

High-level azithromycin resistance in *Neisseria gonorrhoeae* clinical isolates in Nanjing, China, 2013-2014

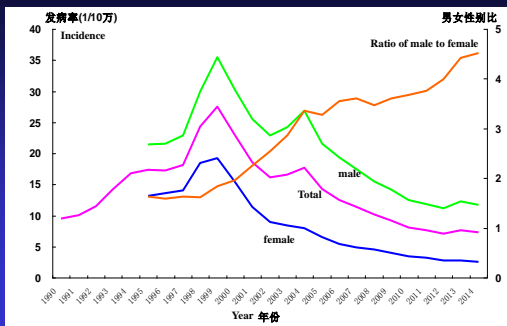


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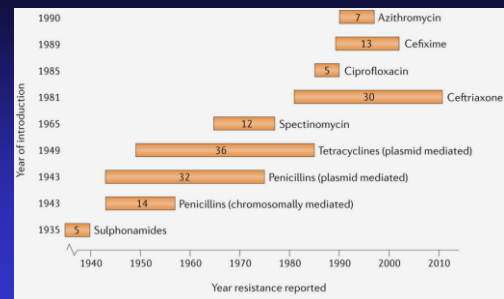
Background

- Gonorrhea is one of the most common bacterial sexually transmitted infections (STIs) worldwide.
- WHO estimated that in 2008, 106.1 million of new cases of gonorrhea occurred globally
- Nearly 100,000 cases of gonorrhea were reported in 2014, making it the fifth most commonly reported communicable disease in China

Incidence of gonorrhea in China



Antimicrobial resistance in *N. gonorrhoeae*



From: Goire N, et al. Molecular approaches to enhance surveillance of gonococcal antimicrobial resistance. Nat Rev Microbiol. 2014; 13(3): 223-229.

Surveillance of *N. gonorrhoeae* AMR

- Gonococcal strains with reduced susceptibility or resistance to ESCs have emerged
- Treatment failures with the oral agent cefixime and injectable ceftriaxone have been documented in several countries.
- Gonococcal strains with high level resistance to ceftriaxone (MIC 1-2µg/ml) have also been isolated from pharyngeal, urethral and rectal specimens

Case reports of treatment failure

- Clinical failures to treat pharyngeal gonorrhea with injectable ceftriaxone have been documented in several countries.

1. Tapsall J, et al. J Med Microbiol 2009; 58:683-7.
2. Ohnishi M, et al. Emerg Infect Dis 2011; 17:148-9.
3. Unemo M, et al. Euro Surveill 2010; 16: 19792.
4. Unemo M, et al. Euro Surveill 2012; 17: 20200.
5. Golparian D, et al. Euro Surveill 2014; 19: 20862.

Action plans



Treatment Guidelines

US CDC 2015	UK 2011	Canada 2013	China 2014
Ceftriaxone 250mg	Ceftriaxone 500mg	Ceftriaxone 250mg or	Ceftriaxone 250mg
Plus	Plus	Cefixime 800mg, plus	or
Azithromycin 1g or	Azithromycin 2g	Azithromycin 1g	Spectinomycin
Doxycycline (7d)			

1. Workowski KA, Bolan GA. Sexually Transmitted Diseases Treatment Guidelines, 2015. MMWR Recomm Rep. 2015; 64 (RR-03) :1-137.
 2. Bignell C, Fitzgerald M; Guideline Development Group. UK national guideline for the management of gonorrhoea in adults. Int J STD AIDS 2011; 22:541-7.
 3. Public Health Agency of Canada. Canadian guidelines on sexually transmitted infections. <http://www.phac.aspc.gc.ca/std/mte/sti/its/gsti-0/cits/section-5-6-eng.php#toc-61210473>.

Objectives

- Emergence of high-level azithromycin resistance in *N. gonorrhoeae* have been reported in many countries.
- No high-level azithromycin-resistant isolates of *N. gonorrhoeae* have been reported in China.
- Azithromycin has been added into antibiotic susceptibility panel since 2013.
- In this study, we present the results of antimicrobial susceptibility testing of 384 gonococcal strains isolated between 2013 and 2014 and evidence of high-level azithromycin resistance in Nanjing, China.

