International Journal of Antimicrobial Agents 61 (2023) 106785

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Multicentre Clinical Evaluation of a Molecular Diagnostic Assay to Identify *Neisseria gonorrhoeae* Infection and Detect Antimicrobial Resistance

Leshan Xiu[a,b,1], Liqin Wang[a,c,1], Yamei Li[a,c], Lihua Hu[d], Jia Huang[d], Gang Yong[e], Youwei Wang[e], Wenling Cao[f], Yuan Dong[g], Weiming Gu[h,**], Junping Peng[a,c,*]

[a] *NHC Key Laboratory of Systems Biology of Pathogens, Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China*
[b] *School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[c] *Key Laboratory of Respiratory Disease Pathogenomics, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*
[d] *Zhejiang Provincial Institute of Dermatology, Deqing, China*
[e] *Institute of Dermatology and Venereology, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, China*
[f] *Guangzhou Institute of Dermatology, Guangzhou, China*
[g] *Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China*
[h] *Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai, China*

ARTICLE INFO

Article history:
Received 3 February 2023
Accepted 7 March 2023

Editor: Dr Shabbir Simjee

Keywords:
Neisseria gonorrhoeae
Antimicrobial resistance
High-resolution melting
Molecular diagnostics
Clinical evaluation
FC428 clone

ABSTRACT

Objectives: Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* (*N. gonorrhoeae*) is an urgent threat to public health, with the emergence of highly resistant strains such as the FC428 clone. This study aimed to evaluate the high-resolution melting assay of *N. gonorrhoeae* AMR (HRM-NG-AMR) for diagnosing *N. gonorrhoeae* infection and detecting extended-spectrum cephalosporins and azithromycin resistance.
Methods: A multicentre collection of 1488 samples, including 770 isolates and 718 urogenital swabs, was used to evaluate the performance of the HRM-NG-AMR assay. The presence of *N. gonorrhoeae* was confirmed by culture. Minimum inhibitory concentrations of antibiotics against the tested isolates were determined using the agar dilution method.
Results: Regarding *N. gonorrhoeae* identification, HRM-NG-AMR had a sensitivity of 95.15% (95% CI 91.65–97.28) and a specificity of 96.44% (95% CI 94.17–97.89) using culture as standard. Regarding AMR detection, the specificity ranged from 96.29% (95% CI 94.57–97.50) for cefixime to 99.52% (95% CI 98.68–99.85) for azithromycin. Additionally, the sensitivity ranged from 31.34% (95% CI 20.87–43.97) for azithromycin to 79.10% (95% CI 63.52–89.42) for ceftriaxone. It was determined that 664 of 672 (98.81%) and 615 of 672 (91.52%) *N. gonorrhoeae* isolates were susceptible to ceftriaxone and cefixime, respectively, by detecting non-mosaic *penA*. Lastly, 40 genotypic FC428-related strains with the *penA*-60.001 allele were accurately identified.
Conclusions: The HRM-NG-AMR assay showed promising diagnostic performance for detecting *N. gonorrhoeae* infection and predicting AMR. This study aimed to evaluate the application of this assay in the clinical setting to enhance AMR surveillance and treatment intervention.

Introduction

Gonorrhoea, caused by *Neisseria gonorrhoeae* (*N. gonorrhoeae*), is the second most common sexually transmitted bacterial infection worldwide [1,2]; consequently, efforts have been made to prevent the spread and eliminate the infection using antimicrobial treatment. Extended-spectrum cephalosporins (ESCs), such as cefixime (CFM) and ceftriaxone (CRO), are considered as the last effective option for empirical monotherapy [2,3]; however, ESCs resistance is increasing worldwide [2,4] and compromising its effectiveness.

* Corresponding authors: No. 6 Rongjing Eastern Street, BDA, Beijing 100176, China. Tel.: 86-10- 67878493; fax: 86-10- 67878493
** Corresponding authors: No. 1278 Bao De Rd., Shanghai 200443, China. Tel.: 86-021-36803177; fax: 86-021-36803177
E-mail addresses: weiming_gu2003@aliyun.com (W. Gu), pengjp@hotmail.com (J. Peng).
[1] Leshan Xiu and Liqin Wang contributed equally to this work

https://doi.org/10.1016/j.ijantimicag.2023.106785
0924-8579/

Table 1
Tested samples from four distinct geographical locations.

Sample source	Clinical isolates			Urogenital swabs					
	Year			Gender			No. of *N. gonorrhoeae* positive results		
	2019	2020	Total	Male	Female	Total	Culture[a]	HRM[b]	RT-PCR[c]
Deqing	102	105	207	31	73	104	24	27	27
Chengdu	92	68	160	33	5	38	18	23	23
Guangzhou	100	100	200	129	78	207	31	32	31
Shanghai	101	102	203	280	89	369	195	189	188
Total	395	375	770	473	245	718	268	271	269

[a] Culture, bacterial culture.
[b] HRM, the HRM-NG-AMR assay.
[c] RT-PCR, an in-house real-time PCR assay.

