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Surveillance for antimicrobial resistant *Neisseria gonorrhoeae* in Japan - disseminating of a ceftriaxone resistant clone.

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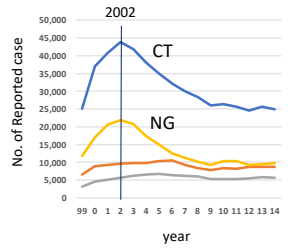
Introduction

- The infectious disease surveillance system in Japan mainly consists of (1) pathogen reporting (laboratory-based surveillance) and (2) patient reporting.
- Information about infectious diseases in Japan is collected and published, and occurrence and trends are assessed, based on reporting from physicians.
- Surveillance for *Neisseria gonorrhoeae* infection is conducted as “patient reporting surveillance” in STI sentinel surveillance (CT, NG, genital Herpes, condyloma acuminatum) at STI sentinel sites.

Introduction



genital Herpes
condyloma acuminatum

- STI sentinel sites (approx. 1,000 medical facilities of obstetrics and gynecology, urology, dermatology, etc. across Japan); should be reported 4 STI and submitted on a monthly basis.

  Limitation:

  Selection of sentinel site is depends on prefectural governments.

  Acquisition of personal information is restricted.

- The number of patients peaked in 2002 and then declined.
- Now It is stable at about 10,000 from 1,000 sentinel sites.
- The sentinel surveillance does NOT include any requirements to perform antimicrobial susceptibility testing.

Introduction

- In 2009, we identified the first high-level ceftriaxone-resistant *Neisseria gonorrhoeae* (H041) in Kyoto; MIC of ceftriaxone (2 μg/mL).

  Ohnishi M et al. Emerg Infect Dis. 2011 17:148-9.

  Ohnishi M et al. Antimicrob Agents Chemother. 2011 55:3538-45.

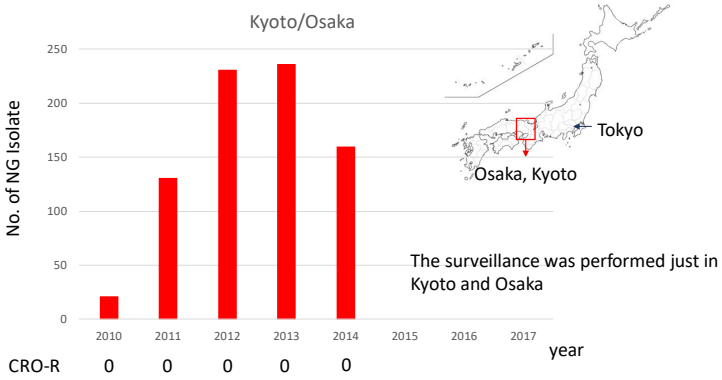
- After the identification of H041, NIID initiated a pathogen-based surveillance (antimicrobial resistance and molecular typing) of *Neisseria gonorrhoeae* in Kyoto and its neighboring prefecture Osaka.

  Collecting mainly urethral discharge from private clinics

  → Isolation, identification and Susceptibility testing in NIID

  Shimuta K et al. Antimicrob Agents Chemother. 2013 57:5225-32.

Pathogen-based surveillance 2010~1014 (NIID)



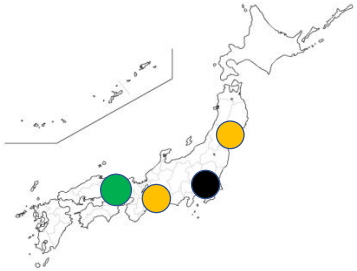
NOT identify the ceftriaxone-resistant *N. gonorrhoeae*.

Pathogen-based surveillance for *Neisseria gonorrhoeae* (from 2015)

In Japan, several research groups collected *N. gonorrhoeae* and published their own data independently.

