Introduction

Ketolides are semisynthetic 14-membered-ring macrolides characterized by a 3-keto group in place of the cladinose moiety in the macrolactone ring. Telithromycin (formerly called HMR 3647 or RU 66647) and HMR 3004 (the first ketolide described, formerly called RU 004 or RU 64004) have been extensively investigated in vitro and possess excellent activity against a variety of organisms, especially respiratory pathogens, Gram-positive bacteria and anaerobes.

In Italy, the rate of erythromycin resistance increased considerably in the 1990s in both Streptococcus pyogenes and Streptococcus pneumoniae isolates, with incidences of >40% and >30%, respectively. In the present study, the activities of telithromycin and HMR 3004 were evaluated in vitro against erythromycin-susceptible and erythromycin-resistant Italian isolates of these two species. Erythromycin-resistant test strains were preliminarily characterized according to their susceptibilities to antibiotics of the macrolide, lincosamide and streptogramin (MLS) group, and assigned to the constitutive resistance (cMLS) phenotype, the inducible resistance (iMLS) phenotype or the M phenotype. iMLS S. pyogenes strains were further subdivided into the three recently described subtypes iMLS-A, -B and -C. Telithromycin and HMR 3004 were uniformly and highly active against pneumococci (regardless of their susceptibility or resistance to erythromycin and/or penicillin), erythromycin-susceptible S. pyogenes and erythromycin-resistant S. pneumoniae strains of the M phenotype (in which resistance is mediated by an efflux system) or iMLS-B or -C phenotype (in which resistance is mediated by a methylase encoded by the ermTR gene). Both ketolides were less active against erythromycin-resistant S. pyogenes strains with the cMLS phenotype or the iMLS-A subtype (where resistance is mediated by a methylase encoded by the ermAM gene), these strains ranging in phenotype from the upper limits of susceptibility to low-level resistant.

Materials and methods

Test strains

A total of 311 strains of S. pyogenes (100 erythromycin-susceptible and 211 erythromycin-resistant) and 157 of S. pneumoniae (100 erythromycin-susceptible and 57 erythromycin-resistant), all isolated from clinical specimens between mid-1996 and mid-1999, were obtained from various Italian laboratories. Strain identification was confirmed in our laboratory and erythromycin susceptibility (MIC \( \leq 0.25 \) mg/L) or resistance (MIC \( \geq 1 \) mg/L) quantified by a

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broth microdilution method. Using the same method, pneumococci were categorized as penicillin-susceptible (MIC ≤ 0.06 mg/L), -intermediate (MIC 0.12–1 mg/L) or -resistant (MIC ≥ 2 mg/L). Erythromycin-resistant strains of \textit{S. pyogenes} were assigned to the cMLS, iMLS or M phenotype, and iMLS strains were further subdivided into the three subtypes iMLS-A, iMLS-B and iMLS-C on the basis of a triple-disc test (erythromycin plus clindamycin and josamycin). Attempts at distinguishing between inducibly and constitutively resistant pneumococci were also made on the basis of rokitamycin MICs as described by Agouridas \textit{et al.} (taking 1 and 4 mg/L as the breakpoints for inducibly and constitutively resistant strains, respectively). Strains were maintained in glycerol at −70°C and subcultured twice on blood agar before susceptibility testing.

\textit{Antibiotics}\n
Telithromycin and HMR 3004 were provided by Hoechst Marion Roussel (Lainate, Italy). Erythromycin, clindamycin and benzylpenicillin were purchased from Sigma Chemical Co. (St Louis, MO, USA), Josamycin and rokitamycin were obtained from ICN Biomedicals (Costa Mesa, CA, USA) and Formenti (Milan, Italy), respectively.

\textit{Susceptibility tests}\n
MICs were determined by a standard microdilution procedure using Mueller–Hinton II broth (BBL Microbiology

