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ORIGINAL RESEARCH

Increase in Multidrug Resistant *Neisseria gonorrhoeae* FC428-Like Isolates Harboring the Mosaic penA 60.001 Gene, in Nanjing, China (2017-2020)

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Background: Since the first Chinese report of the ceftriaxone-resistant *Neisseria gonorrhoeae* FC428 clone in 2016, additional FC428-like, *penA* 60.001 isolates have been identified in China.

Objective: To document the rise in *penA* 60.001 isolates in Nanjing, China, and characterize their molecular and epidemiological features.

Methods: *N. gonorrhoeae* minimum inhibitory concentrations (MICs, mg/L) for ceftriaxone, cefixime, penicillin, tetracycline, ciprofloxacin, azithromycin, spectinomycin, gentamicin and zoliflodacin were determined by agar dilution. MICs for ertapenem were measured by E-test. N. *gonorrhoeae* antimicrobial sequence typing (NG-STAR) of seven loci (*penA, mtrR, porB, ponA, gyrA, parC* and *23S rRNA*) was analyzed together with *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) and multilocus sequence typing (MLST). Phylogenetic analysis was also performed using whole genomic sequencing (WGS).

Results: Fourteen FC428-related *penA* 60.001 *N. gonorrhoeae* infections were identified out of 677 infections from 2017 to 2020, in Nanjing, representing an incremental yearly rise in the percentage of the city's *N. gonorrhoeae* isolates that were FC428-related. Seven FC428-related N. *gonorrhoeae* infections were acquired in Nanjing, proper; four others in eastern Chinese cities and three from unknown locations. All FC428-related isolates were resistant to ceftriaxone, cefixime, ciprofloxacin, tetracycline and penicillin but susceptible to spectinomycin, gentamicin, ertapenem and zoliflodacin; three strains were resistant to azithromycin. *penA* 60.001 isolates displayed closely related MLST types and NG-STAR types but relatively distant NG-MAST types. WGS showed a phylogenetic analysis that intermingled with other international isolates.

Conclusion: *penA* 60.001 *N. gonorrhoeae* isolates emerged in Nanjing, China, beginning in 2017, and have continued to rise.

Keywords: *Neisseria gonorrhoeae*, *penA* 60.001, ceftriaxone-resistance, FC428, WGS phylogenetic analysis

Introduction

Gonorrhea is the second most common bacterial sexually transmitted infection (Chlamydia is first), with a worldwide estimated incidence of new gonococcal infections in 2020 of 82.4 million cases.[1] If untreated, gonorrhea in women can lead to severe complications, such as endometritis and salpingitis (pelvic inflammatory disease [PID]) that may result in substantial morbidity including infertility and ectopic pregnancy; in men epididymitis can also result in infertility.[2] Furthermore, *Neisseria gonorrhoeae* has developed antimicrobial resistance to almost all antibiotic classes used for treatment that now requires intensive surveillance of gonococcal isolates for antibiotic susceptibility.[3]

Presently, ceftriaxone, an extended-spectrum cephalosporin (ESC) represents first-line treatment for gonorrhea. However, individual strains of *N. gonorrhoeae* with high-level resistance to ESCs have emerged sporadically outside of China; in Japan (eg, *N. gonorrhoeae* strains H041, GU140106 and most recently FC428),[4–6] Australia (strain A8806),[7] France (strain F89),[8] Italy (strain G2891).[9] Notably, the FC428 clone remains prominent; first described in Japan in

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2015;[6] since then, FC428-related sub-clones or FC428-like isolates (with a close phylogenomic relationship to FC428) have been identified in Australia (strains A7846 and A7536),[10] Canada (47,707),[11] Denmark (GK124),[12] France (F90)[13] and Ireland (IR72),[14] thereby confirming international spread of FC428-related sub-clones that possess ESC resistance. A common feature of FC428 and FC428-like strains is possession of an ESC-resistant genetic determinant; the mosaic *penA* 60.001 gene.[6,10–14] At present, the surveillance of FC428/FC428-like isolates is a focus in tracking emergence and spread of resistant *N. gonorrhoeae* strains. Notably, infections with FC428/FC428-related sub-clones have been associated with travel to the Asia-Pacific region, now including its identification in several distinct regions in China.[15–21] Here, we report 14 cases of *N. gonorrhoeae* that possessed the FC428-associated *penA* 60.001 gene, isolated from 2017 to 2020 in Nanjing, China. We compared their molecular types, also including whole genome sequences (WGSs), with other FC428-like isolates and identified their contribution, overall, to the reported emergence of FC428-like *N. gonorrhoeae* isolates in China.

