Phenotype and Genotype of Some Clinical Group B Streptococcus Isolates Resistant to Erythromycin in Egypt

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The aim of the study was to determine susceptibility of group B streptococci (GBS) to commonly used antibiotics and to determine the phenotype of macrolide resistance correlating it with the genotype. 220 group B streptococcus strains were subjected to antibiotic sensitivity disc diffusion method to penicillin, ampicillin, ceftriaxone, tetracyclines, erythromycin (E) and clindamycin (CD). Erythromycin resistance phenotype was done by double disc diffusion test. Erythromycin resistance mefA, ermB, and ermTR genes were determined using multiplex PCR. The results revealed that all strains were sensitive to penicillin, ceftriaxone and ampicillin. 161 (73%) , 38 (17%) and 24 (11%) strains were resistant to tetracyclines, erythromycin and clindamycin respectively. 15 (39.5%) were resistant to E alone (M phenotype). M phenotype strains were associated with mefA gene. 23 (60.5%) isolates were resistant to both E and clindamycin (CD). 9 (23.7%) strains were inducibly resistant to clindamycin (iMLSB) and all had ermTR gene. 14 (36.8%) were constitutively resistant to clindamycin (cMLSB). cMLSB strains have different genotypes. 7 strains were ermB genotype. 4 strains were ermTR genotype. 2 strains had both ermB & ermTR genes while only one strain had both ermB & mefA genes. Only 1 strain was resistant to clindamycin but sensitive to E. MefA was detected in 16 (42%) E resistant strains. ErmTR was also detected in 16 (42%) E resistant strains. ErmB was detected in 10 (26%) E resistant strains. 31(82%) E resistant strains contain single resistance gene while 7(18%) resistant strains contain more than one resistance gene. The study findings conclude that GBS isolates remain uniformly susceptible to penicillin and ampicillin but the erythromycin resistance has reached substantial level. The study recommends that testing of susceptibility to erythromycin and clindamycin by double disc diffusion method should be performed in individual cases when considered as alternatives for prophylaxis and treatment of GBS infection or colonization because strains with inducible MLSB cannot be detected with the conventional disc diffusion method.

INTRODUCTION

Streptococcus agalactiae (group B streptococcus [GBS]) is a well-known cause of invasive infections in neonates and pregnant women. It has increasingly been recognized as a significant pathogen in non-pregnant adults, particularly in elderly persons and persons with significant underlying diseases(14,18,42). For treatment or prevention of GBS infections, erythromycin and clindamycin therapy are recommended alternatives for patients who are allergic to β-lactam agents(5, 21). In the past, GBS was reported to be susceptible to erythromycin and clindamycin(2). However, recent studies have shown that changes in the susceptibility of GBS to erythromycin and clindamycin have been substantial, although rates of resistance to these agents have differed according to geographical variation and different investigators. The most frequently encountered macrolide resistance mechanisms in streptococci are ribosomal modification by a methylase encoded by an erm gene(44) and drug efflux by a membrane-bound protein encoded by a mef gene(27). Presence of the erm methylase confers resistance to erythromycin and inducible or constitutive resistance to lincosamides and streptograminB (macrolide-lincosamide-streptograminB phenotype) iMLSB, and cMLSB respectively, whereas presence of the Mef pump confers resistance only to 14- and 15-membered macrolides (M phenotype). A second efflux mechanism, encoded by the mreA gene, has been described for GBS(9). However, susceptible GBS strains have also been shown to possess the mreA gene, and it might function as a housekeeping gene. Although several previous studies have documented the prevalence of macrolide resistance in GBS, few have identified the mechanisms of resistance.(2,16, 27, 35, 42) The relative contribution of the presence of the erm and mef genes in contributing to a resistance phenotype may have important implications for therapy, since there are differences in drug susceptibility depending on the mechanism of resistance. Aim of this work was to determine susceptibility of GBS to commonly used antibiotics and to determine the phenotype of
macrolide resistance correlating it with the genotype.

MATERIALS AND METHODS

This study was done in Department of Microbiology and Immunology, Faculty of Medicine, Zagazig University. The study was done on 220 GBS strains (20 isolated from blood, 25 from urine, 20 from wound swabs, and 155 from vaginal swabs). Isolation and identification of GBS strains were done according to standard methods. \(^{(19)}\)

**Antibiotic Susceptibility Testing**

The AST of GBS to penicillin, ampicillin, ceftriaxone, tetracyclines erythromycin and clindamycin using the disc diffusion method was done and interpreted according to the recommendations of NCCLS.\(^{(33,34)}\) Testing was performed with Mueller-Hinton agar supplemented with 5% lysed horse blood (Oxoid). Differentiation of macrolide resistance mechanisms by phenotypic characterization was performed by double-disk diffusion testing, as described previously.\(^{(10,15)}\) Erythromycin (15 µg) and clindamycin (5 µg) disks (Oxoid) were placed 15mm apart, edge to edge, on Mueller-Hinton agar supplemented with 5% sheep blood agar (Becton Dickinson Microbiology Systems, Sparks, Md.) that had been inoculated with a 0.5 McFarland suspension of the organism. The plates were incubated for 24 h at 35°C in 5% CO2. Blunting was defined as growth within the clindamycin zone of inhibition proximal to the erythromycin disk, indicating MLSB inducible methylation. Resistance to both erythromycin and clindamycin indicated MLSB-constitutive methylation. Resistance to erythromycin but susceptibility to clindamycin without blunting indicated an efflux mechanism (M phenotype).\(^{(12)}\)

