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Surveillance

The second nationwide surveillance of the antimicrobial susceptibility of *Neisseria gonorrhoeae* from male urethritis in Japan, 2012–2013

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ABSTRACT

Worldwide, the most important concern in the treatment of sexually transmitted infections is the increase in antimicrobial resistant *Neisseria gonorrhoeae* strains including resistance to cephalosporins, penicillins, fluoroquinolones or macrolides. To investigate the trends of antimicrobial susceptibility among *N. gonorrhoeae* strains isolated from male patients with urethritis, a Japanese surveillance committee conducted the second nationwide surveillance study. Urethral discharge was collected from male patients with urethritis at 26 medical facilities from March 2012 to January 2013. Of the 151 specimens, 103 *N. gonorrhoeae* strains were tested for susceptibility to 20 antimicrobial agents. None of the strains was resistant to ceftriaxone, but the minimum inhibitory concentration (MIC) 90% of ceftriaxone increased to 0.125 μg/ml, and 11 (10.7%) strains were considered less susceptible with an MIC of 0.125 μg/ml. There were 11 strains resistant to cefixime, and the MICs of these strains were 0.5 μg/ml. The distributions of the MICs of fluoroquinolones, such as ciprofloxacin, levofloxacin and tosufloxacin, were bimodal. Sitafloxacin, a fluoroquinolone, showed strong activity against all strains, including strains resistant to other three fluoroquinolones, such as ciprofloxacin, levofloxacin and tosufloxacin. The azithromycin MICs in 2 strains were 1 μg/ml.

1. Introduction

The antimicrobial resistance of *Neisseria gonorrhoeae* strains against penicillins, tetracyclines, fluoroquinolones, cephalosporins and macrolides is increasing worldwide. Surveillance of the antimicrobial susceptibilities of *N. gonorrhoeae* provides important information for treating gonococcal infections. We have reported the antimicrobial susceptibilities of *N. gonorrhoeae* strains, which were collected at 2009–2010, in the first national surveillance study in Japan [1]. In summary, the rate of less susceptible strains to ceftriaxone was 8.4%; the susceptibility rate to cefixime according to the criteria of the Clinical and Laboratory Standards Institutes (CLSI) [2] was 98.8%; the minimum inhibitory concentrations (MICs) of fluoroquinolones, such as ciprofloxacin, showed a bimodal distribution and resistance rates were 78.3%; sitafloxacn showed low MICs of ≤0.5 μg/ml against ciprofloxacin-resistant strains; the proportion of strains with azithromycin MICs of more than 0.5 μg/ml was 3.6%.

After a ceftriaxone-resistant *N. gonorrhoeae* strain was discovered in a pharyngeal specimen of a female commercial sex worker in Kyoto, Japan [3], other ceftriaxone-resistant *N. gonorrhoeae* strains were discovered in France [4] and Spain [5], but not otherwise in Japan [1,6].

Since 2009, a 2-g azithromycin dose has been accepted by Japanese national insurance for the treatment of both gonococcal and chlamydial urethritis. High-level azithromycin-resistant strains have emerged in many sites worldwide [7–10], and a relationship between the use of azithromycin and an increase in the azithromycin MIC has been described [11]. The Japanese Association of Sexually Transmitted Infections [12] has not recommended the use of azithromycin for treating gonococcal infections. However, some physicians prefer to use azithromycin to treat gonococcal infections, and we anticipate the emergence of high-level azithromycin-resistant strains of *N. gonorrhoeae* in Japan.

In this report, the antimicrobial susceptibility patterns of *N. gonorrhoeae* strains collected from 2012 to 2013 are compared to the patterns from 2009 to 2010 [1]. This national surveillance was conducted by the surveillance committee of three Japanese societies including the Japanese Association of Infectious Diseases, the Japanese Society of Chemotherapy and the Japanese Society of Clinical Microbiology. The committee has previously performed and published other surveillance studies regarding the antimicrobial susceptibilities of pathogens causing respiratory infections, urinary tract infections, urethritis and surgical site infections. The present surveillance study was the second study performed on *N. gonorrhoeae* strains collected from male patients with gonococcal urethritis.

