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*In vitro* activity of the ketolide cethromycin in multidrug-resistant clinical *Neisseria gonorrhoeae* isolates and international reference strains

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Antimicrobial resistance in *Neisseria gonorrhoeae* is a major public health problem, which compromises the treatment of gonorrhoea globally. We evaluated the *in vitro* activity of the ketolide cethromycin against a large panel of clinical gonococcal isolates and international reference strains (n = 254), including numerous multi-drug-resistant and extensively drug-resistant isolates. Cethromycin showed potent *in vitro* activity against most of the gonococcal isolates with the following modal MIC, MIC\(_{50}\) and MIC\(_{90}\): 0.064 mg/L, 0.125 mg/L and 0.5 mg/L, respectively. However, cross-resistance between azithromycin and cethromycin was identified (Spearman’s rank correlation coefficient 0.917) and isolates displaying high-level resistance to azithromycin (MIC >256 mg/L; n = 9) also showed high MICs of cethromycin (32–256 mg/L). In conclusion, the cross-resistance with azithromycin indicates that cethromycin may not be considered for empirical first-line monotherapy of gonorrhoea. However, cethromycin might be valuable in combination antimicrobial therapy and for second-line therapy e.g. for cases with ceftriaxone resistance or allergy.

**Keywords:** gonorrhoea, treatment, ketolide, cethromycin, ABT-773, antimicrobial resistance

Introduction

*Neisseria gonorrhoeae*, causing the sexually transmitted infection (STI) gonorrhoea, remains a significant global public health concern.\(^1\) Worryingly, treatment of gonorrhoea is jeopardised by the incredible ability of *N. gonorrhoeae* to develop antimicrobial resistance (AMR) to all therapeutic antimicrobials that have been introduced.\(^2,3\) The first treatment failure with the currently-recommended dual antimicrobial therapy (ceftriaxone plus azithromycin), which is also the last remaining option for empiric first-line therapy, was reported in 2016.\(^4\) In 2018, the first gonococcal strain with ceftriaxone resistance combined with high-level resistance to azithromycin was also reported from England,\(^5\) followed by two similar cases in Australia.\(^6\) In response to this AMR threat which could lead to untreatable gonorrhoea, the World Health Organization (WHO) has advocated for the development of novel antimicrobials for gonorrhoea treatment.\(^7\)

Ketolides belong to a subclass of macrolides derived from erythromycin A, with a broader antibacterial spectrum and increased ribosomal binding affinity compared to other macrolides. Ketolides have affinity to both domain II and V of the target 23S rRNA of the 50S ribosomal subunit, while traditional macrolides have affinity to only domain V.\(^7\) The first-in-class fluoroketolide solithromycin has recently been evaluated in a phase 3 non-inferiority randomised clinical controlled trial (RCT) of uncomplicated urogenital gonorrhoea. However, in the primary efficacy analysis microbiological cure was achieved in only 92%, 94 and 83% of urogenital, pharyngeal and rectal specimens, respectively.\(^8\) Cethromycin (trade name Restanza and initially known as ABT-773) is also a ketolide, which showed *in vitro* activity against a number of selected Gram-positive, Gram-negative and atypical bacteria.\(^9\) The *in vitro* activity of cethromycin against *N. gonorrhoeae* has not been evaluated, i.e.
with exception of testing 35 antimicrobial susceptible gonococcal isolates reported in 2001.  

This study examined the in vitro activity of the ketolide cethromycin against a large collection of clinical N. gonorrhoeae isolates (n = 220) and international gonococcal reference strains (n = 34), including numerous multidrug-resistant (MDR; n = 57) and extensive drug-resistant (XDR; n = 14) gonococcal isolates.

Materials and methods

N. gonorrhoeae isolates

The examined isolate collection represented a large geographically (mainly global representativeness), temporally (obtained from 1991 to 2018), phenotypically and genetically diverse selection. It included the 2016 WHO reference strains (n = 14), 11,12 20 additional international gonococcal reference strains, 100 consecutive clinical Swedish gonococcal isolates obtained in 2016 and 120 isolates selected for their resistance phenotype including a high proportion (45%) of isolates containing azithromycin-resistance mechanisms, XDR gonococcal isolates (n = 14), isolates with in vitro or clinical resistance to extended-spectrum cephalosporins (ESCs), as well as other high-level clinical resistance and MDR (n = 57) to other antimicrobials previously used for treatment of gonorrhoea.