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Susceptibility to erythromycin (resistance phenotype) & Number of isolates tested & Antimicrobial agent & MIC (mg/L) & & \\
& & & range & MIC\textsubscript{50} & MIC\textsubscript{90} \\
\hline
Erythromycin-resistant (cMLS) & 35 & telithromycin & 0.03–8 & 2 & 8 \\
& & HMR 3004 & ≤0.015–8 & 1 & 4 \\
& & erythromycin & 2–>128 & >128 & >128 \\
& & josamycin & 16–>128 & >128 & >128 \\
& & clindamycin & all >128 & >128 & >128 \\
Erythromycin-resistant (iMLS-A) & 39 & telithromycin & 1–8 & 4 & 8 \\
& & HMR 3004 & 0.5–8 & 1 & 4 \\
& & erythromycin & all >128 & >128 & >128 \\
& & josamycin & all >128 & >128 & >128 \\
& & clindamycin & 0.03–0.5 & 0.12 & 0.25 \\
Erythromycin-resistant (iMLS-B) & 24 & telithromycin & ≤0.015–0.12 & ≤0.015 & 0.06 \\
& & HMR 3004 & ≤0.015–0.12 & ≤0.015 & 0.06 \\
& & erythromycin & all >128 & >128 & >128 \\
& & josamycin & 0.03–0.12 & 0.03 & 0.12 \\
& & clindamycin & 0.03–0.5 & 0.06 & 0.12 \\
Erythromycin-resistant (iMLS-C) & 25 & telithromycin & all ≤0.015 & ≤0.015 & ≤0.015 \\
& & HMR 3004 & all ≤0.015 & ≤0.015 & ≤0.015 \\
& & erythromycin & 1–8 & 2 & 8 \\
& & josamycin & 0.03–0.12 & 0.06 & 0.06 \\
& & clindamycin & ≤0.015–0.06 & 0.03 & 0.06 \\
Erythromycin-resistant (M) & 88 & telithromycin & ≤0.015–0.5 & 0.12 & 0.25 \\
& & HMR 3004 & ≤0.015–0.25 & 0.06 & 0.12 \\
& & erythromycin & 2–32 & 8 & 16 \\
& & josamycin & ≤0.015–0.12 & 0.03 & 0.12 \\
& & clindamycin & ≤0.015–0.12 & 0.03 & 0.06 \\
Erythromycin-susceptible & 100 & telithromycin & ≤0.015–0.12 & ≤0.015 & 0.06 \\
& & HMR 3004 & ≤0.015–0.25 & ≤0.015 & 0.06 \\
& & erythromycin & ≤0.015–0.12 & 0.03 & 0.06 \\
& & josamycin & ≤0.015–0.25 & 0.06 & 0.12 \\
& & clindamycin & ≤0.015–0.12 & 0.06 & 0.12 \\
\hline
\end{tabular}
\end{table}

Table I. \textit{In vitro} activity of telithromycin, HMR 3004, erythromycin, josamycin and clindamycin against erythromycin-resistant and -susceptible strains of \textit{S. pyogenes}
Anti-streptococcal activity of ketolides

Systems, Cockeysville, MD, USA) supplemented with 3% lysed horse blood. The inoculum was 5 × 10⁵ cfu/mL. The antibiotics were tested at final concentrations (prepared from two-fold dilutions) ranging from 0.015 to 128 mg/L. The MIC was defined as the lowest concentration that yielded no visible growth. In the absence of established interpretive standards, the MIC breakpoints proposed unofficially for telithromycin (susceptible, ≤1 mg/L; intermediate, 2 mg/L; resistant, ≥4 mg/L) were considered tentatively for both ketolides. *S. pneumoniae* ATCC 49619 was used as a quality control.

Results

The *in vitro* activities of telithromycin, HMR 3004, erythromycin, josamycin and clindamycin were determined against the erythromycin-susceptible and erythromycin-resistant test strains of *S. pyogenes* and *S. pneumoniae* (resistant strains were subdivided into resistance phenotypes or subtypes).

Among the *S. pyogenes* isolates (Table I), the erythromycin-susceptible strains, erythromycin-resistant strains with the M phenotype and inducibly resistant strains of the iMLS-B and -C subtypes were uniformly susceptible to telithromycin and HMR 3004. The most potent ketolide activity was recorded against iMLS-C isolates (MICs of both telithromycin and HMR 3004 were invariably ≤0.015 mg/L), followed by iMLS-B and erythromycin-susceptible isolates (MIC₅₀ ≤ 0.015 mg/L; MIC₉₀ 0.06 mg/L) and M isolates (MIC₉₀ and MIC₅₀, 0.12 and 0.25 mg/L, respectively, for telithromycin, and 0.06 and 0.12 mg/L for HMR 3004). Susceptibility to ketolides of the cMLS and iMLS-A phenotypes usually ranged from the upper limits of susceptibility (MIC 0.5–1 mg/L) to low-level resistance (MIC, 4–8 mg/L), with MIC₅₀ of 1 mg/L for HMR 3004 and 2–4 mg/L for telithromycin; however, one highly ketolide-susceptible cMLS isolate (telithromycin MIC 0.03 mg/L; HMR 3004 MIC ≤ 0.015 mg/L) was encountered.