2. Materials and methods

2.1. Patients and participating facilities

The targets were male patients older than 16 years with urethral discharge and symptoms of urethritis, such as pain upon micturition, urethral pain or urethral discomfort. Included patients were diagnosed with gonococcal urethritis by a clinician. The period of specimen collection was between March 2012 and January 2013. The 38 participating facilities included departments of urology in hospitals and private clinics that specialized in urology or urology and dermatology in Japan. The clinicians who participated in this study explained the purpose of the study to the patients orally or through written documents and obtained the written consent of each patient. This study was approved by the ethical committee of each facility. The facilities that did not have an ethical committee submitted this study to the ethical committee of the specific non-profit organization CREC net, Kitakyushu, Japan, which approved it.

2.2. Specimens and patient information

The discharge from the urethral meatus was collected with a sterilized cotton swab, placed in transport agar (SEEDSWAB γNo2, Eiken Chemical Co. Ltd., Tokyo, Japan) and sent at room temperature to the Infection Scientific Control Research Center, The Kitasato Institution, Tokyo, Japan. Only one specimen was collected from each patient. The patient's information, including age, diagnosis and the properties of the discharge were reported for each sample.

2.3. Isolation of N. gonorrhoeae strains and antimicrobial susceptibility testing

The bacterial isolation and antimicrobial susceptibility testing were performed in the Infection Scientific Control Research Center, The Kitasato Institution, Tokyo, Japan. For each specimen, *N. gonorrhoeae* strain isolation and identification was attempted.

The antimicrobial susceptibility testing was performed according to the CLSI Document M100-S22 [2], and the minimum inhibitory concentrations (MICs) were determined by the agar dilution method. Supplemented 1% GC agar (1.1 g L-cysteine, 0.03 g guanine HCL, 3 mg thiamine HCL, 13 mg para-aminobenzoic acid, 0.01 g B12, 0.1 g cocarboxylase, 0.25 g nicotinamide adenine dinucleotide, 1 g adenine, 10 g L-glutamine, 100 g glucose, and 0.02 g ferric nitrate per liter) was used for determining the MICs. When the MIC of carbapenems or clavulanic acid was measured, cysteine was not included in the agar. The range of concentrations for testing included 12 two-fold serial dilutions (128–0.063 μg/ml) of antimicrobials, but the starting concentration fluctuated depending on the particular type of antimicrobial used. The inoculum was adjusted to a 0.5 MacFarland standard by the direct adjustment method. The *N. gonorrhoeae* strains were cultured at 36 ± 1 °C in 5% CO_2 atmosphere overnight. *N. gonorrhoeae* ATCC 49226 was used as the standard control.

The MICs of the following 20 antimicrobial agents were measured: penicillin G, ampicillin, amoxicillin, clavulanic acid-amoxicillin, cefpodoxime, cefdinir, cefixime, cefditoren, ceftriaxone, cefodizime, flomoxef, aztreonam, meropenem, spectinomycin, ciprofloxacin, levofloxacin, tosufloxacin, sitafloxacin, minocycline and azithromycin. The susceptibility or resistance of the isolate to each antibiotic was determined according to CLSI Document M100-S22 [2].The antimicrobial susceptibility data in this surveillance were compared with those in the first surveillance.

β-lactamase activity in the *N. gonorrhoeae* isolates was detected by the nitrocefin method (Cefinase Disk™; BD BBL™). The *N. gonorrhoeae* strains that were resistant to penicillin G (MIC: ≥2 μg/ml) and in which β-lactamase activity was detected were determined to be penicillinase-producing *N. gonorrhoeae* (PPNG). Among β-lactamase-non-producing strains, strains that were resistant to penicillin G (MIC: ≥2 μg/ml) were determined to be chromosomally mediated resistant *N. gonorrhoeae* (CMRNG).

The threshold MICs for antimicrobial resistance are assumed according to the following criteria: ≥2 μg/ml of penicillin G, ≥2 μg/ml of minocycline, ≥1 μg/ml of cefpodoxime, ≥1 μg/ml of ciprofloxacin or ≥0.5 μg/ml of azithromycin. The phenotypes of *N. gonorrhoeae* strains were classified by their resistance to antimicrobial agents.

