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penA, *ponA*, *porB1*, and *mtrR* Mutations and Molecular Epidemiological Typing of *Neisseria gonorrhoeae* with Decreased Susceptibility to Cephalosporins

Kayo Osawa,[a] Katsumi Shigemura,[b,c] Yukie Nukata,[a] Koichi Kitagawa,[d] Fukashi Yamamichi,[e] Hiroyuki Yoshida,[a,f] Toshiro Shirakawa,[b,d,g] Soichi Arakawa,[h] Masato Fujisawa[b]

Department of Biophysics, Kobe University Graduate School of Health Sciences, Kobe, Japan[a]; Department of Urology, Kobe University Graduate School of Medicine, Kobe, Japan[b]; Department of International Health, Kobe University Graduate School of Health Sciences, Kobe, Japan[c]; Division of Translational Research for Biologics, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe, Japan[d]; Department of Urology, Hyogo Prefectural Amagasaki General Medical Center, Amagasaki, Japan[e]; Hyogo Clinical Laboratory Corporation, Himeji, Japan[f]; Division of Advanced Medical Science, Kobe University Graduate School of Science, Technology and Innovation, Kobe, Japan[g]; Department of Urology, Sanda City Hospital, Sanda, Japan[h]

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In recent years, *Neisseria gonorrhoeae* strains with decreased susceptibility (DS; MIC, >0.064 mg/liter) or resistance to cefixime (CFM) or ceftriaxone (CRO), have been classed as "superbugs" and have spread worldwide, resulting in gonorrhea treatment failures (1–3). In *N. gonorrhoeae*, the genetic mechanisms of mosaic alleles of *penA* encoding penicillin-binding protein 2 (PBP2), including the fragments of the *penA* gene from commensal *Neisseria* spp., mutation of *ponA* encoding PBP1, mutation of *porB1* encoding the outer membrane porin, and *mtrR* mutations with overexpression of the MtrCDE efflux pump, have also been reported (4–8). As determined by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), which is a sequence-based epidemiological approach, strains highly resistant to CRO are known as sequence type 4220 (ST4220) and ST1407 with mosaic *penA*-X and -XXXIV in Japan and Europe (3, 9). A total of 73 *N. gonorrhoeae* strains were isolated from male urethritis or female cervicitis cases in Hyogo, Japan, in 2012. The strains were confirmed to be a gonococcal species by testing with the HN-20 Rapid system (Nissui, Tokyo, Japan). The MICs of penicillin G (PEN), CFM, and CRO were determined by the agar dilution method based on CLSI standards (10). Of 73 *N. gonorrhoeae* isolates tested, 36% were PEN I (intermediate), 64% were PEN R (resistant), 62% were CFM DS, 14% were CFM R, 7% were CRO DS, and 0% were CRO R. Mosaic *penA* was seen in 78%, including the *penA*-X family (42%) and the *penA*-XXXIV family (21%). An L421P mutation in *ponA* was seen in 100% of the isolates with mosaic *penA*. The *porB1* mutations were G101K and A102D (36%), G101K and A102N (30%), G101 and A102D (1%), and G101T and A102D (1%). A deletion of the *mtrR* promoter region was seen in 63%, and *mtrR* mutations (A39T, A40D, and G45D) were seen in 40% (A39T, 12%; A40D, 16%; G45D, 12%). Isolates with mosaic *penA* (97%, $P < 0.001$), an L421P mutation in *ponA* (87%, $P < 0.001$), *porB1* mutations (90%, $P < 0.001$), or a deletion of the *mtrR* promoter region (87%, $P = 0.004$) were significantly associated with CFM DS/R (Table 1). Fifty (74%) of 68 CRO S isolates were classified as CFM DS/R,

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Address correspondence to Katsumi Shigemura, katsumi@med.kobe-u.ac.jp.