Therefore, the World Health Organization has recommended CRO and azithromycin (AZM) combination therapy as the first-line antimicrobial [1,5], but this treatment strategy is now in danger as *N. gonorrhoeae* has developed resistance to CRO and AZM [6]. A high-level resistant FC428 clone containing the *penA*-60.001 allele was initially identified in Japan in 2015 and subsequently disseminated to all continents [4].

Genetically, AZM resistance has been strongly linked to specific mutations in the 23S rRNA genes [2,7]. The molecular mechanism of ESCs resistance is complicated and associated with mutations in several genes [2,8]. Resistance determinants include (i) acquisition of a mosaic *penA* allele of homologous recombination from commensal *Neisseria* spp. and (ii) mutations in several target genes (*ponA, porB* and *mtrR*) associated with resistance or decreased susceptibility to ESCs [1,2,8]. Clinical studies have suggested that detecting these principal resistance mutations can guide antimicrobial treatment options [9,10]. Therefore, the importance of developing methods that identify resistance determinants and track the global dissemination of the FC428 clone has been recognised, to enhance AMR surveillance and potentially guide antimicrobial selection for treatment purposes.

A standard strategy for gonococcal AMR surveillance includes a combination of culture-based methods, antimicrobial susceptibility testing (AST) and specific PCR for target genes associated with sequence typing; however, these methods have a high turnaround time, are labour-intensive [11] and preclude the rapid screening of large numbers of samples. Although genomic approaches play an increasingly important role in AMR surveillance [12,13], tedious procedures are impractical for most clinical laboratories [14]. Consequently, there is an urgent need for high-throughput comprehensive approaches to improve the diagnosis of *N. gonorrhoeae* and the detection of AMR. These methods should be suitable for clinical settings, where cost savings and higher efficiency are expected owing to the high volume of samples and treatment requirements.

A previously established method [8], the multiplex high-resolution melting (HRM) assay of *N. gonorrhoeae* AMR (HRM-NG-AMR), is one candidate used to simultaneously detect *N. gonorrhoeae* infection and identify molecular markers responsible for ESCs and AZM resistance. This assay's diagnostic accuracy for AMR determinants has previously been reported [8]; however, its performance in predicting AMR directly in clinical samples has not been thoroughly evaluated. Therefore, this study evaluated the HRM-NG-AMR assay for *N. gonorrhoeae* identification, AMR phenotypes and FC428-related clone detection in isolates and urogenital swabs from four geographically and clinically distinct populations.

Materials and Methods

Neisseria gonorrhoeae *isolates and clinical samples*

In this cross-sectional study, two sets of samples were collected from four geographically distinct hospitals in China (Table 1). The first set of samples included 770 *N. gonorrhoeae* isolates, retrieved from isolate banks, from gonorrhoea patients with symptomatic urethritis (dysuria and/or urethral discharge) between 2019 and 2020 (one isolate per patient). The second set included 718 urogenital swabs from consecutive patients with suspected gonorrhoea or urethritis who visited the clinics of the four hospitals between January and July 2021. For details, see Supplementary Materials and Methods.

Specimen processing and antimicrobial susceptibility testing of Neisseria gonorrhoeae

The nucleic acid was extracted from 770 *N. gonorrhoeae* isolates after isolate resuscitation on the GC medium with 1% IsoVitaleX [15]; the HRM and PCR test were subsequently performed. For each isolate, the MIC test was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. For 718 urogenital swabs, one Dacron swab from each patient was taken and transported to the laboratory at room temperature (ca. 25°C). In order to ensure that the sample source of the culture and HRM was consistent, a portion of the swab was directly inoculated on Thayer-Martin (T-M) medium supplemented with 1% IsoVitaleX (Oxoid, USA) and incubated at 37°C ± 1°C with 5% CO_2 for isolation and culture of *N. gonorrhoeae*, and the residual portion of the swab was treated with PBS solution (pH 7.0) for 30 min and vortexed for 30 s and used for the following nucleic acid extraction and HRM-NG-AMR assay. If *N. gonorrhoeae* grew on the TM medium, the MIC test was subsequently performed in accordance with CLSI guidelines. If no *N. gonorrhoeae* presented, it was interpreted as a negative swab culture. Identification of *N. gonorrhoeae* was based on the oxidase test, Gram staining and glucose utilisation tests during routine clinical testing [15]. Gonococcal colonies were suspended in 15% glycerinum of brain-heart base broth and frozen (−80°C). Bacterial culture was used as the gold standard to detect the presence of *N. gonorrhoeae* in the urogenital swabs.

Although 268 swabs were positive for *N. gonorrhoeae* by culture, 144 were excluded from the AST stage for drug resistance determination because no corresponding active gonococcal strains were available. Therefore, 894 *N. gonorrhoeae* isolates were subjected to AST, comprising 770 isolates retrieved from the bacterial isolate banks and 124 collected from the urogenital swabs. The isolates were categorised as susceptible or resistant using the latest clinical breakpoints or epidemiological cut-off values from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for ESCs and AZM (www.eucast.org/clinical_breakpoints, accessed on 15 February 2022). A sample flow diagram is provided in Figure 1.