3. Results

3.1. Number of specimens and isolated strains

Of the 152 specimens from 26 medical facilities, one was omitted because the patient's age was 14 years. The median age was 32 years (range: 18–65), and 39.7% and 33.8% of specimens were collected from patients in their 20s and 30s, respectively. The urethral discharge was described as purulent for 139 specimens and serous for 11. Of the 151 specimens from 151 patients, 103 strains could be cultured and identified as *N. gonorrhoeae*. From the 139 purulent specimens, 100 strains (71.9%) were isolated. Of these 103 strains, 36, 21, 16, 14, 9 and 7 strains were collected from the Kyushu, Chugoku, Kinki, Chubu, Tohoku and Tokyo areas, respectively.

3.2. Antimicrobial susceptibilities

Antimicrobial susceptibility testing was performed on all 103 isolated strains (Table 1). None was susceptible to penicillin G (MIC: ≤0.06). Only two strains (1.9%) were determined to be PPNG, and the MICs of penicillin G to these strains were 2 and 32 μg/ml. The MIC of clavulanic acid-amoxicillin MIC to PPNG strains was 0.5 μg/ml. Among β-lactamase-non-producing strains, 21 strains (20.8%) had higher MICs to penicillin G (MIC ≥2 μg/ml, range: 2–4 μg/ml) and were determined to be CMRNG. The MIC_{90} of four kinds of penicillins including penicillin G, ampicillin, amoxicillin and amoxicillin-clavulanic acid for β-lactamase-non-producing strains was 2 μg/ml.

The MICs of minocycline for two PPNG strains were also higher (8 and 16 μg/ml). The MIC range for minocycline in the β-lactamase-non-producing strains was ≤0.06–32 μg/ml and 3 strains (2.9%) were resistant to minocycline. (MIC: 16 or 32 μg/ml).

The susceptibility rates of all the strains to oral cephalosporins such as cefixime and cefpodoxime were 89.3% and 59.2% according to CLSI criteria [2], respectively. The susceptibility of cefixime evidently decreased compared to the strains collected in the first surveillance study. The MICs of the parenteral cephalosporins ceftriaxone and cefodizime were relatively low. The high-level ceftriaxone-resistant strains, such as Ohnishi's report [3], were not found in the surveillance. The MIC_{90} of ceftriaxone increased from ≤0.06 μg/ml in the first surveillance study to 0.125 μg/ml in this study. All 11 strains with a ceftriaxone MIC of 0.125 μg/ml had cefixime MICs of 0.25 or 0.5 μg/ml. However, 3 strains with cexifime MIC of 0.5 μg/ml had a ceftriaxone MIC of ≤0.06 μg/ml.

The MIC distribution for fluoroquinolones, such as ciprofloxacin, levofloxacin and tosufloxacin, showed bimodal. The MICs of these three antimicrobials for the 21 susceptible strains were ≤0.06 μg/ml. The MIC of sitafloxacin was lower than that of the other fluoroquinolones. Strains with ciprofloxacin MICs of ≤0.06 μg/ml had also sitafloxacin MIC of ≤0.06 μg/ml. Strains with ciprofloxacin MICs between 0.5 and 32 μg/ml showed sitafloxacin MIC of ≤0.06, 0.125 or 0.25 μg/ml. Only one strains with sitafloxacin MIC of 0.5 μg/ml and ciprofloxacin MIC of 64 μg/ml was newly identified.

Strains with resistance to spectinomycin were not identified in this study. No high-level azithromycin resistant strains were found, but two strains had an MIC of 1 μg/ml.

3.3. Phenotypes of antimicrobial resistance among N. gonorrhoeae strains

Table 2 shows the antimicrobial resistance phenotypes of the strains. Among the isolated strains, 83 (80.6%) met resistance criteria to one or more tested antimicrobials and 34 were resistant to a single antimicrobial (ciprofloxacin: 33 azithromycin 1). Forty-nine strains (47.5%) showed resistance to more than two types of antimicrobials such as penicillins, cephalosporins, tetracyclines, fluoroquinolones and macrolides.

Table 1
Antimicrobial MIC distribution for 103 *N. gonorrhoeae* strains.