Materials and Methods

Isolation of *Neisseria gonorrhoeae*

Gonococcal isolates were collected from male subjects with symptomatic urethritis (urethral discharge and/or dysuria) and their female sex partners who were patients attending the STD Clinic at the Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, China. Urethral or cervical exudates were collected using cotton swabs and plated immediately onto Thayer-Martin medium (Zhuhai DL Biotech, China) and incubated in candle jars at 36°C for 24 to 48 h. Colonial morphology, Gram's stain, and oxidase testing were used to identify *N. gonorrhoeae*, which was then sub-cultured onto GC medium (chocolate agar base) (Difco, Detroit, MI) supplemented with 1% Isovitalex (Oxoid, USA). Gonococcal colonies were suspended in tryptone-based soy broth and stored frozen (−80°C).

Antimicrobial Susceptibility Testing

Mean inhibitory concentrations (MICs) for penicillin, tetracycline, ciprofloxacin, azithromycin, cefixime, ceftriaxone, spectinomycin, gentamicin and zoliflodacin were determined by agar dilution, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. For ertapenem, the E-test method was used.[22,23] Using CLSI[24] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[25] criteria for antimicrobial resistance (AMR), the following MIC breakpoints were used to ascertain resistance: ≥0.25 mg/L, ceftriaxone and cefixime; ≥1 mg/L, azithromycin and ciprofloxacin; ≥128 mg/L, spectinomycin and ≥2 mg/L, penicillin and tetracycline. Breakpoints for gentamicin, ertapenem and zoliflodacin have not been established formally by CLSI but we used MICs ≥32 mg/L for gentamicin and ≥0.5 mg/L for zoliflodacin, which have been used previously.[26,27] Using *Haemophilus influenzae* criteria defined by CLSI, an MIC ≤ 0.5 mg/L for ertapenem was considered susceptible.[28] β-lactamase production was determined by paper acidometric testing.[29]

Molecular Epidemiologic Typing

We employed molecular epidemiologic typing: NG-MAST (http://www.ng-mast.net); MLST (http://pubmlst.org/neisseria/) and NG-STAR (https://ngstar.canada.ca), which were analyzed as described previously;[30–32] for NG-MAST; *porB* and *tbpB*, for MLST; *abcZ, adk, aroE, fumC, gdh, pdh* and *pgm* and for NG-STAR; *penA, mtrR, porB, ponA, gyrA, parC* and *23S rRNA*.

Whole Genome Sequencing (WGS) and Phylogenetic Analysis

Genomic DNA was extracted using QIAxtractor DX Kits (QIAGEN, Hilden, Germany). Whole genomes of 13/14 strains were successfully sequenced using the HiSeq (Illumina, San Diego, CA, USA) and PacBio RSII SMRT (Pacific Biosciences, Menlo Park, CA, USA) platforms at Genewiz Inc. (Suzhou, China). The complete genomic sequences of the thirteen isolates were uploaded to NCBI (BioProject ID: PRJNA553852, PRJNA553854 and PRJNA916595). Phylogenetic analysis was performed as previously described.[16] Sequences were aligned with the FC428 genome (BioProject ID: PRJDB5915) and single nucleotide polymorphisms (SNPs) identified using MUMmer software. Repetitive regions and SNPs in putative recombinogenic regions were removed using SyRI software. All available

whole-genome sequences or short-read sequences of FC428-related clones were similarly analyzed. Iqtree was used to analyze the phylogenetic relationship (maximum-likelihood method, bootstrap 1000) and to visualize the phylogenetic tree.

Statistical Analyses

SPSS 23 was used to perform statistical analyses. Comparisons of the rates of isolation of FC428-like isolates over time were performed using a logistic regression test of trend; $P \leq 0.05$ (2-sided) was considered significant.

Results

Between 2017 and 2020, we identified 14 *N. gonorrhoeae* FC428-like isolates with high-level resistance to ceftriaxone and cefixime in Nanjing, which represented an incremental yearly rise in the percentage of the city's *N. gonorrhoeae* isolates that were FC428-like: 0.44% in 2017 (1/229); 0.49% (1/204) in 2018; 4.73% in 2019 (7/148) and 5.21% in 2020 (5/96), (χ^2=12.467, P < 0.001) (Figure 1). Characteristics of subjects infected with FC428-like *N. gonorrhoeae* are shown in Table 1. Thirteen isolates were from men and one from a woman (sexual contact of a man with gonococcal infection with positive nucleic amplification test result for *N. gonorrhoeae*); none had traveled outside of China. All subjects had uncomplicated gonococcal infection and reported 1 or 2 sex partners in the recent month; partners were from Nanjing, Shanghai, Suzhou, Taizhou and Yancheng, indicating the presence of ESC-resistant FC428-like *N. gonorrhoeae* in (certain) eastern Chinese cities. Ten men provided sexual histories: five indicated coitus with regular partners only, five with non-regular partners. 9/14 took antibiotics (levofloxacin or cephalosporin) before attending the clinic. Microscopic examination of gram stained urethral (or cervical) smears revealed >10 polymorphonuclear neutrophils (PMNs)/per high-power field and gram-negative intracellular diplococci in all 14. All subjects were treated with spectinomycin: two that were coinfected with *Chlamydia trachomatis* were also treated with doxycycline (0.1g, twice a day) for ten days. Twelve