HRM-NG-AMR assay

The HRM-NG-AMR assay was prepared and performed as described in the Supplementary Methods. Each sample was tested

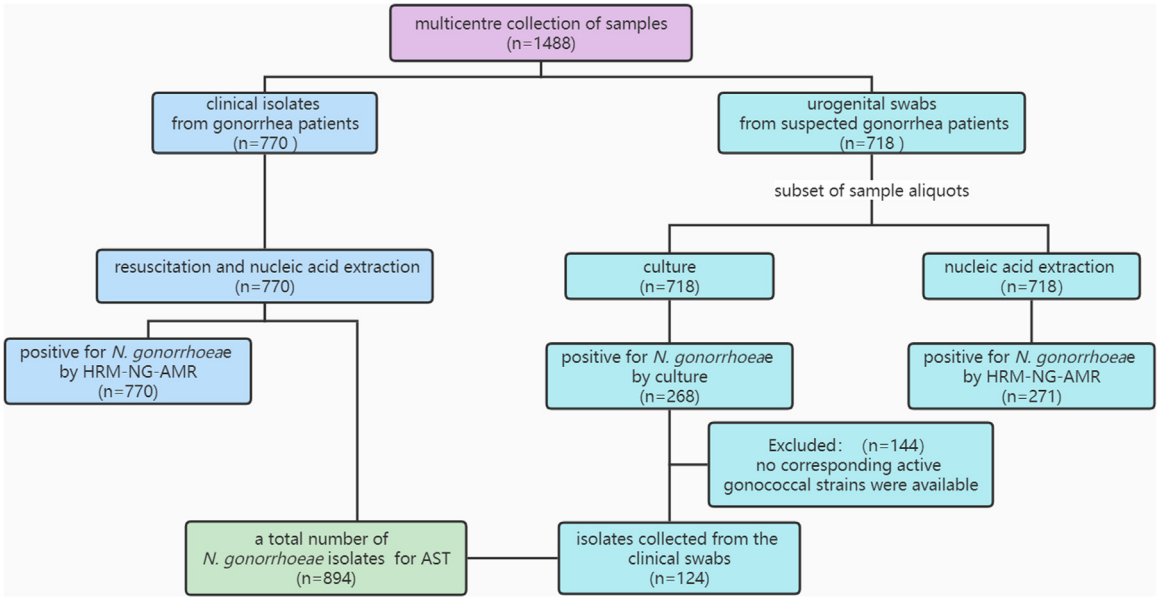

Figure 1. Study flow diagram.

Table 2
Performance of the HRM-NG-AMR assay for *N. gonorrhoeae* identification in 718 urogenital swabs compared with that of bacterial culture.

Sample source	No. of samples with the following result [a]					Analytical performance (%) [b]			
	TP	FP	FN	TN	Total	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Deqing	23	4	1	76	104	95.83 (76.88-99.78)	95 (87.00-98.39)	85.19 (65.39-95.14)	98.7 (91.99-99.93)
Chengdu	18	5	0	15	38	100 (78.12-100)	75 (50.59-90.41)	78.26 (55.79-91.70)	100 (74.65-100)
Guangzhou	31	1	0	175	207	100 (86.27-100)	99.43 (96.39-99.97)	96.87 (82.00-99.84)	100 (97.32-100)
Shanghai	183	6	12	168	369	93.85 (89.24-96.64)	96.55 (92.30-98.59)	96.83 (92.90-98.70)	93.33 (88.37-96.35)
Combined	255	16	13	434	718	95.15 (91.65-97.28)	96.44 (94.17-97.89)	94.10 (90.40-96.48)	97.09 (94.95-98.38)

[a] TP, true positive; FP, false positive; FN, false negative; TN, true negative.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

for 11 targets (*penA*-G545S, *penA*-D345del, *penA*-A501, *mtrR*-G45D, *mtrR*-H105Y, *ponA*-L421P, *porB*-120&121, 23S-A2059G, 23S-C2611T, *porA, opa*). The target *penA*-G545S was originally designed to identify mosaic *penA* alleles in HRM-NG-AMR assay. Interestingly, several strains were clearly differentiated from the mosaic *penA* patterns during the evaluation stage, displaying a change in melting temperature and a shift in the melting peak. Detailed analysis revealed that these strains were all FC428 clones containing the *penA*-60.001 allele. To determine whether the target *penA*-G545S is an indicator for detecting the FC428 clone, five FC428-associated isolates harbouring *penA*-60.001 were used, based on previous studies [4,9,16], to evaluate the effectiveness of the HRM-NG-AMR assay.

PCR and bidirectional sequencing analysis

To verify the discordant results for the presence of *N. gonorrhoeae* in clinical swabs between HRM-NG-AMR and *N. gonorrhoeae* cultures, an in-house real-time PCR assay was performed according to a previous study [17]. To further confirm all FC428-related strains identified by the HRM-NG-AMR assay, full-length *penA* sequencing was performed as described in Supplementary Methods. Similarly, the resistance-determining regions were sequenced for strains with the resistant phenotype of AZM, using specific primers applied in a previous study [8].

Statistical analysis

The assay's specificity, sensitivity, positive (PPV) and negative predictive values (NPV) with their respective 95% confidence interval (CI) were calculated for all tested strains using the VassarStats software (http://www.vassarstats.net/).

