Journal of Microbiology, Immunology and Infection (2015) 48, 510—516

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

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

Wen-Tsung Lee [a,b], **Mei-Chin Lai** [b,*]

[a] *Department of Laboratory Medicine, Kuo General Hospital, Tainan, Taiwan, ROC*
[b] *Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan, ROC*

Received 9 September 2013; received in revised form 18 January 2014; accepted 11 March 2014
Available online 22 April 2014

KEYWORDS
drug resistance;
ermA gene;
erythromycin;
levofloxacin;
macrolide;
mef gene;
quinolone;
vaginal infection

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.

* Corresponding author. Department of Life Sciences, National Chung Hsing University, Number 250, Kuo-Kuang Road, Taichung 40227, Taiwan, ROC.
E-mail address: mclai@dragon.nchu.edu.tw (M.-C. Lai).

http://dx.doi.org/10.1016/j.jmii.2014.03.002

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

amplified using the primers and conditions as described by Murayama et al,[6] whereas amplification of the *gyrB* and *parE* genes was conducted with modified primers based on the method suggested by Wehbeh et al.[12] For the 52 GBS isolates resistant to erythromycin, the *ermA*, *ermB*, and *mefA* genes were amplified using primers and conditions previously described.[19,20] PCR amplification was performed using a TPersonal PCR cycler (Biometra, Göttingen, Germany).

DNA sequencing and analysis

The PCR products of the resistance genes were sequenced using an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA), and the resulting sequences were compared with the relevant sequences of *S. agalactiae* GD201008-001 (accession number: CP003810.1) using DNAMAN software (Lynnon, Quebec, Canada).

Results

Isolation rate of GBS

Bacteria were isolated from 388 of the 1088 patients. Hemolytic reaction and biochemical identification indicated that 190 isolates were *S. agalactiae*. Among them, 114 isolates were from pregnant women and the remaining 76 were from nonpregnant women. Further confirmation testing using MALDI-TOF MS analysis showed that two of them (1 isolate each from the pregnant and nonpregnant groups) were actually *Enterococcus faecalis*. The isolation rate of GBS from pregnant women was 113/519 (21.8%) and that from nonpregnant women was 75/569 (13.2%). There was a significant difference between the two groups in GBS isolation rate by Chi-square test ($p < 0.01$). Overall, 188 (48.4%) isolates were identified to be GBS, forming the majority, followed by *Candida albicans* (95; 24.4%), *E. coli* (58; 15.0%), *Klebsiella pneumoniae* (9; 2.4%), and *E. faecalis* (7; 1.8%); in addition, another 31 species (8.0%) were also identified in this study.

Drug-sensitivity testing

We conducted antibiotic susceptibility testing on all 188 GBS isolates by determining the MICs of nine commonly used antibiotics including β-lactam, macrolide, quinolone agents, and vancomycin. As shown in Table 1, the GBS strain showed the highest resistance to tetracycline (TE) with a nonsusceptibility rate close to 87%, followed by erythromycin and clindamycin (CC), which had nonsusceptibility rates of 50% and 41%, respectively, whereas only 4.8% of the 188 tested were nonsusceptible to levofloxacin. By contrast, the GBS isolates were highly susceptible to cefepime, chloramphenicol, meropenem, penicillin, and vancomycin. The vast majority of isolates ($n = 65$, 34.6%) were identified as TE nonsusceptible but susceptible to all other antibiotics; the second type was nonsusceptible to TE, erythromycin, and CC but susceptible to all other antibiotics ($n = 48$; 25.5%). However, 9.6% ($n = 18$) of isolated strains were susceptible to all antibiotics. There was no significant difference between the pregnant and nonpregnant groups in GBS antibiotic susceptibility, except for erythromycin (Table 2), as indicated by Chi-square test ($p < 0.01$).

