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Original Article

# Emergence of a predominant sequence type ST7363 and the increasing trend of resistance to cefixime and ceftriaxone in *Neisseria gonorrhoeae* in Southern Taiwan, 2019–2021



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**KEYWORDS**

Antimicrobial  
susceptibility;  
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**Abstract** *Background/purpose:* Multi-drug resistance and the presence of epidemic lineages of *Neisseria gonorrhoeae* locally and globally were important clinical and public health issues. We aimed to investigate the molecular epidemiology and the antimicrobial susceptibility profiles of *N. gonorrhoeae* in Southern Taiwan.

*Methods:* Between 2019 and 2021, adult patients who had suspected gonorrhea and attended a urology clinic in southern Taiwan were recruited to participate in this study. Clinical data from medical records and a questionnaire, antimicrobial susceptibility testing using a disk diffusion test in accordance with the guidelines by the Clinical and Laboratory Standards Institute, and Multi-locus sequence typing (MLST) were analyzed.

*Results:* A total of 500 patients participated in the surveillance study. Among them, 232 *N. gonorrhoeae* isolates were identified, but only 164 isolates were recovered for further research. ST7363 (n = 83, 50.61%) was found to be the predominant sequence type, followed by ST1583 (n = 24, 14.63%), ST1588 (n = 13, 7.93%), and ST7827 (n = 12, 7.32%). 100% resistance to penicillin and 99.4% non-susceptible rate of ciprofloxacin were observed. The azithromycin resistant rate being 15.24% and the cefixime non-susceptible rate being 17.07% were alarming, both with decreasing trends in susceptibilities during 2019–2021. The 25 azithromycin resistant isolates were mainly belonged to ST7363 (n = 12) and ST7827 (n = 3). Seven (4.2%) isolates were ceftriaxone non-susceptible. Among them, four were assigned to be ST 7827 and three belonged to ST7363.

*Conclusion:* We observed the emergence of a predominant sequence type ST7363 in southern Taiwan. Compared with previous Taiwan studies, the increasing trend of resistance to cefixime and ceftriaxone necessitates clinicians' alertness for clinical treatment response of the extended spectrum cephalosporins and the further surveillance monitor.

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**Introduction**

Gonorrhea is a common sexual transmission disease which have infected 87 million annually in the world.<sup>1</sup> The convenience of traveling and the complex sexual networks might facilitate its spread.<sup>2–4</sup> During 2019–2020, the overall rate of reported gonorrhea increased rapidly in US.<sup>5</sup> Azithromycin, fluoroquinolone and extended-spectrum cephalosporins (ESCs), including ceftriaxone and cefixime, were the first-line treatment for *Neisseria gonorrhoeae*, the pathogen of gonorrhea.<sup>6–8</sup> However, the emergence of antimicrobial resistance among *N. gonorrhoeae* limited the choices of empirical antibiotics to treat gonorrhea. In view of high fluoroquinolone resistance globally,<sup>9</sup> ESCs and azithromycin have become the recommended options to treat gonococcal infections.<sup>10,11</sup> With the increasing consumption of ESCs and azithromycin, there were an increasing resistance trend for the ESCs (up to 6–30% in Asia–Pacific region) and azithromycin (up to 6–30% in China, Russia, Canada, and Oceania).<sup>1</sup> During 2017–2018, the increasing trend of resistance to ceftriaxone and azithromycin in the United Kingdom, Japan, and China was also reported.<sup>12,13,14</sup>

The multi-locus sequence typing (MLST) using the sequences of seven housekeeping genes in *N. gonorrhoeae* is one commonly-used molecular typing tool.<sup>15</sup> Previous researches have reported<sup>16</sup> that ST7367 has been found as representative type of multidrug-resistant strains and associated with reduced ceftriaxone susceptibility.<sup>17</sup> Four clones including ST7363, ST1583, ST7827 and ST1901 were linked to possession of mosaic *penA* alleles.<sup>4,6,18</sup> The ST7363 and ST1901 have been reported widely spreading in Asia among

the men who have sex with men (MSM) groups,<sup>4</sup> while the ST1583 and ST7827 have been reported in Norway with phylogeographic analyses indicated as derived from Asian origin.<sup>18</sup> Based on the previous studies,<sup>4,6,19–21</sup> it was speculated that the antibiotic resistance and the transmission route were strongly related to specific sequence types. Further evidence using genome-based phylogeographical analysis revealed a multi-resistant and a multi-susceptible lineage for 419 gonococcal isolates from 58 countries during 1960–2013.<sup>4</sup> The multi-susceptible lineage was more associated with the heterosexual orientation, whereas the multi-resistant lineage was found to evolve from the introduction of Asia and was strongly related to the group of men who have sex with men (MSM) and are in high-risk sexual networks.<sup>4</sup> This triggered the concern of relationship between high prevalence of multi-resistant *N. gonorrhoeae* infection among the homosexual orientation group.