Ethical statement

The study was approved by the Medical Ethical Committee of the Shanghai Skin Disease Hospital (2021-20KY) and registered in the Chinese Clinical Trial Registry (ChiCTR2100048771).

Results

Analytical performance for Neisseria gonorrhoeae *identification in clinical swabs*

Among the 718 clinical swabs, 271 (37.74%) tested positive for *N. gonorrhoeae* by the HRM-NG-AMR assay. As described above, 268 swabs were positive for *N. gonorrhoeae* by culture. Overall, the sensitivity, specificity, PPV and NPV determined by the bacterial culture were 95.15% (95% CI 91.65–97.28), 96.44% (95% CI 94.17–97.89), 94.10% (95% CI 90.40–96.48) and 97.09% (95% CI 94.95–98.38), respectively (Table 2). Among the 16 HRM-NG-AMR-positive and culture-negative samples, 14 (87.5%) were confirmed positive by a real-time PCR assay, while two (12.5%) remained unverified. After confirmatory testing, the overall accuracy of *N. gonorrhoeae* identification by the HRM-NG-AMR assay was 99.26% (269 of 271). Another 13 swabs grew *N. gonorrhoeae* in culture but tested negative by the HRM-NG-AMR assay and confirmatory testing (Table S1), indicating low organism burden with only a subset of sample aliquots harbouring bacteria.

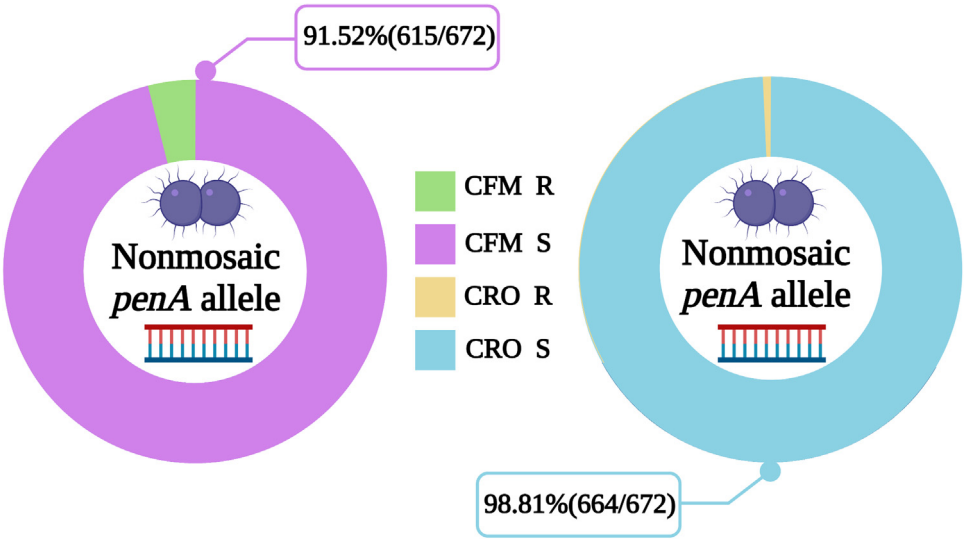

Figure 2. Diagnostic performance of the HRM-NG-AMR assay for the detection of ESCs susceptibility by detecting non-mosaic *penA* allele. Abbreviations: CFM R, cefixime resistance; CFM S, cefixime susceptibility; CRO R, ceftriaxone resistance; CRO S, ceftriaxone susceptibility.

Table 3
Analytical performance of the HRM-NG-AMR assay compared with reference antimicrobial susceptibility testing.

Antimicrobials [a]	AST results [b]			The HRM-NG-AMR assay results [c]					
	R	S	Total	R	S	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CRO	43	851	894	40	854	79.10 (63.52–89.42)	99.30 (98.40–99.72)	85.00 (69.48–93.75)	98.95 (97.93–99.48)
CFM	167	727	894	98	796	42.52 (34.98–50.40)	96.29 (94.57–97.50)	72.45 (62.35–80.76)	87.94 (85.43–90.08)
AZM	67	827	894	25	869	31.34 (20.87–43.97)	99.52 (98.68–99.85)	84.00 (63.08–94.75)	94.71 (92.95–96.06)

[a] CRO, ceftriaxone; CFX, cefixime; AZM, azithromycin.
[b] The isolates were classified as susceptible and resistant using the EUCAST clinical breakpoints for ESCs and AZM (http://www.eucast.org/); AST, antimicrobial susceptibility testing; S, number of susceptible isolates; R, number of resistant isolates.
[c] CRO resistance depended upon the presence of the *penA*-60.001 allele in *penA*; CFM resistance depended upon the presence of a mosaic allele in *penA*, an amino acid change (DA/KD/KG/KA) in *porB*, and the L421P substitution in *ponA*; AZM resistance depended upon the presence of C2611T or A2059G mutations in 23S rRNA; S, number of susceptible isolates; R, number of resistant isolates; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Antimicrobial susceptibility and genetic determinants of 894 Neisseria gonorrhoeae *isolates*

The results of antimicrobial susceptibility and genetic determinants of the 894 *N. gonorrhoeae* isolates are shown in the Supplementary Results (Figures S1 and S2, Tables S2 and S3). Notably, on comparing the tested 894 bacterial strains, 672 (75.17%) isolates were found to contain a non-mosaic *penA* allele by HRM-NG-AMR testing. When tested using culture-based AST, 98.81% (664 of 672) and 91.52% (615 of 672) were labelled as susceptible phenotypes for CRO and CFM, respectively (Figure 2). Based on these results, it was deducted that most *N. gonorrhoeae* isolates would be susceptible to ESCs (CRO and CFM) by detecting non-mosaic *penA*.

