

# ANTIMICROBIAL SUSCEPTIBILITIES OF *NEISSERIA GONORRHOEAE* STRAINS FROM MALE URETHRITIS IN JAPAN - THE FIRST NATIONAL SURVEILLANCE

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## Background

Resistance of *Neisseria gonorrhoeae* to any antimicrobials emerged and spread in the world. Especially, antimicrobials as penicillins, narrow-spectrum cephalosporins, tetracyclines, fluoroquinolones, cannot be used as the first line treatment to gonorrhea in Japan. Only three drugs as ceftriaxone, cefodizime and spectinomycin are recommended to gonococcal infection in the guideline of the Japanese Society for Sexually Transmitted Infection. In addition, ceftriaxone-resistant *N. gonorrhoeae* strain was emerged in Kyoto, Japan and susceptibility of cefixime has been decreased. In this situation, necessity of the national surveillance for susceptibilities of *N. gonorrhoeae* has been emphasized.

## Purpose

In the three Japanese societies as Japanese Society of Chemotherapy, Japanese Association of Infectious Diseases and Japanese Society of Clinical Microbiology conducted the first national surveillance for *N. gonorrhoeae* to know the antimicrobial susceptibilities of *N. gonorrhoeae* in Japan.

## Materials and methods

### 1) Study design, patients and participating facilities

Patients: male patients older than 16 years with urethral discharge and symptoms of urethritis. The patients were diagnosed with gonococcal urethritis by clinicians.  
The period of specimen collection: **from April 2009 to October 2010**.  
Participated facilities: **51 medical facilities** in Japan.  
Ethics: The study was approved by the ethical committee of each facility or by the ethical committee of the specific non-profit organization CREC net, Kitakyushu, Japan. The clinicians explained the purpose of the study to the patients orally or through written documents and obtained the written consent of each patient.

### 2) Specimens, isolation of *N. gonorrhoeae* strains and antimicrobial susceptibility testing

The **discharge from the urethral meatus** was collected with a sterilized cotton swab, placed in transport agar (BD BBL Cultureswab EZII™, Becton Dickinson) and sent to the Laboratory of Infection Control & Research Center for infections and Antimicrobials The Kitasato Institution, Tokyo, Japan, at room temperature.  
*N. gonorrhoeae* strains were isolated and the isolated strains were identified by a nucleic acid amplification test (NAAT, Amplicore STI-1, Rosh).

The antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) Document M7-A8 (M100-S21) , and the minimum inhibitory concentrations (MICs) were determined **by the agar dilution method**. *N. gonorrhoeae* ATCC 49226 was used as the standard control.

The MICs of **18 antimicrobial agents** (Table 1) were measured. **β-lactamase activity** in the isolated *N. gonorrhoeae* was detected by the nitrocefin method (Cefinase Disk™, Becton Dickinson). The *N. gonorrhoeae* strains which were resistant to penicillin G (MIC: ≥2 µg/ml) and were detected β-lactamase activity were determined as PPNG. Among β-lactamase non-producing strains, strains which were resistant to penicillin G (MIC: ≥2 µg/ml) were determined as 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, ≥0.5 µg/ml of cefixime, ≥1 µg/ml of ciprofloxacin or ≥0.5 µg/ml of azithromycin**, *N. gonorrhoeae* strains was classified by combination of resistance to antimicrobial agents.

Table 1 Antimicrobial MIC distribution for 83 *N. gonorrhoeae* strains

Antibacterial agent	MICs (µg/ml)													
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	MIC <sub>50</sub>	MIC <sub>90</sub>
Penicillin G	1	12	9	8	25	19	4	3	1		1		1	4
Ampicillin		12	8	6	24	24	3	1	3	1		1	1	4
Amoxicillin - clavulanic acid			19	7	31	26							1	2
Cefpodoxime	34	5	5	7	21	10	1						0.25	2
Cefdinir	42	1	4	28	8								≤0.06	0.5
Cefixime	46	13	23	1									≤0.06	0.25
Cefditoren	29	22	25	7									0.125	0.25
Ceftriaxone	76	7											≤0.06	≤0.06
Cefodizime	52	24	7										≤0.06	0.125
Flomoxef		5	14	11	9	38	6						2	2
Aztreonam	3	17	11	10	3	4	23	12					1	8
Spectinomycin							9	65	9				8	16
Ciprofloxacin	18					3	10	19	27	6			8	16
Levofloxacin	18					10	24	30	1				4	8
Tosufloxacin	18				1	9	38	7	10				4	16
Sitafloxacin	30	16	37										0.125	0.25
Minocycline	5	14	16	36	7		1	1	3				0.5	1
Azithromycin	10	44	26	1		2							0.125	0.25