In Taiwan, gonorrhea has been listed as one of notifiable communicable diseases since the 1950s. The disease incidence has grown from 9.79 cases per 100,000 populations in 2010 to 19.00 cases per 100,000 populations in 2016.<sup>2</sup> In recent decades, high antibiotic-resistant rates to fluoroquinolone and penicillin were also reported.<sup>2,22,23</sup> In an early study conducted in Northern Taiwan from 1999 to 2003, Hsueh et al. reported that the overall ciprofloxacin resistance was 78%.<sup>22</sup> In another research conducted in Northern Taiwan in 2001–2013, the antimicrobial resistance rates for cefixime, spectinomycin, cefpodoxime, ciprofloxacin, and penicillin were 0.4%, 0.4%, 13.3%, 91.6%, and 87.6%, respectively.<sup>2</sup> In one Taiwan nationwide study, the resistant rate to azithromycin (MIC  $\geq 1$   $\mu\text{g/ml}$ ) was 8.7% in 2001–2018.<sup>23</sup> Azithromycin-high-level-resistant (MIC  $\geq 256$   $\mu\text{g/ml}$ ) *N.*

*gonorrhoeae* in Northern Taiwan belonged to specific MLST sequence type 12,039/10,899.<sup>23</sup> Nevertheless, little is known for the antimicrobial susceptibility and disease transmitted route in Southern Taiwan. We sought to investigate the molecular epidemiology and the antimicrobial susceptibility profiles of *N. gonorrhoeae* in Southern Taiwan.

## Materials and methods

### Collection of *N. gonorrhoeae* specimens

This study was approved by the Institutional Review Board of KMUH (IRB No. KMUHIRB-SV(I)-20180,072). Between March 2019 and July 2021, adult patients ( $\geq 20$  years old) who had suspected *N. gonorrhoeae* infection and attended a Urology Clinic in Kaohsiung, Taiwan were included in this study. A total of 500 patients including 495 men and 5 women, were enrolled in this study. The specimens were collected from each patient through swab culture of penis/urethra with Amies transport medium, along with their clinical data including age, sex, risk behavior, comorbidities, sites of collection, and date of collection. 500 specimens were subjected to inoculation on chocolate agar which was incubated with 5% CO<sub>2</sub> at 37 °C. The colonies were isolated and then transferred for the MALDI-TOF MS system (Bruker Biotyper). A total of 164 gonococcal isolates were collected and stored at –80 °C.

### Molecular epidemiologic typing

The multi-locus sequence typing (MLST) was performed as described on MLST website.<sup>15</sup> The sequence types (STs) of 164 *N. gonorrhoeae* isolates were assigned based on seven housekeeping genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdh*, and *pgm*).

### Antibiotic susceptibility testing

The antibiotic susceptibilities of *N. gonorrhoeae*, including penicillin, ciprofloxacin, spectinomycin, azithromycin and extended-spectrum cephalosporins: ceftriaxone, cefotaxime and cefuroxime was determined using a disk diffusion test (Becton Dickinson Caribe, Ltd.) in accordance with the guideline recommended by Clinical and Laboratory Standards Institute (CLSI).<sup>24</sup> Briefly, the overnight colonies (20–24 h) were suspended in 0.9% PBS and adjusted to 0.5 McFarland. Followed, the bacterial suspension was plated onto GC agar plates using swab, and the disks were placed on the bacterial lawn with sterile tweezers. The plates were incubated at 36 °C  $\pm$  1 °C in a 5% CO<sub>2</sub> atmosphere for 20–24 h, and eventually, the diameter of inhibition zone was measured. The interpretation of the results was mostly according to the criteria recommended by CLSI,<sup>24</sup> except for azithromycin by CDC standard.<sup>25</sup>

### Statistical analysis

The statistical analysis for categorical clinical information was assessed by SPSS version 20.0 (SPSS Inc., Chicago, IL) using Fisher's exact test or Chi square test, as appropriate.