Drug-sensitivity testing of quinolone- and macrolide-resistant GBS

To determine the resistance mechanisms and genes involved in quinolone and erythromycin resistance, we selected 30 isolates from the Kuo General Hospital, including nine levofloxacin-nonsusceptible isolates, which were also nonsusceptible to erythromycin. We also collected 30 GBS isolates from the Women's Healthcare Hospital (Jiaxing, China) and the Second Affiliated Hospital of Zhejiang University with similar resistance profiles. Further drug-susceptibility evaluations were conducted for nine clinically common antibiotics. The results are shown in Table 3, which indicated that most of the isolates were highly susceptible to β-lactams. The susceptibility rates for the GBS isolates from both Taiwan and Zhejiang to penicillin were 100%, whereas the susceptibility rates to cefepime were 86.7% and 93.3%, respectively. Both the isolates from Taiwan and Zhejiang showed susceptibility rates of 13.3% and 70% to erythromycin and levofloxacin, respectively. Statistical analysis conducted using the Fisher's exact probability method indicated that there was no significant difference between the samples from the two regions in terms of the resistance profile.

Table 1 Susceptibility of the 188 *Streptococcus agalactiae* isolates to nine common antibiotics

Antibiotic	Isolates tested	Resistance (%)	Intermediate (%)	Susceptible (%)
Cefepime	188	10 (5.3)	—[a]	178 (94.7)
Chloramphenicol	188	13 (6.9)	11 (5.9)	164 (87.2)
Clindamycin	188	74 (39.4)	3 (1.6)	111 (59.0)
Erythromycin	188	92 (48.9)	2 (1.1)	94 (50.0)
Levofloxacin	188	7 (3.7)	2 (1.1)	179 (95.2)
Meropenem	188	2 (1.1)	—[a]	186 (98.9)
Penicillin	188	0 (0.0)	—[a]	188 (100.0)
Tetracycline	188	162 (86.2)	1 (0.5)	25 (13.3)
Vancomycin	188	0 (0.0)	—[a]	188 (100.0)

[a] CLSI without an intermediate range.
CLSI = Clinical and Laboratory Standards Institute.

Table 2 Analysis of GBS susceptibility of 188 isolates from the pregnant and nonpregnant groups of Taiwan

Antibiotic	Pregnant women ($n = 113$)		Nonpregnant ($n = 75$)		p
	S[a]	Nonsusceptible[b]	S[a]	Nonsusceptible[b]	
Cefepime	105	8	73	2	0.187
Chloramphenicol	98	15	66	9	0.798
Clindamycin	73	40	38	37	0.057
Erythromycin	64	49	30	45	0.025*
Levofloxacin	109	4	70	5	0.325
Meropenem	112	1	74	1	0.769
Penicillin	113	0	75	0	—
Tetracycline	17	96	8	67	0.387
Vancomycin	113	0	75	0	—

[a] Susceptible.
[b] Combined intermediate and resistant.
* $p < 0.05$, Chi-square test.
GBS = group B streptococcus.

Detection and analysis of quinolone-resistance genes

No mutations were detected in the QRDRs for the *parE* and *gyrB* genes. The results of the mutation analysis for the *parC* and *gyrA* genes and levofloxacin resistance of the 24 GBS isolates tested (15 resistant, 3 intermediate, and 6 susceptible) are summarized in Table 4. Sequences were deposited in the GenBank database and accession numbers for *gyrA* and *parC* were KF285457—KF285459. Among the 15 levofloxacin-resistant and three intermediate isolates, one intermediate and 13 resistant isolates were detected to have mutations at either *parC* or *gyrA*. The majority of the isolates with mutations (10 of 14) displayed the same types of mutations, that is, Ser79Tyr and Ser81Leu. There were two isolates that had Ser79Phe and Ser81Leu mutations. In addition, there were two isolates that had an Ser79Tyr mutation in the *parC* gene or an Ser81Leu mutation in the *gyrA* gene. By contrast, no mutations were detected in the six levofloxacin-susceptible isolates. However, no mutations were detected from the four isolates (Isolates 1113, 1319, 1679, and 2272), which were phenotypically demonstrated to be either resistant or intermediate to levofloxacin.

