J Antimicrob Chemother 2014; **69**: 1568–1571
doi:10.1093/jac/dkt537 Advance Access publication 2 February 2014

Journal of Antimicrobial Chemotherapy

Evaluation of the activity of ertapenem against gonococcal isolates exhibiting a range of susceptibilities to cefixime

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Received 15 October 2013; returned 15 November 2013; revised 18 December 2013; accepted 19 December 2013

Objectives: There is a need for new or alternative antimicrobial agents for the treatment of gonorrhoea as antimicrobial resistance emerges to current therapies. The aim was to investigate the activity of ertapenem against isolates of *Neisseria gonorrhoeae* with decreased susceptibility to cefixime.

Methods: A panel of 52 clinical isolates and 10 control strains of *N. gonorrhoeae* were selected to represent a range of susceptibilities to cefixime. Susceptibility testing was performed using the methodology used for the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP). The isolates were typed by *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST).

Results: The isolates comprised 42 different molecular types as defined by NG-MAST. The susceptibility of the clinical isolates to ertapenem was similar to that of cefixime, with a Pearson's correlation coefficient of $R=0.89$. The MIC_{90} and MIC_{50} values of ertapenem were 0.25 and 0.12 mg/L, respectively, while those of cefixime were 0.12 and 0.06 mg/L, respectively. However, these isolates were more susceptible to ceftriaxone than ertapenem, with a Pearson's correlation coefficient of $R=0.65$ and ceftriaxone MIC_{90} and MIC_{50} values of 0.03 and 0.016 mg/L, respectively. The isolates that were least susceptible to ertapenem were all non-producers of penicillinase. However, one isolate that was highly resistant to cefixime and ceftriaxone was more susceptible to ertapenem than either cefixime or ceftriaxone.

Conclusions: This study has shown that ertapenem is not a suitable alternative for first-line treatment for gonorrhoea but that it may be useful for the treatment of highly resistant infections.

Keywords: gonorrhoea, cephalosporins, carbapenems

Introduction

There is global concern that the threat of antimicrobial resistance will compromise the public health control of gonorrhoea, the second most common bacterial sexually transmitted infection in the UK[1] and worldwide.[2] Historically, a series of antimicrobial agents have been used successively over six decades for the treatment of gonorrhoea. The choice of first-line treatment has been changed when resistance has reached >5% to an alternative agent to which resistance is not documented in *Neisseria gonorrhoeae*.[3] As cefixime and ceftriaxone, which are the currently recommended treatments, have begun to show drift towards decreased susceptibility and episodes of treatment failure have been reported,[4,5] there is concern that alternative options are minimal, which may lead to the worldwide spread of resistant strains.

In the absence of any new therapeutic agents for gonorrhoea, the approach in some countries has been to change the recommended treatment from the oral cephalosporin cefixime to ceftriaxone, which is given intramuscularly at a higher dose and in combination with azithromycin, at a dose of either 1 or 2 g.[6,7] Another option is to investigate antimicrobial agents currently used for other infections. One such drug is ertapenem, a carbapenem that is used against other Gram-negative bacteria, is given intramuscularly once daily and has a plasma half-life of around 4 h.[8] To date ertapenem has only been evaluated against *N. gonorrhoeae in vitro* and has been compared with other antimicrobials, including third-generation cephalosporins.[9,10] The objective of this study was to test the *in vitro* activity of ertapenem against a range of gonococcal strains, including those with decreased susceptibility to cefixime and ceftriaxone.

Materials and methods

A representative panel of 62 gonococcal isolates were tested, of which 44 were clinical isolates collected as part of the national surveillance

programme, GRASP (Gonococcal Resistance to Antimicrobials Surveillance Programme), 7 were clinical isolates that had been referred to the Sexually Transmitted Bacteria Reference Unit as part of the reference service between 2008 and 2011, 1 (strain F89), known to have high-level resistance to cefixime and ceftriaxone, was from a patient known to have failed therapy in France,[11] and 10 were control strains, including eight WHO reference strains.[12]

The 51 UK clinical isolates were chosen to represent a range of susceptibilities to cefixime: MIC ≥0.12 mg/L, $n=21$; MIC 0.03–0.06 mg/L, $n=24$; and MIC 0.002–0.016 mg/L, $n=6$ (isolates displaying full susceptibility to cefixime). These isolates were known to belong to 42 different sequence types as defined by *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST).[13]

