J Antimicrob Chemother 2015; **70**: 2536–2542
doi:10.1093/jac/dkv146 Advance Access publication 17 June 2015

Journal of Antimicrobial Chemotherapy

Emergence of decreased susceptibility and resistance to extended-spectrum cephalosporins in *Neisseria gonorrhoeae* in Korea

Hyukmin Lee[1], Magnus Unemo[2], Hyo Jin Kim[3], Younghee Seo[3], Kyungwon Lee[3]* and Yunsop Chong[3]

[1]*Department of Laboratory Medicine, International St Mary's Hospital, Catholic Kwandong University College of Medicine, Incheon, Korea;* [2]*WHO Collaborating Centre for Gonorrhoea and Other STIs, Department of Laboratory Medicine, Clinical Microbiology, Faculty of Health and Medicine, Örebro University, Örebro, Sweden;* [3]*Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea*

*Corresponding author. Tel: +82-2-2228-2446; Fax: +82-2-313-0908; E-mail: leekcp@yuhs.ac

Received 8 February 2015; returned 12 April 2015; revised 6 May 2015; accepted 7 May 2015

Objectives: Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is a major concern globally; however, no comprehensive AMR data for gonococcal isolates cultured after 2006 in Korea have been published internationally. We determined the susceptibility of *N. gonorrhoeae* isolates cultured in 2011–13, the mechanism of extended-spectrum cephalosporin (ESC) resistance and the molecular epidemiology of gonococcal strains in Korea.

Methods: In 2011–13, 210 gonococcal isolates were collected in Korea and their AMR profiles were examined by the agar dilution method. The *penA*, *mtrR*, *penB*, *ponA* and *pilQ* genes were sequenced in 25 isolates that were resistant to ESCs and 70 randomly selected isolates stratified by year. For molecular epidemiology, *N. gonorrhoeae* multiantigen sequence typing and MLST were performed.

Results: None of the *N. gonorrhoeae* isolates was susceptible to penicillin G and most were resistant to tetracycline (50%) and ciprofloxacin (97%). The rates of resistance to ceftriaxone, azithromycin, cefpodoxime and cefixime were 3%, 5%, 8% and 9%, respectively. However, all isolates were susceptible to spectinomycin. Twenty-one (84%) of the 25 ESC-resistant isolates contained the non-mosaic PBP2 XIII allele; however, the remaining 4 (16%) possessed the mosaic PBP2 X allele, which has been previously associated with ESC resistance including treatment failures.

Conclusions: In Korea, susceptibility to spectinomycin remains high. However, the recent emergence of ESC-resistant *N. gonorrhoeae* strains, including strains possessing the PBP2 mosaic X and non-mosaic XIII alleles, is a major concern and enhanced AMR surveillance is necessary to prevent transmission of these strains.

Keywords: gonorrhoea, antimicrobial treatment, antimicrobial resistance, ceftriaxone, cefixime, *penA*, NG-MAST, MLST

Introduction

Neisseria gonorrhoeae is the causative agent of various infections ranging from uncomplicated urethritis or cervicitis to ascending infections, resulting in pelvic inflammatory disease, ectopic pregnancy and infertility, or disseminated infections. According to the WHO, in 2008 the burden of gonococcal infections was estimated as 106 million new cases among adults globally.[1] In Korea, it is difficult to measure the exact number of gonococcal infections because gonorrhoea cases are under-reported to protect patients following the introduction of antiprostitution legislation in 2004. However, ~50000 cases (incidence of ~100 cases per 100000 inhabitants) of gonococcal infection remain reported to the Korea Health Insurance Review and Assessment Service annually.[2]

N. gonorrhoeae has developed resistance to all previously used first-line antimicrobials for treatment of gonorrhoea and in many countries the extended-spectrum cephalosporins (ESCs) are the only remaining options for empirical antimicrobial monotherapy.[3,4] Worryingly, the ESCs are now also threatened due to evolving resistance. Treatment failures with cefixime have been reported in several countries[3,5–7] and rare cases of treatment failures as well as high-level *in vitro* resistance to the more potent ceftriaxone have been verified in a few countries.[3,5,8–12] Due to this developing situation, dual antimicrobial therapy with ceftriaxone and azithromycin is now recommended in Europe and the USA.[13,14] In Korea, antimicrobial resistance in *N. gonorrhoeae* has been a serious problem for many years. However, no comprehensive antimicrobial resistance data for isolates cultured after 2006 have been published internationally.[2]

In recent years, spectinomycin and ceftriaxone have been used mainly as monotherapy to treat gonococcal infections. However, in the 2011 Korean guideline, dual antimicrobial therapy (250 mg of ceftriaxone plus 1 g of azithromycin or 250 mg of ceftriaxone plus 100 mg of doxycycline twice daily for 7 days) has been introduced as the recommended first-line therapy for uncomplicated gonococcal infections.[15] Nevertheless, the high selective pressure resulting from widespread use can lead to the development of resistance. Accordingly, national and international quality-assured surveillance of antimicrobial resistance are essential to identify the emerging resistance, trends in resistance and provide appropriate data supporting timely revisions of treatment guidelines.

