Infection and Drug Resistance

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SHORT REPORT

Evaluating Chelerythrine Chloride as a Potential Treatment for Multidrug-Resistant Gonorrhea

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Objective: *Neisseria gonorrhoeae* (*N. gonorrhoeae)* is responsible for the sexually transmitted infection (STI) gonorrhea, which has an estimated global annual incidence of 82.4 million cases among adults. The recommended first-line treatment typically involves a single-dose systemic therapy, comprising injectable ceftriaxone and oral azithromycin. Nonetheless, the first-line treatment failures caused by antimicrobial resistance represent a major global public health concern, threatening the efficacy of current gonorrhea treatments and highlighting the urgent need for the development of alternative therapeutic approaches.

Methods: A total of 54 clinical strains of *N. gonorrhoeae* were collected in Nanchang City, 2021. To assess the efficacy of antibiotics and chelerythrine chloride, we determined the minimum inhibitory concentrations (MICs) using agar dilution and broth microdilution methods, respectively. To explicitly evaluate the potential for resistance induction, the ATCC49226 strain was subjected to continuous passaging for 30 days in sub-MIC concentrations of chelerythrine chloride, with MIC assessments every 5 days.

Results: In clinical samples, antimicrobial resistance was observed for penicillin (67.27%), tetracycline (81.82%), ciprofloxacin (98.18%), azithromycin (5.45%), and spectinomycin (0%), with decreased susceptibility for ceftriaxone (16.36%) and cefixime (20.00%). High-throughput screening of a natural product library identified chelerythrine chloride as exhibiting significant inhibitory activity against *N. gonorrhoeae*, including strains with decreased susceptibility to cephalosporins. The MIC range was 0.002–8 mg/L, with both the MIC_{50} and MIC_{90} values at 8 mg/L. Furthermore, *N. gonorrhoeae* did not develop resistance, maintaining a stable MIC of 4 mg/L over a 30-day treatment period.

Conclusion: In this study, we have established a novel association between chelerythrine chloride and *N. gonorrhoeae*, demonstrating for the first time its preliminary efficacy in eradicating multidrug-resistant strains of *N. gonorrhoeae*. Considering the significant resistance challenges posed by *N. gonorrhoeae*. chelerythrine chloride emerges as a promising antibacterial agent with substantial potential for clinical development.

Keywords: *N. gonorrhoeae*, antimicrobial resistance, chelerythrine chloride, antimicrobial agent

Introduction

Neisseria gonorrhoeae (*N. gonorrhoeae*), a pathogen primarily targeting the human urogenital tract, is responsible for the sexually transmitted infection gonorrhea. Clinically, *N. gonorrhoeae* infects the mucosal epithelium of the human genitourinary system, leading to symptoms such as urethritis and cervicitis, and if left untreated, it may cause co-infections such as syphilis, AIDS, chlamydia, and also enhances the transmission of HIV, thereby posing a significant threat to public health security.[1] According to the World Health Organization (WHO), an estimated 82 million new cases reported in 2020,[2] with incidence rates escalating due to growing antibiotic resistance (AMR). In the absence of an effective vaccine against *N. gonorrhoeae*, antibiotics such as ceftriaxone remain the mainstay of treatment. While in the

Received: 7 May 2025
Accepted: 15 September 2025
Published: 24 September 2025

past decade, confirmed failure to cure gonorrhoea with ceftriaxone alone or combined with azithromycin or doxycycline was reported in Japan, China, Australia, France, Slovenia, Sweden and the United Kingdom of Great Britain and Northern Ireland.[2] Moreover, the international spreading ceftriaxone-resistant gonococcal strain "FC428" has presented a critical challenge to first-line drug's efficacy.[3] As ceftriaxone resistance rates continue to rise, the WHO has emphasized the need for enhanced global surveillance of AMR and the development of novel antibacterial agents to combat AMR.

