In Vitro Activity of Ertapenem versus Ceftriaxone against Neisseria gonorrhoeae Isolates with Highly Diverse Ceftriaxone MIC Values and Effects of Ceftriaxone Resistance Determinants: Ertapenem for Treatment of Gonorrhea?

Magnus Unemo, a Daniel Golparian, a Athena Limnios, b David Whitey, c Makoto Ohnishi, d Monica M. Lahra, b and John W. Tapsall b†

WHO Collaborating Centre for Gonorrhoea and Other STIs, National Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, Örebro, Sweden; WHO Collaborating Centre for STD, Prince of Wales Hospital, Sydney, New South Wales, Australia; Queensland Paediatric Infectious Diseases Laboratory, Queensland Children’s Medical Research Institute, Children’s Health Service District, Brisbane, Queensland, Australia; and National Institute of Infectious Diseases, Tokyo, Japan

Clinical resistance to the currently recommended extended-spectrum cephalosporins (ESCs), the last remaining treatment options for gonorrhea, is being reported. Gonorrhea may become untreatable, and new treatment options are crucial. We investigated the in vitro activity of ertapenem, relative to ceftriaxone, against N. gonorrhoeae isolates and the effects of ESC resistance determinants on ertapenem. MICs were determined using agar dilution technique or Etest for international reference strains (n = 17) and clinical N. gonorrhoeae isolates (n = 257), which included the two extensively drug-resistant (XDR) strains H041 and F89 and additional isolates with high ESC MICs, clinical ESC resistance, and other types of clinical high-level and multidrug resistance (MDR). Genetic resistance determinants for ESCs (penA, mtrR, and penB) were sequenced. In general, the MICs of ertapenem (MIC50 = 0.032 µg/ml; MIC90 = 0.064 µg/ml) paralleled those of ceftriaxone (MIC50 = 0.032 µg/ml; MIC90 = 0.125 µg/ml). The ESC resistance determinants mainly increased the ertapenem MIC and ceftriaxone MIC at similar levels. However, the MIC ranges for ertapenem (0.002 to 0.125 µg/ml) and ceftriaxone (<0.002 to 4 µg/ml) differed, and the four (1.5%) ceftriaxone-resistant isolates (MIC = 0.5 to 4 µg/ml) had ertapenem MICs of 0.016 to 0.064 µg/ml. Accordingly, ertapenem had in vitro advantages over ceftriaxone for isolates with ceftriaxone resistance. These in vitro results suggest that ertapenem might be an effective treatment option for gonorrhea, particularly for the currently identified ESC-resistant cases and possibly in a dual antimicrobial therapy regimen. However, further knowledge regarding the genetic determinants (and their evolution) conferring resistance to both antimicrobials, and clear correlates between genetic and phenotypic laboratory parameters and clinical treatment outcomes, is essential.

Gonorrhea (etiological agent, Neisseria gonorrhoeae) remains a major public health concern. In 2005, the World Health Organization (WHO) estimated 88 million gonorrhea cases among adults globally, which placed this infection as the second most prevalent bacterial sexually transmitted infection (59). Resistance of N. gonorrhoeae to previously recommended first-line antimicrobials for treatment of gonorrhea is prevalent worldwide. During the last decade, the susceptibility to the extended-spectrum cephalosporins (ESCs) cefixime (oral) and ceftriaxone (parenteral), the current first-line antimicrobials in most countries, has decreased rapidly globally (3, 6, 8, 12, 16, 21, 22, 24, 29, 31, 39, 41, 52, 57, 58). Clinical failures with cefixime have also been verified in Japan since 2003 (14, 60) and, more recently, in European countries such as Austria (50), Norway (51), and the United Kingdom (18). Accordingly, ceftriaxone is essentially the last remaining treatment option and, worryingly, there have also been a few cases of confirmed failure treating pharyngeal gonorrhea with ceftriaxone in Australia (44) and in Sweden (48). These cases, however, likely also reflect that pharyngeal gonorrhea is harder to treat than urogenital gonorrhea (3, 5, 31, 41, 58). Recently, and of grave concern, the two first extensively drug-resistant (XDR [41]) gonococcal strains, H041 (32, 33) and F89 (49), which have been confirmed to have high-level resistance to ceftriaxone, were described. If these strains spread locally, nationally, or globally, gonorrhea may become untreatable in certain circumstances and in the affected settings (32, 41, 49, 52). Consequently, for effective future treatment of gonorrhea, it is imperative to promptly develop new treatment strategies and, in particular, new treatment options.

