Macrolide- and tetracycline-resistance determinants of colonizing group B streptococcus in women in Egypt

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INTRODUCTION

Streptococcus agalactiae or group B streptococcus (GBS), a commensal bacterium of the human gastrointestinal and genital tracts, is a leading cause of neonatal sepsis and meningitis. GBS has also been recognized as an important pathogen in pregnant women and the elderly (Phares et al., 2008). Penicillin is the antibiotic of choice for the prophylaxis and treatment of GBS infections. Macrolides and related drugs provide useful alternative therapies for penicillin-allergic patients. However, the emergence of GBS strains resistant to macrolides and tetracycline has been increasingly reported (Verani et al., 2010).

Macrolide resistance in GBS is commonly mediated by two major mechanisms. First, target-site modification by a ribosomal methylase known as macrolide–lincosamide–streptogramin B (MLSB) phenotype, which is associated with erm genes (ermA and ermB). MLSB can be inducible (iMLSB) or constitutive (cMLSB). Second, a macrolide-specific efflux mechanism (M phenotype) encoded by mef genes (Arpin et al., 1999; Leclercq, 2002). Tetracycline-resistance mechanisms in streptococci mostly involve a ribosomal protection protein that is mainly encoded by the tetM or tetO genes, and efflux pumps for tetracycline encoded by the tetK or tetL genes (Chopra & Roberts, 2001; Chopra et al., 1992).

No data are available on the antibiotic-resistance mechanisms of GBS in Egypt, and the aim of the current study was to fill this gap. We have previously reported on the distribution of serotypes and surface proteins of GBS (Shabayek et al., 2014). In this study, we investigate...
mechanisms of resistance to macrolides and tetracycline for the first time.

METHODS

A total of 100 previously identified GBS strains isolated from pregnant and non-pregnant women attending the Gynaecological Clinic at Ismailia General Hospital (400 beds) and the University Hospital of Suez Canal University (415 beds) (Shabayek et al., 2014) were used in this study. All strains were screened for susceptibility to penicillin G, ampicillin, cefotaxime, erythromycin, azithromycin, clindamycin, vancomycin, levofloxacin, tetracycline and chloramphenicol using the disk-diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2006a, 2007). Macrolide-resistance phenotypes were investigated using a double-disk method and were further classified as having the cMLSb, iMLSb or M phenotype (Figueira-Coelho et al., 2004). The MICs of erythromycin and clindamycin were measured using the agar dilution method for all erythromycin-resistant isolates (CLSI, 2006b, 2007). A multiplex PCR assay was used to detect three macrolide (ermA, ermB and mefA/E) and four tetracycline (tetM, tetO, tetK and tetL) resistance determinants, as described previously (Malhotra-Kumar et al., 2005).

RESULTS AND DISCUSSION

All 100 isolates were uniformly susceptible to penicillin G, ampicillin, cefotaxime, vancomycin and levofloxacin. The resistance rates to erythromycin, clindamycin, azithromycin, tetracycline and chloramphenicol were 17, 14, 16, 98 and 1%, respectively.

In comparison to neighbouring Arabian states, our erythromycin- and clindamycin-resistance rates were higher than those reported in Kuwait (Boswihi et al., 2012), but much lower than those in Tunisia (Hraoui et al., 2012). For tetracycline, our rate was similar to that found in Tunisia (Hraoui et al., 2012) and slightly higher than that in Kuwait (Boswihi et al., 2012). Our results for chloramphenicol resistance were quite similar to the 3.1% reported in Tunisia (Hraoui et al., 2012), but much lower than the 38.4% reported in Kuwait (Boswihi et al., 2012). Considering previous studies from Europe and the USA, our erythromycin-resistance rate was similar to that reported in Poland (Brzychczy-Wloch et al., 2010; Sadowy et al., 2010), higher than that in Portugal (Florindo et al., 2010) and much lower than the rates reported in France (Tazi et al., 2011) and the USA (Phares et al., 2008). For clindamycin, our rate was similar to that reported in the USA (Phares et al., 2008), higher than the rates reported in Poland (Brzychczy-Wloch et al., 2010; Sadowy et al., 2010) and Portugal (Florindo et al., 2010), and lower than that in France (Tazi et al., 2011). For tetracycline, however, our data almost correspond with the results from Poland (Sadowy et al., 2010).

The erythromycin-resistance level observed in our study (17%) indicates the need for careful surveillance in our region, as the first penicillin-non-susceptible GBS has been reported in the USA and Japan (Verani et al., 2010). This constitutes a threat to the use of penicillin, and may lead to further limited treatment options and a potential risk of failure of intrapartum antibiotic prophylaxis with β-lactams for GBS, especially with the increasing resistance to macrolides, which comprise the second-line choices.

Among the 17 erythromycin-resistant isolates, 14 (82.4%), one (5.9%) and two (11.8%) had cMLSb, iMLSb and M resistance phenotypes, respectively. MIC ranges of the erythromycin-resistant isolates are shown in Table 1.