However, the discovery of new drugs is typically time-consuming, costly, and has a low success rate. The development of new antimicrobial drugs is progressing at a significantly slower pace than the emergence of antimicrobial resistance. To address this challenge, high-throughput screening technology has been employed to rapidly identify a variety of drugs. High-throughput screening can complete antimicrobial activity screening in a short period of time that would otherwise take months using traditional methods, clearly demonstrating its overwhelming advantages in terms of speed, throughput, and hit rate.[4] In this study, we successfully collected 54 clinical isolates of *N. gonorrhoeae*. Through susceptibility testing, we observed significant AMR. To enhance the efficiency of therapeutic drug screening, we employed a high-throughput drug screening platform, identifying chelerythrine chloride as a potential antibacterial agent (data to be published).

Papaveraceae – chelerythrine chloride, with the chemical formula $C_{21}H_{18}CINO_4$, a benzophenanthridine alkaloid derived from the poppy family, is known for its anti-inflammatory, antibacterial, and antitumor properties. Previous studies have demonstrated that chelerythrine chloride inhibits the growth of Streptococcus agalactiae by disrupting cell membrane integrity and cell morphology.[5] It can inhibit a post-entry step of the Zika virus replication cycle, thereby blocking RNA synthesis and protein expression,[6] and it has been shown to overcome fluconazole resistance in drug-resistant Candida albicans and inhibit biofilm formation.[7] However, its efficacy against *N. gonorrhoeae* infections has not been previously reported.

To investigate the efficacy and stability of chelerythrine chloride in treating multidrug-resistant *N. gonorrhoeae*, we employed drug susceptibility test and drug development assays. The results demonstrated that chelerythrine chloride exhibited significant inhibitory activity against *N. gonorrhoeae*. Beyond assessing in vitro efficacy, a core aim of this study was to evaluate the propensity for resistance induction—a critical predictor of clinical longevity—through prolonged sub-MIC exposure. Notably, it maintained a stable minimum inhibitory concentration (MIC) over a 30-day treatment period, highlighting its potential as an effective antimicrobial agent against *N. gonorrhoeae*. This study offers new insights into drug development in this field and presents potential treatment options for gonorrhea.

Materials and Methods

We collected 55 *N. gonorrhoeae* strains, comprising 54 clinical isolates from Nanchang People's Hospital in 2021, along with a quality control strain "ATCC 49226" for antibiotic susceptibility testing (AST). Following WHO recommendations, clinical isolates were identified by gram staining, oxidase testing, catalase testing, and sugar fermentation testing. The sub-cultured *N. gonorrhoeae* strains were incubated at 37°C with 5% CO_2 for 18 h on Thayer-Martin and gonococcal blood agar (supplemented with 10% defibrinated sheep blood). Bacterial suspensions were adjusted to 0.5 McFarland standard and diluted in broth medium. The AST was performed using the agar dilution method, the antimicrobial agent is incorporated into a series of twofold dilutions in supplemented GC agar. *N. gonorrhoeae* strains are cultured overnight on GC agar, suspended in MH broth/saline, and inoculated (~10^4 CFU/spot) onto plates containing antibiotics and antibiotic-free controls using an inoculation loop. The plates are incubated overnight, and growth is examined to determine the MIC.[8] The MIC of chelerythrine chloride was determined using the broth microdilution method.[9] Distribute 100 μL per well of *N. gonorrhoeae* suspension into a 96-well plate preloaded with chelerythrine chloride. Incubate at 35°C/5% CO_2 for 24 hours, then read the MICs.

In the course of evaluating the antimicrobial efficacy of chelerythrine chloride against clinical strains, we utilized the ATCC49226 quality control strain to perform both agar dilution and GC Broth microdilution tests. Meanwhile, the DMSO solvent group and antibiotics group served as negative and positive control in chelerythrine chloride relevant drug experiment. These two tests were employed to determine the MICs of seven antibiotics—specifically, penicillin, tetracycline, ciprofloxacin, spectinomycin, ceftriaxone, cefixime, and azithromycin—as well as chelerythrine chloride. The adoption of chelerythrine chloride MICs data in clinical samples only occurred when the MIC values for the seven

antibiotics identified by both agar dilution and GC Broth microdilution methods simultaneously fell within the correct range as defined by the WHO in ATCC49226 quality control strain.