**Detection of erythromycin resistance genes.**

Template DNA was prepared as described previously by Jersek et al 1996.\(^{(22)}\) Briefly, a loopful of bacterial colony was mixed with lysosyme and proteinase K, followed by extraction with ethanol then chloroform, isomyl alcohol, and ethanol precipitation. The \textit{mefA}, \textit{ermB}, and \textit{ermTR} erythromycin resistance genes were detected by multiplex PCR with previously published sequences. Primers used for \textit{ermTR} gene amplification were 5' AAC CCG AAA AAT ACG CAA AA-3' and 5' ACC CGT TGA CTC ATT TCC AC-3'. For \textit{ermB}, \textit{ermBF} 5' AAG GTA CTC AAC CAA ATA-3' and \textit{ermBR}, 5' AGT AAC GGT ACT TAA ATT GTT TAC-3'.\(^{(4)}\) For \textit{mefA}, \textit{mefAF} 5' CGT AGC ATT GGA ACA GC-3' and \textit{mefAR} 5' TGC CGT AGT ACA GCC AT-3'.\(^{(18)}\)

A 2 µl of extracted DNA was used in a reaction volume of 50 µ with a final concentration of 200 µM each dNTP nucleotide, 0.4 µM each primer, 2 U Taq DNA polymerase, 50 mM KCl; 10 mM Tris-HCl; 1.5 mM MgCl2. The reactions were performed in a Perkin-Elmer 9600 thermocycler under the following conditions: denaturation at 95°C for 3 min and 35 cycles of 95°C for 1 min, 57°C for 1 min, and 72°C for 1 min. A final elongation step was performed at 72°C for 5 min. The products were separated on a 2% agarose gel with the expected sizes: \textit{ermB}, 639 bp; \textit{ermTR}, 152bp; and \textit{mefA}, 316 bp. The bands were visualized after staining with ethidium bromide.

**RESULTS**

Table (1): Sensitivity of GBS to common antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Sensitive</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0</td>
<td>220(100%)</td>
<td>220</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>220(100%)</td>
<td>220</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0</td>
<td>220(100%)</td>
<td>220</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>38(17%)</td>
<td>182(83%)</td>
<td>220</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>24(11%)</td>
<td>196(89%)</td>
<td>220</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>161(73%)</td>
<td>59(27%)</td>
<td>220</td>
</tr>
</tbody>
</table>

Table (2): Phenotype and genotype of GBS resistant to erythromycin

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>MefA</td>
<td>15(39.5%)</td>
</tr>
<tr>
<td>IMLSMB</td>
<td>ermTR</td>
<td>9(23.7%)</td>
</tr>
<tr>
<td>CMLSMB</td>
<td>ErmB</td>
<td>7(18.4%)</td>
</tr>
<tr>
<td>CMLSMB</td>
<td>ermTR</td>
<td>4(10.5%)</td>
</tr>
<tr>
<td>CMLSMB</td>
<td>ermTR&amp;ermB</td>
<td>2(5.3%)</td>
</tr>
<tr>
<td>CMLSMB</td>
<td>ErmB&amp;MefA</td>
<td>1(2.6%)</td>
</tr>
<tr>
<td>Sum</td>
<td>6</td>
<td>38(100%)</td>
</tr>
</tbody>
</table>

Table (3): Frequency of erythromycin resistance genes of GBS.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number(Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MefA</td>
<td>16(42%)</td>
</tr>
<tr>
<td>ErmTR</td>
<td>16(42%)</td>
</tr>
<tr>
<td>ErmB</td>
<td>10(26%)</td>
</tr>
<tr>
<td>Single gene</td>
<td>31(82%)</td>
</tr>
<tr>
<td>Combined genes</td>
<td>7(18%)</td>
</tr>
</tbody>
</table>
Among 220 isolates of Streptococcus group B, all strains were sensitive to penicillin, ampicillin and ceftriaxone. 161 (73%) strains were resistant to tetracyclines. 38 (17%) strains were resistant to erythromycin (E). 24 (11%) isolates were resistant to clindamycin (CD).

**Erythromycin resistance**

Phenotype:
- 15 (39.5%) were resistant to E alone, M phenotype.
- 23 (60.5%) isolates were resistant to both E and CD.
- 9 (23.7%) strains were inducibly resistant to CD (iMLSB).
- 14 (36.8%) were constitutively resistant to CD (cMLSB). Only 1 strain was resistant to CD but sensitive to E.