TABLE 1 Distribution of *penA*, *ponA*, *porB1*, and *mtrR* mutations among *N. gonorrhoeae* isolates in different antibiotic susceptibility groups[a]

Mutation and allele or presence/absence	No.[b] (%) of isolates					No.[b] (%) of isolates		
	PEN S	PEN I/R	CFM S	CFM R/DS	*P* value[h]	CRO S	CRO R/DS	*P* value
penA								
Mosaic[c]	0 (0)	57 (100)	2 (4)	55 (97)	<0.001	52 (92)	5 (9)	0.579
Nonmosaic	0 (0)	16 (100)	16 (100)	0 (0)		16 (100)	0 (0)	
ponA[d]								
Yes	0 (0)	63 (100)	8 (13)	55 (87)	<0.001	58 (92)	5 (8)	1.000
No	0 (0)	10 (100)	10 (100)	0 (0)		10 (100)	0 (0)	
porB1[e]								
Yes	0 (0)	50 (100)	5 (10)	45 (90)	<0.001	45 (90)	5 (10)	0.173
No	0 (0)	23 (100)	13 (57)	10 (43)		23 (100)	0 (0)	
mtrR promoter[f]								
Yes	0 (0)	46 (100)	6 (13)	40 (87)	0.004	41 (89)	5 (11)	0.150
No	0 (0)	27 (100)	12 (44)	15 (56)		27 (100)	0 (0)	
mtrR[g]								
Yes	0 (0)	30 (100)	12 (40)	18 (60)	0.014	30 (100)	0 (0)	0.074
No	0 (0)	43 (100)	6 (14)	37 (86)		38 (88)	5 (12)	

[a]PEN MICs: S, ≤0.06 mg/liter; I, 0.12 to 1 mg/liter; R, ≥2 mg/liter. CFM MICs: S, ≤0.06 mg/liter; DS, 0.125 and 0.25 mg/liter; R, >0.25 mg/liter. CRO MICs: S, ≤0.06 mg/liter; DS, 0.125 and 0.25 mg/liter; R, >0.25 mg/liter.
[b]No. of isolates.
[c]X (including A486V, L353W, and L404S), XXXIV (including P551S), XXVI, and XXVII (including A159V and S172N).
[d]L421P.
[e]G101K and A102D; G101K and A102N; G101 and A102D; G101T and A102D.
[f]Mutations with deletions.
[g]A39T, A40D, and G45D.
[h]For CFM S versus R/DS.

and all 50 isolates had a mosaic mutation. Furthermore, five isolates with DS to CRO that were typed as NG-MAST ST6798 with *penA*-X or ST14150 with *penA*-XXXIV-P551S had *ponA* and *porB1* mutations and a deletion of the *mtrR* promoter region, respectively (Table 2). ST14150 strains were assigned to genogroup G1407 (11). ST6798 strains, similar to ST2958, were CFM and CRO R in Fukuoka, Japan (12). Shimuta et al. had shown that NG-MAST ST1407 isolates showed a CRO MIC of 0.125 mg/liter and included *penA*-XXXIV-P551S (1). Genogroup G1407 strains were widespread in China and Spain between 2012 and 2014 (7, 13) and isolated in Hyogo, Japan. In conclusion, on the basis of our findings, even though we have no data on the patients' backgrounds, including demographic data, NG-MAST ST6789 and ST14150 strains in genogroup G1407 had *penA* mosaic alleles and *ponA*, *porB1*, and *mtrR* mutations and DS to CRO (MIC, >0.064 mg/liter), and these factors may lead to DS to cephalosporins in *N. gonorrhoeae* in Hyogo, Japan.

TABLE 2 Characteristics of *N. gonorrhoeae* strains with DS to CRO[a]

Strain	NG-MAST ST	*penA* mosaic	Mutation(s)				MIC (mg/liter)		
			ponA	*porB1*	*mtrR* promoter	*mtrR*	PEN	CFM	CRO
KG12041	ST6798	X	L421P	G101K, A102D	A with deletion	None	4	0.25	0.125
KG12015	ST6798	X-A486V	L421P	G101K, A102D	A with deletion	None	8	0.5	0.125
KG12057	ST6798	X-A486V	L421P	G101K, A102D	A with deletion	None	8	0.5	0.125
KG12051	ST14150	XXXIV-P551S	L421P	G101K, A102N	A with deletion	None	8	0.5	0.125
KG12014	ST14150	XXXIV-P551S	L421P	G101K, A102N	A with deletion	None	8	0.25	0.125

[a]MIC of >0.064 mg/liter.