Due to the non-normal distribution in variables of age and numbers of sexual partner in the recent 6 months, Kruskal–Wallis test was applied to analyze the significance. The statistical significance was defined as *P* value < 0.05. The eBURST algorithm<sup>26,27</sup> implemented in the PUBMLST website<sup>15</sup> was utilized for cluster analysis of our data. Strains were divided into groups using the allelic profiles, showing the possible ancestral type. The heatmap of profiles was constructed using the "heatmap.plus" package in RStudio software version 1.1.453. The year of isolation and MLST of 164 *N. gonorrhoeae* isolates were annotated alongside the heatmap to visualize the relationship between genotype and antimicrobial susceptibility.

## Result

### Clinical characteristics

Among 500 participants, 232 isolates from 232 cases were identified as *N. gonorrhoeae* by MALDI-TOF system. We failed to recover 68 isolates from storage. The following analysis was according to data of the remaining 164 cases. 164 cases were all male, and their median age was 28.5 years old (Table 1). Most patients (138/164, 84.15%) were heterosexually oriented, and the median number of sexual partners in the recent 6 months was 1. All participants denied to have the sexually transmitted diseases, including chlamydia, condyloma acuminata, hepatitis A virus (HAV), hepatitis B virus (HBV) and hepatitis C virus (HCV). However, this finding is limited by the lack of laboratory testing for STDs for the participants so we cannot report correct data on mixed STDs in the article. Among 164 cases, 55.49% (91/164) were living with HIV, and 57.93% (95/164) had syphilis infection history. We divided the time of the specimen was collected into two parts by July 30, 2020. The association of the time of collection with age, sexual orientation, past gonorrhea infection, and sexual partners in 6 months, respectively, were analyzed with the *P* values being 0.645, 0.968, 0.053 and 0.059. The past gonorrhea infection and sexual partners were not significant related to the time of collection but a decreasing trend can be found. The history of sexual transmitted diseases in the recent 6 months was significantly related to the time of collection (*P* < 0.001). Further analysis of the characteristics of 232 patients revealed significant difference in age, recent STD, past gonorrhea infection, HIV, syphilis and sexual partners (Supplement Table 4).

### Antimicrobial susceptibility testing

Among 164 isolated, no isolate was susceptible to penicillin (Table 2), and only one of the isolates showed susceptibility to ciprofloxacin. For the third generation cephalosporins, ceftriaxone exhibited susceptibilities in 94.37–96.77% (overall 95.73%) isolates and cefotaxime exhibited susceptibilities in 98.59–98.92% (overall 98.18%) isolates in 2019–2021, whereas the susceptible level of cefixime showed decreasing trend from 84.95% to 81.69% (overall 82.93%). The high susceptible rates were also revealed for

**Table 1** Clinical characteristics of 164 patients enrolled in this study.

	Total (N = 164)	%	2019 ~ Mid 2020 (N = 93)	%	Mid 2020–2021 (N = 71)	%	P
<b>Biological status</b>							
Age							0.645 <sup>c</sup>
Median (IQR)	28.5 (13)		29 (9)		28 (16)		
<b>Sexual status</b>							
Sexual orientation							0.968 <sup>b</sup>
Heterosexual	138	84.15	78	83.87	60	84.51	
Bisexual	2	1.22	1	1.08	1	1.41	
Homosexual	24	14.63	14	15.05	10	14.08	
History of STD <sup>a</sup> in 6 months							0.000 <sup>b</sup>
Yes	56	53.85	55	59.14	1	1.41	
No	108	65.85	38	40.86	70	98.59	
History of gonorrhea infection							0.053 <sup>b</sup>
Yes	12	7.32	10	10.75	2	2.82	
No	152	92.68	83	89.24	69	97.18	
HIV							<0.001 <sup>b</sup>
Yes	91	55.49	26	27.96	65	91.55	
No	6	3.66	0	0	6	8.45	
Unknown	67	40.85	67	72.04	0	0	
Syphilis							<0.001
Yes	95	57.93	26	27.96	69	97.18	
No	2	1.22	0	0	2	2.82	
Unknown	67	40.85	67	72.04	0	0	
Sexual partners in 6 months							0.059 <sup>c</sup>
Median (IQR)	1 (0)		1 (0)		1 (0)		

<sup>a</sup> Sexual-transmitted disease.

<sup>b</sup> P value of Chi-squared test.

<sup>c</sup> P value of Kruskal–Wallis test.

