Infection and Drug Resistance

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ORIGINAL RESEARCH

Antimicrobial Susceptibility of Ertapenem in *Neisseria gonorrhoeae* Isolates Collected Within the China Gonococcal Resistance Surveillance Programme (China-GRSP) 2018

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Purpose: This study aimed to determine the minimum inhibitory concentrations (MICs) of ertapenem on *Neisseria gonorrhoeae* collected from eight Chinese provinces in 2018.

Methods: The MICs of ertapenem on 503 *Neisseria gonorrhoeae* isolates (415 isolates selected randomly and 88 isolates selected with preference) were measured using the agar dilution method. For comparison, the MICs of ceftriaxone and azithromycin were detected.

Results: Among 415 randomly selected isolates, the MIC range for ertapenem was from ≤0.008 mg/L to 0.5 mg/L. The corresponding MIC_{50} and MIC_{90} were 0.06 and 0.125 mg/L, respectively. Twelve of 415 isolates (2.9%) exhibited MIC values ≥0.25 mg/L, and only one isolate (0.2%) had a MIC of 0.5 mg/L. By comparing all 503 tested isolates, a correlation of $r = 0.487$ ($P < 0.001$) between ertapenem and ceftriaxone MIC was observed, and the correlation between MICs of ertapenem and azithromycin was low ($r = -0.12$, $P = 0.007$). In 24 ceftriaxone-decreased susceptibility isolates, four isolates (16.7%) showed a MIC ≥0.25 mg/L for ertapenem. In 85 azithromycin resistant isolates, three isolates (3.5%) showed a MIC ≥0.25 mg/L for ertapenem.

Conclusion: The in vitro results suggest that ertapenem has satisfactory susceptibility in isolates collected from eight provinces in China; hence, it might be a promising treatment option for resistant gonococcal infections.

Keywords: *Neisseria gonorrhoeae*, treatment, ertapenem, antimicrobial resistance

Introduction

Gonorrhea, caused by *Neisseria gonorrhoeae*, is one of the commonly seen sexually transmitted diseases with a total global incidence of approximately 86.9 million per year.[1] *N. gonorrhoeae* can cause urogenital infection and extragenital infection. It usually leads to dysuria and urethral discharge in men and frequently causes asymptomatic genital tract infection in women. If left untreated, it may lead to severe sequelae such as pelvic inflammatory disease and infertility.[2] Moreover, it can even facilitate the risk of HIV acquisition and transmission.[3] To prevent complications and control the transmission, successful treatment is of great significance.

Ceftriaxone and azithromycin in various doses are recommended as first-line treatments in guidelines in America,[4] Australia,[5] Canada,[6] Europe,[7] New Zealand,[8] and by the World Health Organization (WHO).[9] Monotherapy with ceftriaxone (1 g intramuscularly in a single dose) for uncomplicated gonococcal infections is recommended as the first-line

Received: 20 August 2021
Accepted: 30 September 2021
Published: 12 October 2021

treatment according to the current guidelines in China.[10] However, *N. gonorrhoeae* has developed resistance to all previously used antimicrobial therapies and shows decreased susceptibility to ceftriaxone (DSC, defined as isolates with ceftriaxone MIC ≥0.25 mg/L in China currently) which poses a significant global healthcare burden.[11–13] The emergence of *N. gonorrhoeae* isolates with a multidrug resistant or extremely drug-resistant profile highlights the importance of considering alternate therapy. Noteworthy, one treatment failure with 1 g ceftriaxone was reported in UK, but that infection was cleared with intravenous ertapenem 1 g once daily for 3 days.[14]