Antimicrobial susceptibility testing

The MICs (mg/L) of cethromycin (Advanced Life Sciences Inc., Woodridge, Illinois, USA) were determined by agar dilution technique, according to current CLSI guidelines (www.clsi.org). The MICs (mg/L) of ceftriaxone, cefixime, azithromycin, spectinomycin, ciprofloxacin, ampicillin and tetracycline were determined by the Etest method (AB bioMérieux, Marcy l’Etoile, France), which is highly comparable to agar dilution technique. With exception of azithromycin for which no clinical resistance breakpoint exists, all MICs were interpreted into susceptibility (S), intermediate susceptibility (I) and resistance (R) according to the EUCAST clinical breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v.9.0_Breakpoint_Tables.pdf). EUCAST does not any longer state any clinical breakpoint for azithromycin. However, an ECOFF of 1 μg/mL is recommended for testing purposes to detect acquired azithromycin resistance mechanisms (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v.9.0_Breakpoint_Tables.pdf).

Table 1. MIC range, MIC50, MIC90 and modal MIC value for the ketolide cethromycin and therapeutic antimicrobials currently or previously recommended for Neisseria gonorrhoeae isolates

<table>
<thead>
<tr>
<th>Antimicrobial isolate group (No.)</th>
<th>MIC range (mg/L)</th>
<th>MIC50 (mg/L)</th>
<th>MIC90 (mg/L)</th>
<th>Modal MIC (mg/L)</th>
<th>S/I/R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cethromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All isolates (254)</td>
<td>0.002–256</td>
<td>0.125</td>
<td>0.5</td>
<td>0.064</td>
<td>ND²</td>
</tr>
<tr>
<td>Consecutive isolates (100)</td>
<td>0.002–0.25</td>
<td>0.064</td>
<td>0.125</td>
<td>0.064</td>
<td>ND²</td>
</tr>
<tr>
<td>Selected isolates (120)</td>
<td>0.002–256</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>ND²</td>
</tr>
<tr>
<td>Reference strains (34)</td>
<td>0.004–128</td>
<td>0.064</td>
<td>0.25</td>
<td>0.064</td>
<td>ND²</td>
</tr>
<tr>
<td>Isolates with azithromycin-resistance mechanisms (144)³</td>
<td>0.002–0.125</td>
<td>0.032</td>
<td>0.064</td>
<td>0.064</td>
<td>ND²</td>
</tr>
<tr>
<td>Isolates lacking azithromycin-resistance mechanisms (144)³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone (254)</td>
<td>&lt;0.002–4</td>
<td>0.008</td>
<td>0.064</td>
<td>0.004</td>
<td>97.2/ND¹/2.8</td>
</tr>
<tr>
<td>Cefixime (254)</td>
<td>&lt;0.016–8</td>
<td>&lt;0.016</td>
<td>0.125</td>
<td>&lt;0.016</td>
<td>90.9/ND¹/9.1</td>
</tr>
<tr>
<td>Azithromycin (254)⁴</td>
<td>0.016–256</td>
<td>0.5</td>
<td>16</td>
<td>16</td>
<td>56.1/ND⁴/44.9</td>
</tr>
<tr>
<td>Spectinomycin (254)</td>
<td>4–1024</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>98.0/ND²/2.0</td>
</tr>
<tr>
<td>Ciprofloxacin (254)</td>
<td>&lt;0.002–&gt;32</td>
<td>0.25</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>48.4/0.0/51.6</td>
</tr>
<tr>
<td>Ampicillin (254)</td>
<td>0.016–256</td>
<td>0.5</td>
<td>8</td>
<td>0.125</td>
<td>37.0/48.0/15.0</td>
</tr>
<tr>
<td>Tetracycline (254)</td>
<td>0.125–256</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>25.6/22.4/52.0</td>
</tr>
</tbody>
</table>

Data is presented as absolute values for all isolates (n = 254), and for different sub-groups of isolates, as well as proportion of susceptible, intermediate susceptible and resistant to antimicrobials.