In this study, the antimicrobial susceptibility of *N. gonorrhoeae* isolated between 2011 and 2013 to eight relevant antimicrobials and the antimicrobials used for treatment of gonorrhoea in Korea from 2009 to 2012 were investigated. The genetic determinants of resistance to ESCs and the molecular epidemiological relatedness of the *N. gonorrhoeae* isolates were also elucidated.

Materials and methods

The work was performed at the Department of Laboratory Medicine, Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea.

Gonorrhoea patients and N. gonorrhoeae isolates

A total of 210 *N. gonorrhoeae* isolates were collected from patients with urethritis (136 males and 47 females) and female commercial sex workers ($n=27$) in 2011 ($n=60$), 2012 ($n=91$) and 2013 ($n=59$) in Korea, through a national surveillance programme for gonococcal resistance supported by the Korean Centers for Disease Control and Prevention. Most (71.4%) of the *N. gonorrhoeae* isolates were cultured from specimens obtained from 35 primary urological clinics, which were widely distributed across Korea. However, *N. gonorrhoeae* isolates were also collected from public health centres, secondary care hospitals and tertiary care hospitals.

Transgrow media and BD CultureSwab MaxV (+) (Becton Dickinson, Cockeysville, MD, USA) were used for specimen transportation. Transgrow media was prepared in-house with GC agar base (Becton Dickinson, Sparks, MD, USA), haemoglobin, IsoVitaleX (Becton Dickinson) and VCNT inhibitor (Becton Dickinson), which contains vancomycin, colistin, nystatin and trimethoprim. This medium was distributed into screw-cap tubes, subsequently solidified in a horizontal position and, finally, a CO_2-enriched atmosphere was introduced into the tubes.

Modified Thayer–Martin agar plates (Becton Dickinson) were used for culture isolation and conventional biochemical tests including Gram's stain and the Vitek Neisseria-Haemophilus Identification (NHI) system (bioMérieux, Marcy-l'Étoile, France) were used for species identification. All isolates were stored in 20% skimmed milk (Difco, Detroit, MI, USA) at −70°C prior to analysis.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the agar dilution method according to guidelines published by the CLSI.[16] MICs of ceftriaxone (Hanmi, Seoul, Korea), cefixime (Dong-A, Seoul, Korea), cefpodoxime (Pfizer Korea, Seoul, Korea), spectinomycin (Kuk Je, Seoul, Korea), azithromycin (Pfizer Korea), penicillin G (Sigma Chemical, St Louis, MO, USA), tetracycline (Pfizer Korea) and ciprofloxacin (Bayer Korea, Seoul, Korea) were determined using a GC II agar base supplemented with 1% IsoVitaleX (Becton Dickinson). Briefly, $\sim 10^4$ cfu of each isolate were applied to the agar plates with a Steer's replicator (Craft Machine, Chester, PA, USA) and the inoculated plates were then incubated in a 5% CO_2-enriched atmosphere at 35°C for 24 h. A cefinase disc test was performed to determine β-lactamase production. The resistance breakpoints stated by EUCAST (www.eucast.org) were applied. However, EUCAST does not state any resistance breakpoint for cefpodoxime and, accordingly, the cefpodoxime breakpoint from the CLSI was applied. *N. gonorrhoeae* reference strains ATCC 49226, WHO A, WHO B, WHO G, WHO J, WHO K and WHO L[17] were used for quality control.

Identification of genetic determinants associated with resistance to β-lactam antimicrobials

The entire *penA* gene was sequenced in all isolates that were resistant to at least one of the ESCs ($n=25$) and for 70 randomly selected isolates stratified by year using a statistically random method. The PBP2 sequences, encoded by the *penA* gene sequences, were assigned allele numbers according to previously published nomenclature.[8] Other genetic determinants responsible for resistance to ESCs (ceftriaxone, cefixime or cefpodoxime) and/or penicillin G were also sequenced, including the *mtrR*, *penB* (in the *porB1b* gene), *ponA* and *pilQ* genes. The primers and conditions used for amplifications were described in a previous study.[18] The amplification process consisted of 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C (*mtrR*), 46°C (*penB*), 56°C (*ponA*) or 52°C (*pilQ*) for 30 s and elongation at 72°C for 1 min. The size of the amplified product was confirmed by electrophoresis and DNA was extracted from the gel and sequenced at a commercial laboratory (Macrogen, Seoul, Korea).

Molecular epidemiological typing

To determine the molecular epidemiological relatedness, *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) was carried out for all isolates and MLST was performed for the 25 isolates that showed resistance to at least one of the ESCs examined. NG-MAST and MLST were performed according to the guidelines outlined on the respective database web site (NG-MAST, http://www.ng-mast.net;[19] MLST, http://pubmlst.org/neisseria/) with minor modifications. Phylogenetic tree analysis for the NG-MAST *porB* and *tbpB* alleles was performed by MEGA6 software using the neighbour-joining method.

