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In Vitro Activity of Iclaprim against Respiratory and Bacteremic Isolates of Streptococcus pneumoniae

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Iclaprim, a novel dihydrofolate reductase inhibitor, inhibited 90% of the clinical isolates (MIC\textsubscript{90}) of Streptococcus pneumoniae (n = 785) collected by a national surveillance program at a concentration of 1 μg/ml. The MIC\textsubscript{90} for iclaprim was 7 doubling dilutions lower for trimethoprim-sulfamethoxazole-susceptible isolates (n = 670; MIC\textsubscript{90}, 0.06 μg/ml) than for trimethoprim-sulfamethoxazole-resistant isolates (n = 115; MIC\textsubscript{90}, ≥8 μg/ml). The potential clinical utility of iclaprim to treat patients with pneumococcal infections may depend upon the current prevalence of resistance to trimethoprim-sulfamethoxazole in this pathogen.

Antimicrobial resistance remains an important consideration when treating patients with suspected or laboratory-proven bacterial respiratory tract infections. Streptococcus pneumoniae is recognized as the most common cause of community-acquired pneumonia, bacterial meningitis, and acute otitis media, and resistance to penicillin and macrolides, as well as emerging fluoroquinolone resistance, is an important consideration for this pathogen (8, 14). Iclaprim, formerly AR-100, is an investigational racemate of 2,4-diaminopyrimidine known to inhibit dihydrofolate reductase (DHFR) and is currently being developed to attempt to address issues of antimicrobial resistance in Staphylococcus aureus and several other pathogens (6, 10, 11). Only limited data have been published on gram-positive pathogens other than S. aureus that may potentially be susceptible to iclaprim (6, 10, 11). The purpose of this study was to assess the in vitro activity of iclaprim against a representative collection of respiratory and bacteremic isolates of S. pneumoniae to determine its in vitro potential for use in treating infections attributable to this pathogen.

Isolates of S. pneumoniae from 2002 were obtained from frozen stocks of the previously described Canadian Respiratory Organism Surveillance Study (CROSS) (14); 540 isolates from respiratory sources and 245 bacteremic isolates were tested. Stock antibiotic solutions were prepared and dilutions made according to Clinical and Laboratory Standards Institute (CLSI) guidelines (4). Following two subcultures from frozen stock, the MICs for iclaprim (Arpida, Reinach, Switzerland), trimethoprim-sulfamethoxazole (SXT), penicillin, clarithromycin, doxycycline, and ciprofloxacin were determined using the CLSI broth microdilution method (4). The SXT, penicillin, clarithromycin, and doxycycline MICs were interpreted according to CLSI guidelines (5). The ciprofloxacin MICs were interpreted as follows: ≤1 μg/ml, susceptible; 2 μg/ml, intermediate; and ≥4 μg/ml, resistant. The results for iclaprim testing were compared directly with those for SXT, given that trimethoprim is currently the most widely used DHFR inhibitor. Trimethoprim is most frequently used in combination with the sulfonamide sulfamethoxazole to treat infections caused by a wide variety of pathogens.

The collection of 785 isolates of S. pneumoniae tested in this study was 14.7, 11.8, 6.8, 1.7, and 0.9% resistant to SXT, clarithromycin, penicillin, ciprofloxacin, and doxycycline, respectively. Against SXT-susceptible isolates (n = 670), iclaprim had a MIC\textsubscript{90} (0.06 μg/ml) that was 3 doubling dilutions lower than that of SXT (0.5 μg/ml) (Table 1). Iclaprim demonstrated a MIC\textsubscript{50} of ≤0.03 μg/ml and a MIC\textsubscript{90} of 1 μg/ml against the 785 isolates tested; the MIC\textsubscript{50} and MIC\textsubscript{90} for iclaprim were lower for isolates from blood than from respiratory sources. The MIC\textsubscript{90} for iclaprim was 7 doubling dilutions lower for SXT-susceptible isolates (MIC\textsubscript{90}, 0.06 μg/ml) than for SXT-resistant isolates (≥8 μg/ml). Iclaprim also demonstrated lower MIC\textsubscript{90}s against penicillin- and clarithromycin-susceptible isolates than against isolates with penicillin- and clarithromycin-resistant phenotypes as a function of their underlying SXT susceptibility. Isolates resistant to penicillin and/or clarithromycin and concurrently susceptible to SXT demonstrated a MIC\textsubscript{90} of 0.06 μg/ml (data not shown). The relationship between the MIC for iclaprim and the in vitro susceptibility of isolates to SXT, penicillin, and clarithromycin is depicted in Fig. 1.

