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SHORT REPORT

Emergence and Characterization of a Ceftriaxone-Resistant *Neisseria gonorrhoeae* FC428 Clone Evolving Moderate-Level Resistance to Azithromycin in Shenzhen, China

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Abstract: We here described a ceftriaxone-resistant *Neisseria gonorrhoeae* FC428 clone (YL201) with moderate-level resistance to azithromycin in Shenzhen, South China in 2020. The NG-STAR type of YL201 is ST2238, containing a mosaic *penA*-60.001 allele, which is a typical characteristic of FC428 clone. YL201 harbours four copies of the 23S rRNA C2611T mutation, conferring moderate-level resistance to azithromycin. The MLST type is ST1600, identical with two *N. gonorrhoeae* FC428 clones identified in Hangzhou. Genome-wide phylogeny analysis demonstrates that YL201 is clustered with other FC428 clones from Hangzhou (South-east China) and Chengdu (South-west China). Isolates within this cluster have relatively higher MIC for ceftriaxone and display closely related MLST STs (ST1600 and ST7363) but are different from the ST of typical FC428 clone (ST1903). As ST1600 and ST7363 are common STs in Shenzhen, the further spread of FC428 clones may increase the severity of gonococcal resistance. In summary, identifying a multidrug-resistant (MDR) *N. gonorrhoeae* isolate in Shenzhen showed FC428 clones have undergone further transmission in China and presented more extensive and concerning antimicrobial resistance (AMR) characteristics during the spread.

Keywords: *Neisseria gonorrhoeae*, ceftriaxone, azithromycin, phylogeny, antimicrobial resistance

Introduction

With the emerging resistance of *N. gonorrhoeae* to nearly all antibiotics, effective antimicrobials for gonorrhoea have become increasingly scarce, including first-line dual therapy with ceftriaxone (CRO) and azithromycin (AZM) recommended by WHO.[1] To date, the MDR *N. gonorrhoeae* isolates have been reported in Ireland,[2] Denmark,[3] UK[4] and Australia.[5] In China, *N. gonorrhoeae* isolates with decreased susceptibility or resistance to both CRO and AZM have been reported,[6–8] and here in Guangdong Province (South China), we describe a ceftriaxone-resistant *N. gonorrhoeae* FC428 clone with a higher level of macrolide resistance than previously reported.

The patient was a heterosexual male in his late twenties. He visited the sexually transmitted diseases clinic in Shenzhen Center for Chronic Disease Control in August, 2020 with urethritis symptoms. He reported this was his third infection, and all infections were due to sexual intercourse with commercial sex workers. *N. gonorrhoeae* (isolate YL201) was cultured from urethral secretions.

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Table 1 Phenotypic Characteristics and Molecular Characteristics of Isolates Related to the FC428 Clone

Isolate	Country	Patient Gender	Sexual Orientation	Sampling Site	MIC (mg/L)						PPNG	bla_{TEM} Type	Reference
					CRO	TET	SPT	AZM	CIP	PEN			
YL201	China	Male	Hetero	Urethral	0.75	4	12	12	32	1.5	Yes	1	This study
BJ16148	China	Male	Hetero	Urethral	0.5	4	16	0.25	>32	NA	NA	NA	[12]
GC185	China	NA	NA	Urethral	1	NA	NA	0.5	NA	NA	Yes	135	[6]
GC250	China	Male	hetero	Urethral	0.5	NA	NA	2	NA	NA	Yes	1	[6]
SC18-25	China	Male	Hetero	Urethral	≥0.5	NA	16.0	0.5	≥16.0	2	No	No	[13]
SC18-26	China	Male	Hetero	Urethral	≥0.5	NA	16.0	1.0	≥16.0	≥8.0	Yes	1	[13]
SC18-68	China	Male	Hetero	Urethral	≥0.5	NA	16.0	0.5	≥16.0	4.0	No	No	[13]
SRRSH214	China	NA	NA	Urethral or vaginal	1	NA	NA	0.1	NA	NA	NA	NA	[7]
SRRSH229	China	NA	NA	Urethral or vaginal	1	NA	NA	0.3	NA	NA	NA	NA	[7]
SZ2017191	China	NA	NA	Urethral	0.5	8	16	0.5	16	1	NA	NA	[14]
FC428	Japan	Male	NA	Urethral	0.5	0.5	8	0.25	>32	>32	Yes	135	[15]
FC460	Japan	Male	NA	Urethral	0.5	0.5	8	0.25	>32	>32	Yes	NA	[15]
FC498	Japan	Male	NA	Urethral	0.75	NA	8	0.5	>32	1.5	NO	NO	[16]
KU16054	Japan	Male	NA	Urethral	0.5	NA	8	0.19	>32	0.5	NO	NO	[16]
KM383	Japan	Male	NA	Urethral	0.5	NA	12	0.125	>32	1	NO	NO	[16]
A7846	Australia	Male	Hetero	Urethral	0.5	2	8	0.25	>32	≥32	Yes	NA	[17]
A7536	Australia	Male	Hetero	Urethral	0.5	4	8	0.25	>32	≥32	Yes	NA	[17]
GK124	Denmark	Male	Hetero	Urethral	0.5	NA	8	0.5	>32	>256	NA	NA	[3]
47707	Canada	Female	Hetero	NA	1	4	16	0.5	32	≥256	Yes	NA	[18]
IR72	Ireland	Male	Hetero	Urethral	0.5	0.5	16	0.38–0.5	>32	NA	NA	NA	[2]
A2543	Australia	Female	NA	NA	0.5	NA	NA	>256	NA	NA	NA	NA	[5]
H18-502	UK	Female	Hetero	Vaginal	1	2	8	0.5	>32	2	No	No	[4]
51742	Canada	Male	Hetero	Urethral	0.5	2	16	0.25	32	2	NA	NA	[19]