¹MIC was determined using agar dilution technique for cethromycin and the Etest for the additional antimicrobials.
²ID = intermediate susceptible; R = resistant; S = susceptible. The EUCAST breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v.9.0_Breakpoint_Tables.pdf) were applied for all antimicrobials.
³Not determined due to lack of interpretative criteria.
⁴EUCAST does not any longer state any clinical breakpoint for azithromycin. However, an ECOFF of 1 μg/mL is recommended for testing purposes to detect acquired azithromycin resistance mechanisms (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v.9.0_Breakpoint_Tables.pdf). This ECOFF has been used to indicate resistance to azithromycin in the present study.

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This ECOFF was used to indicate resistance to azithromycin in the present study. No interpretative criteria exist for cethromycin. Only whole MIC dilutions are reported in the present manuscript.

**Results**

Cethromycin showed potent *in vitro* activity against the vast majority of tested *N. gonorrhoeae* isolates (*n* = 254). The susceptibility results for cethromycin and seven antimicrobials currently or previously recommended for gonorrhoea treatment are summarised in Table 1. The isolates were divided into different groups, i.e. all isolates, consecutive isolates, selected isolates, international reference strains and isolates containing azithromycin-resistance mechanisms as well as lacking azithromycin-resistance mechanisms (Table 1).

Briefly, the modal MIC, MIC<sub>50</sub>, MIC<sub>90</sub> and MIC range of cethromycin was 0.064 mg/L, 0.125 mg/L and 0.5 mg/L and from 0.002 to 256 mg/L, respectively. With the exception of the ESCs (ceftiraxone and cefixime), the modal MIC, MIC<sub>50</sub> and MIC<sub>90</sub> of the additional antimicrobials tested were all substantially higher than those observed for cethromycin (Table 1). However, cross-resistance was observed between cethromycin and the macrolide azithromycin, which also targets the 23S rRNA of the 50S ribosomal subunit. The MIC distributions for cethromycin and azithromycin and a direct comparison of MIC values are shown in Figure 1a and b, respectively. Accordingly, the correlation between the MICs of cethromycin and azithromycin was high, i.e. a Spearman’s rank correlation coefficient of 0.917. Furthermore, all nine isolates with high-level resistance to azithromycin (MIC > 256 mg/L; due to the A2059G mutation at 124 of the four 23S rRNA gene alleles) displayed MIC values of 32–256 mg/L for cethromycin (Figure 1b). Finally, the *in vitro* activity of cethromycin against isolates containing azithromycin-resistance mechanisms (*n* = 114) was lower compared to its activity against isolates lacking azithromycin-resistance mechanisms (*n* = 140). The modal MIC, MIC<sub>50</sub>, MIC<sub>90</sub> and MIC range of cethromycin for these groups were 0.25 mg/L, 0.25 mg/L, 0.5 mg/L, 0.002–256 mg/L and 0.064 mg/L, 0.032 mg/L, 0.064 mg/L, 0.002–0.125 mg/L, respectively (Table 1).
For the non-macrolide antimicrobials, no cross-resistance with cethromycin was identified and cethromycin MICs were similar among resistant and susceptible isolates. For the isolates with in vitro resistance to the currently recommended ESCs and isolates resulting in verified ESC treatment failures included in the material (n = 23, 9.1%), the MICs of cethromycin were low and ranged from 0.064 mg/L to 0.5 mg/L.