Antimicrobial agents used to treat gonorrhoea in Korea

Given that all people in Korea are covered by the National Health Insurance System and antimicrobial agents are not available without prescription, the prescription data for treatment of gonorrhoea were acquired from the Korea Health Insurance Review and Assessment Service to analyse the recent selective antimicrobial pressure for development of resistance in *N. gonorrhoeae*. The percentage of patients (diagnosed with gonococcal infection without any accompanying additional sexually transmitted infection) treated with each specific antimicrobial from 2009 to 2012 was analysed.

Ethics statement

All examined gonococcal isolates were cultured and stored as part of routine diagnostics (standard care) and no patient identification information was available in the study. This study was also granted an exemption from requirement for ethics approval because it was performed as one of the projects of the national surveillance programme supported by the Korean Centers for Disease Control and Prevention (2013-E44003-00).

Results

Antimicrobial susceptibility in N. gonorrhoeae isolates from Korea during 2011–13 (n = 210)

The results of the antimicrobial resistance testing for all isolates are summarized in Table 1. From 2011 to 2013, the MIC

Table 1. Antimicrobial susceptibility of *N. gonorrhoeae* isolates collected from 2011 to 2013 in Korea ($n=210$)

Antimicrobial	MIC (mg/L)			Susceptibility/resistance (percentage of isolates)		
	range	50%	90%	S	I	R
Ceftriaxone	≤0.008–0.25	0.03	0.12	97		3
Cefpodoxime	≤0.008–4	0.12	0.5	92		8
Cefixime	≤0.008–0.5	0.03	0.12	91		9
Spectinomycin	≤16–32	32	32	100	0	0
Azithromycin	≤0.06–8	0.25	0.5	62	33	5
Penicillin G	0.12 to >128	1	4	0	71	29
Tetracycline	≤0.12–64	2	32	26	24	50
Ciprofloxacin	≤0.008–64	4	16	3	0	97

MICs were determined using the agar dilution technique.
S (susceptible), I (intermediate resistant) and R (resistant) were interpreted in accordance with the EUCAST breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf). However, EUCAST (www.eucast.org) does not state any resistance breakpoints for cefpodoxime and, accordingly, the breakpoint from the CLSI (www.clsi.org) was applied.

distributions and susceptibility to the surveyed antimicrobials did not differ greatly by year. All *N. gonorrhoeae* isolates were susceptible to spectinomycin and the MIC range, MIC_{50} and MIC_{90} were ≤16–32, 32 and 32 mg/L, respectively. The rates of resistance to ceftriaxone, cefpodoxime and cefixime were 3.3%, 8.1% and 9.0%, respectively. The MIC_{90} (MIC range) of ceftriaxone, cefpodoxime and cefixime was 0.12 mg/L (≤0.008–0.25 mg/L), 0.5 mg/L (≤0.008–4 mg/L) and 0.12 mg/L (≤0.008–0.5 mg/L), respectively. The rate of azithromycin-resistant isolates was 4.8% and an additional 33% of the isolates showed intermediate resistance. However, the MICs for the azithromycin-resistant isolates ranged from 1 to 8 mg/L and, accordingly, no isolates with high-level resistance to azithromycin (MIC ≥256 mg/L) were identified. All isolates were resistant (29%) or intermediate (71%) to penicillin G. The proportion of penicillinase-producing *N. gonorrhoeae* (PPNG) was 6.2% (13/210). Most of the isolates were resistant to tetracycline (50%), including 17% with high-level resistance, and ciprofloxacin (97%). Furthermore, 27% of isolates were resistant to penicillin G, ciprofloxacin and tetracycline and 12% of these MDR isolates were also resistant to azithromycin. Coresistance to ESCs and azithromycin was noted in one isolate that was resistant to ceftriaxone (MIC = 0.25 mg/L), cefpodoxime (MIC = 2 mg/L) and azithromycin (MIC = 2 mg/L).

Antimicrobial agents used to treat gonorrhoea in Korea, 2009–12

During 2009–12, more than half of the patients with gonorrhoea in Korea were treated with spectinomycin (51.7%–72.8%). However, the usage rate decreased from 72.8% in 2009 to 51.7% in 2012. By contrast, the proportion of patients treated with ceftriaxone significantly increased from 23.8% in 2009 to 45.3% in 2012. Cefixime, penicillin G and ciprofloxacin were also used; however, each was prescribed in only <1% of cases. Other cephalosporins including cefotaxime, cefpodoxime and ceftizoxime were used to treat 0.9%–2.1% of gonococcal infections (Table 2).