Abbreviations: CRO, ceftriaxone; TET, tetracycline; SPT, spectinomycin; AZM, azithromycin; CIP, ciprofloxacin; PEN, penicillin; PPNG, penicillinase producing *Neisseria gonorrhoeae*; NA, not available.

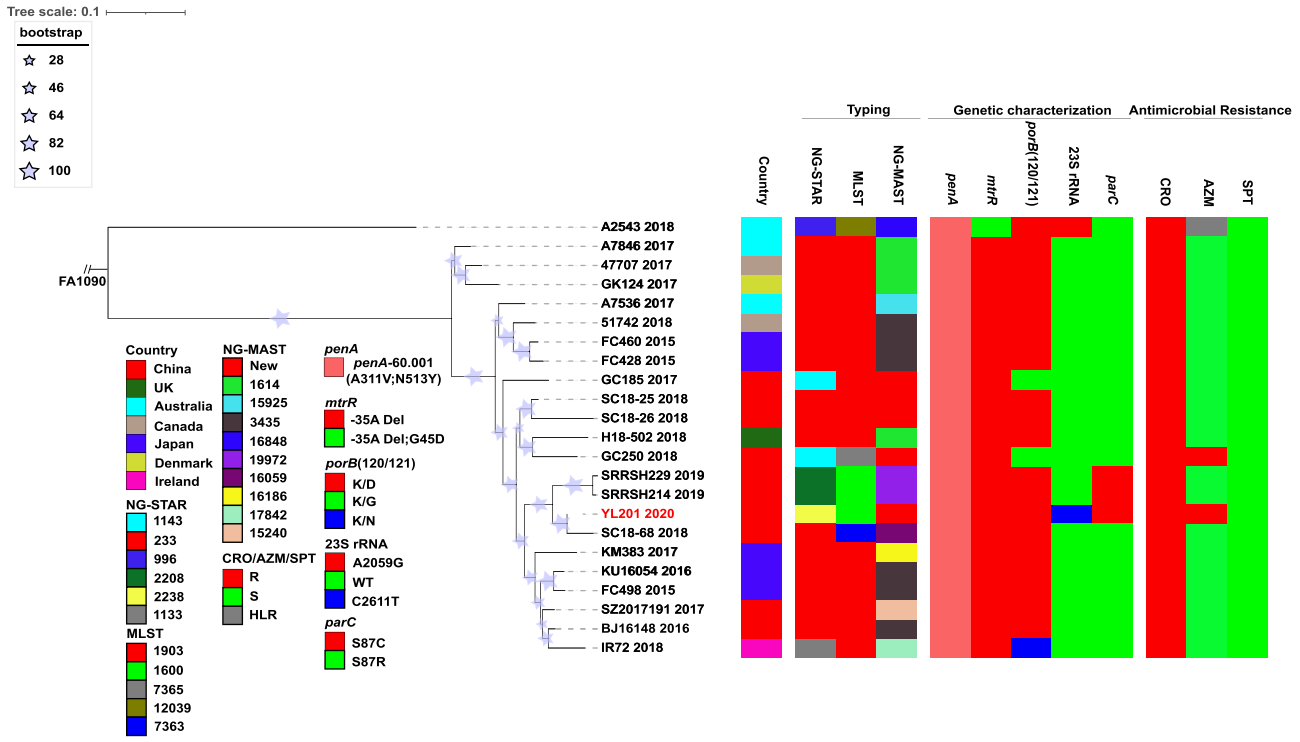

Figure 1 Maximum-likelihood tree based on 13,236 SNPs extracted from whole-genome sequences. FA1090 was placed as the outgroup. STs, antimicrobial resistance determinants and antimicrobial susceptibility are also shown. For YL201 and A2543, they contain four copies of the 23S rRNA C2611T and A2059G mutation respectively. Isolate YL201 described in this study is shown in red. The color coding of AMR phenotype and AMR-related alleles are indicated in the columns on the bottom left.
Abbreviations: R, resistance; S susceptibility; HLR, High-level resistance; WT, Wild type.