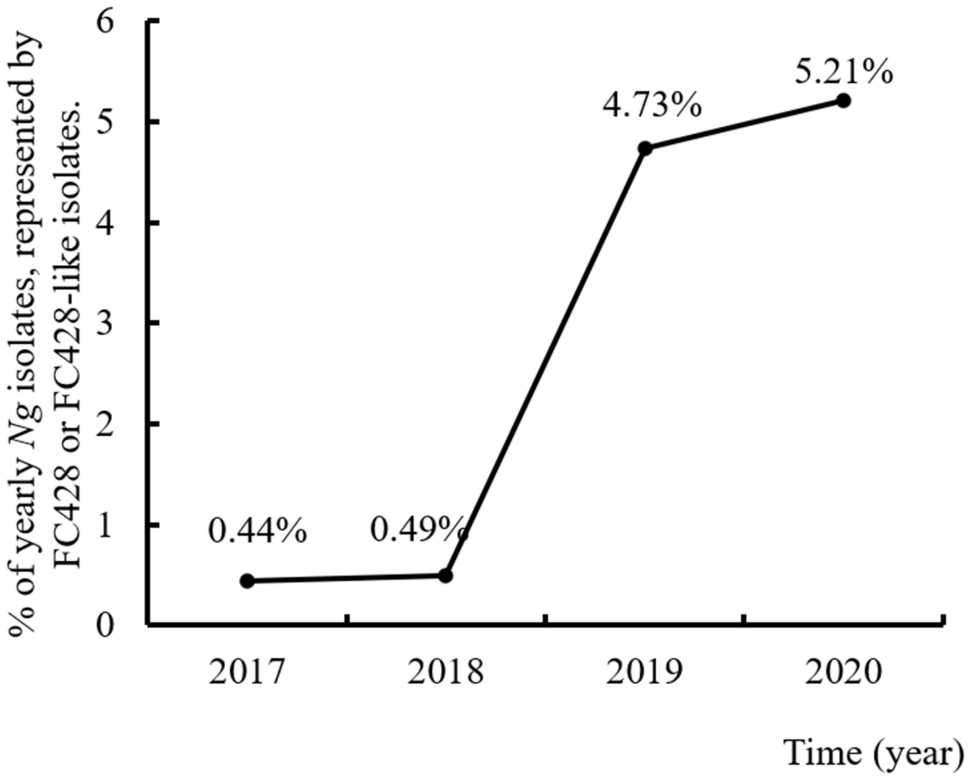

Figure 1 % of yearly *N. gonorrhoeae* (*Ng*) isolates, represented by FC428 or FC428-like isolates (2017–20). Rate (%) of FC428 or FC428-like isolates increased yearly across the 4 years, taken as a whole (P < 0.001; χ^2=12.467; Test of Trend) (between 2017 and 2020): linear association of the rates of FC428 isolates and year examined by logistic regression.

Table 1 Characteristics of 14 Subjects with Uncomplicated Gonococcal Infection, Nanjing, China

Characteristics	1	2	3	4	5	6	7
Subject							
Date	Sept, 2017	July,2018	May, 2019	June, 2019	June, 2019	Sept, 2019	Nov, 2019
Gender	Male	Male	Male	Male	Male	Male	Male
Age	24	34	59	19	23	16	47
City of origin	Nanjing	Nanjing	Nanjing	Nanjing	Nanjing	Nanjing	Nanjing
Foreign travel	No	No	No	No	No	No	No
History of STIs	No	No	No	NGU	CT, PPLO infection	No	HPV, PPLO infection
Administered antibiotics prior to the clinic visit	LVFX	LVFX	LVFX	No	LVFX Cephalosporin	Cephalosporin	Yes, type undetermined
Microscopic exam							
Gram's stain of exudate	>10 PMN /hpf + GNIDs	>20 PMN /hpf + GNIDs	>20 PMN /hpf + GNIDs	>10 PMN /hpf + GNIDs	>10 PMN /hpf + GNIDs	>10 PMN /hpf + GNIDs	>10 PMN /hpf + GNIDs
Microbiology							
NG isolate	NJ 1711654	NJ 189125	NJ 195417	NJ 196610	NJ 197542	NJ 1911400	NJ 1913940
MG (PCR)	NA	–	–	+	–	–	–
CT (PCR)	–	–	–	–	+	–	–
Treatment	Spe	Spe	Spe	Spe	Spe	Spe	Spe
Follow-up	Resolution of symptoms and signs	Resolution of symptoms and signs	Resolution of symptoms and signs	Urethral discomfort No sign	Urethral discomfort No sign	Resolution of symptoms and signs	Resolution of symptoms and signs
Sex-contact(s)							
No. of Contacts (recent month)	1	1	1	1	1	2	2
City/ies where contact occurred	Unknown	Shanghai	Nanjing	Unknown	Taizhou	Nanjing	1. Nanjing 2. Suzhou
Nature of the sex contact	Unknown	Unknown	Sex-worker	Regular sex-partner	Non-Regular sex-partner	Non-Regular sex-partner	1. Wife 2. Non-Regular sex-partner
Sex-contact treated	Unknown	Unknown	Unknown	Yes	Unknown	Unknown	1. Yes 2. Unknown