**Table 2** Antimicrobial susceptibility of 164 tested isolates.

	2019 ~ Mid 2020 (N = 93) n (%)			Mid 2020–2021 (N = 71) n (%)		
	S	I	R	S	I	R
Penicillin	0 (0%)	28 (30.11%)	65 (69.89%)	0 (0%)	20 (28.17%)	51 (71.83%)
Cefixime	79 (84.95%)	—	—	58 (81.69%)	—	—
Ceftriaxone	90 (96.77%)	—	—	67 (94.37%)	—	—
Cefotaxime	92 (98.92%)	—	—	70 (98.59%)	—	—
Ciprofloxacin	1 (1.08%)	14 (15.05%)	78 (83.87%)	0 (0%)	8 (11%)	63 (88.73%)
Spectinomycin	92 (98.92%)	0 (0%)	1 (1.08%)	69 (97.18%)	1 (1.41%)	1 (1.41%)
Azithromycin	—	—	14 (15.05%)	—	—	11 (15.49%)

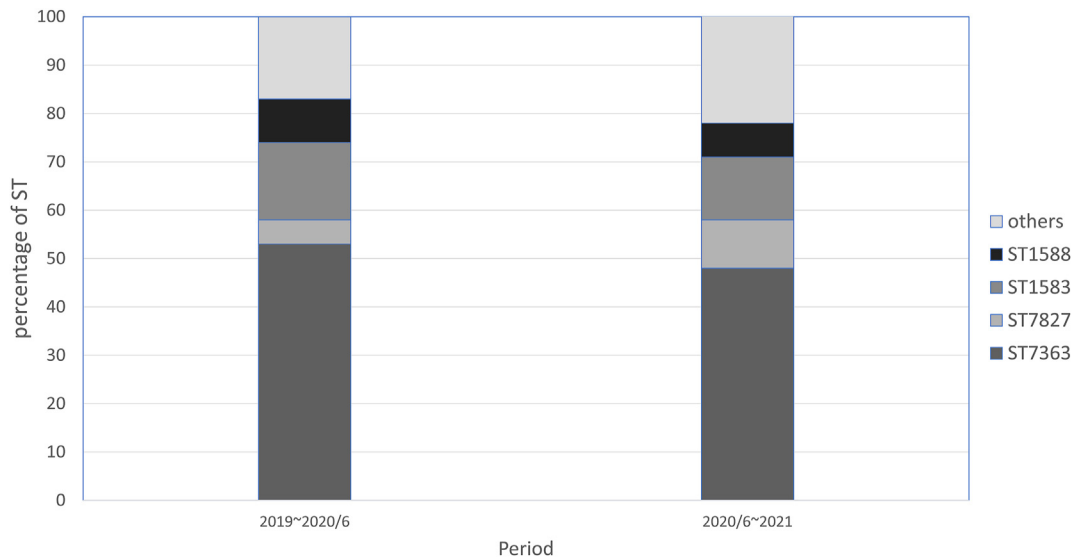
S, susceptible; I, intermediate-resistant; R, resistant.

spectinomycin, with 98.59–98.92% susceptible during 2019–2021 (overall 98.78%). In accordance with the breakpoint recommended by CDC, an alarming resistant rate, 15.24%, was found for azithromycin against the isolates tested in this study, with 15.05–15.49% resistance between 2019 and 2021.

### MLST genotyping

The 164 isolates were assigned to 23 STs, including the 3 new types found in 3 isolates in the study. The loci