Antibacterial agent	MIC (μg/ml)														
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256	MIC_{50}	MIC_{90}
Penicillin G		6	24	17	33	20	2			1				1	2
Ampicillin		8	14	11	29	33	7				1			1	2
Amoxicillin			21	8	20	52	1				1			2	2
Clavulanic acid-amoxicillin		1	21	10	25	46								1	2
Cefpodoxime	38	3	5	15	23	17	2							0.5	2
Cefdinir	41		2	25	35									0.5	1
Cefixime	42	8	42	11										0.25	0.5
Cefditoren	52	24	22	5										≤0.06	0.25
Ceftriaxone	92	11												≤0.06	0.125
Cefodizime	79	21	3											≤0.06	0.125
Flomoxef		3	20	8	22	41	9							1	2
Aztreonam	10	15	14	2	1	1	38	22						4	8
Meropenem	68	35												≤0.06	0.125
Spectinomycin								3	95	5				16	16
Ciprofloxacin	21			1		1	13	18	36	12	1			8	32
Levofloxacin	21			1	1	6	26	38	10					4	8
Tosufloxacin	21			3	4	31	14	19	11					2	16
Sitafloxacin	38	32	32	1										0.125	0.25
Minocycline	2	29	26	40	1			1	3	1				0.25	0.5
Azithromycin	15	44	39	3	2									0.125	0.25

4. Discussion

The guideline of the Japanese Association of Sexually Transmitted Infections recommends that gonococcal urethritis and cervicitis be treated with 1 g of ceftriaxone in a single intravenous dose, 1 g of cefodizime in a single intravenous dose and 2 g of spectinomycin in a single intramuscular dose [12]. This recommendation did not change during the first and second surveillance studies. The second surveillance study revealed that the patient demographics and clinical features of the urethral discharges were the same as in the first study, although the antimicrobial susceptibility data were different.

The prevalence of PPNG decreased compared to the first surveillance data. Previous to 2009, the prevalence of PPNG in Japan had been approximately 1% [13]. However, the prevalence was 7.2% in the first surveillance study. The second surveillance data showed the prevalence of PPNG to be 0.2%, which is a return to historic levels. We are not able to explain why the prevalence was higher in 2009–2010. One explanation is that patients who were infected in foreign countries were included in the first study. Penicillins are not available for the treatment of gonococcal infections in Japan. No strain was sensitive to penicillin G in this study. In addition, penicillin with a β-lactamase inhibitor cannot be recommended to treat gonococcal urethritis, because the MICs of amoxicillin are relatively higher and the MIC of clavulanic acid-amoxicillin was not low for PPNG strains (0.5 μg/ml).

The most serious problem for the treatment of gonococcal infections worldwide is the increase in cephalosporin-resistant strains. We did not find further evidence of high-level ceftriaxone-resistant strains in Japan. However, ceftriaxone-resistant strains were reported from France and Spain [4,5] and there are reports of ceftriaxone treatment failure in patients with *N. gonorrhoeae* strains isolated from the pharynx [14–17]. In particular, Swedish case-report described a *N. gonorrhoeae* strain with a ceftriaxone MIC of 0.25 μg/ml that was detected from the pharynx of a male patient after treatment with 250 mg and 500 mg of ceftriaxone; this patient was most likely infected through unprotected oral sex with a female partner in Japan [14]. This indicates that some women in Japan have *N. gonorrhoeae* strains with a high ceftriaxone MIC, but these strains were not found in any surveillance. Indeed, the MIC_{90} of ceftriaxone increased from ≤0.06 μg/ml in the first surveillance study to 0.125 μg/ml in this study. The strains with a ceftriaxone MIC of 0.125 μg/ml, those are termed as "less susceptible strains," increased. In addition, the strains sensitive to cefixime with an MIC ≤0.06 μg/ml, which Deguchi recommended as the breakpoint MIC of cefixime in Japan [18], decreased from 55.4% to 40.8%. These resistance or those less susceptibility in *N. gonorrhoeae* to cephalosporins are closely related to the mosaic structure of *penA* gene which codes for PBP2 [4,5,19,20]. Our data are evidence that this type of resistance has been spreading in Japan.

The MICs of fluoroquinolones did not differ between the first and second surveillance studies. The fluoroquinolone-resistant rates of strains have shown nearly the same level as in Tanaka's reports [21]. This likely means that physicians treating gonococcal infections are complying with the guidelines of the Japanese Society of Sexually Transmitted Infections. The activity of sitafloxacin remains stable in two our surveillances. As described previously [1], sitafloxacin has activity against ciprofloxacin-resistant *N. gonorrhoeae* strains. Sitafloxacin can be one of treatment options for untreatable *N. gonorrhoeae* strains. However, one strains with sitafloxacin MIC of 0.5 μg/ml was found and further observation or clinical study would be necessary.