The susceptibility of all isolates was determined by the agar dilution method as described for the GRASP.[13] The agents tested included ertapenem (range 0.002–1 mg/L), cefixime (0.002–0.25 mg/L), ceftriaxone (0.002–0.12 mg/L), penicillin (0.25–4 mg/L), azithromycin (0.12–2 mg/L), ciprofloxacin (0.25–8 mg/L) and spectinomycin (32–64 mg/L). The MIC of each antimicrobial was obtained after 48 h of incubation. β-Lactamase activity for each isolate was detected using the nitrocefin test (Oxoid, Basingstoke, UK).

All data were handled in Excel (Microsoft). The correlation coefficient was determined using Pearson's *R*. The data for F89 were excluded for determination of the correlation coefficient and for the MIC_{50} and MIC_{90}, as this strain was highly resistant and gave outlying results. The breakpoints described in the GRASP protocol[13] were used to determine decreased susceptibility to cefixime (MIC ≥0.12 mg/L) and ceftriaxone (MIC ≥0.12 mg/L) and resistance to penicillin (MIC ≥1 mg/L), ciprofloxacin (MIC ≥1 mg/L), azithromycin (MIC ≥1 mg/L) and spectinomycin (MIC >64 mg/L).

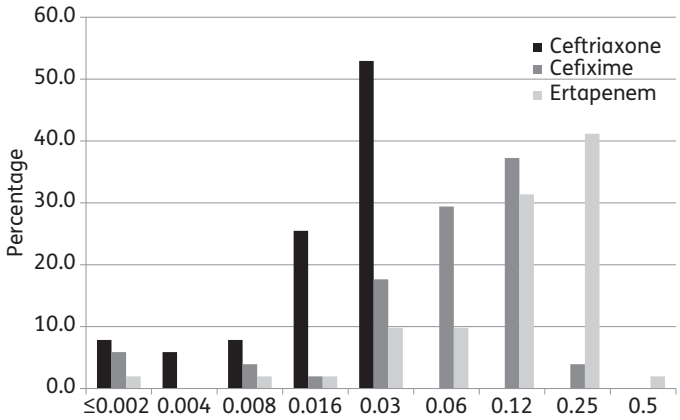

Figure 1. Susceptibility (MIC, mg/L) to ertapenem, cefixime and ceftriaxone.

Results

Pearson's correlation coefficient was used to compare susceptibilities of the UK clinical isolates to ertapenem with susceptibility to cefixime and ceftriaxone, and was $R=0.89$ for cefixime, but was lower for ceftriaxone at $R=0.65$. The MIC ranges of ertapenem, cefixime and ceftriaxone were 0.002–0.5, ≤0.002–0.25 and ≤0.002–0.03 mg/L, respectively (Figure 1). The MIC_{90} and MIC_{50} values of ertapenem were 0.25 mg/L and 0.12 mg/L, respectively, while the MIC_{90} and MIC_{50} values of cefixime were 0.12 and 0.06 mg/L, respectively, and the MIC_{90} and MIC_{50} values of ceftriaxone were 0.03 and 0.016 mg/L, respectively.

The MIC profiles for the control strains are shown in Table 1. In addition, strain F89 had cefixime and ceftriaxone MICs of >0.25 and >0.12 mg/L, respectively, consistent with previous data showing an MIC of 4 mg/L for cefixime and 1–2 mg/L for ceftriaxone,[11] and had an ertapenem MIC of 0.03 mg/L.

The 22 isolates exhibiting the highest ertapenem MICs (0.25–0.5 mg/L) were all non-penicillinase-producing *N. gonorrhoeae* (non-PPNG); 86% (19/22) showed decreased susceptibility to cefixime, all (22/22) were resistant to ciprofloxacin and 91% (20/22) were resistant to penicillin. All isolates were susceptible to azithromycin, ceftriaxone and spectinomycin.

Three isolates of penicillinase-producing *N. gonorrhoeae* were included in this study and the MICs ranged between 0.008 and 0.12 mg/L for ertapenem, between 0.002 and 0.03 mg/L for ceftriaxone and between ≤0.002 and 0.06 mg/L for cefixime.