Characteristics	8	9	10	11	12	13	14
Subject							
Date	Nov, 2019	Dec, 2109	Jun, 2020	July, 2020	Oct, 2020	Oct, 2020	Nov, 2020
Gender	Male	Male	Male	Female	Male	Male	Male
Age	20	42	20	26	20	21	20
City of origin	Nanjing	Nanjing	Nanjing	Nanjing	Nanjing	Unknown	Nanjing
Foreign travel	No	No	No	No	No	No	No
History of STIs	No	No	No	No	No	No	NG infection
Administered antibiotics prior to the clinic visit	Yes, type undetermined	Cefdinir	No	No	No	LVFX	No
Microscopic exam							
Gram's stain of exudate	>10 PMN /hpf + GNIDs	>10 PMN /hpf + GNIDs	>20 PMN /hpf + GNIDs	>20 PMN /hpf + GNIDs	>20 PMN /hpf + GNIDs	>20 PMN /hpf + GNIDs	>10 PMN /hpf + GNIDs
Microbiology							
NG isolate	NJ 1914215	NJ 1914646	NJ 203279	NJ 204705	NJ 208430	NJ 208756	NJ 209469
MG (PCR)	–	–	–	–	NA	–	–
CT (PCR)	–	–	+	–	NA	–	–
Treatment	Spe	Spe	Spe	Spe	Spe	Spe	Spe
Follow-up	Resolution of symptoms and signs	Resolution of symptoms and signs	Resolution of symptoms and signs	Resolution of symptoms and signs TOC (-)	Resolution of symptoms and signs TOC (-)	Resolution of symptoms and signs	Resolution of symptoms and signs
Sex-contact(s)							
No. of Contacts (recent month)	1	1	1	1	1	2	1
City/ies where contact occurred	Nanjing	Yancheng	Nanjing	Nanjing	Nanjing	Unknown	Nanjing
Nature of the sex contact	Regular Sex-partner	Non-Regular sex-partner	Regular Sex-partner	Regular Sex-partner	Unknown	Unknown	Regular Sex-partner
Sex-contact treated	Yes	Yes	No	Yes	Unknown	Unknown	No

Abbreviations: STIs, Sexually transmitted infections; NGU; non-gonococcal urethritis; MG, Mycoplasma genitalium; PPLO, mycoplasma; CT, Chlamydia trachomatis; HPV, human papilloma virus; LVFX, levofloxacin; NA, not available; TOC, test of cure; PMN, polymorphonuclear neutrophils; hpf, high power field; GNID, Gram-negative Intracellular Diplococci; Spe, spectinomycin.

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had total resolution of symptoms and signs; 2 had residual urethral symptoms but no discharge. Two subjects had tests-of -cure; both of which were negative (Table 1).

Antimicrobial susceptibility testing of the 14 isolates showed similar resistance profiles (Table 2); all were resistant to ceftriaxone (MICs ≥0.5 mg/L), cefixime (MICs ≥2 mg/L), ciprofloxacin (MICs ≥16 mg/L), tetracycline (MICs ≥1 mg/L) and penicillin (MICs ≥2 mg/L) but were susceptible to spectinomycin, gentamicin, ertapenem and zoliflodacin. Three strains (NJ1914215, NJ203279 and NJ209649) were resistant to azithromycin (MIC, 8 mg/L); the remaining isolates were sensitive (0.25 mg/L).

