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ORIGINAL ARTICLE

High prevalence of *Streptococcus agalactiae* from vaginas of women in Taiwan and its mechanisms of macrolide and quinolone resistance

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Background/Purpose: *Streptococcus agalactiae* (GBS), is the most common pathogen causing infections among perinatal women and neonatal babies. Nonetheless, there are few studies on the occurrence of GBS among the pregnant women and the mechanisms of GBS resistance to quinolones and macrolides in Taiwan.
Methods: GBS were isolated from vaginas of the pregnant and non-pregnant symptomatic women in Taiwan. The prevalence, antimicrobial susceptibility, and mechanisms of resistance against erythromycin and quinolone of total 188 isolates were studied.
Results: The isolation rate of GBS from pregnant women was significantly higher at 21.8% compare with the non-pregnant women of 13.2%. Antibiotic susceptibility test of the 188 GBS isolates revealed a high non-susceptible rate for erythromycin (50.0%) while the rate for levofloxacin was only 4.8%. Among 94 erythromycin non-susceptible GBS isolates, *ermB* gene was detected 83.1% (59/71) for those GBS that were non-susceptible to both clindamycin and tetracycline, which was significantly higher than GBS that are susceptible to clindamycin but resistant to tetracycline at 43.8% (7/16). No *ermA* or *mef* gene was detected in any isolate. Mutations were detected in the *parC* and *gyrA* genes in 14 out of 18 levofloxacin non-susceptible isolates. The predominant mutation type was the combination of Ser79Tyr in *parC* and Ser81Leu mutations in *gyrA*.
Conclusion: GBS is the most common isolated pathogens in vaginal infections in Taiwan, resistance to tetracycline and erythromycin is higher than the rate observed for other regions of the world, while the resistance rate for levofloxacin was relatively lower in Taiwan.

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Introduction

Streptococcus agalactiae, also known as group B streptococcus (GBS), usually resides in the human vagina and/or intestines. GBS is a predominant group of pathogens that causes perinatal infections. It has been proven that a high percentage of neonatal infections due to *S. agalactiae* (especially among newborns) occur through vertical transmission from a GBS-colonized mother to the newborn during labor and birth. Some 5—40% of women carry GBS in their genital tracts, and 10—30% of pregnant women have transient vaginal carriage.[1—3] GBS also causes bacteremia, endocarditis, skin and soft-tissue infections, and osteomyelitis among patients with compromised immune systems.[4—6] Therefore, maternal intrapartum prophylaxis for pregnant women colonized with GBS is recommended. While β-lactam antibiotics are the most commonly used antibiotics for treatment of GBS infection, the macrolide antibiotics are selected alternatives for patients with allergies to β-lactam agents or with less severe infections. Notably, GBS resistance to the macrolides and other antibiotics has increased gradually during the past several years.[7,8] In addition, since the first report by Kawamura et al[9] in 2003 on quinolone-resistant GBS, these GBS isolates have also been identified in the United States, Spain, Brazil, and several other countries.[10—12]

Two principal mechanisms are involved in the resistance of GBS to erythromycin. One mechanism involves the methylation of the target gene, which is regulated by the *erm* gene, whereas the other is the efflux pump mediated by the *mef* gene.[5] Mutations of the *gyrA* and *parC* genes, which are located in the quinolone resistance-determining regions (QRDRs), are the main mechanisms for resistance to quinolones.[6,9,12—14] Nonetheless, there are only a few studies on the occurrence of GBS in pregnant women in Taiwan as well as on the mechanisms of GBS resistance to quinolones and macrolides in Taiwan. Therefore, we surveyed the pathogens isolated from pregnant women in Taiwan during a 1.5-year period to investigate the prevalence of GBS and antibiotic susceptibility and resistance of the isolated GBS strains. Moreover, for the erythromycin- and quinolone-resistant GBS strains, we investigated the resistance mechanisms and associated genes in comparison with isolates from China.

Materials and methods

Sources of bacteria

Bacteria were isolated from vaginal swabs from 1088 patients during the period between January 1, 2011 and May 31, 2012 at the Kuo General Hospital in Tainan, Taiwan. The patients were 519 asymptomatic pregnant women with gestation period of 35—37 weeks and 569 nonpregnant but symptomatic women outpatients with vaginitis who were treated with drugs recommended by a clinician. Vaginal secretion was collected using the BBL CultureSwab Plus Collection and Transport Systems (Becton, Dickinson and Company, Sparks, MD, USA) following the manufacture's instruction. All isolates were identified using the Phoenix 100 fully automated bacterial identification and susceptibility testing system (Becton, Dickinson and Company). A total of 188 isolates were identified as *S. agalactiae*. The identities of these isolates were further confirmed by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS; Bruker Biotyper, Bremen, German) analysis. The MALDI-TOF MS analysis produced significantly quick and reliable results and the species-level identification was 100%.[15,16] *Escherichia coli* DH5α was used as a standard strain for the MALDI-TOF MS analysis.

To reveal and compare the resistance profiles and possible mechanisms of resistance from among those isolated at the Kuo General Hospital, 30 GBS isolates, including four erythromycin-susceptible isolates as controls and 26 erythromycin-nonsusceptible isolates, which had various kinds of minimum inhibitory concentration (MIC) levels (among them, 9 isolates were quinolone nonsusceptible), were selected for gene analysis. In addition, another 30 GBS isolates from the posterior vaginal fornix secretions of pregnant or healthy nonpregnant women, amniotic fluid of women in delivery, which had resistance profiles similar to those from the Kuo General Hospital were provided by the Second Affiliated Hospital of Zhejiang University, Hangzhou, China. All participants gave prior informed written consent to participate in the study.

Drug-susceptibility testing

Antibiotic susceptibility was determined using the Phoenix 100 system (Becton, Dickinson and Company) by the MIC method, and susceptibility judgment was based on the criteria set by the Clinical and Laboratory Standards Institute.[17]

Determination of efflux pump effects

An efflux mechanism was believed to be present when the MIC of an agent in the presence of reserpine was at least fourfold less (2 doubling dilutions) than the MIC in the absence of reserpine.[18] Efflux pump experiments were conducted with the modified broth dilution method of Bast et al[18] for the isolates that showed quinolone or erythromycin resistance but no mutations in QRDR (Isolates 1113, 1319, 1679, 2272) or in the *ermA, ermB,* and *mefA* genes (Isolates S8, S9, S11 from Zhejiang and Isolates 1113, 1319, 2272 from Taiwan). In brief, 100 μL of GBS strains at 1.5×10^7 colony forming units/mL were inoculated in 2 mL of broth (with or without 10 mg/mL reserpine) containing different levels of levofloxacin. After incubation, the MICs of the strains were determined for levofloxacin or erythromycin resistance in the absence and presence of reserpine.

Polymerase chain reaction analysis

For the 24 isolates (9 nonsusceptible and 3 susceptible to levofloxacin from Taiwan and China, respectively) among the 60 GBS isolates used for comparison, polymerase chain reaction (PCR) of the relevant genes was conducted to detect mutations in *parC, parE, gyrA,* and *gyrB* for quinolone resistance. In this study, the *gyrA* and *parC* genes were