Ertapenem belongs to the class of carbapenem antibiotics, which interacts with penicillin binding proteins (PBPs) located on the bacterial cell wall. In particular, its inhibitory effect to PBPs 2 and 3 disturbs the final transpeptidation step in the synthesis of peptidoglycan, an essential component of the bacterial cell wall. Subsequently, dysfunctional cell wall causes cell death of Gram-positive and Gram-negative aerobic and anaerobic pathogens. According to the elimination half-life of ertapenem in healthy volunteers (3.8 hours), it is suitable for both intramuscular injection and intravenous.[15] Besides its stability for DHP-1 (dehydropeptidase-1), one injection per day without combination of cilastatin is sufficient in clinical practice. Furthermore, there has been research proving that ertapenem can be regarded as a promising alternative for the treatment of ceftriaxone resistance both in vitro and in vivo. For instance, in the UK and Australia, ertapenem has been successfully applied to patients with multidrug resistant gonorrhea.[14,15] Additionally, in vitro studies have shown that isolates resistant to ceftriaxone, including isolates with high levels of resistance to ceftriaxone, were still sensitive to ertapenem.[16,17]

Based on successful clinical treatment cases and data of previous in vitro studies,[16,17] in this study, we analyzed the susceptibility of ertapenem of 503 *N. gonorrhoeae* isolates. Those isolates were collected in 2018 from eight different Chinese provinces.

Materials and Methods

Gonococcal Isolates

The ethics approval for the China Gonococcal Resistance Surveillance Programme (China-GRSP) was obtained from the Medical Ethics Committee at the Institute of Dermatology, the Chinese Academy of Medical Sciences & Peking Union Medical College and the National Center for Sexually Transmitted Disease Control at Nanjing (2014-LS-026) for the use of specimens collected annually from patients attending local dermatology or STD clinics. Participants no less than 18 years of age who signed an informed consent to provide urine, vaginal and rectal swabs were enrolled in the study. The isolates detected in our study were randomly selected from the China-GRSP sample bank collected in 2018. Samples were inoculated, identified, preserved, and transferred as previously described.[18]

Clinical gonococcal isolates from eight provinces (Beijing, Guangdong, Guangxi, Liaoning, Sichuan, Shaanxi, Tianjin and Yunnan) were used. In total, there are 503 isolates, which were all collected from the urogenital tract. Among them, 415 isolates were selected out of random sampling for MIC distribution analysis in different provinces, and the other 88 isolates were additionally selected with preference (isolates with relatively high MICs of ceftriaxone or azithromycin) for cross resistance analysis.

Antimicrobial Susceptibility Tests by Agar Dilution

Antimicrobial susceptibility tests to ertapenem, ceftriaxone, and azithromycin were determined for all isolates by the agar dilution method as previously described.[19] For ertapenem, the concentrations were 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, and 1 mg/L, for ceftriaxone they were 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, and 1 mg/L, and for azithromycin they were 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, and 4 mg/L. The lowest antibiotic concentration that inhibited growth was defined as the MIC of isolates. Resistance to azithromycin (RTA) was defined as MIC ≥ 1.0 mg/L, and DSC was defined as MIC ≥ 0.25 mg/L. So far, no standardized criteria for ertapenem to *N. gonorrhoeae* has been published from EUCAST and Clinical and Laboratory Standards Institute (CLSI). Quality assurance was taken using WHO *N. gonorrhoeae* reference isolates (G, J, K, L, and P) and ATCC 49226.[20]

Data Analysis

The MIC of the reference strain was within the reference range whereas the batch of results was included in the analysis. 415 isolates from eight provinces were used to evaluate the MIC distribution in various province in China. Other analyses were carried out using all 503 isolates. Here, we describe the eight provinces' MICs distribution of collected *N. gonorrhoeae* isolates to ertapenem. Correlation between MICs of ertapenem and ceftriaxone or

azithromycin was calculated using log_2-transformed MIC data by linear regression among 503 isolates. For MICs detected as ≤ or ≥ a specific value, these specific values were used for the calculation of log_2-transformed MIC data. A *p*-value <0.05 was considered statistically significant. Statistical analyses were carried out utilizing SPSS software (IBM, New York, USA), and Excel (Microsoft, Washington, USA). Figures were made using R (GNU System).