Analytical performance for antimicrobial resistance detection in Neisseria gonorrhoeae

Overall, the HRM-NG-AMR assay demonstrated 99.30% (95% CI 98.40–99.72) specificity and 79.10% (95% CI 63.52–89.42) sensitivity for CRO resistance detection compared with the phenotype-based susceptibility data (Table 3). Based on the HRM results, the specificity and sensitivity for CFM resistance detection were 96.29% (95% CI 94.57–97.50) and 42.52% (95% CI 34.98–50.40), respectively. Compared with reference AST, the HRM-NG-AMR assay displayed a specificity of 99.52% (95% CI 98.68–99.85) and sensitivity of 31.34% (95% CI 20.87–43.97) for AZM resistance detection.

Characteristics of FC428-related strains screening

The HRM-NG-AMR assay accurately identified FC428-associated strains, which were easily differentiated from the mosaic allele (Figure 3A). Therefore, *penA*-G545S could be used as a target for screening FC428-related strains from clinical samples. Of the 894 *N. gonorrhoeae* isolates, 40 were classified as genotypic FC428-related strains with the *penA*-60.001 allele by the HRM-NG-AMR assay, confirmed by *penA* sequencing. Among the 40 genotypic FC428-related strains, 39 of 40 (97.5%) isolates had a phenotypic profile of resistance or decreased susceptibility to ESCs (Figure 3B).

Discussion

This study performed a large-scale multicentre clinical evaluation of a molecular diagnostic assay, including 1488 individuals from four distinct geographical locations in China. The HRM-NG-AMR assay showed promising diagnostic performance for identifying *N. gonorrhoeae* infection and detecting AMR; it also showed an impressive capacity for screening FC428-related strains when used to examine clinical isolates and secretion swabs.

Several molecular assays have been successfully developed to detect *N. gonorrhoeae* infection [18,19]; however, some of these assays use only one or two targets, leading to the possibility of false-negative results [20], owing to mutations or genetic diversity within targets. High specificity rates are significant in low-