Sequence typing (ST) analysis showed that the 14 isolates displayed closely related MLST and NG-STAR types but relatively distant NG-MAST types (Table 3). Three strains (NJ189125, NJ195417 and NJ1911400) belonged to ST3435, identical to FC428; the NG-MAST of NJ1711654 (ST17114) differed in *porB* by a single base pair (*tbpB* was identical). However, the remaining 10 isolates belonged to 6 different MG-MAST types and displayed relatively large differences in the number of SNPs in *porB* and *tbpB* compared to FC428: ST19387 (22 SNPs in *porB* [1 isolate]); ST19389 (23 SNPs in *porB* [1isolate]); ST19489 (24 SNPs in *porB* [2 isolates]); ST20048 (25 SNPs in *porB* and 53 SNPs in *tbpB* [3 isolates]); ST20051 (16 SNPs in *porB* and 81 SNPs in *tbpB* [1 isolate]); ST22091 (29 SNPs in *porB* [1 isolate]) and ST21683 (25 SNPs in *porB* and 81 SNPs in *tbpB* [1 isolate]). Nonetheless, the 14 isolates belonged to only 3 MLST types: 10 were MLST1903, identical to FC428; three were MLST 7363 (1 SNP in *abcZ* and 1 SNP in *fumC*) and one was MLST 11710 (7 SNPs in *aroE* and 1 SNP in *fumC*). NG-STAR types were identified as: ST 233 (5 isolates); ST 2238 (3 isolates); ST 1961 and ST 1143 (2 isolates each) and ST 3523 and ST 2239 (1 isolate each). A few SNPs were identified in *porB* (ST 1143, ST 2239 and ST 3523), *parC* (ST 2238), *23S rRNA* (ST 2238) and *gyrA* (ST 1961) alleles. NG-STAR typing identified mosaic *penA* 60.001 in all 14 strains. Notably, 3 strains (NJ1914215, NJ203279 and NJ209649) possessed a C2611T mutation in *23S rRNA* and were resistant to azithromycin.

Table 2 Antimicrobial Susceptibilities of Mosaic penA 60.001 Multidrug Resistant *N. Gonorrhoeae* Strains Isolated in Nanjing, China

MIC (mg/L)	NJ1711654	NJ189125	NJ195417	NJ196610	NJ197542	NJ1911400	NJ1913940	NJ1914215
Ceftriaxone	1	1	1	1	1	1	0.5	1
Cefixime	2	≥4	≥4	≥4	≥4	2	2	2
Spectinomycin	16	32	32	32	32	32	16	16
Tetracycline	4	4	2	2	2	2	1	1
Ciprofloxacin	≥16	≥16	≥32	≥32	≥32	≥32	≥32	16
Azithromycin	0.25	0.25	0.5	0.5	0.25	0.25	0.25	8
Penicillin	4	4	4	8	4	4	4	2
Gentamicin	≤4	8	8	8	8	8	8	≤4
AZD	0.03	0.06	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125
PPNG	–	–	–	–	–	–	–	–
TRNG	–	–	–	–	–	–	–	–

MIC (mg/L)	NJ1914646	NJ203279	NJ204705	NJ208430	NJ208756	NJ209649	FC428
Ceftriaxone	0.5	1	1	0.5	1	1	0.5
Cefixime	2	2	2	2	2	2	1
Spectinomycin	32	32	16	32	32	16	8
Tetracycline	1	2	≥32	1	2	1	NA
Ciprofloxacin	16	16	16	≥32	≥32	16	>32
Azithromycin	0.25	8	0.25	0.5	0.5	8	0.25
Penicillin	2	2	4	≥16	4	2	NA
Gentamicin	8	8	8	8	8	8	NA
AZD	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	NA
PPNG	–	–	–	+	–	–	NA
TRNG	–	–	+	–	–	–	NA

Table 3 Molecular Characteristics of Mosaic penA 60.001 Multidrug Resistant *N. Gonorrhoeae* Strains Isolated in Nanjing, China

Characteristics	NJ1711654	NJ189125	NJ195417	NJ196610	NJ197542	NJ1911400	NJ1913940	NJ1914215
NG-MAST	**17114**	**3435**	**3435**	**19,387**	**19,389**	**3435**	**19,489**	**20,048**
porB	2183	1053	1053	11,301	11,303	1053	3531	206
tbpB	21	21	21	21	21	21	21	3021
MLST	**1903**	**1903**	**1903**	**1903**	**1903**	**1903**	**1903**	**7363**
abcZ	126	126	126	126	126	126	126	59
adk	39	39	39	39	39	39	39	39
aroE	67	67	67	67	67	67	67	67
fumC	157	157	157	157	157	157	157	78
gdh	148	148	148	148	148	148	148	148
pdhC	153	153	153	153	153	153	153	153
pgm	65	65	65	65	65	65	65	65
NG-STAR	**233**	**233**	**233**	**1961**	**1961**	**233**	**1143**	**2238**
penA	60.001	60.001	60.001	60.001	60.001	60.001	60.001	60.001
mtrR	1	1	1	1	1	1	1	1
porB	8	8	8	8	8	8	12	8
ponA	1	1	1	1	1	1	1	1
gyrA	7	7	7	101	101	7	7	7
parC	3	3	3	3	3	3	3	53
23S rRNA	100	100	100	100	100	100	100	2