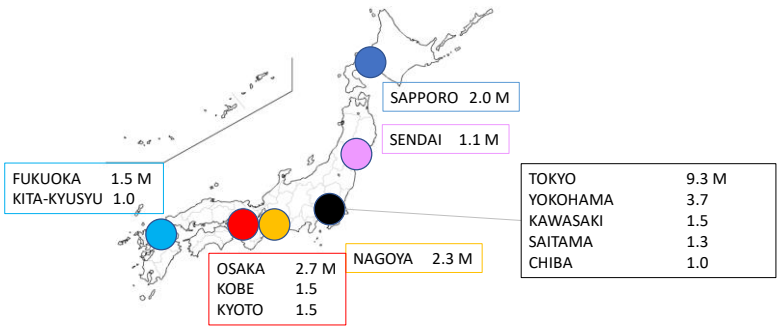
- Gifu University (Prof. Deguchi group, Dr. Yasuda M)
- Toho University (Prof. Kobayashi)
- Kobe University (Prof. Arakawa group, Dr Osasa K)

Japan Agency for Medical Research and Development (AMED) initiated supports for integrating the data from NIID and the other groups.

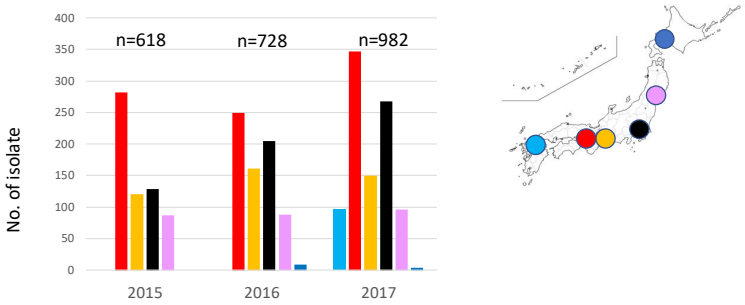


Pathogen-based surveillance for *Neisseria gonorrhoeae* (from 2015)

Pathogen-based surveillance system is focusing on Major cities

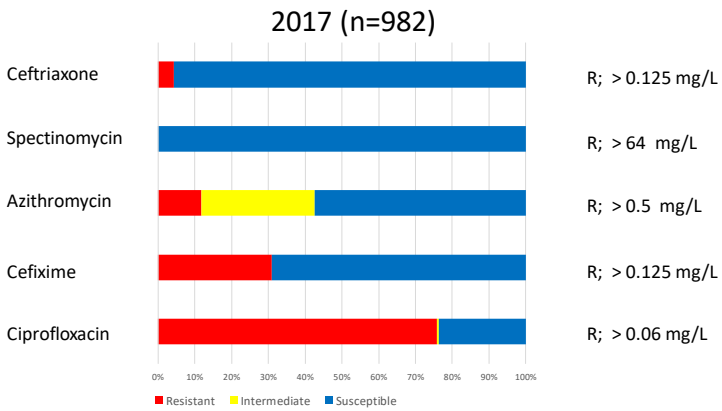


AMR surveillance for *Neisseria gonorrhoeae*



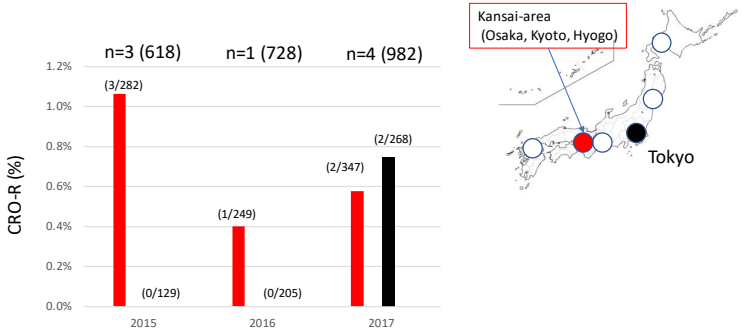
- The number of clinics and commercial Labs participating in this system has increased.
- The number of strains has increased, and the area has expanded.
- JAPAN Agency for Medical Research and Development (AMED) continue to support to the surveillance (2018-2020).