## Results

### 1) Number of specimens, isolated strains, patients characteristics

In this study, **156 patients** were participated (median age : **29 years old**, range: **16 to 66 years old**). From specimens, **144 were positive for *N. gonorrhoeae* by NAAT**, but only **84 strains could be isolated by culture** .

### 2) Antimicrobial susceptibilities (Table 1).

Among the 84 isolated strains, **83 strains** were available for antimicrobial susceptibility testing. Rates of **PPNG** and **CMRNG** were **7.2%** and **26.5%**, respectively. The value of MIC<sub>90</sub> of minocycline in the β-lactamase-producing or and non-producing strains were 16 µg/ml or 0.5 µg/ml, respectively. The susceptibility rates of all the strains to cefixime and cefpodoxime, were 98.8% and 61.4%, respectively. Only one strain had a MIC of 0.5 µg/ml for cefixime. None of the strains were resistant to ceftriaxone, but 7 strains (8.4%) had a MIC of 0.125 µg/ml for this antimicrobial. The MIC distribution for fluoroquinolones, such as ciprofloxacin, levofloxacin and tosufloxacin, showed bimodal. Eighteen strains were susceptible to fluoroquinolones and these strains were β-lactamase non-producing. Sitafloxacin showed the strongest activity to *N. gonorrhoeae* (**details are shown in P1. 017**).

No strains with spectinomycin-resistance were found. Regarding azithromycin, 2 strains had a MIC of 2 µg/ml, but no high-level resistant strains were found.

### 3) Antimicrobial resistance patterns among *N. gonorrhoeae* strains

*N. gonorrhoeae* strains could be classified by the combinations of resistance to antimicrobial agents, then 65 *N. gonorrhoeae* strains met the resistance criteria for some antimicrobials (Table 2)

Table 2 Classification of antimicrobial resistance among *N. gonorrhoeae* strains

Combinations of resistance to antimicrobial agents	n
ciprofloxacin	22
ciprofloxacin + penicillin G	6
ciprofloxacin + penicillin G,+ minocycline	3
ciprofloxacin + penicillin G,+ minocycline + cefpodoxime	1
ciprofloxacin + penicillin G,+ cefpodoxime	14
ciprofloxacin + penicillin G,+ cefpodoxime + cefixime	1
ciprofloxacin + penicillin G,+ azithromycin	2
ciprofloxacin + cefpodoxime	15
ciprofloxacin + azithromycin	1
total	65

## Discussion and conclusion

The first national surveillance of the antimicrobial susceptibility of *N. gonorrhoeae* was performed and antimicrobial susceptibilities of 83 strains to 18 antimicrobials were examined. Compared with previous reports in Japan, the percentage of PPNG in this study was higher, but percentage of CMRNG was almost same. Only one strain was resistant to cefixime according to category by CLSI. None of the strains were resistant to ceftriaxone, but 8.4% of tested strains had a MIC of 0.125 µg/ml. In fluoroquinolones, sitafloxacin had the strongest activity to *N. gonorrhoeae*. In all strains, 51.8 % were resistant to more than 2 antimicrobials and all of these isolates were resistant to ciprofloxacin. Currently, *N. gonorrhoeae* is one of the most difficult bacterial infections to treat in Japan. The surveillance of *N. gonorrhoeae* for antimicrobial susceptibility should be continued.

**The contents of this study will be published in *Journal of Infections and Chemotherapy* (available as “ Online first” on website).**  
<http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s10156-013-0637-2>