Results

Antimicrobial Susceptibility Test of 415 Isolates

Geographically, the 415 isolates were collected from Beijing, Guangdong, Guangxi, Liaoning, Sichuan, Shaanxi, Tianjin, and Yunnan. The specific numbers of isolates for each province were 40, 59, 53, 38, 67, 52, 48, and 58, respectively.

All isolates were determined for their MIC values to ertapenem, ceftriaxone, and azithromycin. MIC values obtained from the WHO *N. gonorrhoeae* reference strains were identical or within 1 MIC dilution of those previously reported in every batch of tests.[20] It supported the reliability of the MIC values to those three antimicrobials.

Ertapenem MICs ranged from ≤0.008 to 0.5 mg/L. The MIC proportions of ertapenem in different provinces is shown in Figure 1. Strains (12 out of 415, 2.9%) with MIC greater than 0.25 mg/L were found in six provinces. Among them, the largest proportion (4 out of 12) were strains from Guangdong Province. Meanwhile, no strains with a MIC ≥ 0.25 mg/L were found in Liaoning and Tianjin (Table 1). In addition, there was one strain with MIC greater than 0.5 mg/L found in Shaanxi province (Table 1 and Figure 1). Overall, the MIC_{50} of the eight provinces was 0.06 mg/L, with the exception of Sichuan and Yunnan provinces, those of which were both 0.03 mg/L (Table 1). While the overall MIC_{90} of the eight provinces was 0.125 mg/L, although those of Liaoning, Shaanxi, Tianjin and Yunnan were 0.06 mg/L, the other four provinces were consistent with the overall value (Table 1). The difference between MIC_{50} and MIC_{90} values in different provinces suggested that the MIC distribution of ertapenem could be inconsistent among regions.

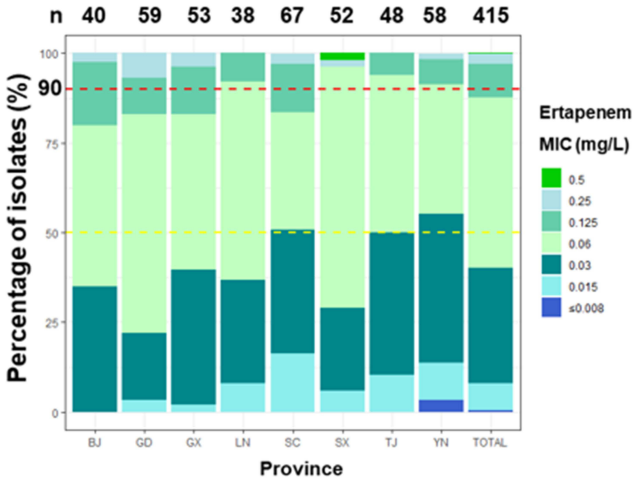

Figure 1 The percentage of *N. gonorrhoeae* isolates with different MICs (mg/L) for ertapenem. The MIC_{50} (yellow-dotted line), MIC_{90} (red-dotted line) and number of isolates per province are shown.

Abbreviations: BJ, Beijing; GD, Guangdong; GX, Guangxi; LN, Liaoning; SC, Sichuan; SX, Shaanxi; TJ, Tianjin; YN, Yunnan; n, number.

Correlation Between MICs of Ertapenem and Ceftriaxone or Azithromycin

MIC distribution and correlation analyses for ertapenem with ceftriaxone and azithromycin are shown in Figure 2. A correlation of $r = 0.487$ ($P < 0.001$) was observed between ertapenem and ceftriaxone (Figure 2A). In comparison, the MIC values of ertapenem and azithromycin correlated poorly with $r = -0.12$ ($P = 0.007$) (Figure 2B). These results indicated a cross resistance between ertapenem and ceftriaxone. However, azithromycin resistance might have limited impact on ertapenem susceptibility.