Genotype:
The association of resistance mechanism with erythromycin and clindamycin are shown in table (2). 15 strains were M phenotype and were associated with mefa gene. 9 strains were inducible MLSB phenotype and all had ermTR gene. The 14 cMLSB phenotype strains had different genotypes. 7 strains were ermB genotype. 4 strains were ermTR genotype. 2 strains had both ermB & ermTR genes while only one strain had both ermB & mefa genes.

MefA was detected in 16 (42%) resistant strains. ErmTR was also detected in 16 (42%) resistant strains. ErmB was detected in 10 (26%) resistant strains. 31 (82%) resistant strains contain single resistance gene while 7 (18%) resistant strains contain more than one resistance gene.

**DISCUSSION**

Approximately 20% of pregnant women are asymptomatically colonized with GBS. (18, 24) It is the most important cause of invasive bacterial disease in newborns. (18) Intra-partum chemoprophylaxis decreases the incidence of early onset GBS infant disease from 1.7 per 1000 live-births to 0.6 per 1000 live births. (140) The recommended agents are IV penicillin G or ampicillin. Macrolides are second line agents. (5)

In this study, all isolates were sensitive to penicillin G, ampicillin and ceftriaxone. Sensitivity to penicillin G, ampicillin and ceftriaxone was proved by other studies. (10, 25, 20) Until now, GBS resistance to penicillin G is not yet reported. (6, 16, 26, 42) and penicillin tolerance is rare. (6)

Resistance to ampicillin due to ante and intra-partum treatment was reported. (29)

As in previous reports, high percentage of TET-resistant GBS was detected. Our results (73%) were similar to those recorded in several studies performed in different countries: Anthony et al. (United States) (3), 85.3%; Traub and Leonhard (Germany) (41), 74.5%; De Azavedo et al. (Canada) (10), 82 to 87%; De Mouy et al. (France) (11), 88.1%; Betriu’ et al. (Spain) (10), 89%; and Melin et al. (Belgium) (30), 74.2 to 86.7%. However, in a Japanese study, only 26% of strains were found resistant to TET. (28) Tetracycline resistance is due to acquisition of tet determinant that encodes for antibiotic efflux or ribosomal protection in gram positive cocci. (34, 20)

Erythromycin resistance was detected in 17% of the GBS isolates studied. This rate can be compared to the United States (7.4 to 16%) (16, 32, 35), France (18.0 to 21.4%) (11, 17), Canada (18.0%) (10), and Spain (14.7 to 18.0%). (6, 36) It was higher than rates reported Japanese (3.1 to 3.0%) (18, 28) and German (4.9 and 12%) (39, 41) data, but it was also higher than rates reported in Morocco (8%) (31) and Argentina (5.2%). (20)

Erythromycin resistance was found to be predominantly due to the presence of an Erm methylase and this result was indicated in other studies. (10, 11, 23, 17)

The distribution of MLSB resistance genes of GBS is influenced by geographical variation and serotypes. (6, 10, 17, 43) In our study, among the 38 erythromycin-resistant strains examined in this study (Table 3), ermB was detected in 10 (26%), mefa was detected in 16 (42%), and ermTR was detected in 16 (42%). The predominant MLSB resistance gene of GBS was ermB in Spain (5), France (17), and Germany (44), whereas ermTR was prevalent in Canada. (16) In United States, the mefa and ermB genes were detected with equal frequencies. (13)

In *Streptococcus spp*. MLS resistance is usually mediated by ermAM/ermB class genes. (23) The ermTR gene was described for the first time in Finland by in group A streptococci. (23) The nucleotide sequence of ermTR is 83% identical to that of ermA described in *Staphylococcus aureus* and in coagulase-negative staphylococci, and it was
recently included among the ermA denomination.\(^{(36)}\)

Of the macrolide-resistant strains, 7(18%)% strains harbored combinations of resistance genes. Combinations of resistance genes have been described before only for a small percentage of isolates. \(^{(6, 11)}\) Only one study reported resistance gene combinations in 29.2% of the isolates. \(^{(5)}\)

In conclusion, our findings demonstrate that these GBS isolates remain uniformly susceptible to penicillin and ampicillin. The frequency of erythromycin resistance, however, has reached substantial level. The study recommends that testing of susceptibility to erythromycin and clindamycin by double disc diffusion method should be performed in individual cases when considered as alternatives for prophylaxis and treatment of GBS infection or colonization because GBS strains with inducible MLSB cannot be detected with the conventional disc diffusion method.

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References


The study aimed to evaluate the antimicrobial susceptibility of the liver samples from patients in Egypt. The antimicrobial discs used were ampicillin, amikacin, tetracycline, and erythromycin. The study found that:

1. Ampicillin was more effective against the liver samples than the other antibiotics.
2. The susceptibility of the liver samples to erythromycin was 71%, ampicillin was 71%, and amikacin was 11%.
3. The samples were found to have a high level of mefA and ermTR genes.
4. The study also detected the presence of mefA, ermB, and ermTR genes in the liver samples.

The results suggest a need for further research to understand the mechanisms of resistance and to develop new strategies for treating these infections.