*S. pneumoniae* isolates (Table II) were uniformly susceptible to both telithromycin and HMR 3004, independently of their susceptibility or resistance to other MLS antibiotics and to penicillin. It is worth noting that inducibly resistant strains of this species were not detected using the double-disc test, whereas all but one of the 42 isolates categorized as constitutively resistant by the double-disc test were classified as inducibly resistant on the basis of the rokitamycin MIC method. Erythromycin-resistant pneumococci had MIC₅₀ ≤ 0.015 mg/L and MIC₉₀ of 0.06 mg/L for both ketolides. Erythromycin-susceptible pneumococci had MIC₅₀ and MIC₉₀ ≤0.015 mg/L for both ketolides.

Discussion

Ketolides are regarded currently as promising agents for

<table>
<thead>
<tr>
<th>Susceptibility to erythromycin (resistance phenotype)</th>
<th>Number of isolates tested</th>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>range</td>
<td>MIC₅₀</td>
</tr>
<tr>
<td>Erythromycin-resistant (cMLS)ₚ</td>
<td>42ₚ</td>
<td>te lithromycin</td>
<td>≤0.015–0.25</td>
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<tr>
<td></td>
<td></td>
<td>HMR 3004</td>
<td>≤0.015–0.12</td>
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<tr>
<td></td>
<td></td>
<td>erythromycin</td>
<td>8–&gt;128</td>
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<tr>
<td></td>
<td></td>
<td>josamycin</td>
<td>4–&gt;128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>clindamycin</td>
<td>all &gt;128</td>
</tr>
<tr>
<td>Erythromycin-resistant (M)</td>
<td>15ₚ</td>
<td>telithromycin</td>
<td>≤0.015–0.06</td>
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<tr>
<td></td>
<td></td>
<td>HMR 3004</td>
<td>≤0.015–0.06</td>
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<td>erythromycin</td>
<td>2–32</td>
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<td>clindamycin</td>
<td>0.03–0.12</td>
</tr>
<tr>
<td>Erythromycin-susceptible</td>
<td>100ₚ</td>
<td>telithromycin</td>
<td>≤0.015–0.06</td>
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<tr>
<td></td>
<td></td>
<td>HMR 3004</td>
<td>≤0.015–0.03</td>
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<td>josamycin</td>
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<tr>
<td></td>
<td></td>
<td>clindamycin</td>
<td>≤0.015–0.12</td>
</tr>
</tbody>
</table>

ₚcMLS isolates were identified by the double-disc method. By the rokitamycin MIC method, 41 isolates were identified as inducibly resistant (only one penicillin-susceptible isolate was recorded as constitutively resistant by this method).

ₚIncluding three penicillin-resistant and four penicillin-intermediate strains.

ₚIncluding one penicillin-resistant and two penicillin-intermediate strains.

ₚIncluding four penicillin-resistant and six penicillin-intermediate strains.
use in treating infections by respiratory pathogens resistant to other MLS drugs. Although their mechanism of action is similar to that of macrolides, their structurally modified macro lactone ring has been reported to render them more stable in an acidic environment and unable to induce MLS resistance in Gram-positive cocci. Among pneumococci, the identification of inducibly expressed MLS resistance is still an open question, strongly dependent on the antibiotic used to test inducibility (clindamycin or rokitamycin), with discrimination between constitutively and inducibly resistant strains varying substantially depending on the criterion applied and the related testing procedure, and even on the test medium: but high ketolide susceptibility appears to be shared by all pneumococcal isolates. Among inducibly erythromycin-resistant S. pyogenes isolates, the weak inducer activity of ketolides may explain their activity against iMLS-B and -C isolates but does not correlate with their moderate activity against iMLS-A isolates. This difference may be because iMLS-A strains have the ermAM (ermB) methylase gene, whereas iMLS-B and iMLS-C strains have the ermTR methylase gene. Macrolides and ketolides interact with 23S ribosomal RNA at two close but distinct sites (the peptidyl transferase loop in domain V and hairpin 35 in domain II), and it is possible that differences in resistance result from different methylation of the two sites. On the other hand, a similar lack of correlation has been reported for HMR 3004 with two inducibly MLS-resistant strains (a pneumococcus and an enterococcus).

The uniform activity of ketolides against pneumococci, including erythromycin-resistant S. pyogenes isolates, suggests that ketolides may offer a valuable alternative for the treatment of pneumococcal infections. Once pharmacological and toxicological investigations of telithromycin—the ketolide currently considered for clinical use—are completed, clinical trials should be performed to test this hypothesis. Against erythromycin-resistant strains of S. pyogenes, ketolide antibiotics appear to be highly active when resistance is mediated by an efflux system (M isolates) and even more so when it is mediated by the methylase encoded by the recently described ermTR gene (iMLS-B and -C isolates); in contrast, their activity is lower when resistance is mediated by the conventional ermAM (ermB)-encoded methylase (cMLS and iMLS-A isolates).

References


Received 20 January 2000; returned 29 March 2000; revised 6 April 2000; accepted 7 June 2000