High Incidence of Erythromycin Resistance among Clinical Isolates of *Streptococcus agalactiae* in Taiwan

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The in vitro susceptibilities of 266 isolates of *Streptococcus agalactiae* determined by the agar dilution method showed that 6% of isolates were nonsusceptible to penicillin and 46% was resistant to erythromycin. Of the erythromycin-resistant isolates, 86.3% had the macrolide-lincosamide-streptogramin (MLS) resistance phenotype (constitutive MLS, 85.5%; inducible MLS, 0.8%) and 13.7% had the M phenotype.

*Streptococcus agalactiae* (a group B streptococcus [GBS]) is a leading cause of meningitis and bacteremia in newborns and also causes a variety of invasive diseases in pregnant women and nonpregnant adults (3, 8, 24, 30, 32). Widespread use of antibiotics, particularly penicillin, β-lactams, and erythromycin (and its newly available semisynthetic derivatives), in various clinical conditions, as well as the widely accepted efficacy of intrapartum antimicrobial prophylaxis in decreasing early-onset neonatal GBS infections, has potentiated the emergence of antibiotic resistance (4, 17, 19, 22, 29, 31). Although isolates with lower susceptibility to penicillin have been reported, penicillin is still the recommended drug of choice for the treatment or prophylaxis of GBS infections (6, 7, 19, 23, 29). However, recent reports of the increasing incidence of macrolide and clindamycin resistance in GBS in different countries have raised concerns about the possibility of inadequate prophylaxis or treatment with these antibiotics as alternative agents in patients allergic to penicillin (1, 5, 6, 21, 29). We describe herein the antimicrobial susceptibility patterns and serotype distribution of GBS isolates recovered from various clinical specimens from patients treated at the National Taiwan University Hospital (NTUH).

From April 1998 to October 2000, 266 isolates of *S. agalactiae* recovered from various clinical specimens from 266 patients treated at NTUH, a tertiary-care referral center with 2,000 beds in northern Taiwan, were studied. Of these isolates, 29 were recovered from respiratory sources (throat swab and sputum), 38 were from blood samples, 141 were from urine, 30 were from genital secretion (endocervical swab and vaginal discharge), and 28 were from other sources (wound pus, cerebrospinal fluid, and central venous catheters).

Antimicrobial susceptibility testing was performed by the standard agar dilution method (20). The following organisms were included as control strains: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619. Organisms were categorized as susceptible, intermediate, or resistant to the antimicrobial agents tested based on the interpretive guidelines for *Streptococcus* species other than *S. pneumoniae* provided by the NCCLS (20).

To understand the secular trend of resistance, data on the disk diffusion susceptibilities to erythromycin and clindamycin of *S. agalactiae* isolates recovered from 1994 to 2000 at NTUH were also analyzed.

The resistance phenotypes of erythromycin-resistant *S. agalactiae* were determined by the double-disk test as described previously (31). The presence of *ermAM, mefE*, and *mefA* gene sequences in chromosomal DNA of erythromycin-resistant *S. agalactiae* was investigated by PCR with primers as described previously (2, 25). Serotyping of each isolate was performed by the agglutination test (Denka Seiken, Tokyo, Japan). Antisera to polysaccharide antigens Ia, Ib, II, III, IV, and V were used.

Figure 1 shows the annual incidence of erythromycin and clindamycin resistance among the 978 isolates of *S. agalactiae* obtained from 1994 to 2000. A stepwise increase in erythromycin (clindamycin) resistance was found, from 19% (18%) in 1994 to 46% (37%) in 1997, and the level has remained stable over the past 4 years.

As shown in Table 1, 15 (6%) of the 266 isolates were nonsusceptible to penicillin: the MIC for 11 was 0.25 μg/ml, for 3 it was 0.5 μg/ml, and for 1 it was 2 μg/ml. The last isolate was also nonsusceptible to cefotaxime and cefepime (the MIC of both drugs was 4 μg/ml). Of the 266 isolates, 48 to 55% and

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The MICs for 50 and 90% of the isolates with the MLS phenotype isolates possessed both the M phenotype (Table 2). The isolates with MLS resistance phenotype (constitutive MLS [cMLS], 85.5%); inducible MLS [iMLS], 0.8%) and 17 (13.7%) had the 85.5%; inducible MLS [iMLS], 0.8%) and 17 (13.7%) had the MLS phenotype (Table 2). The MIC distribution of erythromycin-resistant S. agalactiae according to the resistance phenotype. The values above the bars are the numbers of isolates.

TABLE 1. In vitro antimicrobial susceptibility testing results for 266 isolates of S. agalactiae at NTUH from April 1998 to October 2000

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>% of isolates in category*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>For 50% of isolates</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.03–2</td>
<td>0.12</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.03–4</td>
<td>0.06</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.03–4</td>
<td>0.12</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.25–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.03–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2–128</td>
<td>64</td>
</tr>
<tr>
<td>Ciprofloxacine</td>
<td>0.12–4</td>
<td>1</td>
</tr>
<tr>
<td>Tovacloxacin</td>
<td>0.06–0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.06–0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0.03–&gt;32</td>
<td>0.12</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.06–&gt;128</td>
<td>0.25</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.12–&gt;128</td>
<td>1</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.06–&gt;128</td>
<td>0.25</td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>0.06–4</td>
<td>1</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.5–2</td>
<td>1</td>
</tr>
</tbody>
</table>

* S, susceptible; I, intermediate; R, resistant.

