Accuracy of the Broth Microdilution and Etest Methods for Detecting Chloramphenicol (CM) Acetyltransferase-Producing Strains of *S. pneumoniae* (SPN) Including World-Wide Geographic Variations in the Prevalence of Resistance Observed in the SENTRY Program

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**Abstract**

High antimicrobial resistance rates in SPN has caused a need for alternate therapies. CM is currently being reconsidered as an empiric treatment for respiratory tract infections particularly in developing countries. In this study, we assessed the ability of the reference NCCLS broth microdilution (BMD) and Etest (AT; AB BIODISK) methods to detect CM resistance among SPN as compared to the CM acetyltransferase (CAT) assay (ASM Manual of Clinical Microbiology). In the 1999 SENTRY Program, 1,671 SPN from respiratory tract infections were collected from 49 participants located in the Americas and Europe. The rates of penicillin and macrolide-non-susceptibility were 35.1 and 22.9%, respectively. All of the CM-resistant (≥ 8 µg/ml) isolates (n=193, 9.2%) and 53 CM-susceptible isolates were selected for further intermethod comparisons. The CM resistance rates were (%/sites): Canada (4.5); USA (10.6); Latin America (4.3%) and Europe (12.7%).

**Introduction**

Pneumococcal pneumonia is a leading cause of community-acquired pneumonia and other serious/orbital infections, including meningitis, sinusitis and otitis media. Occurrence of penicillin non-susceptible strains harboring resistance to other classes of drugs is on the rise around the world. Chloramphenicol has been widely used as therapy for nearly five decades. However, its use as a veterinary product was prohibited in Europe in 1994 because of emerging resistance, and its use in humans has been severely limited due to potentially serious toxicities. Currently it is recommended as a therapy for bacterial meningitis caused by *S. pneumoniae*, *meningococci* and *Haemophilus influenzae* for patients with severe penicillin allergy. Chloramphenicol retains relatively good antimicrobial activity in many parts of the world, with resistance rates ranging from as little as 1% in Austria and 18.0% in Bulgaria, Romania and Slovakia.

**Results**

1. In the 1999 SENTRY Antimicrobial Surveillance Program, a total of 1541-671 (3.2%) *S. pneumoniae* isolates were selected from respiratory tract infections that were resistant to chloramphenicol (MIC ≥ 8 µg/ml).

**Results (continued)**

- Resistance to chloramphenicol and other key antimicrobial classes varied widely between the different geographic regions.
- The highest CM resistance rates were observed in Latin America (4.3%) and Canada (4.5%). Much higher resistance rates were detected in the US (10.6%) and Europe (12.7%).
- A larger number of resistant isolates in the US were collected from the states of Nebraska (345 strains; 20.0%), New York (189/0 from two sites; 20.0%), Texas (739 strains; 17.5%), and Pennsylvania (1039 strains; 25.6%).
- The highest rate of chloramphenicol resistance in Europe was noted in Barcelona, Spain (31/2 strains; 38.5%).
- The highest penicillin and macrolide resistance rates were detected in the US and Europe. Latin America demonstrated elevated resistance to penicillin (27.5%).
- Overall, the lowest resistance rates were found in Canada (12.8% to erythromycin and 15.6% to penicillin).
- All the chloramphenicol resistant pneumococci produced a positive result by the CAT test, whereas the susceptible isolates did not exhibit the presence of enzyme by this assay (ASM, 1992).
- Induction with CM prior to the assay enhanced the positive test qualitatively. However, induction did not turn a negative test (with a susceptible strain at MIC ≤ 4 µg/ml) into a false-positive reaction.
- The results from relatively short-ranged miniaturized MIC tests (2 - 16 µg/ml) were confirmed by Etest analysis of all the resistant (154) and 53 additional susceptible isolates.
- Eight *H. influenzae* isolates (31/344 isolates; 0.5%) resistant to chloramphenicol were isolated from patients with *H. influenzae* respiratory tract infections in the SENTRY Antimicrobial Surveillance Program, (1999).

**Materials and Methods**

**Bacterial isolates**

All *S. pneumoniae* strains were isolated from patients with respiratory tract infections in medical centers in the United States (USA; 27 sites), Canada (five sites), Latin America (eight sites) and Europe (seven sites), as part of the SENTRY Program during the winter respiratory season of 1999. Eight *Haemophilus influenzae* isolates with MICs of ≥ 8 µg/ml (resistant) were also selected and tested for the production of CAT.

**Reagents**

Chloramphenicol Etest strips were prepared at AB BIODISK (Solna, Sweden). Broth microdilution trays (5% lysed horse blood Mueller-Hinton broth) for minimum inhibitory concentration (MIC) tests were manufactured by TREK Diagnostics (Westlake, OH, USA); Mueller-Hinton plates with 5% sheep blood for use in the Etest were manufactured by Remel (Lenexa, KS, USA).

**Procedure**

Microdilution and Etest MIC determinations were performed according to methods described by the National Committee for Clinical Laboratory Standards (NCCLS) or the product package insert (AB BIODISK). The CAT assay was performed as described in the American Society for Microbiology Manual of Clinical Microbiology ([1992]. All the chloramphenicol-resistant (154) strains were isolated and an additional 53 susceptible strains were selected for intermethod comparisons. These isolates were subject to chloramphenicol Etest assay procedures. The bacterial cultures were “induced” by growing in the presence of chloramphenicol before determining the CAT activity. The results from relatively short-ranged miniaturized MIC tests (2 - 16 µg/ml) were confirmed by Etest analysis of all the resistant (154) and 53 additional susceptible isolates.

**Table 1**

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of Strains</th>
<th>MIC (µg/ml)</th>
<th>% Susceptible</th>
<th>% Intermediate</th>
<th>% Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>27</td>
<td>1029</td>
<td>10.6 (0-25.6)</td>
<td>26.6 (1-44.6)</td>
<td>29.8 (37-55)</td>
</tr>
<tr>
<td>Latin America</td>
<td>19</td>
<td>257</td>
<td>4.3 (0-9.0)</td>
<td>12.0 (0-25.0)</td>
<td>84.3 (27-95)</td>
</tr>
<tr>
<td>Europe</td>
<td>7</td>
<td>213</td>
<td>12.7 (0-68.6)</td>
<td>16.7 (0-43.8)</td>
<td>70.6 (41-57)</td>
</tr>
</tbody>
</table>

**Conclusions**

- The reference and Etest MIC methods proved to be very sensitive in predicting the CAT phenotype of chloramphenicol resistance in *S. pneumoniae* and *H. influenzae*.
- The CAT enzyme remains responsible for the vast majority of chloramphenicol resistance in pneumococci.
- Monitoring of chloramphenicol susceptibility patterns is needed as resistance rates vary widely and as high-level enzyme-mediated chloramphenicol resistance is often present in nasopharyngeal and pneumococcal isolates.