Discussion

This study has shown that the isolates tested were more susceptible to ceftriaxone than to ertapenem and cefixime and confirms previous findings.[9,10] The clinical isolates tested were selected on the basis that they exhibited a range of susceptibilities to cefixime and belonged to diverse molecular types. While this targeted group ensured inclusion of isolates with representative susceptibility profiles, it is a limitation of the study that a larger group was not tested. Previous studies tested a large number of

Table 1. Susceptibility (MIC, mg/L) of control strains to a range of antimicrobial agents

Strain	β-Lactamase	Ertapenem	Cefixime	Ceftriaxone	Azithromycin	Ciprofloxacin	Penicillin	Spectinomycin
1336	−	0.06	0.016	0.016	≤0.12	0.5	2.0	≤32
A24	−	0.06	0.06	0.03	≤0.12	0.5	1.0	≤32
WHO A	−	0.016	≤0.002	≤0.002	≤0.12	≤0.25	≤0.25	>64
WHO F	−	0.016	0.004	≤0.002	≤0.12	≤0.25	≤0.25	≤32
WHO G	−	0.06	0.016	0.008	≤0.12	≤0.25	1.0	≤32
WHO K	−	0.5	0.25	0.06	0.25	>8.0	2.0	≤32
WHO M	+	0.03	0.008	0.004	0.25	2.0	>4.0	≤32
WHO N	+	0.03	0.008	0.004	≤0.12	8.0	>4.0	≤32
WHO O	+	0.06	0.016	0.008	0.25	≤0.25	>4.0	64
WHO P	−	0.03	0.008	0.004	2.0	≤0.25	0.5	≤32

consecutive isolates from GRASP in 2003[9] at a time when decreased susceptibility to cefixime was uncommon, and a selection of isolates from the Australian surveillance programme,[10] using a different methodology from this study.

The correlation between susceptibility to ertapenem and cefixime and ceftriaxone is unsurprising given that they are both β-lactam antimicrobial agents and target the penicillin-binding proteins. Acquisition of *penA* mosaic alleles, resulting in alteration to the PBP2 target in isolates with decreased susceptibility to cefixime, is likely to be responsible for the association with decreased susceptibility to ertapenem. The lower level of correlation between ceftriaxone and ertapenem susceptibility is probably related to the contribution of other mechanisms of resistance, such as *mtr* and *penB*, which are thought may be different between these two extended-spectrum cephalosporins.[14]

The study by Unemo *et al*.[10] showed ertapenem to be highly active against isolates with high-level clinical resistance or exhibiting multidrug resistance to a number of antimicrobials. However, in this study ertapenem does not appear to be highly effective against strains with penicillin or ciprofloxacin resistance, but does appear to show activity against the small number of β-lactamase-positive isolates in this study.

Ertapenem appears to have insufficient *in vitro* activity against strains exhibiting decreased susceptibility to cefixime for it to be considered as first-line treatment. However, it has been previously documented that two strains, H041 and F89, that were highly resistant to both cefixime (MIC 4–8 mg/L) and ceftriaxone (MIC 2–4 mg/L), gave lower MICs of ertapenem, of 0.06 and 0.016 mg/L, respectively,[12] and this was confirmed for F89 in this study. This suggests that there may be a place for ertapenem for the treatment of strains that exhibit high-level resistance to the extended-spectrum cephalosporins cefixime or ceftriaxone.

Ceftriaxone remains the drug of choice for treating gonorrhoea, but is one of the last remaining treatment options available. The use of increased dosage in an attempt to prolong the useful life of this drug appears to have slowed the drift to resistance.[4] However, it is probable that full resistance will emerge over time and alternative antimicrobial agents for treatment of gonorrhoea, such as JNJ-Q2, a novel quinolone,[15] and solithromycin, a fluoroketolide,[16,17] which have recently shown promise, need to be investigated.

Acknowledgements

We would like to thank Patrice Sednaoui for supplying *N. gonorrhoeae* strain F89.

Funding

This work was supported by a grant to C. A. I. from Merck.

Transparency declarations

C. A. I. has received funds from Merck to test ertapenem as an alternative agent for the treatment of gonorrhoea. N. Q. and M. J. C. have no conflicts of interest to declare. The funder had no role in the design or analysis of this study or the preparation of this manuscript.

Author contributions

The study was initiated by C. A. I. and M. J. C., the laboratory work was carried out by N. Q. and the first draft of the manuscript was prepared by N. Q. All authors edited and approved the final manuscript.

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