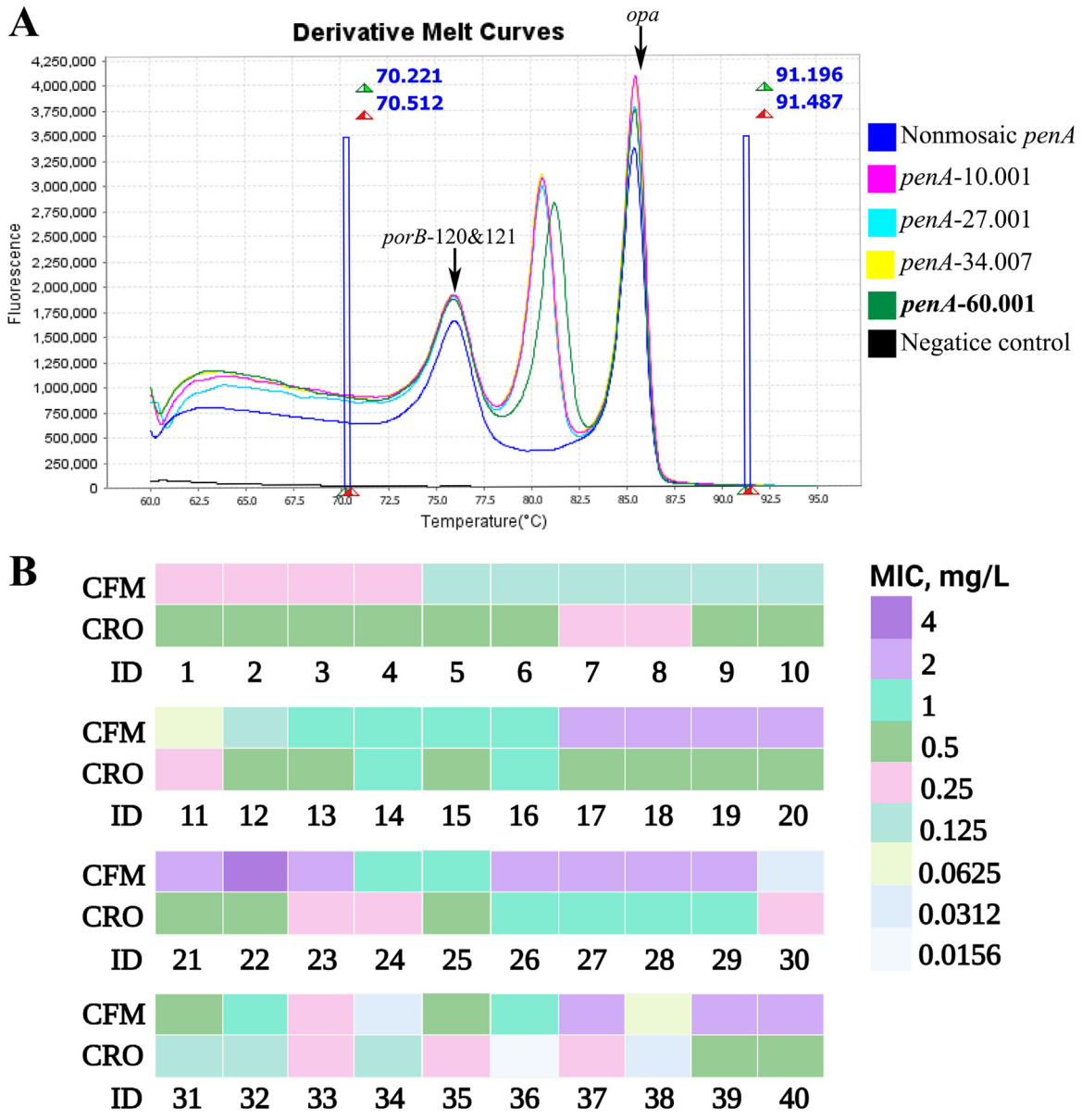

Figure 3. The characteristic for screening of FC428-related strains using the HRM-NG-AMR assay. (A) Derivative melt curves of the target for *penA*-G545S in the HRM-NG-AMR assay. (B) MIC values among 40 FC428-related strains containing *penA*-60.001 allele identified by the HRM-NG-AMR assay.
Abbreviations: CFM, cefixime; CRO, ceftriaxone.

prevalence settings to reach a high PPV and reliably discriminate between *N. gonorrhoeae* and commensal *Neisseria* species [20]. To address this dilemma, dual-target (*porA* and *opa*) strategies were adopted to increase specificity. Compared with the culture method, the sensitivity, specificity, PPV and NPV of HRM-NG-AMR were 95.15%, 96.44%, 94.10% and 97.09%, respectively. This result further illustrates the importance of including dual targets in investigating gonorrhoea cases with false-negative results using molecular methods [21].

Given that ESCs and AZM are commonly used in treatment regimens [1,2,4], direct detection of AMR determinants in clinical samples will rapidly identify resistance and facilitate the judicious use of appropriate antibiotics in treating *N. gonorrhoeae*. An additional benefit of the HRM-NG-AMR assay is that it concomitantly detects resistance-associated determinants [8]. Compared with culture-based AMR testing, the current study obtained a similarly high specificity of 99.30% with a lower sensitivity of 79.10% for detecting CRO resistance. The data demonstrated that detecting the non-mosaic *penA* allele could have predicted that the majority (98.81% and 91.52%) of isolates were susceptible to ESCs (CFM and CRO). Although this assay could enhance the effective use of ESCs in treating gonorrhoea, relying solely on non-mosaic *penA* to determine susceptibility has its limitation. Another advantage of the HRM-NG-AMR assay is its ability to detect AZM resistance markers [8]. This assay accurately detected mutation patterns (A2059G and C2611T) in the 23S rRNA gene, which was confirmed by PCR and sequencing. C2611T is still the predominant determinant of AZM resistance, which is consistent with previous results [22,23]. The current assay showed suboptimal sensitivity for detecting phenotypic AZM resistance; there are several possible reasons for this, one of which is that AZM resistance can also be caused by other mechanisms [24] such as overexpression of the MtrCDE efflux pump, presence of *erm* genes, inactivation of macrolides by *ere* and *mph* genes, ribosomal gene mutations in *rplD* and *rplV*, and presence of mosaic-*mtrR* substitutions. Therefore, further optimisation is required to increase the prediction

accuracy of AZM resistance, including the detection of additional AZM resistance determinants.

The widespread dissemination of the FC428 clone, particularly when combined with AZM resistance, has created significant therapeutic challenges [16,25]. Tracking the spread of this clone has been made possible through traditional and complicated genotyping techniques. To address this challenge, an HRM-NG-AMR assay was used to determine whether specific genetic marker identification can accurately predict the dissemination of FC428 clones. It was found that the level of specific melting pattern concordance in FC428-related strains containing the *penA*-60.001 allele was 100%, suggesting that the *penA*-G545S target might be a good indicator for detecting FC428 clones. Furthermore, a nucleic acid change (T to C) in the target region was found, compared with general mosaic alleles, which was responsible for the difference in melting profiles. This study found that the genotypic FC428-related isolate was susceptible to ESCs. The findings suggest that FC428-related strains might change during transmission and may have enabled the emergence of diversity in the infection reservoir. Given that the HRM-NG-AMR assay can screen for FC428-related strains, it could potentially be used in clinical laboratories in conjunction with diagnostic methods that are now commonplace for FC428-related strain screening. Additionally, the FC428 clone has spread to several geographically-distinct regions within China, and its prevalence might be increasing [15,26–28]; therefore, prioritising and facilitating FC428 clone identification in China by implementing the HRM-NG-AMR assay would fundamentally improve the detection and management of the disease.