Antimicrobial susceptibility interpretations were conducted in accordance with the most recent guidelines established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2025)[10] and the WHO Western Pacific Region Gonococcal Antimicrobial Surveillance Program.[11] According to EUCAST and WHO standards, its antibiotic breakpoints are shown in Supplemental Table 1.

Finally, our research previously used high-throughput screening (data to be published), identifying the promising bactericidal drugs: chelerythrine chloride. The concentrations of chelerythrine chloride used in the sensitivity test ranged from 0.001 to 16 mg/L. The chelerythrine chloride was purchased from Target Mol Chemicals Inc and dissolved in dimethyl sulfoxide according to its physicochemical properties and the manufacturer's instructions. To directly address the risk of resistance development—a key study goal—we inoculated the ATCC49226 strain into GC Broth containing sub-MIC (2 mg/L) concentrations of chelerythrine chloride. Cultures were passaged every 24 hours for 30 consecutive days. MIC values were determined every 5 days using the broth microdilution method to monitor stability or shifts in susceptibility.

Results

As detailed in Supplemental Table 2, the 54 *N. gonorrhoeae* strains exhibited multidrug resistance (MDR). Further analysis presented in Table 1 revealed that 67.27% of the strains were resistant to penicillin, with MIC_{50} and MIC_{90} values exceeding 32 mg/L. For tetracycline, the resistance rate reached 81.82%, with an MIC_{50} of 2 mg/L and an MIC_{90} greater than 32 mg/L. Resistance to ciprofloxacin was observed in 98.18% of the strains, with an MIC_{50} of 8 mg/L and an MIC_{90} of 16 mg/L. Notably, 5.45% of the strains demonstrated resistance to azithromycin, an antibiotic currently employed in dual therapy regimens, with MIC_{50} and MIC_{90} values of 2 mg/L. All clinical isolates remained susceptible to spectinomycin, with MIC values ranging from 1 to 16 mg/L. Furthermore, 16.36% of the strains exhibited decreased

Table 1 MICs Profiles of Penicillin, Tetracycline, Ciprofloxacin, Spectinomycin, Ceftriaxone, Cefixime and Azithromycin Against 54 Clinical *N. Gonorrhoeae* and ATCC49226 Quality Control Strain

	MIC range (mg/L)	MIC Interpretation	No. of Isolate (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Penicillin	≤0.06	S	3.64	0.06	0.06
	0.12–1	I	29.09	1	1
	>1	R	67.27	>32	>32
Tetracycline	≤0.5	S	18.18	0.5	0.5
	>0.5	R	81.82	2	>32
Ciprofloxacin	≤0.03	S	1.82	0.008	0.008
	0.06	I	0	0	0
	>0.06	R	98.18	8	16
Azithromycin	<1	S	94.55	0.25	0.5
	≥1	R	5.45	2	2
Spectinomycin	≤64	S	100	16	16
	>64	R	0	0	0
Ceftriaxone	<0.125	S	83.64	0.03	0.06
	≥0.125	DS	16.36	0.5	0.5
Cefixime	<0.25	S	80.00	0.03	0.125
	≥0.25	DS	20.00	1	>1