Active efflux pump analysis

For the isolates that had quinolone nonsusceptibility but with no mutations on the QRDRs, the MICs of levofloxacin in the presence and absence of reserpine were determined. Similar experiments were also conducted for the six erythromycin-resistant isolates from which the *ermA*, *ermB*, and *mefA* genes were not identified. However, reserpine did not provide greater than a fourfold decrease in MIC for the tested isolates, indicating that the active pump mechanism was not functioning (data not shown).

Detection and analysis of erythromycin-resistant genes

From the 26 erythromycin-resistant GBS from Zhejiang, the *ermB* gene was detected in six (23.1%) isolates, whereas 14 of the 26 (53.8%) erythromycin-resistant GBS from Taiwan had the *ermB* gene (Table 5). Neither the *mefA* nor *ermA* gene was detected in any of these investigated isolates. Because the 26 erythromycin-resistant GBS from Taiwan did not have either *mefA* or *ermA*, we further examined all

Table 3 Analysis of GBS susceptibility of 30 isolates from Taiwan with 30 matched cases from Zhejiang, China to nine clinically common antibiotics

Antibiotics	Taiwan		Zhejiang		p	χ^2 value
	Susceptible (%)	Nonsusceptible (%)	Susceptible (%)	Nonsusceptible (%)		
Cefepime	26 (86.7)	4 (13.3)	28 (93.3)	2 (6.7)	0.671	—[a]
Chloramphenicol	21 (70.0)	9 (30.0)	24 (80.0)	6 (20.0)	0.371	0.800
Clindamycin	11 (36.7)	19 (63.3)	8 (26.7)	22 (73.3)	0.405	0.693
Erythromycin	4 (13.3)	26 (86.7)	4 (13.3)	26 (86.7)	1.000	—[a]
Levofloxacin	21 (70.0)	9 (30.0)	21 (70.0)	9 (30.0)	—[b]	—[b]
Meropenem	28 (93.3)	2 (6.7)	29 (96.7)	1 (3.3)	1.000	—[a]
Penicillin	30 (100)	0 (0.0)	30 (100)	0 (0.0)	—[b]	—[b]
Tetracycline	3 (10.0)	27 (90.0)	5 (16.7)	25 (83.3)	1.000	—[a]
Vancomycin	30 (100)	0 (0.0)	30 (100)	0 (0.0)	—[b]	—[b]

[a] Indicates that the data were analyzed using the Fisher's exact probability analysis, and use only a p but no χ^2.
[b] Indicates that no statistical analysis was conducted.
GBS = group B streptococcus.

Table 4 Analysis of quinolone-resistance-related mutations

Sources	Serial	Code	Levofloxacin resistance	*parC* gene	*gyrA* gene
Taiwan	1	737	S	—	—
Taiwan	2	788	S	—	—
Taiwan	3	934	S	—	—
Taiwan	4	1113	I	—	—
Taiwan	5	1192	R	Ser79Tyr	Ser81Leu
Taiwan	6	1319	R	—	—
Taiwan	7	1492	R	Ser79Tyr	Ser81Leu
Taiwan	8	1679	I	—	—
Taiwan	9	1749	R	Ser79Phe	Ser81Leu
Taiwan	10	1798	R	Ser79Tyr	Ser81Leu
Taiwan	11	2272	R	—	—
Taiwan	12	2280	R	Ser79Tyr	Ser81Leu
Zhejiang	13	S1	S	—	—
Zhejiang	14	S3	R	Ser79Tyr	Ser81Leu
Zhejiang	15	S8	R	Ser79Tyr	Ser81Leu
Zhejiang	16	S9	R	—	Ser81Leu
Zhejiang	17	S11	R	Ser79Tyr	Ser81Leu
Zhejiang	18	S12	S	—	—
Zhejiang	19	S13	S	—	—
Zhejiang	20	S15	R	Ser79Phe	Ser81Leu
Zhejiang	21	S17	I	Ser79Tyr	Ser81Leu
Zhejiang	22	S22	R	Ser79Tyr	—
Zhejiang	23	1	R	Ser79Tyr	Ser81Leu
Zhejiang	24	120411074	R	Ser79Tyr	Ser81Leu

Leu = leucine; Phe = phenylalanine; Ser = serine; Tyr = tyrosine.