Characteristics	NJ1914646	NJ203279	NJ204705	NJ208430	NJ208756	NJ209649	FC428
NG-MAST	**20051**	**20,048**	**21,683**	**22,091**	**19,489**	**20,048**	**3435**
porB	6485	206	206	12,096	3531	206	1053
tbpB	110	3021	3131	21	21	3021	21
MLST	**1903**	**7363**	**11,710**	**1903**	**1903**	**7363**	**1903**
abcZ	126	59	126	126	126	59	126
adk	39	39	39	39	39	39	39
aroE	67	67	170	67	67	67	67
fumC	157	78	78	157	157	78	157
gdh	148	148	148	148	148	148	148
pdhC	153	153	153	153	153	153	153
pgm	65	65	65	65	65	65	65
NG-STAR	**2239**	**2238**	**233**	**3523**	**1143**	**2238**	**233**
penA	60.001	60.001	60.001	60.001	60.001	60.001	60.001
mtrR	1	1	1	1	1	1	1
porB	4	8	8	48	12	8	8
ponA	1	1	1	1	1	1	1
gyrA	7	7	7	7	7	7	7
parC	3	53	3	3	3	53	3
23S rRNA	100	2	100	100	100	2	100

Whole genomic sequencing (WGS) and resultant phylogenetic analysis demonstrated that 13 FC428-related clones were derived from five clades (A-E in Figure 2 [sequencing of NJ203279, the 14th isolate, was unsuccessful]). NJ1711654, NJ189125, NJ195417 were in clade A (similar to BJ16148 isolated in Beijing)[15] and possessed 100–200 SNPs compared to FC428. NJ196610, NJ197542 and NJ1911400 belonged to a newly identified clade in our collection (clade B) and possessed 283–438 SNPs compared to FC428. NJ208430, NJ208756 and NJ1913940, also a new clade in our collection (clade C), possessed 297–566 SNPs compared to FC428. NJ1914215, NJ206649, NJ1914646 (clade D) represented isolates similar to seven additional isolates in the phylogenetic tree from Hangzhou.[19] Genomic sequences of NJ20204705 and FC428 differed by >1000 SNPs; NJ20204705 was more closely related to G97687 and G7944, which