Notes: For EUCAST criteria, resistance (R) thresholds were defined as: azithromycin MIC ≥1 mg/L, ciprofloxacin MIC >0.06 mg/L, penicillin MIC >1 mg/L, spectinomycin MIC >64 mg/L and tetracycline MIC >0.5 mg/L. Intermediate susceptibility (I) applied to penicillin (MIC=0.12–1 mg/L) and ciprofloxacin (MIC =0.06 mg/L), while susceptibility (S) was assigned to azithromycin (MIC <1 mg/L), penicillin (MIC ≤0.06 mg/L), tetracycline (MIC ≤0.5 mg/L), ciprofloxacin (MIC ≤0.03 mg/L), and spectinomycin (MIC ≤64 mg/L). For cephalosporins, WHO criteria defined decreased susceptibility (DS) as ceftriaxone MIC ≥0.125 mg/L and cefixime MIC ≥0.25 mg/L, with susceptibility (S) thresholds at <0.125 mg/L and <0.25 mg/L, respectively.

https://doi.org/10.2147/IDR.S538954

Table 2 MIC Feature of Chelerythrine Chloride

(A) MIC_{50} and MIC_{90} analysis of chelerythrine chloride against 54 clinical *N. gonorrhoeae* and ATCC49226 quality control strain.							
Antimicrobial	**MIC Range (mg/L)**		**MIC_{50} (mg/L)**		**MIC_{90} (mg/L)**		
Chelerythrine chloride	0.002-8		8		8		
(B) *N.gonorrhoeae* was continuously cultured at sub-MIC of chelerythrine chloride for 30 days.							
Chelerythrine chloride MIC Daily Variability (mg/L)							
	Day 0	**Day 5**	**Day 10**	**Day 15**	**Day 20**	**Day 25**	**Day 30**
ATCC49226	4	4	4	4	4	4	4

Notes: Drug concentration range were set at 0, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 mg/L.

susceptibility to ceftriaxone (MIC_{50} = 0.5 mg/L, MIC_{90} = 0.5 mg/L), while 20.00% showed decreased susceptibility to cefixime (MIC_{50} = 1 mg/L, MIC_{90} >1 mg/L).

To address the AMR of the aforementioned strains and to identify compounds capable of eradicating multi-drug-resistant *N. gonorrhoeae*, we conducted high-throughput screening and identified chelerythrine chloride as a promising antibacterial agent. As indicated in Supplemental Table 3A and Table 2A, the MIC_{50} and MIC_{90} values for chelerythrine chloride were both determined to be 8 mg/L, with a MIC range of 0.002–8 mg/L. In Supplemental Table 3B, it is noteworthy that all cephalosporins resistant isolates remained susceptible to spectinomycin, with MIC values ranging from 8 to 16 mg/L. In contrast, chelerythrine chloride demonstrated comparable or superior efficacy, particularly against strains resistant to cephalosporins, with MIC values between 0.5 and 8 mg/L. Addressing the core objective of evaluating resistance induction, chelerythrine chloride maintained a stable MIC of 4 mg/L over 30 days of continuous sub-MIC exposure (Table 2B). No increase in MIC was observed across six successive measurements, indicating a low propensity for resistance development under sustained selective pressure (Table 2B). Overall, chelerythrine chloride emerges as a potential candidate for the development of antibacterial therapies targeting drug-resistant *N. gonorrhoeae*.

Discussion

To prevent gonorrhea from reverting to a pre-antibiotic era characterized by the absence of effective antibiotic treatments, it is imperative to expedite the drug development process. A pivotal finding of this study is the negligible induction of resistance to chelerythrine chloride, evidenced by stable MIC values over 30 days of continuous sub-MIC exposure. This contrasts sharply with cephalosporins and azithromycin, where resistance emerges rapidly. By explicitly framing resistance induction as a primary endpoint, our data suggest chelerythrine chloride may have a higher genetic barrier to resistance—a critical advantage for sustained clinical efficacy against MDR strains like FC428. Especially in the context of the widespread dissemination of the ceftriaxone-resistant strain FC428, the compound chelerythrine chloride demonstrated significant inhibitory effects on strains resistant to ceftriaxone.