Table 2. Antimicrobials used for treatment of gonococcal infections in Korea from 2009 to 2012

Antimicrobial	Percentage of patients treated in			
	2009	2010	2011	2012
Spectinomycin	72.8	68.9	64.8	51.7
Ceftriaxone	23.8	27.6	32.5	45.3
Cefixime	0.3	0.4	0.4	0.5
Other cephalosporin[a]	1.8	2.1	0.9	1.5
Ciprofloxacin	0.9	0.7	0.9	0.5
Penicillin G	0.5	0.4	0.4	0.4
Total	100.0	100.0	100.0	100.0

[a]Cefotaxime, cefpodoxime and ceftizoxime.

Mechanisms of resistance to β-lactam antimicrobials

The most common PBP2 allele in the 25 isolates that were resistant to at least one of the ESCs (ceftriaxone 7, cefixime 19 and cefpodoxime 17) and 70 randomly selected ESC-susceptible isolates was the non-mosaic PBP2 XIII allele (56/95, 59%; Table 3), which contains the A501V and P552S alterations that both might increase the MICs of ESCs.[3,4,20,21] Twenty-one (84%) of the ESC-resistant isolates contained this non-mosaic PBP2 XIII allele; however, the remaining 4 (16%) of the ESC-resistant isolates possessed the mosaic PBP2 X allele, which has been previously associated with ESC treatment failures and high MICs of ESCs.[3,8] Many ESC-susceptible isolates that had MICs exactly at the ESC-resistance breakpoints also contained the XIII allele, while the non-mosaic IV, V, XII, XVI, XVII and XVIII alleles were mostly found in isolates with full susceptibility to these ESCs. The isolates ($n=4$) with the mosaic PBP2 X allele showed an MIC range of 0.06–0.25 mg/L for ceftriaxone, 0.25–0.5 mg/L for cefixime and 2–4 mg/L for cefpodoxime (Table 3). Additional genetic resistance determinants for β-lactam antimicrobials were found in most of the *N. gonorrhoeae* isolates, e.g. *mtrR* (A deletion in promoter: 99%; MtrR A39T mutation: 1%), *penB* (71%) and *ponA* (L421P, 94%), the last one mainly involved in penicillin G resistance.[3,22–24] In the *pilQ* gene, an N648S mutation was found in three isolates and one isolate had both an N648S mutation and the previously described E666K mutation,[23,25,26] but all of these isolates were susceptible to the ESCs examined.

Molecular epidemiology of N. gonorrhoeae isolates resistant to ESCs

According to the epidemiological analysis of the 25 ESC-resistant *N. gonorrhoeae* isolates, 21 (84%) were collected at clinics and hospitals that were located in geographically closely related regions (Seoul, Incheon and Gyeonnggi province). The four isolates containing the mosaic PBP2 X allele were isolated in 2011

Table 3. MIC distributions of ceftriaxone, cefixime and cefpodoxime for 95 *N. gonorrhoeae* isolates according to PBP2 allele[8]

		MIC (mg/L) (number of isolates)									
Antimicrobial	PBP2 allele[8]	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4
Ceftriaxone	IV[a]		2	5	1						
	V[a]	6	1	2	2	1					
	X[b]				1	2	**1**				
	XII[a]			1		1					
	XIII[a]	1	1	7	14	27	**6**				
	XVI[a]	1									
	XVII[a]				1	1					
	XVIII[a]	1		3	1	5					
Cefixime	IV		7		1						
	V	4	4	1	2	1					
	X						**3**	**1**			
	XII			1		1					
	XIII	1		2	15	23	**14**	**1**			
	XVI	1									
	XVII			1	1						
	XVIII		1	4	4	1					
Cefpodoxime	IV				1	6	1				
	V			6	2	2	1	1			
	X									**3**	**1**
	XII						1	1			
	XIII			1		4	13	25	**10**	**2**	**1**
	XVI			1							
	XVII						1	1			
	XVIII			1	1	2	5	1			

Bold formatting indicates resistant *N. gonorrhoeae* isolates.
[a]Non-mosaic PBP2 allele.[8]
[b]Mosaic PBP2 allele.[8]

(one isolate) and 2013 (three isolates). All of these four isolates were resistant to cefixime and cefpodoxime; however, only one of them was also resistant to ceftriaxone. All four isolates belonged to the MLST ST1901, which has been spreading globally and accounted for the majority of the ESC resistance internationally.[3,4] Three of them were assigned to NG-MAST ST2958 and one to NG-MAST ST9669. Among the 21 ESC-resistant isolates containing the non-mosaic PBP2 XIII allele, 3 isolates were resistant to all three ESCs, i.e. ceftriaxone, cefixime and cefpodoxime (Table 4). The NG-MAST STs of the 25 ESC-resistant *N. gonorrhoeae* isolates varied, but most (76%) of them had the NG-MAST *tbpB* 21 allele and the majority of the isolates were also genetically related by phylogenetic analysis of the *porB* gene (Figure 1). In NG-MAST analysis of the 10 azithromycin-resistant *N. gonorrhoeae* isolates, 5 had the identical *tbpB* 328 allele (3 ST2066, 1 ST5699 and 1 ST8162) and their *porB* gene sequences were genetically related (data not shown).