This study had some limitations. First, some molecular markers insufficiently contributed to AMR detection, indicating that resistant isolates cannot be efficiently screened. Hence, to optimise the performance of this algorithm, the diagnostic strategy should be further adjusted by incorporating markers that contribute significantly. Additionally, the accuracy and precision of AZM resistance detection based on molecular determinants were primarily limited by the proportion of mutations in the 23S rRNA gene used to test samples. There was a low degree of sensitivity (31.34%) but higher specificity for resistance detection (99.52%) because of the relatively large number of AZM-resistant strains (62.22%, 28 of 45) that did not carry any mutations in the 23S rRNA genes. However, these limitations can be reduced by regularly updating the original assay with newly discovered resistance determinants and adjusting the diagnostic strategy by replacing it with significantly contributing markers.

In conclusion, this analysis is the first step towards bridging the gap between molecular diagnostic assays and ARM detection in *N. gonorrhoeae*. Furthermore, it showed that the HRM-NG-AMR assay has promising diagnostic performance when used in appropriate scenarios, consequently improving patient outcomes, clinical decisions, infection control and AMR surveillance. In view of results derived from this study, future studies that focus on treatment intervention are planned to validate whether this assay will inform antimicrobial treatment decisions in the clinical setting.

Funding

This study was supported by grants from the CAMS Innovation Fund for Medical Sciences (2021-I2M-1-038), Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2019PT310029), the Fundamental research Funds for the Central Universities (3332021092), the Outstanding Youth Training Program of Shanghai Municipal Health Commission (2018YQ57), and sponsored by Shanghai Pujiang Program (22PJD035).

Competing Interests

Junping Peng and Leshan Xiu have a patent (People's Republic of China) related to this article. The authors have declared that no other competing financial interests exist.

Ethical Approval

The study was approved by the Medical Ethical Committee of the Shanghai Skin Disease Hospital (2021-20KY) and registered in the Chinese Clinical Trial Registry (ChiCTR2100048771).

Sequence Information

Not applicable.

Author contributions

JP, WG and LX were involved in the concept of the study. JP and WG contributed to the study design and formulating the research question. LX performed the experiments, analysed the results and wrote the manuscript. YD, LW, YL, LH, JH, GY, YW and WC conducted the experiments and analysed the results. All authors reviewed and revised the final version of manuscript.

Acknowledgements

We give special thanks to Prof. Tianci Yang for his constructive suggestions on our evaluation study. We would like to thank Dr Mingmin Liao for assistance in data analysis and the manuscript preparation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2023.106785.

References

[1] Golparian D, Unemo M. Antimicrobial resistance prediction in *Neisseria gonorrhoeae*: current status and future prospects. Expert Rev Mol Diagn 2022;22:29–48.
[2] Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev 2014;27:587–613.
[3] Weston EJ, Wi T, Papp J. Strengthening Global Surveillance for Antimicrobial Drug-Resistant *Neisseria gonorrhoeae* through the Enhanced Gonococcal Antimicrobial Surveillance Program. Emerg Infect Dis 2017;23(13):S47–52.
[4] Xiu L, Yuan Q, Li Y, Zhang C, Tang L, Peng J. Emergence of ceftriaxone-resistant *Neisseria gonorrhoeae* strains harbouring a novel mosaic penA gene in China. J Antimicrob Chemother 2020;75:907–10.
[5] Gianecini RA, Golparian D, Zittermann S, Litvik A, Gonzalez S, Oviedo C, et al. Genome-based epidemiology and antimicrobial resistance determinants of *Neisseria gonorrhoeae* isolates with decreased susceptibility and resistance to extended-spectrum cephalosporins in Argentina in 2011–16. J Antimicrob Chemother 2019;74:1551–9.
[6] Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, et al. Failure of Dual Antimicrobial Therapy in Treatment of Gonorrhea. New Eng J Med 2016;374:2504–6.
[7] Wind CM, Bruisten SM, Schim van der Loeff MF, Dierdorp M, de Vries HJC, van Dam AP. A Case-Control Study of Molecular Epidemiology in Relation to Azithromycin Resistance in *Neisseria gonorrhoeae* Isolates Collected in Amsterdam, the Netherlands, between 2008 and 2015. Antimicrob Agents Chemother 2017;61(6) e02374–16.
[8] Xiu L, Li Y, Wang F, Zhang C, Li Y, Zeng Y, et al. Multiplex High-Resolution Melting Assay for Simultaneous Identification of Molecular Markers Associated with Extended-Spectrum Cephalosporins and Azithromycin Resistance in *Neisseria gonorrhoeae*. J Mol Diagn 2020;22:1344–55.
[9] Xiu L, Zhang C, Li Y, Wang F, Peng J. High-resolution melting analysis for rapid detection of the internationally spreading ceftriaxone-resistant *Neisseria gonorrhoeae* FC428 clone. J Antimicrob Chemother 2020;75:106–9.
[10] Lin EY, Adamson PC, Ha SM, Klausner JD. Reliability of Genetic Alterations in Predicting Ceftriaxone Resistance in *Neisseria gonorrhoeae* Globally. Microbiol Spectr 2022;10:e0206521.