MICs Distribution of Ertapenem in Isolates with Decreased Susceptibility to Ceftriaxone or Resistance to Azithromycin

Out of all 503 tested isolates, 24 isolates showed decreased susceptibility to ceftriaxone (DSC), 85 isolates were resistant to azithromycin (RTA), three isolates possessed a resistance to both ceftriaxone and azithromycin. Ertapenem MICs distributions for DSC or RTA isolates are shown in Table 2. Among the 24 DSC isolates, only four isolates showed a MIC ≥ 0.25 mg/L in ertapenem. Regarding RTA isolates, only three out of 85 showed MIC ≥ 0.25 mg/L for ertapenem.

Discussion

The antimicrobial resistance of *N. gonorrhoeae* remains a severe issue for public health. Ceftriaxone-resistant isolates have been reported in many countries over the world,[2,3,12,13,21,22] which suggests the importance of finding alternative treatments. The recommended strategies from the World Health Organization include "increasing

Table 1 The Proportions of the Isolates with Different MICs of Ertapenem by Provinces

Province	Isolates Detected	MIC (mg/L)			MIC ≥ 0.25 mg/L	
		Range	MIC_{50}	MIC_{90}	n (%)	*P*-value
Beijing	40	0.03–0.25	0.06	0.125	1 (2.5%)	1.0000
Guangdong	59	0.015–0.25	0.06	0.125	4 (6.8%)	0.1252
Guangxi	53	0.015–0.25	0.06	0.125	2 (3.8%)	0.6655
Liaoning	38	0.015–0.125	0.06	0.06	0	0.6109
Sichuan	67	0.015–0.25	0.03	0.125	2 (3.0%)	1.0000
Shaanxi	52	0.015–0.5	0.06	0.06	2 (3.9%)	0.6617
Tianjin	48	0.015–0.125	0.03	0.06	0	0.6230
Yunnan	58	≤0.008–0.25	0.03	0.06	1 (1.8%)	1.0000
Total	415	≤0.008–0.5	0.06	0.125	12 (2.9%)	

Notes: *P*-value was the chi-square of isolates with MIC ≥ 0.25 mg/L in different provinces to total.

the therapeutic dose", "new antimicrobial drugs development", "recycle "old" antimicrobial agents", etc. The gonorrhea treatment guidelines in China suggest an increase of ceftriaxone dose in the treatment of gonorrhea (from 250 mg in 2014 to 1 g in 2020). However, this can only temporarily alleviate the dilemma of gonorrhea treatment. With the evolution of gonococcal drug resistance, highly ceftriaxone-resistant isolates may continue to emerge. Therefore, it is urgent to find other effective therapeutic drugs.

For the time being, new antibiotics are also under intensive development, such as zoliflodacin and gepotidacin.[23,24] However, the development of new drugs is known to be extremely time and cost-consuming. It is estimated that the research and development of new antimicrobials requires high socio-economic costs from target to product, which may take 15 years and $2.5 billion.[25] Compared with the drug R & D returns of other non-infectious and chronic diseases, the R & D of antimicrobials development has more market risks. Therefore, the reassessment of developed drugs is of great significance. It is not only one of the alternative treatment strategies recommended by the WHO, but also the fastest treatment for gonococcal resistance.

Studies have shown that ertapenem may be a good alternative to ceftriaxone-resistant isolates both in vitro and in vivo. In vitro studies showed that ertapenem was still sensitive to ceftriaxone-resistant isolates (including highly ceftriaxone-resistant isolates).[16] In addition, there are reports about ertapenem successfully curing multidrug-