Iclaprim has been previously reported to be active in vitro against S. aureus, including methicillin-resistant and vancomycin-resistant strains (2, 3, 11, 12, 13), and is a promising agent in phase III clinical trials for the treatment of complicated skin and soft tissue infections. In studies with limited numbers of isolates, iclaprim has also been reported to be active in vitro against S. pneumoniae, Streptococcus pyogenes, and viridans group streptococci, as well as Haemophilus influenzae, Moraxella catarrhalis, Chlamydia trachomatis, and Chlamyophila pneumoniae (7, 11). The intent of the current study was to determine the in vitro activity of iclaprim against a larger, clinically relevant collection of S. pneumoniae isolates to assess its potential for development against this pathogen. In this study, our major finding was that iclaprim was active against S. pneumoniae from both respiratory and bacteremic sources (MIC\textsubscript{90}, 1 μg/ml), including isolates resistant to penicillin and macrolides, but that its in vitro activity was a function of the...
underlying susceptibility or resistance to SXT. Iclaprim was more active in vitro against SXT-susceptible isolates (MIC$_{90}$, 0.06 µg/ml) than against SXT-resistant isolates (MIC$_{90}$, ≥8 µg/ml). This finding was not surprising given that iclaprim, like trimethoprim, targets bacterial DHFR (6, 10).

Iclaprim is a racemic mixture composed of two enantiomers with similar antimicrobial activities (6, 10). Whether the equipotent activity of both of these enantiomers is responsible for the greater activity of iclaprim compared to trimethoprim (or SXT) versus both SXT-susceptible and SXT-resistant *S. pneumoniae* is currently unknown. Trimethoprim resistance in *S. pneumoniae* has been shown to be due primarily to point mutations in DHFR, especially at position 100 with an isoleucine to leucine change (13); whether iclaprim is less likely than SXT or trimethoprim to select for these mutations is unknown (15). Data published for *S. aureus* suggests that it is very difficult to select for resistance to iclaprim in vitro (6, 10). However, it should be noted that the emergence of resistance to SXT in

<table>
<thead>
<tr>
<th>Isolate parameter</th>
<th>No. of isolates</th>
<th>Iclaprim</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>All isolates tested</td>
<td>785</td>
<td>≤0.03</td>
<td>1</td>
</tr>
<tr>
<td>Specimen sources</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>540</td>
<td>0.03</td>
<td>2</td>
</tr>
<tr>
<td>Blood</td>
<td>245</td>
<td>≤0.03</td>
<td>0.5</td>
</tr>
<tr>
<td>Antimicrobial susceptibility phenotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SXT susceptible</td>
<td>670</td>
<td>≤0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>SXT resistant</td>
<td>115</td>
<td>2</td>
<td>≥8</td>
</tr>
<tr>
<td>Penicillin susceptible</td>
<td>620</td>
<td>≤0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Penicillin resistant</td>
<td>53</td>
<td>2</td>
<td>≥8</td>
</tr>
<tr>
<td>Clarithromycin susceptible</td>
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<td>0.06</td>
<td>0.12</td>
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<tr>
<td>Clarithromycin resistant</td>
<td>93</td>
<td>0.06</td>
<td>2</td>
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<tr>
<td>Ciprofloxacin susceptible*</td>
<td>772</td>
<td>≤0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Ciprofloxacin resistant*</td>
<td>778</td>
<td>≤0.03</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Fewer than 20 isolates of *S. pneumoniae* resistant to ciprofloxacin or doxycycline were available; therefore, these data were not included in the table.

FIG. 1. Iclaprim MIC distributions for isolates of *S. pneumoniae* with SXT-, penicillin-, and clarithromycin-susceptible and resistant phenotypes.
pneumococci was not directly associated with SXT use but rather was due to its coselection in isolates resistant to penicillin and macrolides and was associated with the use of these latter two agent groups. It is also presently unknown whether the addition of sulfamethoxazole to iclaprim would enhance iclaprim’s pharmacodynamic properties against *S. pneumoniae*, although it is known that iclaprim functions synergistically in combination with a sulfonamide (6).

So what is the future for iclaprim for respiratory tract infections? Currently, SXT is infrequently prescribed for the treatment of respiratory tract infections, such as acute exacerbations of chronic bronchitis and acute bacterial sinusitis (8), and rates of resistance to SXT in North American isolates of pneumococci exceed 20% (9, 14). Even though iclaprim is more active in vitro than SXT against *S. pneumoniae*, a key respiratory pathogen, iclaprim’s utility in respiratory tract infections may depend upon its pharmacokinetic and pharmacodynamic parameters and is worthy of further study. Recently published pharmacokinetic data indicate that alveolar macrophage and epithelial lining fluid concentrations of iclaprim were 24.5 and 12.61, 7.16 and 6.38, and 5.28 and 2.66 g/ml, respectively, at 1 to 2 h, 3 to 4 h, and 5.5 to 7 h following a single, 60-min intravenous infusion of iclaprim at a dose of 1.6 mg/kg (1). These data suggest that iclaprim concentrations in alveolar macrophages and in epithelial lining fluid exceed the MIC<sub>50</sub> for isolates of *S. pneumoniae* isolated from the respiratory tract (MIC<sub>50</sub> 2 µg/ml) and from blood (MIC<sub>50</sub> 0.5 µg/ml) for 5.5 to 7 h following intravenous administration; similarly, ≥50% of SXT- and penicillin-resistant isolates (MIC<sub>50</sub> 2 µg/ml) may be inhibited at this dose of iclaprim (Table 1). Together, these data suggest that iclaprim’s potential to treat patients with community-acquired pneumonia caused by *S. pneumoniae* may depend heavily upon the current prevalence of SXT resistance for this pathogen.

## REFERENCES