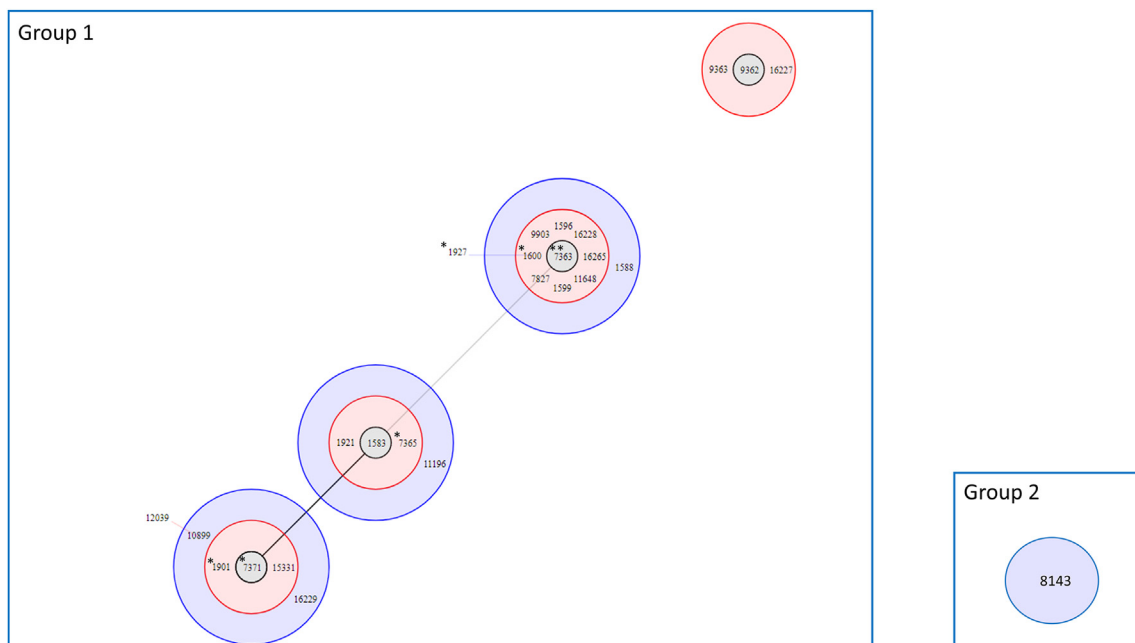
sequences of these 3 new types were deposited in PubMLST database as ST16227, ST16228, ST16229 (Supplementary Table 1). Among 164 isolates, the predominant type was ST7363 (n = 83; 50.61%), followed by ST1583 (n = 24; 14.63%), ST1588 (n = 13; 7.93%), and ST7827 (n = 12; 7.32%) (Supplementary table 2). The distribution of ST in 2019–2021 was illustrated in Fig. 1. The ST7363 was found as the predominant type throughout 2019–2021, with a decreasing trend from 53% (49/93) to 48% (34/71). The ST7363 and ST1583 were noticed with various proportions in the period, while the



**Figure 1.** Distribution of sequence types during 2019–2021. The dominant ST was defined as ( $n \geq 10$  isolates), while the STs with  $n < 10$  isolates were classified as “Others”.

ST7827 presented increasing proportions from 5% in 2019 – mid-2020 to 10% in mid-2020 - 2021 and became one of the dominant MLST types. The remaining 20 STs were observed with a steady proportion around 20% during the two periods.

The cluster analysis was performed using the eBURST on PubMLST web for 164 isolates to investigate the phylogenetic relation. According to the eBURST analysis, the 23 STs were classified into 2 groups: the major group included 160 isolates in 22 STs (Fig. 2), and the other group possessed 4



**Figure 2.** Population of 164 *N. gonorrhoeae* isolates and prevalent STs in previous Taiwan study analyzed via eBURST algorithm. The analysis was adapted for use as a plugin for the BIGSdb database software by Keith Jolley on <https://pubmlst.org/>. The original result illustrated 2 groups, including group 1 ( $n = 160$ ) and group 2 ( $n = 4$ ). The ST7363 was defined as ancestral type during analysis and shown with double asterisk sign next to it. The gray circles were the founder genotypes, and the circles nearby (pink) represented that only one house-keeping gene sequence was different from that of the founder genotype and the outer circle represented two house-keeping gene different (purple). The lines between founder types represented sharing six of seven alleles between the two types. The prevalent type of previous Taiwan study: ST1901, ST7365, ST1927, ST1600, and ST7371 were marked with an asterisk (\*), where the ST1927 and ST7365 weren't found in our study. The sub-group ST9362 is a double-locus variant of ST11196.

isolates belonged to ST8143. The major group was further divided into four clonal complexes (Fig. 2), and the ST1583, ST7363, ST7371, and ST9362 were recognized as the founder genotypes (the center of each complex). The ST1583, ST7363, and ST7371 were noted with the evolutionary relation, sharing six of seven alleles with each other. The ST9362 is a double-locus variant to ST11196 and formed a sub-group in group 1. The STs nearby the founder groups represented that only one house-keeping gene sequence was different from that of the founder genotype, implying the phylogenetic. Eleven of 22 STs in the major group belonged to the clonal complex of ST7363. The new types deposited by this study were noticed that ST16227 was derived from ST9362; ST16228 and ST16230 were from ST7363; ST16229 was from ST7371. Overall, an increasing trend of the clonal complex of ST7363, particularly ST7363, ST7827, and ST1588, was noticed (Figs. 1 and 2). Compared to the previous research in Taiwan, the prevalent STs also belonged to the major group and derived from the ST7363, ST7371, and ST1581 sub-group.