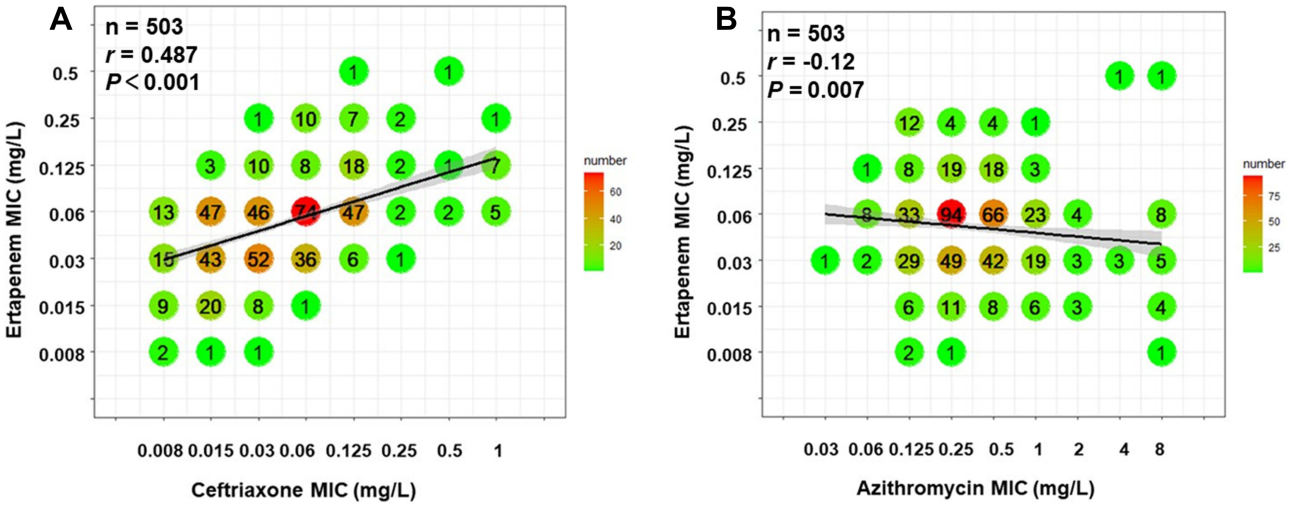

Figure 2 Correlation between MICs of ertapenem and ceftriaxone or azithromycin. Each symbol represents one or multiple isolates, which is also shown with specific numbers and colors. The regression line was calculated using log_2-transformed MIC data. For the MICs ≤ or ≥ a specific value, log_2-transformed this specific value was used for calculating linear regression. (**A**) Correlation between MICs of ertapenem and ceftriaxone, r = 0.487, P <0.001. (**B**) Correlation between MICs of ertapenem and azithromycin, r = −0.12, P = 0.007.

Table 2 Ertapenem MICs Distributions for Decreased Susceptibility to Ceftriaxone (DSC) or Azithromycin-Resistant (RTA) *N. gonorrhoeae* Isolates

Ertapenem MIC (mg/L)	DSC			RTA		
	Number	Percentage	Cumulative %	Number	Percentage	Cumulative %
≤0.008	0	0	0	1	1.2	1.2
0.015	0	0	0	13	15.3	16.5
0.03	1	4.2	4.2	30	35.3	51.8
0.06	9	37.5	41.7	35	41.2	92.9
0.125	10	41.7	83.3	3	3.5	96.5
0.25	3	12.5	95.8	1	1.2	97.6
0.5	1	4.2	100.0	2	2.4	100.0
Sum	24	100.0		85	100.0	

resistant gonorrhea patients.[15] However, *N. gonorrhoeae* can easily evolve antimicrobial resistant variants. Therefore, we suggest a parallel antimicrobial resistance surveillance of ertapenem if it is used in a larger scale.