Disquietingly, there are few promising new antimicrobials or other bactericidal compounds for treatment of gonorrhea in sight (6, 24, 31, 32, 41, 49, 58). In this context, the present study investigated the in vitro activity of ertapenem, a parenteral 1-β-methyl-carbapenem, against N. gonorrhoeae. Ertapenem shares activity with other carbapenems such as imipenem and meropenem against most bacterial species; however, it is less active against nonfermentative Gram-negative bacteria such as Pseudomonas aeruginosa. It has been shown to be safe, well tolerated (few adverse effects), and effective, also in comparison with ceftriaxone, against urinary tract infections (2, 7, 13, 20, 27, 46). However, in
regard to \textit{N. gonorrhoeae}, ertapenem has been evaluated only \textit{in vitro} and was compared to ceftriaxone solely in a sample of gonococci with low ceftriaxone MICs (maximum of 0.032 \textmu g/ml) (26). Accordingly, \textit{N. gonorrhoeae} isolates for which ceftriaxone has high MIC values have not yet been tested for ertapenem resistance.

Similar resistance mechanisms have been shown to affect the MICs of many \beta-lactam antimicrobials, such as penicillins, narrow-spectrum cephalosporins, and ESCs. The main mechanism in \textit{N. gonorrhoeae} for decreased susceptibility and resistance to ESCs is alteration of the \textit{penA} gene encoding the lethal target, penicillin-binding protein 2 (PBP2). Thus, acquisition of a \textit{penA} mosaic allele or single amino acid alterations of \textit{AS01} or possibly G545 and P551 in PBP2 result in a lower affinity for ESCs (1, 16, 18, 19, 23, 25, 32, 38, 43, 45, 48–51, 55, 56, 62). Mutations in the promoter and/or coding sequence of \textit{mtrR} result in the overexpression of the MtrC-MtrD-MtrE efflux pump (\textit{mtrR} resistance determinant), which further increases the MICs of ESCs (16, 17, 23, 25, 32, 37, 48–51, 54, 61, 62), and \textit{parBib} mutations that alter amino acid G101 and A102 in the PorBib porin (the \textit{penB} resistance determinant) result in additionally increased MICs of ESCs (16, 23, 25, 32, 34, 35, 37, 48–51, 62). At least one nontransformable resistance determinant remains unknown (16, 25, 32, 45, 62). The effects of ESC resistance determinants on the \beta-lactam antimicrobial ertapenem are unknown.

In the present study, the \textit{in vitro} activity of the carbapenem ertapenem was compared to the activity of ceftriaxone against \textit{N. gonorrhoeae}, and the effects of ESC resistance determinants on ertapenem were investigated. The examined \textit{N. gonorrhoeae} isolates and international reference strains included the only two confirmed XDR strains, H041 (32,33) and F89 (49), and additional isolates with substantially increased ESC MICs, clinical ESC resistance, and other types of clinical high-level and multidrug resistance (MDR).

**MATERIALS AND METHODS**

\textit{Neisseria gonorrhoeae} isolates. A total of 126 \textit{N. gonorrhoeae} clinical isolates referred to the WHO Collaborating Centre (CC) for STD, Sydney, Australia, 131 \textit{N. gonorrhoeae} clinical isolates referred to the WHO CC for Gonorrhoea and other STIs, Orebro, Sweden, and 17 \textit{N. gonorrhoeae} international reference strains were examined. Some of these clinical gonococcal isolates have been included in previous studies (16, 25, 35), examining their susceptibility to ceftriaxone and their \textit{penA} gene allele. The clinical isolates were obtained from 2002 to 2011 and were selected to represent geographically (mainly global representativeness), phenotypically, and genetically diverse isolates. Thus, the collection included the XDR H041 strain from Japan (32, 33) and the XDR F89 strain from France (49) with clinical high-level resistance to all ESCs, many additional isolates with substantially increased MICs of ESCs, isolates displaying clinical ESC resistance and associated with treatment failure (n = 4 (48, 50, 51)), and isolates with other types of clinical high-level resistance and/or MDR. The international reference strains included the WHO A-E, WHO I, WHO I, MS-11, and FA1090 gonococcal strains, as well as the recently described WHO 2008 \textit{N. gonorrhoeae} reference strains (n = 8) (47). These WHO 2008 reference strains were also used for standardization of the MIC testing in the two WHO CCs (based on giving highly comparable results) as well as forming part of the quality control in all MIC testing. The species of all gonococcal isolates and reference strains were initially verified with a sugar utilization test and/or PhadebaGT GC Monoclonal Test (Bactus AB, Sweden), and then the isolates were stored at −70°C as previously described (53). Prior to the MIC testing, the isolates were cultured on modified Thayer–Martin culture media without any included antimicrobials.