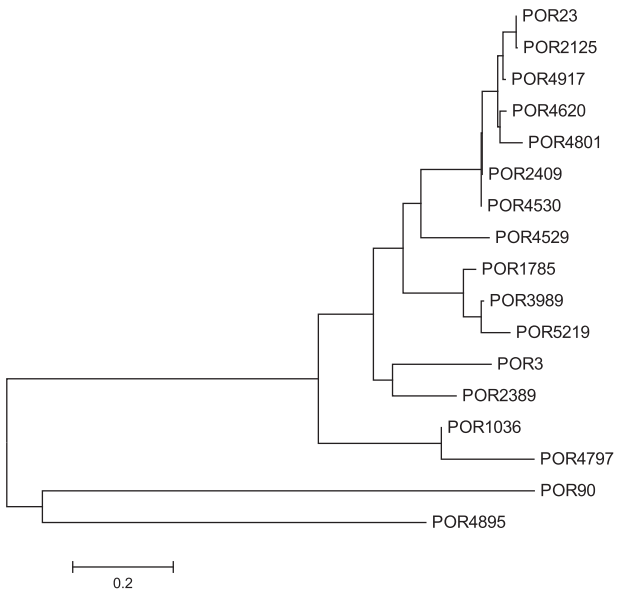
Discussion

Internationally, as well as in Korea, the threat of untreatable or difficult-to-treat gonorrhoea is real. None of the Korean isolates collected between 2011 and 2013 was susceptible to penicillin G, although the PPNG rate remained low (6%). Accordingly, resistance due to chromosomal mutations was common. All isolates contained a PBP2 mosaic allele or a PBP2 allele with an aspartate insertion at position 346[8] and *mtrR* mutation, which collaboratively result in increased penicillin G MICs.[4,22,23] Most isolates also had resistance mutations in the *porB1b* (*penB* resistance determinant) and *ponA* genes. In *porB1b*, the G120K and A121D mutations (38% of isolates) were the most common, which is in agreement with previous reports.[3,22,23] The *ponA* L421P mutation was found in 94% of isolates. Interestingly, three isolates had an N648S mutation and one isolate had both an N648S mutation and the previously described *pilQ* E666K mutation;[23,25] their penicillin G MICs ranged between 0.5 and 1.0 mg/L. SNPs in the *pilQ* gene, e.g. E666K, have been reported in laboratory-derived isolates and associated with high-level penicillin G resistance when accompanied with *penA*, *mtrR* and *penB* mutations.[23] However, these mutations were stated to inhibit proper pili formation, which is essential for virulence of gonococcal strains.[26] Nevertheless, we found the *pilQ* E666K SNP in one clinical isolate, but the penicillin G MIC was only 1 mg/L despite concomitant *penA*, *mtrR*, *penB* and *ponA* mutations. This might indicate that this isolate was lacking the still unknown

Table 4. *N. gonorrhoeae* isolates resistant to any ESC from 2011 to 2013 in Korea

			MIC (mg/L)				NG-MAST			Other genetic determinants			
PBP2[8]	Year	Region	CRO	CFM	CPD	PEN	ST	*porB*	*tbpB*	*mtrR*	*penB*	*ponA*	*pilQ*
X ($n=4$)[a]	2011	Incheon	0.06	0.25	2	2	2958	1785	110	deletion of A	G120K, A121D	L421P	—
	2013	Seoul	0.25	0.5	4	8	2958	1785	110	deletion of A	G120K, A121D	L421P	—
	2013	Gyeonnggi	0.12	0.25	2	4	2958	1785	110	deletion of A	G120K, A121D	L421P	—
	2013	Seoul	0.12	0.25	2	4	9669	4529	110	deletion of A	G120K, A121D	L421P	—
XIII ($n=21$)	2011	Daejeon	0.12	0.25	0.25	2	3968	2409	21	deletion of A	G120K, A121D	L421P	—
	2011	Seoul	0.12	0.25	1	1	8044	3	21	deletion of A		L421P	—
	2011	Seoul	0.12	0.5	4	4	8867	5219	21	deletion of A		L421P	—
	2011	Incheon	0.12	0.12	1	2	7069	3989	21	deletion of A	G120K, A121D	L421P	—
	2011	Seoul	0.12	0.12	1	1	8869	4620	1611	deletion of A	G120N, A121D	L421P	—
	2011	Incheon	0.25	0.12	0.5	2	7548	4530	21	deletion of A	G120K, A121D	L421P	—
	2012	Incheon	0.25	0.25	2	2	7069	3989	21	deletion of A	G120K, A121D	L421P	—
	2012	Incheon	0.12	0.25	1	1	7686	1036	21	deletion of A		L421P	—
	2012	Seoul	0.25	0.25	1	2	7548	4530	21	deletion of A	G120K, A121D	L421P	—
	2012	Incheon	0.12	0.25	1	1	7687	90	620	deletion of A	D120K, A121G	L421P	N27S
	2012	Cheonbuk	0.12	0.25	0.5	1	7688	23	21	deletion of A		L421P	—
	2012	Seoul	0.12	0.25	1	1	8041	2125	21	deletion of A		L421P	—
	2012	Incheon	0.12	0.25	1	1	8193	4917	21	deletion of A	G120K, A121D	L421P	—
	2012	Incheon	0.12	0.12	1	2	7684	2389	21	deletion of A	G120K, A121N	L421P	—
	2013	Cheonbuk	0.25	0.25	1	4	7069	3989	21	deletion of A	G120K, A121D	L421P	—
	2013	Cheonbuk	0.12	0.25	0.5	1	8044	3	21	deletion of A		L421P	—
	2013	Seoul	0.12	0.25	0.5	1	8046	4797	21	deletion of A		L421P	—
	2013	Seoul	0.12	0.25	0.5	1	8052	4801	21	deletion of A		L421P	—
	2013	Seoul	0.03	0.25	0.25	0.5	8164	4895	33	deletion of A	G120N, A121V	L421P	—
	2013	Seoul	0.25	0.12	2	1	7688	23	21	deletion of A		L421P	—
	2013	Incheon	0.25	0.12	0.5	2	4502	1785	21	deletion of A	G120K, A121D	L421P	—

