isolate. In a modelling study, the Ala-501→Val substitution and mosaic structure induced a conformational alteration of the β-lactam-binding pocket in PBP 2 making a major contribution to the reduced susceptibility to cephems.[24] In other transformation experiments,[8] the Ala-501→Val substitution led to a 2- to 4-fold increase in the MICs of cefixime and other cephems. Whiley *et al.*[11] suggested that the Ala-501→Val substitution is more significant as a contribution to ceftriaxone susceptibility than a mosaic sequence. Our data showed the possibility that the Ala-501→Val substitution contributed to the reduced susceptibility to broad-spectrum cephalosporins although the *penA* mosaic allele has received attention with regard to reduced susceptibility to cephalosporins.[4,8,12,13,17] In contrast, two isolates that were fully susceptible to cephalosporins contained PBP 2 pattern XIV, consistent with the previous study.[11] This suggests that pattern XIV is not related to reduced susceptibility to cephalosporins.

The isolates with reduced susceptibility to cephalosporins were not restricted to any particular genotype. The isolates with identical PBP 2 patterns further resolved into multiple NG-MAST sequence types. These results showed the heterogeneity of *N. gonorrhoeae* strains with reduced susceptibility to cephalosporins, as has been observed in a recent study.[11] Both the previous study[11] and our present study included gonococci isolated at different times and in diverse geographical locations and comprised multiple subtypes.[25] The heterogeneity of NG-MAST sequence types suggests that the common occurrence of mutations in the *penA* gene rather than clonal spread of a single strain contributes to the reduced susceptibility to cephalosporins. The NG-MAST sequence type 835, which was reported in the strains with reduced susceptibility to cephalosporins from the nearby countries of Hong Kong[12] and Taiwan,[26] was not found in Korea.

The isolate with a mosaic *penA* allele showed the highest cefixime MIC (0.5 mg/L), but other isolates with non-mosaic *penA* alleles also had increased cefixime MICs. The ceftriaxone MIC for the isolate with the mosaic allele (0.25 mg/L) overlapped with MICs for the isolates with non-mosaic *penA* alleles. These results suggest that non-mosaic *penA* mutations are also able to increase cephalosporins MICs to a level similar to that mediated by the mosaic allele. Recently, a clinical isolate with a different mosaic PBP 2, pattern XXIII, was reported to be fully susceptible to ceftriaxone.[11,20] This indicates that the mosaic PBP 2 is not sufficient for reduced susceptibility to ceftriaxone. Transformation experiments[17] have shown that a significant level of cefixime resistance was conferred by the mosaic *penA* allele, with only a small contribution from *mtrR* and *porB*, whereas ceftriaxone resistance was equally dependent upon both. It is possible that besides *penA*, *mtrR*, *porB* and *ponA*, additional genetic mutations in an as yet unidentified loci also contribute to increased resistance of *N. gonorrhoeae* to cephalosporins.[17]

All isolates with reduced susceptibility to cephalosporins had *mtrR*, *porB* and *ponA* mutations except two, which did not have the *ponA* mutation (Table 3). We set the cefixime or ceftriaxone MIC cut-offs for selecting strains for this study at 0.12 mg/L. This cut-off was chosen based on the study by Ito *et al.*[13] which reported mosaic *penA* (type X) in strains for which the cefixime MIC was ≥0.12 mg/L. This high MIC cut-off would make it very difficult to find different combinations of several gene mutations that can be observed in *N. gonorrhoeae* strains with lower MICs and to deduce the effect of each gene mutation. However, we expect that the *ponA* mutation (*ponA1* allele) is not important for cephalosporin resistance because cephalosporin susceptibility was not different between isolates with *ponA* mutation and isolates without the mutation if other gene mutations were the same, supporting a recent transformation study.[17]

Several new mutations of the *mtrR* gene were found in this study. The two susceptible isolates had an incomplete MtrR protein due to a single nucleotide (G) deletion at position 81. In contrast, the isolates with reduced susceptibility had a deletion in the promoter region (44 isolates) or newly found amino acid substitutions (Ala-39→Thr, Leu-47→Pro) within MtrR (2 isolates). Hagman and Shafer[27] reported that the loss of MtrR resulted in enhanced expression of *mtrC*, but not to the same extent as that caused by the single bp deletion in the *mtrR* promoter region. The *mtrR* promoter overlaps the −35 sequence of *mtrC*, so mutation in the promoter region can enhance binding of either RNA polymerase or an activator to the *mtrC* promoter due to decreased competition for binding on the same region of the DNA, which induces higher resistance.[27]

In conclusion, reduced susceptibility of *N. gonorrhoeae* clinical isolates to ceftriaxone and cefixime was associated with diverse *penA* mutations, particularly PBP 2 pattern XIII containing an Ala-501→Val substitution. The existence of only one isolate having the mosaic *penA* sequence indicated that ceftriaxone and cefixime resistance in Korea is mostly not associated with a mosaic *penA* sequence. The mutations in *mtrR* and *porB* observed in all strains with increased MICs indicate their contribution to ceftriaxone and cefixime resistance. The considerable diversity in NG-MAST sequence types excluded the clonal expansion of a particular subtype.

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Transparency declarations

None to declare.

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