The minimal inhibitory concentrations (MICs) of the isolate were determined using E-TEST method, and the results were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org) interpretative criteria. YL201 showed resistance to CRO (MIC: 0.75 mg/L) and AZM (MIC: 12 mg/L), but was susceptible to spectinomycin (MIC: 12 mg/L) (Table 1).

Whole genome sequencing of YL201 was performed using Illumina HiSeq X Ten and Oxford Nanopore MinION sequencer. *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), multilocus sequence typing (MLST) and *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR) were confirmed using Sanger sequencing. The NG-MAST type was novel with *porB*-3101 and *tbpB*-752. The MLST type was ST1600, identical with SRRSH214 and SRRSH229 identified in Hangzhou.[7] Results of antimicrobial susceptibility testing showed that the three isolates with $MLST_{ST1600}$ have higher MIC for ceftriaxone than most strains with $MLST_{ST1903}$ (Table 1). This finding indicates that although isolates harbor identical *penA* mosaic allele, their MIC values may differ. Such variation can be explained by *penA*-60.001 allele recombined into isolates with certain MLST types associated with CRO decreased susceptibility, and in this case, recombination events happening in $MLST_{ST1600}$ isolates may contribute to a higher MIC value. According to our previous study,[8] $MLST_{ST7363}$ is associated with decreased ceftriaxone susceptibility. Moreover, phylogenetic analysis showed that $MLST_{ST7363}$ isolates (SC18-68) were clustered with $MLST_{ST1600}$ isolates, and that they share 6 identical loci with each other. Therefore, considering the genomic similarity between isolates with the two MLST STs, and the fact that $MLST_{ST7363}$ is a common ST in Shenzhen, the expansion of *penA*-60.001 allele to $MLST_{ST7363}$ isolates may have already happened and resulted in elevated MIC values. YL201 had the NG-STAR type of ST2238, containing a mosaic *penA*-60.001 allele with key resistance-mediating amino acid substitutions A311V and T483S, as well as G545S, I312M and V316T, which is typical characteristics of FC428 clone. YL201 has different NG-STAR type with SRRSH214 and SRRSH229 (ST2238 versus ST2208). The reason for this difference is that YL201 harbours four copies of the 23S rRNA C2611T mutation, while SRRSH214 and SRRSH229 with wild type 23S rRNA allele. Compared