CRO, ceftriaxone; CFM, cefixime; CPD, cefpodoxime; PEN, penicillin G.
[a]All isolates assigned to the MLST ST1901.



tbpB	ST	No. of isolates
21	7688	2
21	8041	1
21	8193	1
1611	8869	1
21	8052	1
21	3968	1
21	7548	2
110	9669	1
110/21	2958/4502	3/1
21	7069	3
21	8867	1
21	8044	2
21	7684	1
21	7686	1
21	8046	1
620	7687	1
33	8164	1

Figure 1. Phylogenetic tree of the *porB* gene sequences, *tbpB* alleles and NG-MAST STs of *N. gonorrhoeae* isolates with resistance to at least one ESC from 2011 to 2013 in Korea.

non-transformable resistance determinant 'Factor X'.[3–5,8,21–23] The significance of *pilQ* mutations, e.g. E666K, in clinical isolates requires further investigation. In Korea, the use of tetracyclines and fluoroquinolones in gonorrhoea treatment was abandoned in the mid-1980s and mid-2000s, respectively. In this study, many isolates were resistant to tetracycline (50%) and ciprofloxacin (97%), which is in agreement with earlier Korean studies.[2] The rate of plasmid-encoded high-level resistance to tetracycline (TRNG) was stable at 0%–4% until the mid-2000s, but then started to increase and reached 17% during 2011–13. Due to the low tetracycline selective pressure in Korea, foreign import of TRNG might be the most plausible explanation for this increase.

Despite spectinomycin having been commonly used in Korea, gonococcal susceptibility to spectinomycin remains exceedingly high. In Korea, the majority of gonorrhoea patients from 2002 to 2006 were treated with spectinomycin[2] and the situation was similar in 2009–12. In many countries, spectinomycin is not produced any more and its use in gonorrhoea treatment was abandoned or at least significantly decreased after the first reports of resistance, e.g. among US military in Korea;[27] however, internationally, spectinomycin resistance has been exceedingly rare in recent decades.[3,4] Even in Korea, spectinomycin resistance has been very rare, i.e. not reported after 1993. The spread of spectinomycin resistance in earlier decades might represent the spread of a few successful gonococcal strains and additional research regarding possible fitness cost of spectinomycin resistance would be valuable, which might explain the reasons for the limited spread of spectinomycin resistance in Korea and globally in modern times.