The antimicrobial susceptibility profile



AMR surveillance for *Neisseria gonorrhoeae*

Ceftriaxone-resistant *N. gonorrhoeae* MIC ≥ 0.5



- Ceftriaxone-resistant *N. gonorrhoeae* (MIC= 0.5 or more) were isolated in Osaka/Hyogo and Tokyo.
- Percentage is less than 1% but in the case of these strains are identical or clonal, “continuously isolation in different areas” might a serious situation.

Nakayama SI et al. Antimicrob Agents Chemother. 2016 60:4339-41.
Lee KI et al. in preparation

AMR surveillance for *Neisseria gonorrhoeae*

Characterization of FC428 isolated in Jan 2015 in Osaka

PPNG (penicillinase-producing NG)

PPNG is rare in Japan (2.4%, 15 in 618 (2015)).

In Nanjing China (2012), 31% were PPNG.
Chen SC, et al. Sexually Transmitted Diseases. 40:872–876

Molecular type

MLST = ST1903

NG-MAST = ST3435

Before FC428 isolation, MLST 1903 or NG-MAST 3435 has never been found in our Japanese strain collection (MLST (n=1,327) and NG-MAST (n=1,476)).

MLST ST1903 strains were identified, for example, in Thailand among PPNG.

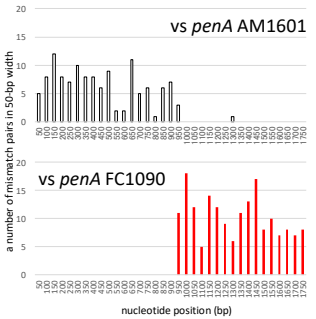
Resistant determinant (*penA* type)

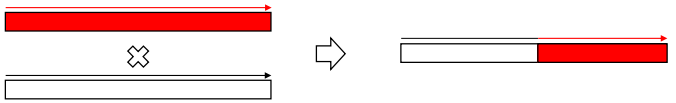
penA-60.001 (Novel *penA* sequence)

Nakayama SI et al. Antimicrob Agents Chemother. 2016 60:4339-41.
Nakayama SI et al. Antimicrob Agents Chemother. 2012 Feb; 56(2): 916-920.

Chimeric structure of *penA*-60.001 (FC428)

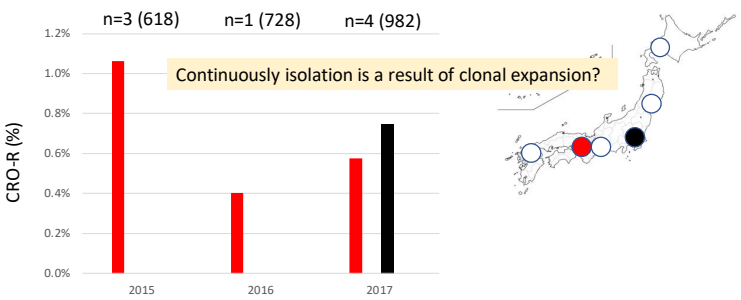
- We could isolate several commensal *Neisseria* spp. with high MIC of ceftriaxone.
- We compared *penA*-60.001 with *penA* of *N. cinerea* AM1601 (ceftriaxone MIC = 1 mg/L), and a wild type *penA* of *N. gonorrhoeae*.





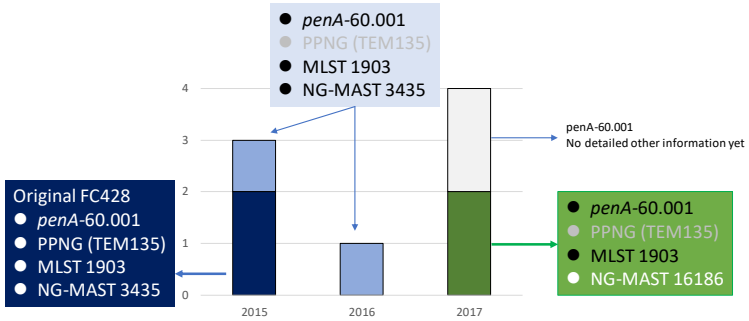
- We could speculate that *in vivo* recombination created the novel *penA* sequence.