### Relation between genotypes and antimicrobial susceptibility profiles and demographical factors

For the heatmap analysis, a total of 164 isolates with contact information of genotype and antimicrobial susceptibility profiles were involved. As shown in Fig. 3, similar heatmap patterns of susceptibility profiles was clustered. Along with the heatmap, the year of isolation and the dominant genotypes were visualized, demonstrating that isolates with multi-drug resistance tendency were all belonging to ST7827 (Fig. 3). Moreover, the 25 azithromycin-resistant isolates belonged to ST7363 (n = 12), ST7827 (n = 3), ST1583 (n = 2), ST9363 (n = 2), and ST8143, ST16229, ST10899, ST12039, ST11196, ST1921 (n = 1, each). Among 7 ceftriaxone-non-susceptible isolates, 4 belonged to ST7827, and 3 belonged to ST7363. There was no specific sequence type to associate with any specific antimicrobial resistance pattern with statistical significance. There was also no specific sequence type to associate with HIV, syphilis history and sexual orientation.

### Discussion

Our results revealed ST7363 accounted for about a half of gonorrhea in southern Taiwan in 2019–2021. ST7828 emerged in 2020 as one of the four major STs in southern Taiwan. Though the 23 STs were classified into 2 groups in eBURST analysis, there was a major group to include 22 STs (161 isolates). The loss of susceptibility to penicillin and a very low susceptibility rate to ciprofloxacin indicated the inactive role of the two antimicrobial agents in treating gonorrhea. The azithromycin resistant rate was 15.24% and cefixime non-susceptible rate was 17.07%. Both alerts the clinicians to watch out for the treatment results when using the two oral antimicrobial agents. The 4.27% non-susceptible rate to ceftriaxone was also of concern.

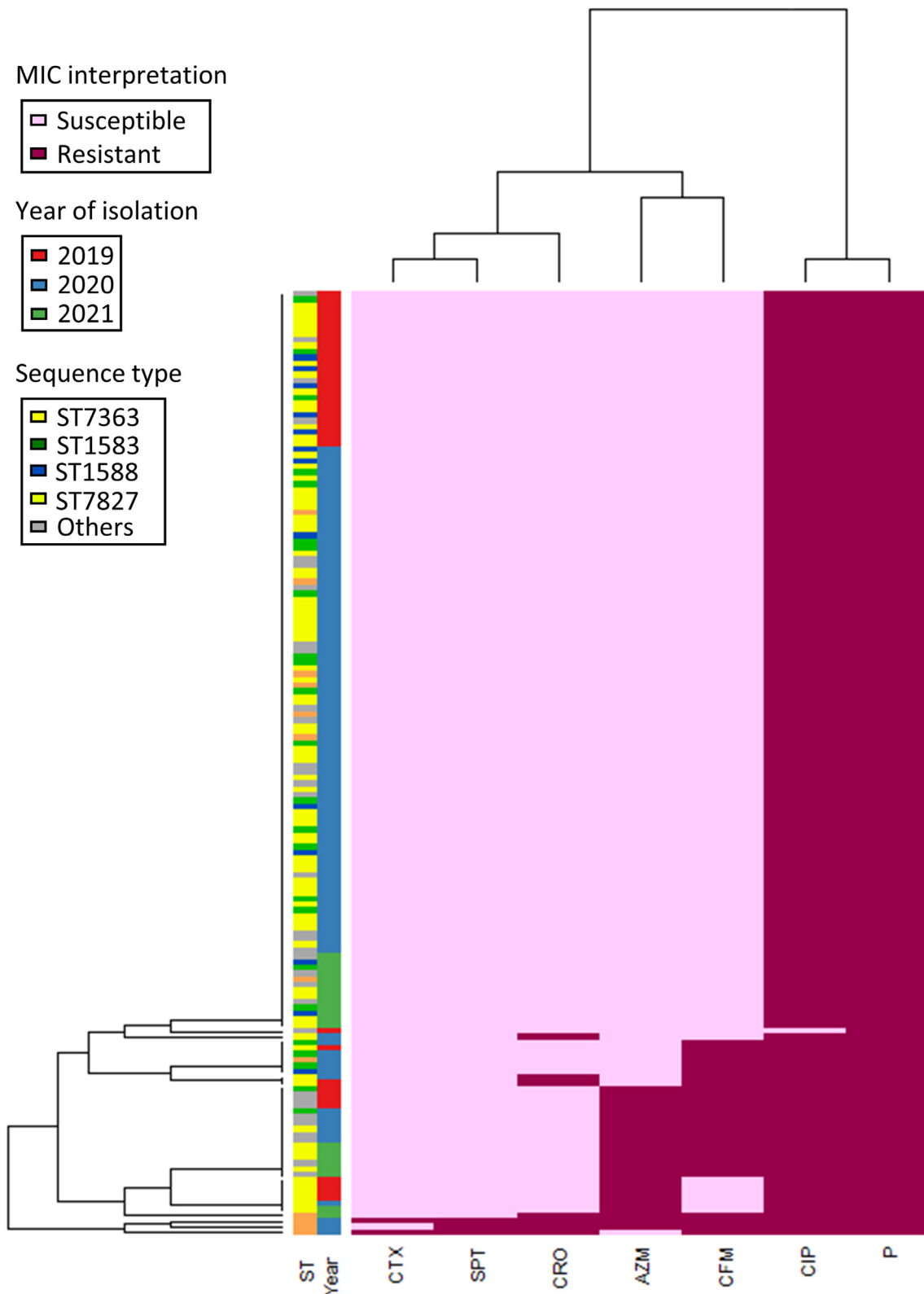
Previous studies reported that 46% of gonococcal cases were transmitted in/between homosexual groups, especially MSM groups in EU/EEA countries, 2016,<sup>28</sup> whereas in a Northern Taiwan hospital which specialized in sex