The recent emergence and spread of ESC-resistant *N. gonorrhoeae* has become a global concern.[3–14] In Korea, the resistance rates from 2011 to 2013 were 3.3% to ceftriaxone, 8.1% to cefpodoxime and 9% to cefpodoxime. The MIC_{90} of ceftriaxone increased 2-fold from 0.06 to 0.12 mg/L compared with a previous report from the mid-2000s.[2] In infections caused by gonococci with a ceftriaxone MIC of 0.12 mg/L, even when 1 g of ceftriaxone is administered, the time period that the concentration of free ceftriaxone exceeds the MIC ($fT_{>MIC}$) may be <20 h in the lower 95% CI.[28] Furthermore, gonococcal isolates with a ceftriaxone MIC ≤0.12 mg/L have resulted in failures to treat pharyngeal gonorrhoea with ceftriaxone.[3,9,10,12] Regarding cefixime, already in 2004 one resistant isolate was reported in Korea.[2] Cefixime resistance has been closely associated with mosaic *penA* alleles worldwide and both the isolate from 2004 and four cefixime-resistant isolates from 2013 had the mosaic PBP2 X allele, which resulted in early cefixime resistance in, e.g. Japan.[3,8,18] Interestingly, the 15 additional cefixime-resistant isolates instead had the non-mosaic PBP2 XIII allele, which also was the most common PBP2 type in Korea. This non-mosaic PBP2 XIII allele has five specific amino acid alterations (A501V, F504L, A510V, A516G and P551S),[8] among which A501V has been verified (and P551S statistically associated) to be important in ESC resistance.[3,4,20,21] However, most of the Korean *N. gonorrhoeae* isolates with the PBP2 XIII allele showed susceptibility to all the ESCs. Further investigation is crucial to determine the differences between these ESC-resistant and -susceptible isolates. The mosaic PBP2 X allele and some additional mosaic PBP2 alleles, e.g. mosaic PBP2 XXXIV allele, have been reported to be also involved in ceftriaxone resistance.[3–5,8] Accordingly, antimicrobial resistance surveillance or screening is very important nationally and internationally. However, in many geographical settings, diagnosis based on culture has been replaced by molecular diagnostics and, consequently, a highly sensitive and specific molecular antimicrobial resistance screening test is essential. Our results indicate that a silent dissemination of *N. gonorrhoeae* with the mosaic PBP2 X allele is initiated and genetic screening and/or surveillance should be performed to prevent the spread of ESC resistance.

Azithromycin has been suggested to have several advantages in the treatment of gonococcal infections. However, selection of azithromycin resistance in *N. gonorrhoeae* and *Mycoplasma genitalium*, which correlates with azithromycin use in monotherapy, has been described internationally.[3,4,29] In Korea, only 62% of isolates were susceptible to azithromycin and the resistance rate was 5%. However, no isolates with high-level resistance to azithromycin (MIC ≥256 mg/L), which have been identified in many other countries worldwide,[4] were identified or have been previously found in Korea.[2] All except one of the azithromycin-resistant *N. gonorrhoeae* isolates were collected from the capital area (Seoul, Incheon and Gyeonnggi). Three of the 10 azithromycin-resistant isolates belonged to NG-MAST ST2066 and two additional isolates possessed the identical *tbpB* 328 allele and showed genetic relatedness with ST2066 in the *porB* phylogenetic tree analysis. This indicates that clonal dissemination of azithromycin-resistant *N. gonorrhoeae* occurred in the capital area. The dissemination not only reduced the clinical usefulness of monotherapy with azithromycin, which should be avoided, but might also reduce the efficacy of the newly introduced combination treatment with ceftriaxone.

In conclusion, the susceptibility of *N. gonorrhoeae* to spectinomycin remains high. However, *in vitro* resistance to the ESCs ceftriaxone, cefixime and cefpodoxime is identified in Korea. The recent emergence of ESC-resistant *N. gonorrhoeae* strains containing the mosaic PBP2 X allele is a major concern and enhanced AMR surveillance is essential to prevent transmission of these strains. However, in Korea, as in many other countries globally, molecular detection of *N. gonorrhoeae* has rapidly been replacing culture and, accordingly, a highly sensitive and specific genetic screening for ESC-resistant isolates would be exceedingly valuable.

Acknowledgements

We are very grateful to Hayeon Kim for collecting the isolates.

Funding

The present work was supported by grants from the National Institute of Health, Korea Centers for Disease Control and Prevention, Ministry of Health and Welfare, Republic of Korea.

Transparency declarations

None to declare.

References

1 WHO. *Global Incidence and Prevalence of Selected Curable Sexually Transmitted Infections—2008*. Geneva: WHO. http://www.who.int/reproductivehealth/publications/rtis/2008_STI_estimates.pdf.

2 Lee H, Hong SG, Soe Y *et al*. Trends in antimicrobial resistance of *Neisseria gonorrhoeae* isolated from Korean patients from 2000 to 2006. *Sex Trans Dis* 2011; **38**: 1082–6.

3 Unemo M, Nicholas RA. Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. *Future Microbiol* 2012; **7**: 1401–22.

4 Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 2014; **27**: 587–613.

5 Unemo M, Golparian D, Nicholas R *et al*. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel *penA* mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012; **56**: 1273–80.

6 Allen VG, Mitterni L, Seah C *et al*. *Neisseria gonorrhoeae* treatment failure and susceptibility to cefixime in Toronto, Canada. *JAMA* 2013; **309**: 163–70.

7 Lewis DA, Sriruttan C, Müller EE *et al*. Phenotypic and genetic characterization of the first two cases of extended-spectrum cephalosporin-resistant *Neisseria gonorrhoeae* infection in South Africa and association with cefixime treatment failure. *J Antimicrobial Chemother* 2013; **68**: 1267–70.