Clonal expansion and spread of the ceftriaxone-resistant *Neisseria gonorrhoeae*



All other resistant strains encoded *penA*-60.001.

Genome sequence of FC428 and the other resistant strains with *penA*-60.001



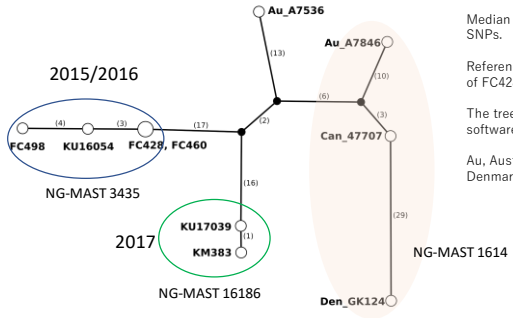
- Based on genetic markers, penA-60.001 strains were divided into 3 types
- Original, PPNG Plasmid negative, or PPNG Plasmid negative and different NG-MAST

Internationally Disseminated Ceftriaxone-Resistant *Neisseria gonorrhoeae* Strains

	Year	Place	MLST/NGMAST(porB.tbpB)/PPNG
FC428/FC460	2015	Osaka	1903/3435(1053.21)/PPNG
FC498/ KU16054	2015/2016	Osaka/Hyogo	1903/3435(1053.21)/non-PPNG
KM383/KU17039	2016/2017	Osaka/Hyogo	1903/ 16186(3457/2601) /non-PPNG
GK124	2017	Denmark	1903/ 1614(1053.33) /PPNG
47707	2017	Canada	1903/ 1614(1053.33) /PPNG
A7846	2017	Australia	1903/ 1614(1053.33) /PPNG
A7356	2017	Australia	1903/ 15925(9300/21) /PPNG
F90	2017	France	1903/3435(1053.21)/NA

Nakayama SI et al. AAC, 2016 ; Terkelsen D et al. Euro Surveill 2017; Lefebvre B et al. EID, 2018; Lahra MM et al. EID, 2018
Poncin T et al.Euro Surveill, 2018

Phylogenomic analysis of *penA*-60.001 type Ceftriaxone-resistant *N. gonorrhoeae*



Median joining tree of the core genome SNPs.

Reference: Complete sequencing genome of FC428.

The tree was generated by using PopART software v. 1.7.

Au, Australia; Can, Canada; Den, Denmark.

- The Japanese ceftriaxone-resistant *penA*-60.001 isolates were subdivided into two clades.
- Isolates from different countries could not form a clear cluster even in the same NG-MAST strains.

Summary

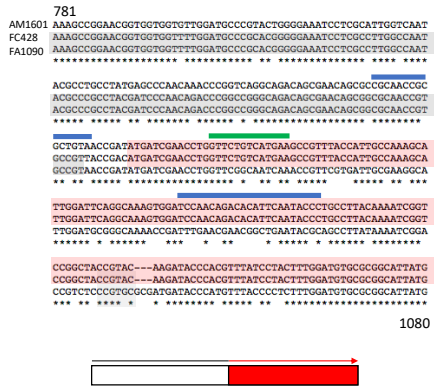
In Japan, pathogen-based surveillance launched in 2015.
Now we try to expand the system.

Using the system, we could detect a new ceftriaxone resistant *N. gonorrhoeae* FC428 and its derivatives in Osaka/Hyogo and Tokyo. The positive rate is still 1% or below.

penA-60.001 is a chimera. Commensal *Neisseria* spp. might play a big role for creating new resistant genes.

N. gonorrhoeae FC428-like strain are spreading worldwide.
Genetically, these are clonal, but already had a diverse population.
It might indicate unknown focus/outbreak region(s).

penA of FC428 (*penA*-60.001)



Thank you for your attention!

Acknowledgements

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