In this study, we found that ertapenem demonstrated a low MIC value (MIC range: ≤0.008–0.5 mg/L) in isolates collected from eight provinces in China. Using the PK-PD (non-species related) breakpoints reported in EUCAST clinical breakpoint of ertapenem (MIC ≤ 0.5 mg/L, susceptible), all isolates were sensitive to ertapenem, which indicated that ertapenem might be a potential therapeutic agent in gonorrhea treatment. Moreover, the MIC_{90} of ertapenem (0.125 mg/L) equaled the value of ceftriaxone in our study, but higher than that (MIC_{90} 0.06 mg/L) reported by Yang et al., who had detected that level with 504 isolates collected from Zhejiang province from 2011–2012 and 2015–2017.[26] This variation might be related to the time periods of the selected isolates, or the differences between provinces, as we found that the MIC_{90} in different provinces ranges from 0.06 to 0.125 mg/L in our study revealed the existence of variations in different provinces. The proportions of MIC ≥ 0.25 mg/L ranged from 0 (Liaoning and Tianjin) to 6.8% (Guangdong). Although this difference was not statistically significant, it could indicate the importance of antimicrobial susceptibility surveillance. Especially before prescribing antibiotics to patients, an antimicrobial sensitivity test is vitally important.

Since both ertapenem and ceftriaxone are located to β-Lactams, we analyzed the correlation between ertapenem and ceftriaxone MIC to evaluate the cross resistance. The MICs of ertapenem and ceftriaxone showed a correlation of $r = 0.487$, which indicated a cross resistance between ertapenem and ceftriaxone. But the MIC ranges of ertapenem in DSC isolates were lower than that of ceftriaxone, and in 24 DSC isolates, only four isolates showed ertapenem MIC ≥ 0.25 mg/L. Two of the 503 isolates had a MIC value of 0.5 mg/L. Whether these isolates will evolve into ertapenem-resistant isolates in the future will need further monitoring. Accordingly, ertapenem had in vitro advantages over ceftriaxone for isolates with ceftriaxone resistance. Generally clinicians are cautious about the use of carbapenem antibiotics, as they are usually used for serious infections in clinical practice. But facing high dose ceftriaxone treatment failure or persistent gonococcal infection patients, the clinicians may draw their attention to the application of ertapenem.

Our in vitro results suggested that ertapenem might be an effective treatment option for gonorrhea, particularly for the DSC isolates currently identified, which could serve as a foundation for further clinical trials in vivo. This study is the first multi-province research reporting the *N. gonorrhoeae* susceptibility to ertapenem in China. Moreover, our results provide a reference for antimicrobial susceptibility data of ertapenem in vitro for gonococcal infections in China. Nevertheless, there are some limitations in this study: the number of isolates accounts for only 0.3% (415/133,156) of the reported cases of gonorrhea in China for 2018, and lacking extragenital isolates in this study may also lead to a potential bias. At the same time, when interpreting the susceptibility data, we refer to the susceptibility break points of PK-PD (non-species related) reported in EUCAST, and there is no break point of antimicrobial susceptibility of ertapenem to *N. gonorrhoeae*, which may also lead to bias in the analysis of results. All these deficiencies may limit the generalizability of this study in China.

Conclusion

The study evaluated ertapenem MICs of *N. gonorrhoeae* isolates in 2018 from eight provinces in China and the correlation was analyzed to see the potential cross resistance between ertapenem and ceftriaxone or azithromycin. The antimicrobial effect of ertapenem on isolates with DSC and resistant to azithromycin were also evaluated. Our results suggest that ertapenem has low MIC values with isolates collected from eight provinces in China. Overall, this study provides new scope for ertapenem in the treatment of gonorrhea, although its effectiveness in vivo needs further study.

Acknowledgments

This work was supported by the National Science and Technology Major Project (2018ZX101010 01-004-003), the Nanjing Incubation Program for National Clinical Research Center (2019060001), and Jiangsu Province Youth Fund Project (BK20180156). The authors thank the patients who provided the specimens and acknowledge LF Yuan, WL Cao, BY Zhu, ZG Zhang, G Yong, CS Zhu, ZJ Zheng and L Bai for their contribution in isolates collecting.

Disclosure

The authors report no conflicts of interest in this work.

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