The ongoing evolution of AMR, particularly cephalosporin resistance in *N. gonorrhoeae*, presents a substantial challenge for healthcare providers responsible for managing the treatment of this prevalent global sexually transmitted infection.[12] Despite this challenge, the WHO continues to recommend single-dose ceftriaxone therapy, as well as dual therapy with cephalosporin and azithromycin, as the first-line treatments for gonorrhea.[13] This study reconfirmed the severity of AMR in *N. gonorrhoeae* samples, with resistance rates of 67.27% to penicillin, 81.82% to tetracycline, and 98.18% to ciprofloxacin. Decreased susceptibility to ceftriaxone and cefixime was 16.36% and 20.00%, respectively. Spectinomycin, though currently effective (0% resistance in this cohort), faces significant clinical limitations: it is ineffective against pharyngeal infections, requires intramuscular injection, and is unavailable in many regions.[14] The AMR levels identified in our study exceed the national average reported in China in 2022,[15] indicating that certain regions may encounter more severe AMR challenges and a heightened need for novel therapeutics. Thus, our study also provides support for the urgency of addressing AMR for this purpose.

While this study provides valuable insights, several limitations should be acknowledged. First, the restricted sample size (n = 54) and single-region sampling (Nanchang City, China) might limit the generalizability of our findings. Given known geographic variations in microbial resistance profiles, future multicenter studies with larger, epidemiologically representative cohorts are needed to validate these results across diverse populations. Second, while our in vitro data demonstrate promising antibacterial activity of chelerythrine chloride, critical gaps remain in its translational characterization: (1) Mechanistic understanding: The precise molecular targets and resistance mechanisms require elucidation through techniques such as structural characterization, whole-genome sequencing of resistant mutants or proteomic profiling. (2) Preclinical validation: Cytotoxicity (eg, CC_{50} in mammalian cell lines), in vivo efficacy (murine infection models), and pharmacodynamic properties (time-kill kinetics, post-antibiotic effect, bacterial death rate) remain unaddressed. (3) Safety and pharmacokinetics: Absorption, distribution, metabolism, and excretion (ADME) parameters must be quantified to assess therapeutic potential. (4) Clinical applicability hinges on phased human trials (Phase I–III) to establish safety margins, dosing regimens, and comparative efficacy against standard therapies. (5) Considering that an MIC_{90} value of 8 mg/L may restrict clinical utility, further investigations could be undertaken to explore the potential formulation of chelerythrine chloride into lotions or suppositories for localized administration, thereby optimizing drug delivery. Until such data are available, the compound's therapeutic utility remains speculative.

Conclusion

AST was performed on 55 drug-resistant strains to assess AMR rates, reaffirming the critical challenge posed by gonococcal infections in treatment contexts. Our in vitro experiments highlighted the potential of chelerythrine chloride as an antimicrobial agent against *N. gonorrhoeae*; however, further research is necessary to elucidate its mechanism of action and evaluate its clinical applicability. Notably, the lack of induced resistance following extended exposure suggests that chelerythrine chloride may be a promising candidate with a substantial barrier to resistance development.

Data Sharing Statement

Ensure that all data is authentic and usable.

Ethics Approval and Consent to Participate

The clinic strains were part of the routine hospital laboratory procedure, and this study has also been approved by the Medical Ethics Committee of the Dermatology Hospital of Southern Medical University/Guangdong Provincial Dermatology Hospital (Approval No. 2021067).

Acknowledgments

We are grateful to Dr. Qinwen Jiang for providing clinical samples.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Consent for Publication

All authors read and revise the article. Agree to publish.

Funding

This work was supported by the Guangzhou Municipal Science and Technology Bureau (No. 202201010917), the Medical Science and Technology Research Foundation of Guangdong Province (No. A2022106), the Natural Science

Foundation of Guangdong Province (No. 2022A1515111148), Shanghai Municipal Health Commission Health Industry Clinical Research Project (20244Y0232) and the National Natural Science Foundation of China (No. 82302578).

Disclosure

The authors report no conflicts of interest in this work.

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