[11] McAuliffe GN, Smith M, Cooper G, Forster RF, Roberts SA. Variability in Azithromycin Susceptibility Results for *Neisseria gonorrhoeae* Obtained Using Gradient MIC Strip and Agar Dilution Techniques. J Clin Microbiol 2019;57(12):e0206521.
[12] Yahara K, Nakayama SI, Shimuta K, Lee KI, Morita M, Kawahata T, et al. Genomic surveillance of *Neisseria gonorrhoeae* to investigate the distribution and evolution of antimicrobial-resistance determinants and lineages. Microb Genom 2018;4(8):e000205.
[13] Peng JP, Yin YP, Chen SC, Yang J, Dai XQ, Zheng HP, et al. A Whole-genome Sequencing Analysis of *Neisseria gonorrhoeae* Isolates in China: An Observational Study. E Clin Med 2019;7:47–54.
[14] Xiu L, Zhang C, Li Y, Wang F, Peng J. Simultaneous detection of eleven sexually transmitted agents using multiplexed PCR coupled with MALDI-TOF analysis. Infect Drug Resist 2019;12:2671–82.
[15] Yang Y, Yang Y, Martin I, Dong Y, Diao N, Wang Y, et al. NG-STAR genotypes are associated with MDR in *Neisseria gonorrhoeae* isolates collected in 2017 in Shanghai. J Antimicrob Chemother 2020;75:566–70.
[16] Yuan Q, Li Y, Xiu L, Zhang C, Fu Y, Jiang C, et al. Identification of multidrug-resistant *Neisseria gonorrhoeae* isolates with combined resistance to both ceftriaxone and azithromycin, China, 2017–2018. Emerg Microbes Infect 2019;8:1546–9.
[17] Hjelmevoll SO, Olsen ME, Sollid JU, Haaheim H, Unemo M, Skogen V. A fast real-time polymerase chain reaction method for sensitive and specific detection of the *Neisseria gonorrhoeae* porA pseudogene. J Mol Diagn 2006;8:574–81.
[18] Alexander DC, Taylor RM, Martin I, Adam H, Lagace-Wiens P, Pieroni P, et al. Evaluation of the Hologic Aptima Combo 2 Assay for Detection of *Neisseria gonorrhoeae* from Joint Fluid Specimens. J Clin Microbiol 2022;60:e0253021.
[19] van Niekerk JM, van der Veer B, Hoebe C, van de Bovenkamp J, van Herk C, van Loo IHM, et al. Despite Excellent Test Characteristics of the cobas 4800 CT/NG Assay, Detection of Oropharyngeal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Remains Challenging. J Clin Microbiol 2021;59(2) e02137–20.
[20] Sukhum KV, Jean S, Wallace M, Anderson N, Burnham CA, Dantas G. Genomic Characterization of Emerging Bacterial Uropathogen *Neisseria meningitidis*, Which Was Misidentified as *Neisseria gonorrhoeae* by Nucleic Acid Amplification Testing. J Clin Microbiol 2021;59(2) e01699–20.
[21] Whiley DM, Tapsall JW, Sloots TP. Nucleic acid amplification testing for *Neisseria gonorrhoeae*: an ongoing challenge. J Mol Diagn 2006;8:3–15.
[22] Demczuk W, Martin I, Peterson S, Bharat A, Van Domselaar G, Graham M, et al. Genomic Epidemiology and Molecular Resistance Mechanisms of Azithromycin-Resistant *Neisseria gonorrhoeae* in Canada from 1997 to 2014. J Clin Microbiol 2016;54:1304–13.
[23] Gianecini RA, Poklepovich T, Golparian D, Cuenca N, Tuduri E, Unemo M, et al. Genomic Epidemiology of Azithromycin-Nonsusceptible *Neisseria gonorrhoeae*, Argentina, 2005–2019. Emerg Infect Dis 2021;27:2369–78.
[24] Pham CD, Sharpe S, Schlanger K, St Cyr S, Holderman J, Steece R, et al. Emergence of *Neisseria gonorrhoeae* Strains Harboring a Novel Combination of Azithromycin-Attenuating Mutations. Antimicrob Agents Chemother 2019;63(4):e02313–18.
[25] Eyre DW, Town K, Street T, Barker L, Sanderson N, Cole MJ, et al. Detection in the United Kingdom of the *Neisseria gonorrhoeae* FC428 clone, with ceftriaxone resistance and intermediate resistance to azithromycin, October to December 2018. Euro Surveill 2019;24(10):1900147.
[26] Wang H, Wang Y, Yong G, Li X, Yu L, Ma S, et al. Emergence and genomic characterization of the ceftriaxone-resistant *Neisseria gonorrhoeae* FC428 clone in Chengdu, China. J Antimicrob Chemother 2020;75:2495–8.
[27] Yan J, Chen Y, Yang F, Ling X, Jiang S, Zhao F, et al. High percentage of the ceftriaxone-resistant *Neisseria gonorrhoeae* FC428 clone among isolates from a single hospital in Hangzhou, China. J Antimicrob Chemother 2021;76:936–9.
[28] Lin X, Qin X, Wu X, Liao Y, Yu Y, Xie Q, et al. Markedly Increasing Antibiotic Resistance and Dual Treatment of *Neisseria gonorrhoeae* Isolates in Guangdong, China, from 2013 to 2020. Antimicrob Agents Chemother 2022;66:e0229421.