**Discussion**

This study is the first comprehensive evaluation of the in vitro activities of the ketolide cethromycin against *N. gonorrhoeae*, i.e. as a potential future treatment option for gonorrhoea. A large geographically, temporally and genetically diverse collection of clinical *N. gonorrhoeae* isolates and international reference strains was used, including various types of high-level resistant, XDR and MDR isolates. The activity of cethromycin was also compared with the activities of seven antimicrobials which are currently or were previously recommended for gonorrhoea treatment, i.e. ceftriaxone, cefixime, azithromycin, spectinomycin, ciprofloxacin, ampicillin and tetracycline. Agar dilution was used to evaluate cethromycin since no Etest strips exist for this antimicrobial otherwise is the Etest methods the most frequently recommended method for *N. gonorrhoeae*, used in routine diagnostics, resistance surveillance and research studies. Highly comparable results between agar dilution and Etest has been shown in previous studies, one study describes, however, minor differences mainly for cefixime and, most importantly, the differences shown was mainly within the inter-assay variation of both agar dilution and Etest (±1 MIC dilution). Cethromycin was highly active against the majority of *N. gonorrhoeae* isolates and displayed an MIC ≤1 mg/L for 96.5% (n = 245) of tested *N. gonorrhoeae* isolates. In the remaining nine isolates with azithromycin MICs of >256 mg/L, cross-resistance with cethromycin, due to the 23S rRNA A2059G resistance mutation, was observed (Spearman’s rank correlation coefficient of 0.917 for the cethromycin and azithromycin MICs). This cross-resistance is of concern as gonococcal isolates with high-level azithromycin resistance have been sporadically reported in many countries, and a sustained transmission of such strains is currently ongoing in the United Kingdom.

Cethromycin has in previous studies displayed in vitro activity against a wide range of both Gram-positive and Gram-negative bacterial species, including penicillin- and macrolide-resistant streptococci, staphylococci, respiratory Gram-negative organisms including β-lactamase producing *Haemophilus influenzae* and *Moraxella catarrhalis*, bacteria commonly associated with atypical pneumonia (*Chlamydia pneumoniae* species, *Legionella* species and *Mycoplasma species*) and even against a small collection of *N. gonorrhoeae* isolates (n = 35). Cethromycin was granted an orphan drug designation by the US FDA in 2007 for the prophylactic treatment of patients exposed to inhalation anthrax (*Bacillus anthracis*) and in 2009 for the prophylactic treatment of plague and tularemia (*Yersinia pestis* and *Francisella tularensis*).

Although until date, cethromycin has not been evaluated in STIs trials, several phase 2 and 3 randomised controlled clinical trials (RCTs) have been conducted to investigate its efficacy and safety in the treatment of community acquired bacterial pneumonia, acute bacterial exacerbation of chronic bronchitis and acute bacterial sinusitis. In these RCTs, cethromycin was shown to be non-inferior to currently used antimicrobials and pharmacokinetic data support a 300 mg daily dosing regimen in further studies. Available data on adverse effects suggest that cethromycin is safe and well tolerated. Adverse effects appear to be dose-related, with gastrointestinal (diarrhea, nausea, vomiting and abdominal pain) and nervous system (headaches) events being most prominent. In contrast to telithromycin, the only ketolide approved for treatment by the European medicines agency (EMA) and the (U.S Food and drug administration) FDA, no cases of severe hepatotoxicity have been reported after treatment with cethromycin.

In conclusion, the ketolide cethromycin demonstrated high in vitro activity against the majority of the *N. gonorrhoeae* isolates tested, including XDR and MDR strains. However, the identified cross-resistance with azithromycin indicates that cethromycin may not be considered for empirical first-line monotherapy of gonorrhoea. Nevertheless, cethromycin could be valuable in combination antimicrobial therapy (e.g. together with ceftriaxone or some new promising antimicrobial such as zoliflodacin) and for second-line therapy e.g. for cases with ceftriaxone resistance or allergy. This could be particularly effective in combination with a molecular test targeting the 23S rRNA A2059G gene mutation causing resistance to cethromycin. Additional studies further evaluating cethromycin including in vitro selection, mechanisms and biological fitness of cethromycin resistance, pharmacokinetic/pharmacodynamic studies to inform optimal dosing as well as RCTs evaluating clinical efficacy in patients with urogenital and extragenital gonorrhoea would be valuable. Clinical activity against
other STIs such as infections due to Chlamydia trachomatis and Mycoplasma genitalium should also be explored.

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