**MIC determination.** MIC determinations (in micrograms per milliliter) using the agar dilution technique for ertapenem and ceftriaxone (126 clinical isolates examined in Sydney, Australia) were performed as previously described (40, 42) and with the Etest method for ertapenem and ceftriaxone (131 clinical isolates and 17 international reference strains examined in Orebro, Sweden) according to the manufacturer’s instructions (AB bioMérieux, Solna, Sweden) and as previously described (4). The MIC testing for each isolate was performed in parallel for both antimicrobials. \beta-Lactamase activity was detected using nitrocefin discs.

For ceftriaxone, interpretative criteria from the Clinical and Laboratory Standards Institute (CLSI [11]) were used. No interpretative criteria have been stated by CLSI (11) or any other organization for ertapenem.

**Genetic characterization.** Molecular epidemiological characterization of all clinical isolates and reference strains by means of \textit{N. gonorrhoeae} multiantigen sequence typing (NG-MAST) was performed as described previously (28, 53). PCR amplification and sequencing of known gonococcal ESC resistance determinants, i.e., \textit{penA}, \textit{mtrR}, and \textit{parBib}, were done as described elsewhere (25, 47, 55).

**RESULTS**

\textit{In vitro} activity of ertapenem, compared to ceftriaxone, against \textit{Neisseria gonorrhoeae} clinical isolates (n = 257) and international reference strains (n = 17). In general, the MICs of ertapenem (MIC\textsubscript{50}, 0.032 \mu g/ml; MIC\textsubscript{90}, 0.064 \mu g/ml) and ceftriaxone (MIC\textsubscript{50}, 0.032 \mu g/ml; MIC\textsubscript{90}, 0.125 \mu g/ml) were similar. For the \beta-lactamase-producing isolates (n = 23), the MICs of ertapenem (MIC\textsubscript{50}, 0.016 \mu g/ml; MIC\textsubscript{90}, 0.032 \mu g/ml) and ceftriaxone (MIC\textsubscript{50}, 0.032 \mu g/ml; MIC\textsubscript{90}, 0.064 \mu g/ml) were also similar. However, the ranges of MIC values for ertapenem (0.002 to 0.125 \mu g/ml) and ceftriaxone (<0.002 to 4 \mu g/ml) substantially differed (Fig. 1).

Four (1.5%) isolates were resistant to ceftriaxone (MIC, 0.5 to 4 \mu g/ml) according to the interpretative criteria stated by the CLSI (11), but these isolates showed MICs from 0.016 \mu g/ml to 0.064 \mu g/ml only for ertapenem. For the XDR \textit{N. gonorrhoeae} strains H041 (32, 33) and F89 (49), both of which are highly resistant to cefixime (MIC = 4 to 8 \mu g/ml) and ceftriaxone (MIC = 2 to 4 \mu g/ml), the ertapenem MICs were significantly lower (0.064 \mu g/ml and 0.016 \mu g/ml, respectively). Furthermore, for the strains causing cefixime treatment failures in Norway (n = 2; MIC, 0.125 \mu g/ml and 0.25 to 0.5 \mu g/ml for ceftriaxone and cefixime, respectively [51]) and in Austria (n = 1; MIC, 0.5 \mu g/ml and 1.0 \mu g/ml for ceftriaxone and cefixime, respectively [50]) and a ceftriaxone treatment failure strain from a pharyngeal gonorrhoea in Sweden (n = 1; MIC, 0.125 to 0.25 \mu g/ml and 0.5 \mu g/ml for ceftriaxone and cefixime, respectively [48]), the corresponding MIC values for ertapenem were lower, ranging between 0.064 and 0.125 \mu g/ml.