1. Chisholm SA, et al. Emergence of high-level azithromycin resistance in *Neisseria gonorrhoeae* in England and Wales. J Antimicrob Chemother 2009; 64:353-8
 2. Katz AR, et al. *Neisseria gonorrhoeae* with high-level resistance to azithromycin: Case-report of the first isolate identified in the United States. Clin Infect Dis 2012; 54:841-3.
 3. Bercot B, et al. High-level azithromycin-resistant *Neisseria gonorrhoeae*: clinical isolate in France, March 2014. Euro Surveill 2014; 19: 20951.
 4. Wamso M, et al. First three *Neisseria gonorrhoeae* isolates with high-level resistance to azithromycin in Sweden: a threat to currently available dual-antimicrobial regimens for treatment of gonorrhoea? Antimicrob Agents Chemother 2014; 58:624-5.

Materials and Methods

Year	No. Screened	No. Positive	Positive rate (%)
2013	432	187	43.28
2014	473	197	41.63
Total	905	384	42.43

384 *N. gonorrhoeae* isolates were isolated sequentially from male adults with symptoms/signs of urethritis attending a single STD clinic in Nanjing, China between 2013 and 2014.

MIC Determination

- Minimum inhibitory concentrations (MICs; µg/ml) of *N. gonorrhoeae* to penicillin, tetracycline, ciprofloxacin, spectinomycin, azithromycin, cefixime and ceftriaxone were determined by agar gel dilution according to the Clinical and Laboratory Standards Institute (CLSI).
- All antibiotics were purchased from Sigma (Aldrich, Saint Louis, MO, USA).

Quality Controls

2013		2014	
ATCC49226	Ceftriaxone S	ATCC49226	
WHO A	Spectinomycin R	WHO G	TRNG/ Penicillin LS
WHO G	TRNG	WHO K	Ciprofloxacin R Ceftriaxone LS
WHO J	Ciprofloxacin R /PPNG	WHO O	Spectinomycin R/PPNG
CTXR	Ceftriaxone R	WHO P	Azithromycin R
		CTXR	Ceftriaxone R

MIC breakpoints

MIC interpretative criteria for categorizing *N.gonorrhoeae* into susceptibility categories according to CLSI, with exception of azithromycin from EUCAST

Antimicrobial	MIC(mg/L)		
	Susceptible	Intermediate susceptible	Resistant
Penicillin	≤0.06	0.12-1	≥2
Tetracycline	≤0.06	0.12-1	≥2
Ciprofloxacin	≤0.06	0.12-0.5	≥1
Spectinomycin	≤32	64	≥128
Ceftriaxone	≤0.25	-	-
Cefixime	≤0.25	-	-
azithromycin	≤0.25	0.5	≥1

1. Clinical and Laboratory Standards Institute. 2012. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second International Supplement M100-S22. CLSI, Wayne, PA, USA.
2. Breakpoint tables for interpretation of MICs and zone diameters: version 1.3. European Committee on Antimicrobial Susceptibility Testing. Accessed January 15, 2012. http://www.eucast.org/clinical_breakpoints/

Antimicrobial susceptibility of *N. gonorrhoeae* (n=384) from Nanjing, 2013-2014

Antimicrobial	No.(%)			MIC(mg/L)		
	Susceptible	Intermediate	Resistant	Range	MIC ₅₀	MIC ₉₀
Penicillin	0	27.9	72.1	0.125->8	2	>8
Tetracycline	0	14.1	85.9	0.5->16	4	>16
Ciprofloxacin	0	0	100	1->8	>8	>8
Spectinomycin	100	0	0	8-32	16	32
Ceftriaxone	100	-	-	≤0.002-0.125	0.03	0.06
Cefixime	100	-	-	≤0.002-0.25	0.03	0.06
azithromycin	40.3	27.3	32.3	≤0.016->256	0.5	64

Reduced susceptibility to extended-spectrum cephalosporins

- 16 isolates (4.3%) had elevated MICs (≥0.125 µg/ml) for cefixime
- 38 isolates (10.1%) had an MIC of 0.125 µg/ml for ceftriaxone

Molecular typing of PPNG and TRNG

- Production of β-lactamase was identified by paper acidometric testing.
- β-lactamase producing plasmids and tetM gene containing plasmids were characterized using PCR

1. Turner A, Gough KR, Leeming JP: Molecular epidemiology of tetM genes in *Neisseria gonorrhoeae*. Sex Transm Infect 1999, 75:60-66.
2. Palmer HM, Leeming JP, Turner A: A multiplex polymerase chain reaction to differentiate beta-lactamase plasmids of *Neisseria gonorrhoeae*. J Antimicrob Chemother 2000, 45:777-782.