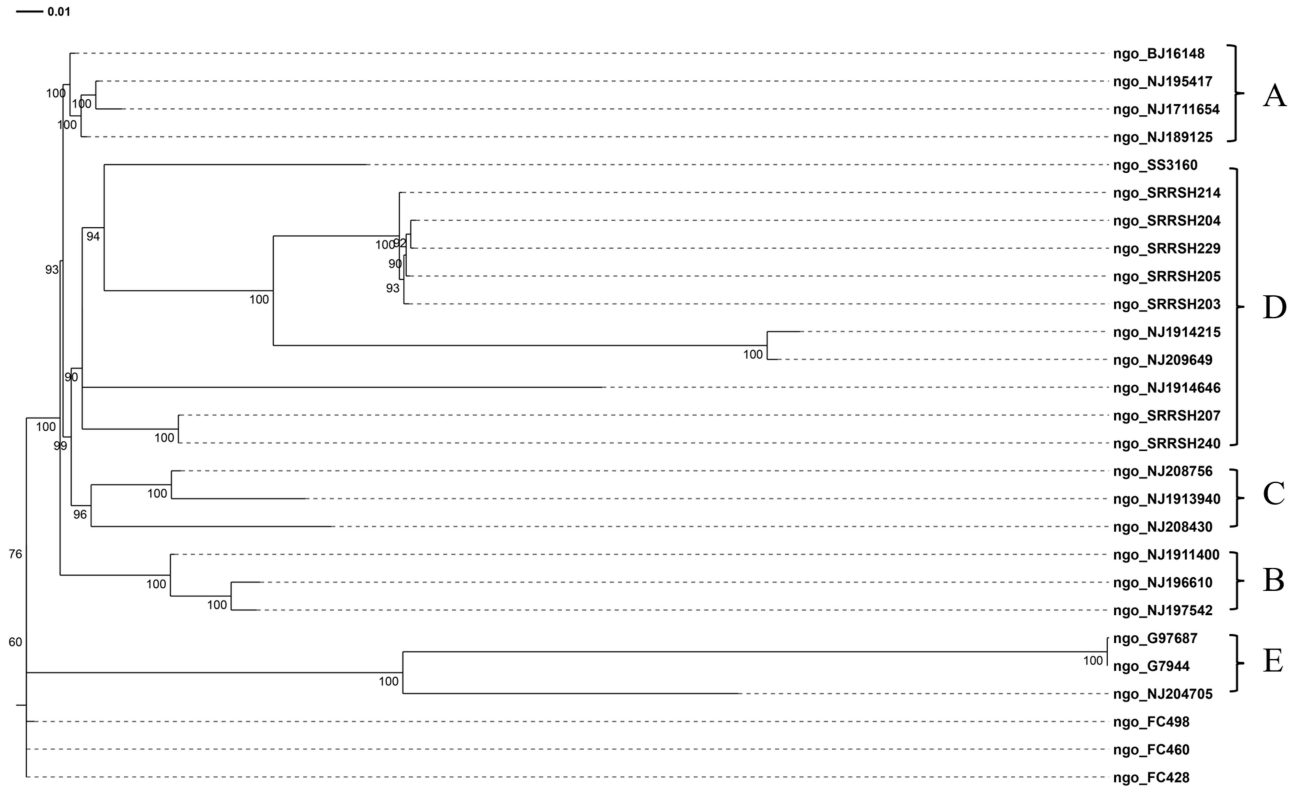

Figure 2 Maximum Likelihood (ML) phylogeny of 13 FC428-like isolates in this study (termed NJ) compared with FC428-like strains from other Chinese and internationals sites: FC428 is placed as the phylogenetic tree root. 13 FC428-related clones were derived from five clades (**A–E**). The scale bar indicates the average number of SNPs per site. Whole genome sequences (WGSs) of Nanjing (NJ) isolates were determined in this study. Other WGSs were obtained from public databases.

are English isolates.[33] However, NJ20204705 was susceptible to azithromycin (MIC 0.25ug/mL) while G97687 and G7944 were highly resistant to azithromycin (and ESCs).

Discussion

Sustained spread of *N. gonorrhoeae* FC428 and FC428-like isolates has been demonstrated by their continued emergence, suggesting a possible fitness advantage in humans. However, experiments with *N. gonorrhoeae* FC428 (in vitro and in vivo in mouse models) have shown ambiguous changes in fitness,[34] distinct from ceftriaxone-resistant gonococcal strains H041 and F89, for example, that harbor mosaic *penA* alleles and where fitness is reduced,[35] possibly explaining why these strains have not been transmitted widely.[35] Resistance of *N. gonorrhoeae* FC428 to ESCs, first reported in Japan in 2015,[6] and identified subsequently in Europe,[10,12–14,36,37] North America,[11,38] Southeast Asia (Vietnam,[39] Singapore[40] but not Thailand[41–43]), is caused by the mosaic *penA*-60.001 allele. In addition to the 14 isolates reported here, thirty-five FC428-like isolates have also been reported from 7 eastern Chinese cities (Figure 3),[15–19,21,44,45] surpassing the 31 isolates that have been reported from other parts of the world.[10–14,36–40,46] We report that FC428/FC428-like gonococcal isolates identified in Nanjing increased markedly from 2017 to 2020, exceeding the numbers of isolates reported from any other Chinese city (Figure 3).

In nearly six years, from 2015 to 2021, nine cases of ceftriaxone-resistant *N. gonorrhoeae* were reported from the United Kingdom (UK); all were associated international travel, including two that possessed documented whole genomic similarity to *N. gonorrhoeae* strain FC428, also exhibiting multilocus sequence type ST-1903; *N. gonorrhoeae* multi-antigen sequence type ST-1614 (*porB* 1053, *tbpB* 33) and antimicrobial resistance sequence type ST233.[36] In just 6 months, between December 2021 and June 2022, 9 mosaic *penA* 60.001 *N. gonorrhoeae* isolates were obtained from patients presenting to sexual health service across the UK.[47] MLST types for the 9 isolates were ST16406 (1 isolate) and ST8123 (8 isolates), differing from ST1903, the most prevalent Nanjing MLST type (11 of 14 isolates). ST8123 was the