**Effects of ESC resistance determinants on ertapenem in \textit{Neisseria gonorrhoeae} clinical isolates (n = 257) and international reference strains (n = 17).** Similar mechanisms affect the MICs of many \beta-lactam antimicrobials; however, the effects of ESC resistance determinants, such as specific \textit{penA}, \textit{mtrR}, or \textit{penB} alterations, on ertapenem resistance are unknown. Of the 274 \textit{N. gonorrhoeae} isolates, 101 (36.9%), 71 (25.9%), 242 (88.3%), and 249 (90.9%) had a \textit{penA} mosaic allele, an alteration of \textit{AS01} in PBP2, an \textit{mtrR} resistance determinant, and a \textit{penB} resistance determinant, respectively (Table 1). The presence of, in particular, a \textit{penA} mosaic allele was associated with increased MICs of ertapenem; e.g., 25 (35.7%), 48 (50.5%), and 21 (91.3%) of all the isolates with an ertapenem MIC of 0.032 \mu g/ml, 0.064 \mu g/ml, and 0.125 \mu g/ml,
respectively, contained a penA mosaic allele. For comparison, only seven (8.1%) of the isolates with an ertapenem MIC < 0.032 μg/ml contained a penA mosaic allele (Table 1 and Fig. 2). Furthermore, the isolates containing a penA mosaic allele and, additionally, the mtrR and penB resistance determinants had the highest ertapenem MICs. Nevertheless, the specific penA mosaic alleles resulting inceftriaxone resistance in four isolates (ceftriaxone MIC, 0.5 to 4 μg/ml) caused a significantly lower MIC of ertapenem, ranging from 0.016 to 0.064 (Table 1). In comparison with the penA mosaic alleles, the alteration of A501 in PBP2 appeared less associated with increased MICs of ertapenem (Table 1 and Fig. 2). In fact, isolates containing both the mtrR resistance determinant and penB resistance determinant, combined with a penA wild-type allele, displayed MICs of ertapenem as high as those seen with the isolates additionally containing an alteration of A501 in PBP2 (Table 1).

**Molecular epidemiological characterization of examined Neisseria gonorrhoeae clinical isolates (n = 257) and international reference strains (n = 17).** In total, the examined N. gonorrhoeae isolates were assigned to 133 different NG-MAST sequence types. ST225 (17 isolates), which is an internationally transmitted clone associated with resistance to ciprofloxacin and a slightly increased MIC of ceftriaxone, and ST1407 (n = 33), comprising a globally spread clone that accounts for a substantial proportion of the isolates showing intermediate susceptibility and resistance to ESCs and MDR internationally (16, 18, 43, 49–51), were the most prevalent sequence types. Among the sequence types represented by more than five isolates (n = 8), ST1407 (n = 33; MIC<sub>50</sub>, 0.064 μg/ml), ST5 (n = 8; MIC<sub>50</sub>, 0.064 μg/ml), ST326 (n = 7; MIC<sub>50</sub>, 0.125 μg/ml), and ST925 (n = 6; MIC<sub>50</sub>, 0.125 μg/ml) appeared to be associated with increased MICs of ertapenem. Each of these sequence type clones contained the mtrR determinant and penB determinant, and all sequence types, with exception of ST5, contained also a penA mosaic allele.