Of the 124 erythromycin-resistant (MIC, ≥1 µg/ml) isolates, 107 (86.3%) had the macrolide-lincosamide-streptogramin (MLS) resistance phenotype (constitutive MLS [cMLS], 85.5%; inducible MLS [iMLS], 0.8%) and 17 (13.7%) had the M phenotype (Table 2). The emmA gene was present in 88.7% of the isolates with the cMLS phenotype, and 64.7% of the M phenotype isolates possessed both the mefE and mefA genes. The MICs for 50 and 90% of the isolates with the MLS phenotype were 128 and >128 µg/ml, and those for the isolates with the M phenotype were 8 and 16 µg/ml, respectively (Fig. 2).

43% were nonsusceptible (including intermediate and resistant isolates) to macrolides and clindamycin, respectively. Four (26.7%) of the 15 penicillin-nonsusceptible isolates were resistant to erythromycin. Fourteen percent of these isolates were nonsusceptible to quinupristin-dalfopristin. All isolates were susceptible to vancomycin, trovafloxacin, and moxifloxacin.

Rates of resistance to erythromycin were highest in isolates from the respiratory tract (69%), followed by those from the genital tract (57%), urine (44%), and blood (34%). Sixty-one percent of 51 isolates recovered from patients aged 21 to 40 years, compared with 27% of 15 isolates from patients aged under 10 years, were resistant to erythromycin.

Of the 124 erythromycin-resistant (MIC, ≥1 µg/ml) isolates, 107 (86.3%) had the macrolide-lincosamide-streptogramin (MLS) resistance phenotype (constitutive MLS [cMLS], 85.5%; inducible MLS [iMLS], 0.8%) and 17 (13.7%) had the M phenotype (Table 2). The emmA gene was present in 88.7% of the isolates with the cMLS phenotype, and 64.7% of the M phenotype isolates possessed both the mefE and mefA genes. The MICs for 50 and 90% of the isolates with the MLS phenotype were 128 and >128 µg/ml, and those for the isolates with the M phenotype were 8 and 16 µg/ml, respectively (Fig. 2).

Of the 266 isolates, 70% belonged to one of six serotypes,
in Taiwan, rates of macrolide resistance in GBS, as in S. pyogenes isolates, seemed to have reached a plateau (40 to 50%) (10; Hsueh et al., unpublished data). This phenomenon differs significantly from that of S. pneumoniae: its rate of macrolide resistance continued to increase (to more than 90%) (11–13; Hsueh et al., unpublished data). Extreme differences in the distribution of the M phenotype in recent isolates of S. pyogenes (>90%), S. pneumoniae (30 to 40%), and GBS (14%) in Taiwan are also impressive. The findings of our study highlight that macrolide or clindamycin is not the drug of choice for intrapartum prophylaxis or for empirical therapy of GBS diseases in Taiwan. In Taiwan, high rates of quinupristin-dalfopristin nonsusceptibility among various gram-positive bacteria have been documented (18). A similar scenario was also present in our GBS isolates.

The distribution of capsular serotypes of GBS strains varies with time, source of isolation, and geographical area (1, 17, 24). In the United States before the 1990s, most perinatal GBS disease was due to strains of serotypes Ia, Ib, and III (1, 16). Recently, serotypes Ia, III, and V have predominated in isolates causing early-onset neonatal sepsis (17). Lachenauer et al. reported that serotypes VI and VIII prevailed among GBS isolates from pregnant Japanese women (15). In this study, the higher incidence of serotype V among isolates from genitourinary sources is impressive.

The frequency of resistant isolates is also related to particular serotypes (9, 17). Previous studies demonstrated that erythromycin resistance was notable for serotypes Ia, III, and V among isolates recovered from neonates (9, 17). Furthermore, a high percentage (up to 30 to 35%) of serotype V isolates was reported to be resistant to erythromycin among isolates from various clinical specimens (15). On the contrary, our study showed that serotypes IV, Ib, and III had higher incidences of erythromycin resistance than did serotype V. This study also demonstrated source-specific resistance patterns, with isolates from respiratory secretions having the highest erythromycin resistance.

GBS strains have been considered uniformly susceptible to penicillin (17, 18). Strains of penicillin-nonsusceptible GBS have been described, and penicillin tolerance linked to treatment failure in patients with serious GBS infection was also observed (6, 31). However, recent reports of penicillin-nonsusceptible GBS isolates are rare (1, 9, 17). The high rate (11.7%) of nonsusceptibility of GBS isolates, as well as the finding of our study (6% of GBS isolates were nonsusceptible to penicillin), indicates that susceptibility surveillance of these isolates should be performed continuously (31). Interestingly, 40% of our penicillin-nonsusceptible GBS isolates belonged to serotype V. The limited number of penicillin-nonsusceptible GBS isolates tested here makes it difficult to determine the propensity of serotype V for penicillin nonsusceptibility.

In conclusion, the results presented herein from the testing of 266 recent isolates of GBS from Taiwan indicate a high incidence of macrolide resistance, particularly of the MLS phenotype, and persistence of GBS strains nonsusceptible to penicillin. Increases in resistance, together with variations in resistance patterns and in those from different sources, make local and extensive antimicrobial susceptibility surveillance crucial in establishing and/or modifying guidelines for the prophylaxis and treatment of GBS infections.

**REFERENCES**