Table 2
The phenotypes of antimicrobial resistance among *N. gonorrhoeae* strains.

Antimicrobials					Numbers
Penicillin G	Minocycline	Cefpodoxime	Ciprofloxacin	Azithromycin	
			R		33
				R	1
R	R				1
R			R		1
		R	R		22
	R		R		3
			R	R	1
R		R	R		17
R	R		R		1
R		R	R	R	3

R: Resistance to antimicrobial agents that was determined by the criteria below. MICs of ≥2 μg/ml of penicillin G, ≥2 μg/ml of minocycline, ≥1 μg/ml of cefpodoxime, ≥1 μg/ml of ciprofloxacin or ≥0.5 μg/ml of azithromycin.

In both the first and second surveillance study, high-level resistant strains to azithromycin were not found. The main mechanism for azithromycin-resistance in *N. gonorrhoeae* is mutations of the macrolide target, 23S *r*RNA as A2059G or C2611T mutations [7–10,20]. *N. gonorrhoeae* has four 23S *r*RNA alleles and the resistance to azithromycin depends on the numbers of alleles with mutations [20]. Unemo described that the azithromycin MIC increase to 4,096 μg/ml in strains with A2059G mutations in three or four alleles, but the MICs of strains with the mutation in one allele are not changed as wild-type strains [10]. Through this theory, at least two strains (1.9%) with an azithromycin MIC of 1 μg/ml have mutations in more than 2 alleles of the 23S *r*RNA. In other Japanese surveillance data, 3.6% (7/193) of strains in the Kyoto and Osaka areas or 6.6% (8/122) of strains in the Tokyo area were resistant to azithromycin (>0.5 μg/ml) [6,22]. In the Tokyo area, higher-resistant strains were found (one and two strains with MIC 16 and 8 μg/ml, respectively). Yasuda et al. showed clinical study using azithromycin 2g in Japan and strains with MIC 2 or 4 μg/ml of azithromycin remained after the treatment [23]. Among strains with MIC 1 or 0.5 μg/ml eradication rates were 58% (7/12) and 97% (31/32), respectively. Even if azithromycin 2g is used, a high dose of azithromycin could covered only strains with MIC ≤0.5 μg/ml and we wonder that azithromycin-resistance would increase.

Table 2 shows the numbers of strains according to the antimicrobial resistant phenotype. Among 103 strains, 20 were classified as "not resistant" to all antimicrobials. These strains were 20 of 21 the strains with ciprofloxacin MIC ≤0.06 μg/ml (another one had an azithromycin MIC of 0.5 μg/ml). Regarding *N. gonorrhoeae* strains in Japan, the susceptibility for fluoroquinolone, which are less frequently used now is important. According to Tapsall's criteria for multi-drug resistant *N. gonorrhoeae* (MDR-NG) [24], 42 strains (40.1%) met the criteria of MDR-NG. In 2009–2010, 39.8% of the strains were classified as MDR-NG, which was quite similar to the proportion in 2011–2012.

The next Japanese antimicrobial surveillance initiative for *N. gonorrhoeae* is planned for 2016. The increase in cephalosporin-resistance is more prominent worldwide. Dual therapies, such as ceftriaxone plus azithromycin or ceftriaxone and effective quinolones are recommended and being used worldwide. The recommendation of Japanese Association of Sexually Transmitted Infections for treating gonococcal infections has remained the same. The purpose of the next surveillance study is to evaluate the appropriateness of the present recommendation for the treatment of gonococcal infection and to determine whether the recommendation should be modified.

Conflicts of interest

Mitsuru Yasuda has received donation from Astellas Pharma Inc. Akira Watanabe has received speaker's honorarium from MSD K.K., Glaxo SmithKline K.K., Shionogi & Co., Ltd., Daiichi Sankyo Co., Ltd., Taisho Toyama Pharmaceutical Co., Ltd. and Pfizer Japan Inc.; grant support from Kyorin Pharmaceutical Co., Ltd., Shionogi & Co., Ltd.

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