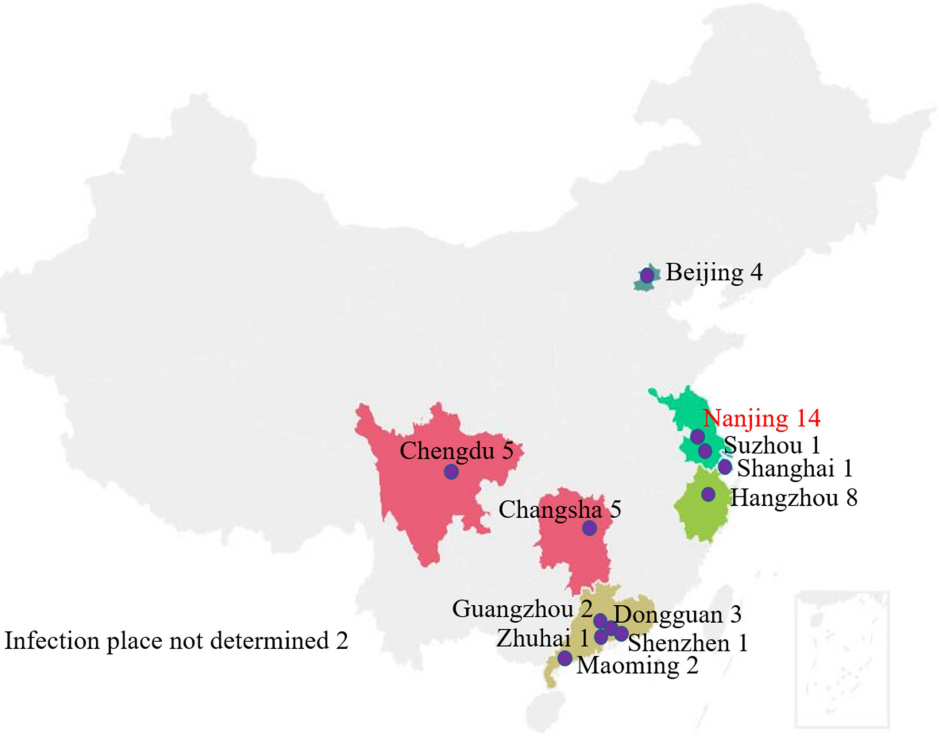

Figure 3 Distribution of FC428-like isolates reported from Chinese cities.

most common ST type among isolates collected in Shenzhen, China, between 2014 and 2018.[48] The rapid emergence of mosaic *penA* 60.001 *N. gonorrhoeae* in two separate areas of the world suggests that mosaic *penA* 60.001 strains are spreading rapidly throughout the world.

Effective antimicrobial treatment of gonorrhea is essential to interrupt transmission of FC428/FC428-like isolates. Although differences in antimicrobial susceptibilities of the 14 isolates in our study were evident, MICs of ceftriaxone for the 14 isolates were similar to those for FC428.[20] Three isolates showed a moderate level of resistance to azithromycin, which has also been reported in FC428-like isolates from several Chinese cities including Hangzhou, Changsha and Shenzhen,[18,19,21] indicating that FC428-related sub-clones have evolved further and spread in China.

FC428-like genotypes (including NG-MAST, MLST and NG-STAR) have evolved to possess additional gene mutations during their spread. The 14 strains displayed relatively distant NG-MAST types; only one other FC428-like isolate, ST3435, has been reported to harbor an NG-MAST type that differed from the FC428 "signature" NG-MAST type. SNPs in *porB* resulted in different NG-MAST types. MLST1903, which is identical to FC428, was the predominant MLST type;[19] however, three isolates were typed as MLST 7363 (identical to SC18-68 isolated in Chengdu, China[17]) and one was typed as MLST 11710 (not yet reported). NG-STAR types were similar to ST 233 (same as the FC428 type). NG-STAR ST1961, ST 2238, ST 2239 and ST 3523 were all new types. NG-STAR ST1143 was identical to FC428-like isolates from Changsha.[18]

Phylogenetic analysis showed that 13/14 of the isolates reported in this study were closely related to other FC428-like isolates (Figure 2); these subdivided into 5 clades, which were linked to other clades from Beijing,[15] Hangzhou[19] and England.[33]

Conclusion

In summary, we describe fourteen recent ceftriaxone-resistant FC428-related *penA* 60.001 *N. gonorrhoeae* infections in Nanjing, China, which represents a significant rise in this infection locally. Other Chinese cities have seen similar cases; however, reporting from nearby SE Asian countries has been sporadic or absent. In the West, isolated infections and even outbreaks have been caused by this organism. The rapid emergence of FC428-related *penA* 60.001 *N. gonorrhoeae* infections in separate areas of the world suggests that this infection is rapidly gaining a foothold worldwide.

Ethics Approval and Informed Consent

Our study complies with the Declaration of Helsinki. The Ethics Committee of the Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College (approval number: 2016-KY 022), and the Institutional Review Board (IRB) at Tufts University School of Medicine (approval number: 12219) approved the study; all enrolled participants provided written informed consent. Participants themselves and a parent or legal guardian of participants under 18 years of age provided written informed consents.

Data Sharing Statement

The data of complete genomic sequences of the thirteen isolates were available from NCBI (BioProject ID: PRJNA553852, PRJNA553854 and PRJNA916595).

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Disclosure

No potential conflict of interest was reported by the authors.

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