transmitted diseases during 2006–2013, the rate of the sexual orientation were 44.95% homosexual (481/1090), 47.85% heterosexual (521/1090), and 7.20% bisexual (77/1090).<sup>29</sup> This study was conducted in a community urology clinic and the proportion of MSM among gonorrhea patients was 15.15%. This lower rate than the rate in EU/EAA countries and the lack of specific sequence type lineage to associate with MSM implied difference in local epidemiology. This lower rate than the rate in a Northern Taiwan hospital indicates different epidemiology in different region but also suggests the need to survey gonococcal infection and transmission in community clinic setting.

The only earlier study of *N. gonorrhoeae* MLST conducted in Taiwan was for isolates from 2001 to 2013 in a northern Taiwan medical center that ST1901 (14.2%), ST7365 (9.3%), ST1927 (8.0%), ST1600 (7.0%), and ST7371 (4.9%) were identified as the main STs.<sup>2</sup> Interestingly, all the above five STs were all different from the major four STs in this study (ST7363 (50.3%), ST1583 (14.6%), ST1588 (7.8%), ST7827 (7.3%)). All 4 of our most prevalent type shares the same 5 alleles. The ST1600 from previous study of Northern Taiwan shares the most alleles with ST7363 from our study, which six out of total seven locus are the same (Supplementary Table 3). So as ST7365, ST7371 with ST1583. Followed by ST1901 with ST1583 shares 5 alleles. The ST7363 being responsible for half of the infection from 2019 to 2021 in southern Taiwan was only present in 3.1% of isolates in a northern Taiwan medical center from 2001 to 2013.<sup>2</sup> From 2014 to 2018 in China, the most prevalent STs was ST8123 (9.79%), followed by ST7363 (n = 85, 9.35%).<sup>30</sup> For isolates in Japan, Yahara et al. reported that among ST7363 (n = 36, 17.82%) and ST1901 (n = 33, 16.34%) were the major STs in 2015.<sup>31</sup> The phylogenetic analysis also revealed that ST8123 was highly related to ST7363 and ST7827.<sup>30</sup> The above revealed that the molecular prevalent lineage of *N. gonorrhoeae* evolved and was mainly attributed to the lineage of ST7363 and ST1901 in East Asia.

The antimicrobial resistance in *N. gonorrhoeae* in East Asia was summarized in Table 3.<sup>2,32–34</sup> The loss of ciprofloxacin susceptibility was revealed in our study, the other Taiwan studies,<sup>2</sup> and even the nearby countries (Korea,<sup>33</sup> China,<sup>32</sup> Philippines,<sup>35,36</sup> and Vietnam<sup>34</sup>). The azithromycin-resistant rates were stable: 14.6%<sup>2</sup> and 15.24% (this study) in Taiwan, in spite of the higher resistance rates in nearby countries (38% in Korea and 30% in Vietnam).<sup>33,34</sup> Though the susceptibility rates to two extended spectrum cephalosporins, including 95.73% for ceftriaxone and 98.78% for cefotaxime, were high, the susceptibility rate to cefixime was 82.93%. Noteworthy, a decreasing trend in cefixime susceptibility was noticed from 84.21% in 2019 to 66.27% in 2021. Besides, the emergence of resistance to ceftriaxone and cefixime in Taiwan, when compared to a previous Taiwan report with no resistance to ceftriaxone and a 0.4% non-susceptible rate to cefixime,<sup>2</sup> implies that the use of cefixime, which was recommended as the first line treatment in Taiwan,<sup>37</sup> should be re-evaluated (Tables 2 and 3).