**DISCUSSION**

This is the first study investigating the in vitro activity of the carbapenem ertapenem relative to ceftriaxone against ESC-susceptible and ESC-resistant N. gonorrhoeae isolates, as well as outlining the effects of ESC resistance determinants on ertapenem. Gonococcal strains from a collection representing geographically and genetically diverse isolates were examined. The collection included the only two confirmed XDR strains, H041 (32, 33) and F89 (49), and additional isolates with substantially increased ESC MICs, ESC resistance associated with ESC treatment failure, and other types of clinical MDR. Ertapenem had no apparent in vitro advantage over ceftriaxone for N. gonorrhoeae isolates with lower ceftriaxone MICs. This is also in full concordance with a previous study by Livermore et al. (26), where ceftriaxone retained superior in vitro activity, compared to ertapenem, against gonococcal isolates with lower ceftriaxone MICs. Nevertheless, for all isolates with resistance to ceftriaxone (MIC, 0.5 to 4 μg/ml), the corresponding MICs of ertapenem were low (0.016 to 0.064 μg/ml). Ertapenem was also highly active against isolates with high-level clinical resistance and MDR to all types of other antimicrobials (data not shown). Accordingly, ertapenem may be an effective treatment option for gonorrhea and, in particular, for the currently identified ESC-resistant cases and possibly in dual antimicrobial therapy for treatment of gonorrhea.

Ertapenem is rapidly bactericidal and, like other β-lactam antimicrobials, derives its activity from binding to specific PBPs and subsequent blocking of cell wall synthesis that result in time-dependent killing. Resistance to ertapenem in bacteria is usually mediated by upregulation of efflux pumps, porin deficiency, production of metallo-β-lactamases/carbapenemases, or PBP changes resulting in decreased affinity for the drug. Ertapenem activity may also be affected by some classical extended-spectrum β-lactamases (ESBL) and hyperproduced AmpC β-lactamases, but the organisms mainly remain clinically susceptible. Nevertheless, resistance can arise when these enzymes are presented with extreme impermeability (7, 13, 27). In the present study, for N. gonorrhoeae isolates with increased MICs of ceftriaxone (but not full resistance), the penA mosaic alleles encoding mosaic PBPs with less affinity for ESCs appeared to increase the MIC of ertapenem to a level similar to that of ceftriaxone. For these penA mosaic isolates, the MIC of ertapenem appeared to further increase also.

**FIG 1** MIC (micrograms per milliliter) distribution of ertapenem and ceftriaxone for clinical Neisseria gonorrhoeae isolates (n = 257) and N. gonorrhoeae international reference strains (n = 17).
TABLE 1 MICs of ertapenem and ceftriaxone, and presence of determinants of resistance to extended-spectrum cephalosporins, for *N. gonorrhoeae* clinical isolates (*n* = 257) and international reference strains (*n* = 17)

<table>
<thead>
<tr>
<th>Isolates with other penA (<em>n</em> = 103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtrR&lt;sup&gt;c&lt;/sup&gt; + penB&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TX 2 (0.7) 8 (2.9) 5 (1.8) 13 (4.7) 30 (11) 14 (5.1) 1 (0.4)</td>
</tr>
<tr>
<td>ETP 13 (4.7) 9 (3.3) 23 (8.4) 27 (9.8) 1 (0.4)</td>
</tr>
<tr>
<td>penB&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TX 4 (1.4) 1 (0.4) 1 (0.4) 2 (0.7)</td>
</tr>
<tr>
<td>ETP 1 (0.4) 3 (1.1) 4 (1.4) 2 (0.7) 1 (0.4)</td>
</tr>
<tr>
<td>Wild type</td>
</tr>
<tr>
<td>TX 5 (1.8) 6 (2.2) 1 (0.4) 1 (0.4) 1 (0.4) 1 (0.4)</td>
</tr>
<tr>
<td>ETP 1 (0.4) 5 (1.8) 5 (1.8) 2 (0.7) 2 (0.7)</td>
</tr>
<tr>
<td>Total TX 11 (3.9) 20 (7.3) 15 (5.5) 25 (9.1) 71 (27) 77 (28) 48 (18) 3 (1.1) 1 (0.4) 1 (0.4) 2 (0.7)</td>
</tr>
<tr>
<td>ETP 2 (0.8) 10 (3.7) 37 (14) 36 (13) 70 (25) 95 (34) 23 (8.5)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only whole MIC dilutions are presented. TX, ceftriaxone; ETP, ertapenem.

<sup>b</sup> penA mosaic, mosaic alleles, which may differ in their exact nucleotide sequences, encoding a mosaic penicillin binding protein 2 (PBP2), which causes decreased susceptibility to extended-spectrum cephalosporins.