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REFERENCES

1. Shimuta K, Unemo M, Nakayama S, Morita-Ishihara T, Dorin M, Kawahata T, Ohnishi M, Antibiotic-Resistant Gonorrhea Study Group. 2013. 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. Antimicrob Agents Chemother 57:5225–5232. https://doi.org/10.1128/AAC.01295-13.
2. Suay-García B, Pérez-Gracia MT. 16 February 2017. Drug-resistant *Neisseria gonorrhoeae*: latest developments. Eur J Clin Microbiol Infect Dis https://doi.org/10.1007/s10096-017-2931-x.
3. Unemo M. 2015. Current and future antimicrobial treatment of gonorrhoea—the rapidly evolving *Neisseria gonorrhoeae* continues to challenge. BMC Infect Dis 15:364. https://doi.org/10.1186/s12879-015-1029-2.
4. Ameyama S, Onodera S, Takahata M, Minami S, Maki N, Endo K, Goto H, Suzuki H, Oishi Y. 2002. Mosaic-like structure of penicillin-binding protein 2 gene (*penA*) in clinical isolates of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime. Antimicrob Agents Chemother 46:3744–3749. https://doi.org/10.1128/AAC.46.12.3744-3749.2002.
5. Ito M, Deguchi T, Mizutani KS, Yasuda M, Yokoi S, Ito S, Takahashi Y, Ishihara S, Kawamura Y, Ezaki T. 2005. Emergence and spread of *Neisseria gonorrhoeae* clinical isolates harboring mosaic-like structure of penicillin-binding protein 2 in central Japan. Antimicrob Agents Chemother 49:137–143. https://doi.org/10.1128/AAC.49.1.137-143.2005.
6. Unemo M, Shafer WM. 2014. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev 27:587–613. https://doi.org/10.1128/CMR.00010-14.
7. Serra-Pladevall J, Barberá MJ, Rodriguez S, Bartolomé-Comas R, Roig G, Juvé R, Andreu A. 2016. *Neisseria gonorrhoeae* antimicrobial susceptibility in Barcelona: *penA*, *ponA*, *mtrR*, and *porB* mutations and NG-MAST sequence types associated with decreased susceptibility to cephalosporins. Eur J Clin Microbiol Infect Dis 35:1549–1556. https://doi.org/10.1007/s10096-016-2696-7.
8. Shigemura K, Osawa K, Miura M, Tanaka K, Arakawa S, Shirakawa T, Fujisawa M. 2015. Azithromycin resistance and its mechanism in *Neisseria gonorrhoeae* strains in Hyogo, Japan. Antimicrob Agents Chemother 59:2695–2699. https://doi.org/10.1128/AAC.04320-14.
9. Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K, Nakayama S, Kitawaki J, Unemo M. 2011. Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrob Agents Chemother 55:3538–3545. https://doi.org/10.1128/AAC.00325-11.
10. Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
11. Chisholm SA, Unemo M, Quaye N, Johansson E, Cole MJ, Ison CA, Van de Laar MJ. 2013. Molecular epidemiological typing within the European Gonococcal Antimicrobial Resistance Surveillance Programme reveals predominance of a multidrug-resistant clone. Euro Surveill 18:20358. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20358.
12. Tanaka M, Furuya R, Irie S, Kanayama A, Kobayashi I. 2015. High prevalence of azithromycin-resistant *Neisseria gonorrhoeae* isolates with a multidrug resistance phenotype in Fukuoka, Japan. Sex Transm Dis 42:337–341. https://doi.org/10.1097/OLQ.0000000000000279.
13. Chen SC, Yin YP, Dai XQ, Unemo M, Chen XS. 2016. First nationwide study regarding ceftriaxone resistance and molecular epidemiology of *Neisseria gonorrhoeae* in China. J Antimicrob Chemother 71:92–99. https://doi.org/10.1093/jac/dkv321.