As expected, nine out of the 14 cMLSb phenotypes carried the ermB gene and the single iMLSb phenotype carried the ermA gene, while one M phenotype carried the mefA/E gene (Table 1). Macrolide resistance was found to be predominantly due to the presence of erm methylase, and the ermB gene was the most prevalent. The mefA/E and ermA genes were rare among our GBS strains. This is in agreement with recent studies from other Arabian states and Europe (Boswihi et al., 2012; Brzychczy-Wloch et al., 2010; Hraoui et al., 2012; Sadowy et al., 2010). As reported previously, combinations of macrolide-resistance determinants were not found in our study (Brzychczy-Wloch et al., 2010; Figueira-Coelho et al., 2004; Fitoussi et al., 2001; Sadowy et al., 2010; Tazi et al., 2011). Phenotypic and genotypic features of erythromycin and clindamycin resistance in GBS isolated from women are shown in Table 1.

Six erythromycin-resistant strains (five cMLSb and one M phenotype) had a negative PCR result for macrolide-resistance genes. PCR inhibition was improbable due to amplification of the internal control (Malhotra-Kumar et al., 2005). Phenotypic-resistant strains with negative genotypic results have been previously reported (Acikgoz et al., 2004; De Mouy et al., 2001; Figueira-Coelho et al., 2004; Fitoussi et al., 2001; Tazi et al., 2011). These strains might possess other macrolide-resistance genes that were not investigated in our study. Other possible resistance mechanisms in β-haemolytic streptococci may be related to mutations in ribosomal proteins, as previously reported for Streptococcus pneumoniae (Malhotra-Kumar et al., 2005). Rarely, mutations in 23S rRNA or in the ribosomal L4 and L22 proteins prevent antibiotic binding, causing macrolide resistance (Diner & Hayes, 2009).

On the other hand, seven macrolide-sensitive strains (MIC <0.03 µg ml⁻¹) were ermB positive and one (MIC <0.03 µg ml⁻¹) harboured the mefA/E gene. Gene inactivation due to insertions and/or deletions is possible (Malhotra-Kumar et al., 2005), and might explain the macrolide susceptibility even in the presence of the positive PCR results. In fact, this result has also been reported by other authors in GBS (Tazi et al., 2011) and in Staphylococcus aureus and Staphylococcus epidermidis (Martineau et al., 2000). According to Martineau et al. (2000), a susceptible strain harbouring but not expressing an antibiotic-resistance gene should be regarded as potentially resistant to that antibiotic.

In concordance with others (Boswihi et al., 2012; Hraoui et al., 2012; Sadowy et al., 2010; Tazi et al., 2011), we found...
high rates of tetracycline resistance due to the presence of the tetM gene (99%). The tetM gene was detected alone (83.7%) or in association with tetL (12.2%), tetK (1%) or tetO (1%). This gene was also detected in combination with both tetL and tetK (1%). Only one strain carried tetO alone. The tetM gene is the most prevalent resistance determinant accounting for tetracycline resistance in Gram-positive bacteria (Roberts, 1996), and combinations of tetracycline-resistance determinants have been reported previously in various Gram-positive bacteria, including S. agalactiae (Boswihi et al., 2012; Hraoui et al., 2012; Roberts, 1996; Sadowy et al., 2010). The distribution of tetracycline-resistance gene clusters among GBS isolates in the current study is shown in Table 2.

Streptococci carrying more than one macrolide/tetracycline-resistance determinant are being increasingly noted (Boswihi et al., 2012; Hraoui et al., 2012; Malhotra-Kumar et al., 2005). There is evidence suggesting genetic linkage of tetO with ermA or mefA (Malhotra-Kumar et al., 2005). In the current study, the two tetO strains were positive for the mefA/E gene, and the tetL and tetK carrier strains harboured the ermB gene.

**Conclusions**

This is the first study to report mechanisms of macrolide and tetracycline resistance in Egypt; however, a notable limitation is the relatively small initial sample size and hence the number of resistant strains. Nevertheless, we detected resistance rates of 17 and 14% for erythromycin and clindamycin, respectively. Tetracycline resistance was detected in 98% of isolates. Vancomycin and levofloxacin are reliable substitutes for erythromycin and clindamycin for the treatment of GBS infections in patients with penicillin allergy.

**REFERENCES**


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**Table 1.** Phenotypic and genotypic features of erythromycin and clindamycin resistance in GBS isolated from women

<table>
<thead>
<tr>
<th>Macrolide-resistance phenotype (no. isolates)</th>
<th>Resistance marker (no. isolates)</th>
<th>MIC range (µg ml⁻¹)</th>
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<tr>
<td></td>
<td>ermB</td>
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<td>cMLS₉ (n=14)</td>
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<td>iMLS₉ (n=1)</td>
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**Table 2.** Distribution of tetracycline-resistance gene clusters among GBS isolates from women

<table>
<thead>
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<th>Resistance marker</th>
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</tr>
<tr>
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<tr>
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<td>1</td>
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<tr>
<td>tetM, tetL, tetK</td>
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</tr>
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<td>1</td>
</tr>
<tr>
<td>tetO</td>
<td>1</td>
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</table>