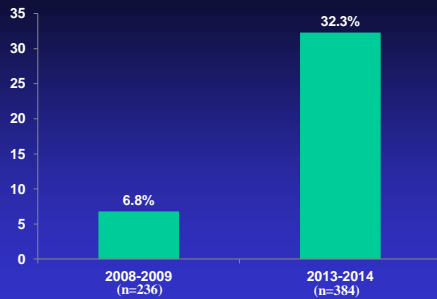
Prevalence of PPNG and TRNG

Year	PPNG		TRNG	
	Positive No.	%	Positive No.	%
2013	87	46.5	68	35.4
2014	93	47.2	65	33
Total	180	46.9	133	34.6

Molecular typing of PPNG and TRNG

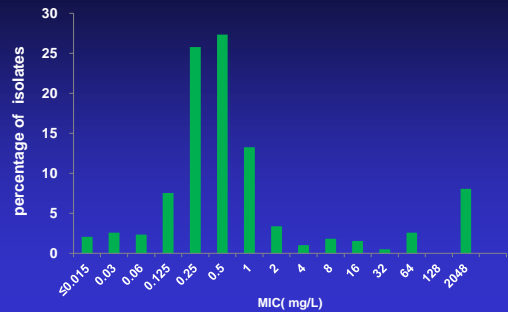
Year	β-lactamase encoding plasmid (n=180)		tetM encoding plasmid (n=133)	
	Asian type	African type	Dutch type	American type
2013	65(74.7%)	22(25.3%)	61(89.7%)	7(10.3%)
2014	67(72%)	26(28%)	61(93.8%)	4(6.2%)
	132(73.3%)	48(26.7)	122(91.7%)	11(8.3%)

Increased azithromycin resistance in Nanjing



Yuan LF, et al Resistance to azithromycin of *Neisseria gonorrhoeae* isolates from 2 cities in China, Sex Transm Dis, 2011, 38(8): 764-768.

Azithromycin MIC distribution (2013-2014, n=384)

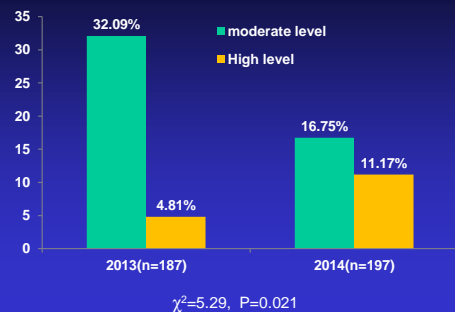


Azithromycin resistance level

- Isolates with AZM MICs of 2 to 8 µg/ml were considered moderately resistant
- Isolates with MICs of 256 µg/ml were considered highly resistant

Allen VG, Seah C, Martin I, Melano RG. 2014. Azithromycin resistance is coevolving with reduced susceptibility to cephalosporins in *Neisseria gonorrhoeae* in Ontario, Canada. Antimicrob Agents Chemother 58:2528-34.

High-level AZM resistance increased



Molecular typing of azithromycin resistant isolates (n=124)

- 69 different NG-MAST STs were identified among 124 isolates.
- The most frequent types found among high-level azithromycin resistant isolates were ST1866 (n=15, %), followed by ST5309 (n=5), and ST11910 (n=4).
- The most prevalent types found among moderate resistant isolates were ST3356 (n=10), ST270 (n=5) and ST4007 (n=4)

NG-MAST STs

- The previously reported STs (ST1133, ST1136, ST1740, ST6798 and ST6800) associated with high level resistance to azithromycin in other countries were not identified in our study
- ST1866, ST3356, ST1766 have been identified in previous study in Nanjing

Conclusions

- A high prevalence of *N. gonorrhoeae* isolates displaying resistance to penicillin, tetracycline, azithromycin and ciprofloxacin and reduced susceptibility to extended-spectrum cephalosporins has been found in Nanjing, China.
- High-level azithromycin resistance in *N. gonorrhoeae* has emerged in Nanjing, China.

Acknowledgements

- This work was supported, in part, by NIH grant AI084048.
- We are very grateful to Jordi Camara for providing the ceftriaxone-resistant *N. gonorrhoeae* isolate.
- We are very grateful to Magnus Unemo for providing WHO reference strains