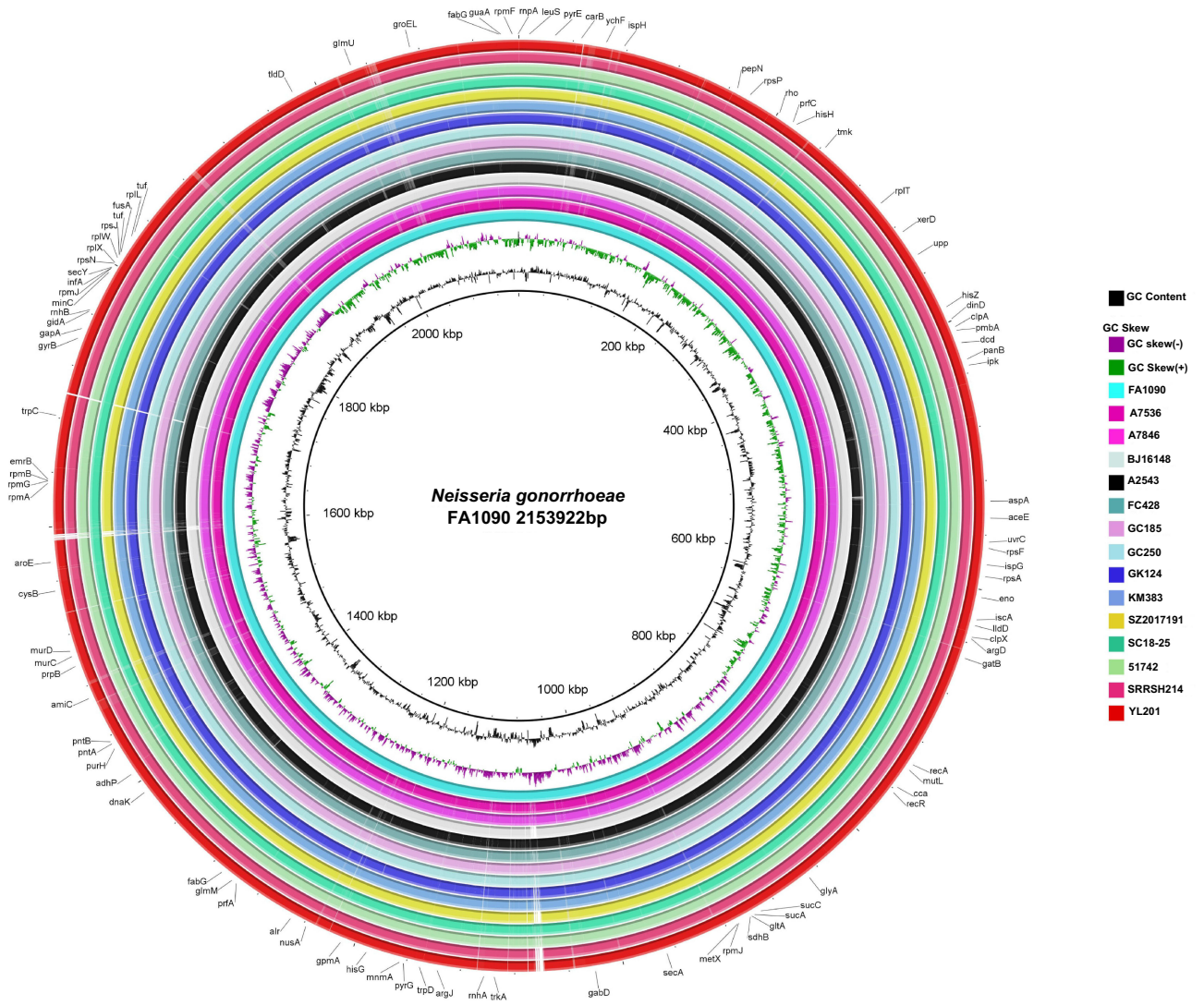

Figure 2 Comparison of YL201 and other 14 strains genomes in the phylogenetic tree. FA1090 (GenBank: AE004969.1) genome was used as the reference. The outermost ring indicates YL201. BLASTn matches with less than 30% identity appear as blank spaces (gaps) in each ring.

with wild-type, four copies of 23S rRNA C2611T mutation increased MICs 40–120-fold,[7] conferring moderate-level resistance to azithromycin.

Raw short-reads or draft genome assemblies of world-wide FC428-related strains were analysed to infer the phylogeny of YL201. A concatenate superset of SNPs relative to NCCP11945 was generated as previously described.[9] Based on the genome-wide SNP sites, a maximum likelihood tree was built using PhyML 3.0[10] and the substitution model was automatically selected using SMS (http://www.atgc-montpellier.fr/phyml/).[11] According to the phylogeny, YL201, SC18-68, SRRSH214 and SRRSH229 formed a clade (Figure 1), indicating FC428 clones originated from distinct regions have undergone further transmission in China. To date, all isolates within this clade have MLST STs different from ST1903, which may confer a higher MIC for ceftriaxone. In future, novel identified isolates belonging to this clade may present similar features. Additionally, including YL201, genomes of FC428-related strains were compared using BLAST Ring Image Generator (BRIG) and showed high similarities in genome structure without large insertions or deletions (Figure 2). Illumina and Nanopore sequencing data of YL201 have been stored in NCBI short read archive under BioProject PRJNA560592.

In conclusion, we have identified an MDR *N. gonorrhoeae* isolate in Shenzhen China with resistance to CRO and moderate-level resistance to AZM. The findings demonstrated that FC428 clones have undergone further transmission in China, and during the spread, they have presented more extensive and concerning AMR

characteristics. More importantly, as a major port with a large floating population, combined with our previous baseline data, we consider Shenzhen possesses the conditions for further transmission of FC428 clones, thus increasing the severity of gonococcal resistance. Therefore, regional surveillance should be highlighted to understand the transmission of emerging gonococcal drug-resistant clones.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and obtained approval from Medical Ethics Committee at the Shenzhen Center for Chronic Disease Control (approval number SZCCC-2021-008-01-PJ). Written informed consent was provided by the patient to allow the case details to be published.

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Disclosure

The authors report no conflicts of interest in this work.

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