The wild type *penA* allele was found to be replaced by a mosaic *penA10* allele in a clade of ST7363 during 1960–2013. The *penA10* allele was found to subsequently mutate into *penA34* in 2 ST1901 isolates. The mosaic *penA* alleles in ST7363 and 1901 led to high MICs for both cefixime (3–4 µg/ml) and ceftriaxone (2 µg/ml).<sup>4</sup> In Norway, ST7827



**Figure 3.** Heatmap visualization of antimicrobial resistant profiles for 164 isolates. The year of isolation and MLST were supplied as rowsides for analysis. The heatmap was constructed with the “heatmap.plus” package in RStudio software version 1.1.453. The legends on the top-left represented the meaning of the colors for MIC interpretation, year of isolation, and sequence type. P, Penicillin; CFM, Cefixime; CRO, Ceftriaxone; CTX, Cefotaxime; CIP, Ciprofloxacin; SPT, Spectinomycin; AZM, Azithromycin. The dendrograms on the heatmap indicated the antibiotic susceptibility patterns along strains (left)/antibiotics (upper).

**Table 3** Antimicrobial susceptibilities of *N. gonorrhoeae* in East Asia.

Country	Collection year	% Non-susceptibility <sup>a</sup>							Ref.
		Ceftriaxone	Cefixime	Spectinomycin	Azithromycin	Penicillin G	Tetracycline	Ciprofloxacin	
Taiwan	2001–2013	0	0.4	0.4	14.6 <sup>b</sup>	100	NR <sup>d</sup>	93.4	2
Korea	2011–2013	3	9	0	38 <sup>c</sup>	100	74	97	33
China	2012–2013	4.4	NR	0.2	NR	NR	42.9	99.8	32
Philippines	2013–2014	0	0	NR	NR	95.2	66.7	90.4	35
Philippines	2015–2017	0	17.4	8.1	NR	100	94.9	95.1	36
Vietnam	2017–2019	0.7	3	0	30	100	99.9	99.9	34
Taiwan	2019–2021	4.2	17	1.8	15.2	100	ND	99.4	this study

<sup>a</sup> Non-susceptibility was defined as those not susceptible to the agent, including intermediate, resistant, and non-susceptible.

<sup>b</sup> The criteria used in the study was EUCAST version 7.1.

<sup>c</sup> 5% resistant and 33% intermediate-resistant rates.

<sup>d</sup> NR, Not reported.

possessed resistance to fluoroquinolones and was associated with both decreased susceptibility of azithromycin and third generation cephalosporin among 2016–2018 isolates.<sup>18</sup> High-level azithromycin resistance (HL-AZMR) were associated with ST10899 in China<sup>38</sup> and was found in 4 isolates in USA, including ST9363 (1 isolate), ST7363 (1), and ST11982 (2).<sup>19</sup> According to previous efforts,<sup>4,6,19–21</sup> the antimicrobial susceptibility of *N. gonorrhoeae* demonstrated relationship with their ST. The heatmap of antimicrobial profiles in the study demonstrated a fraction of ST7827, which is phylogenetically related to ST7363<sup>30</sup> to harbor multidrug resistance, including resistance to ceftriaxone (For the seven ceftriaxone non-susceptible isolates, four were assigned to ST 7827 and three belonged to ST7363). Moreover, Among the 25 azithromycin-resistant isolates, twelve belonged to ST7363 and three were of ST7827. In our study, the increasing trend in the proportion of ST7827 and the high prevalence rate of ST7363 in Taiwan was observed. The emergence of azithromycin or ceftriaxone resistance was also found. To this end, the specific lineages of *N. gonorrhoeae* should be closely monitored for the treatment and infection control of gonorrhea. Further genomic investigation using the whole genome sequencing technique would be needed.

In conclusion, the molecular epidemiology of antibiotic-resistant *N. gonorrhoeae* evolved geographically and timely. Agree with previous studies conducted in other countries,<sup>2,30,31</sup> our finding showed that ST7363 and its relevant sequence type, ST7827, which were in half of the *N. gonorrhoeae* isolates in Southern Taiwan were also found with higher proportions of drug resistance than others. However, further evidence based on genome analyses would be needed to clarify the relation between molecular epidemiology and antimicrobial susceptibility *N. gonorrhoeae* and to monitor the phylogenesis. Besides, this study was limited by the lack of other STD detections, and thus, the concurrent co-infection of *N. gonorrhoeae* and other STDs was unknown.

## Declaration of competing interest

The authors declare no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmii.2023.03.005>.