<sup>c</sup> Characteristic single nucleotide (A) deletion in the inverted repeat of the promoter region of *mtrR* that causes overexpression of the MtrCDE efflux pump, which results in a further decreased susceptibility to extended-spectrum cephalosporins.

<sup>d</sup> Alterations of amino acids 120 and/or 121 in the porin PorB1b that cause a decreased intake of extended-spectrum cephalosporins and, accordingly, a further decreased susceptibility to extended-spectrum cephalosporins.

<sup>e</sup> Alterations of amino acid A501 in PBP2, which has been associated with decreased susceptibility to extended-spectrum cephalosporins.

For future treatment of gonorrhea, the development of new treatment regimens and/or options is essential. Use of an increased dose of ceftriaxone has already been implemented (9, 14, 41). However, this approach provides only a short-term solution. Dual antimicrobial combination treatment has also recently been introduced in the United States (58) and the United Kingdom (5) for uncomplicated anogenital gonorrhea cases. Unfortunately, due to cost issues, combination therapy is challenging in settings of lesser resources and, from a global public health perspective, the need for an effective antimicrobial for single-drug treatment of gonorrhea appears fundamental. However, for future treatment of gonorrhea, there are few promising novel alternatives in sight (6, 24, 31, 32, 41, 49, 58). Nevertheless, gentamicin has been used as a first-line treatment in Malawi, Africa, for nearly 2 decades without any reported emergence of *in vitro* resistance, and *in vitro* susceptibility in the European Union appears high (10). Furthermore, a new fluoroketolide, solithromycin (CEM-101), has also been shown to have high *in vitro* activity against gonococci (15, 36). Finally, the present report shows that ertapenem may also be an effective option for treatment of gonorrhea, in particular, for...
currently identified ESC-resistant cases and possibly in a dual antimicrobial combination therapy regimen. However, all these potential future treatment regimens require up-to-date and comprehensive \textit{in vitro} and \textit{in vivo} evaluations, including appropriately designed, randomized, and controlled treatment studies (evaluating parameters such as efficacy, safety, toxicity, and cost) and pharmacokinetic/pharmacodynamics data for genital and extragenital (especially pharyngeal) gonorrhea. Furthermore, additional knowledge regarding present and future (\textit{in vitro}-selected and \textit{in vivo}-emergent) genetic resistance determinants for these antimicrobials, and clear correlates between genetic and phenotypic laboratory parameters and clinical treatment outcomes, would be very valuable.

In conclusion, clinical resistance to ceftriaxone in \textit{N. gonorrhoeae} has been reported and the widespread concern that gonorrhea may become untreatable in certain circumstances is valid. A major global focus, imperative for public health, is to promptly identify new antimicrobials (or other compounds) for the effective treatment of gonorrhea. The present \textit{in vitro} study showed that ertapenem may be an effective treatment option for gonorrhea and, particularly, for the currently identified ESC-resistant cases and possibly as part of a dual antimicrobial therapy regimen. Additional appropriately designed \textit{in vitro} and surveillance studies and, in particular, \textit{in vivo} clinical efficacy trials for all potentially new therapeutic options for gonorrhea are urgently needed.

\section*{ACKNOWLEDGMENTS}

The present work was supported by grants from the Örebro County Council Research Committee and the Foundation for Medical Research at Örebro University Hospital, Sweden. The WHO Collaborating Centre for STD, Sydney, Australia; and Queensland Paediatric Infectious Diseases Laboratory, Brisbane, Australia.

\section*{REFERENCES}


\begin{figure}
\centering
\includegraphics[width=\textwidth]{activity_of_ertapenem_against_n gonorrhoeae}
\caption{Ceftriaxone and ertapenem MICs, and their relation to \textit{penA} mosaic alleles and alteration of amino acid A501 in PBP2, in all \textit{N. gonorrhoeae} clinical isolates (\textit{n} = 253) and international reference strains (\textit{n} = 17) susceptible to ceftriaxone (MIC \leq 0.25 \mu g/ml [11]).}
\end{figure}


penA alterations and subtypes in *Neisseria gonorrhoeae* strains from Sydney, Australia, that are less susceptible to ceftriaxone. Antimicrob. Agents Chemother. 51:3111–3116.