8 Ohnishi M, Golparian D, Shimuta K *et al*. 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* 2011; **55**: 3538–45.

9 Read PJ, Limnios EA, McNulty A *et al*. One confirmed and one suspected case of pharyngeal gonorrhoea treatment failure following 500 mg ceftriaxone in Sydney, Australia. *Sex Health* 2013; **10**: 460–2.

10 Chen YM, Stevens K, Tideman R *et al*. Failure of 500 mg of ceftriaxone to eradicate pharyngeal gonorrhoea, Australia. *J Antimicrob Chemother* 2013; **68**: 1445–7.

11 Cámara J, Serra J, Ayats J *et al*. Molecular characterization of two high-level ceftriaxone-resistant *Neisseria gonorrhoeae* isolates detected in Catalonia, Spain. *J Antimicrob Chemother* 2012; **67**: 1858–60.

12 Golparian D, Ohlsson A, Janson H *et al*. Four treatment failures of pharyngeal gonorrhoea with ceftriaxone (500 mg) or cefotaxime (500 mg), Sweden, 2013 and 2014. *Euro Surveill* 2014; **19**: pii=20862.

13 Bignell C, Unemo M on behalf of the European STI Guidelines Editorial Board. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. *Int J STD AIDS* 2013; **24**: 85–92.

14 CDC. Update to CDC's *Sexually Transmitted Diseases Treatment Guidelines*, *2010*: oral cephalosporins no longer a recommended treatment for gonococcal infections. *MMWR Morb Mortal Wkly Rep* 2012; **61**: 590–4.

15 Korea Centers for Disease Control and Prevention, and Korean Association of Urogenital Tract Infection and Inflammation. *Korean Guideline for Sexually Transmitted Disease*. Seoul: Inomax, 2011.

16 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fourth Informational Supplement M100-S24*. CLSI, Wayne, PA, USA, 2014.

17 Unemo M, Fasth O, Fredlund H *et al*. Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. *J Antimicrob Chemother* 2009; **63**: 1142–51.

18 Lee SG, Lee H, Jeong SH *et al*. Various *penA* mutations together with *mtrR*, *porB* and *ponA* mutations in *Neisseria gonorrhoeae* isolates with reduced susceptibility to cefixime or ceftriaxone. *J Antimicrob Chemother* 2010; **65**: 669–75.

19 Martin IM, Ison CA, Aanensen DM *et al*. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. *J Infect Dis* 2004; **189**: 1497–505.

20 Whiley DM, Goire N, Lambert SB *et al*. Reduced susceptibility to ceftriaxone in *Neisseria gonorrhoeae* is associated with mutations G542S, P551S and P551L in the gonococcal penicillin-binding protein 2. *J Antimicrob Chemother* 2010; **65**: 1615–8.

21 Tomberg J, Unemo M, Davies C *et al*. Molecular and structural analysis of mosaic variants of penicillin-binding protein 2 conferring decreased susceptibility to expanded-spectrum cephalosporins in *Neisseria gonorrhoeae*: role of epistatic mutations. *Biochemistry* 2010; **49**: 8062–70.

22 Zhao S, Duncan M, Tomberg J *et al*. Genetics of chromosomally mediated intermediate resistance to ceftriaxone and cefixime in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2009; **53**: 3744–51.

23 Ropp PA, Hu M, Olesky M *et al*. Mutations in *ponA*, the gene encoding penicillin-binding protein 1, and a novel locus, *penC*, are required for high-level chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2002; **46**: 769–77.

24 Veal WL, Nicholas RA, Shafer WM. Overexpression of the MtrC-MtrD-MtrE efflux pump due to an *mtrR* mutation is required for chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. *J Bacteriol* 2002; **184**: 5619–24.

25 Chen CJ, Tobiason DM, Thomas CE *et al*. A mutant form of the *Neisseria gonorrhoeae* pilus secretin protein *PilQ* allows increased entry of heme and antimicrobial compounds. *J Bacteriol* 2004; **186**: 730–9.

26 Helm RA, Barnhart MM, Seifert HS. *pilQ* missense mutations have diverse effects on PilQ multimer formation, piliation, and pilus function in *Neisseria gonorrhoeae*. *J Bacteriol* 2007; **189**: 3198–207.

27 Boslego JW, Tramont EC, Takafuji ET *et al*. Effect of spectinomycin use on the prevalence of spectinomycin-resistant and penicillinase-producing *Neisseria gonorrhoeae*. *N Engl J Med* 1987; **317**: 272–8.

28 Chisholm SA, Mouton JW, Lewis DA *et al*. Cephalosporin MIC creep among gonococci: time for a pharmacodynamic rethink? *J Antimicrob Chemother* 2010; **65**: 2141–8.

29 Salado-Rasmussen K, Jensen JS. *Mycoplasma genitalium* testing pattern and macrolide resistance: a Danish nationwide retrospective survey. *Clin Infect Dis* 2014; **59**: 24–30.