Table 5 Comparison the occurrence of *ermB* gene between clindamycin (R/S) and tetracycline (R/S) group among the erythromycin non-susceptible *Streptococcus agalactiae* isolates

	All isolated from Taiwan (n = 94)		Comparison test			
			Taiwan (n = 26)		China (n = 26)	
	ermB (+)	*ermB* (−)	*ermB* (+)	*ermB* (−)	*ermB* (+)	*ermB* (−)
CC(R)/TE(R)	59*	12	12	5	6	12
CC(S)/TE(R)	7*	9	2	5	0	5
CC(R)/TE(S)	3	0	0	0	0	3
CC(S)/TE(S)	0	4	0	2	0	0
Total	69	25	14	12	6	20

* $p < 0.01$, Chi-square test.
CC = clindamycin; R = resistant (included intermediate); S = susceptible; TE = tetracycline.

remaining erythromycin-nonsusceptible GBS (68 strains) for *mefA* and *ermA* and obtained the same results. However, in the 94 erythromycin-nonsusceptible GBS strains obtained in this study, the *ermB* gene was detected in 69 isolates (73.4%), suggesting that the most predominant resistant mechanism may be due to the *ermB* gene product. For antibiotic patterns (1) erythromycin combined with CC and TE-resistant GBS, (2) CC susceptible but TE resistant, (3) CC resistant but TE susceptible, and (4) susceptible to both CC and TE, the *ermB* gene carried rate was 83.1% (59/71), 43.8% (7/16), 100% (3/3), and 0% (0/4), respectively (Table 5). There was a significant difference between the two groups [CC(R)/TE(R) vs. CC(S)/TE(R)] in terms of GBS nonsusceptibility to erythromycin and the *ermB* gene carried rate by Chi-square test ($p < 0.01$; Table 5). Although the rate of erythromycin nonsusceptibility for the GBS strains isolated from nonpregnant women was higher than the pregnant group (45/75 and 49/113, respectively), there was no difference in the occurrence of the *ermB* gene (33/45 and 36/49, respectively; $p = 0.99$).

Discussion

GBS is the most common pathogen that causes infections in perinatal women and neonatal babies. In the United States and Europe, neonatal bacteremia, pneumonia, and meningitis due to GBS infections lead to a mortality rate as high as 4–6%. GBS also causes skin and soft-tissue infection, urinary bladder infection, bacteremia, pneumonia, endocarditis, and osteomyelitis in adults, especially in patients with diabetes and cancer.[4,21] However, little attention has been paid to GBS infections in pregnant women in Taiwan.

We found that GBS accounted for almost half (48.4%) of the colonizing and clinical strains isolated from the vaginal tracts of women. This rate is much higher than that of *C. albicans*, which has been considered the commonest causative pathogen of vaginal infection. Our findings indicate the importance of monitoring GBS, especially in pregnant women.

GBS is reportedly highly resistant to erythromycin, TE, and CC.[7,22–24] We found that the nonsusceptibility rates for erythromycin, TE, and CC among the GBS isolates in Taiwan ranged from 41% to 87%. The resistance rate for TE was as high as 86.2%, which was almost as high as the reported rate (97.3%) in Tunisia,[22] slightly higher than the reported rate of 81.5% in China,[23] and much higher than the reported rate (46.5%) in Japan.[24] The nonsusceptibility rate for erythromycin was approximately 50%, which is close to the reported rate (50.7%) in the USA,[7] but greater than the rate reported in Tunisia (40.0%),[22] and less than the rate in China (71.2%),[23] indicating that the resistance of GBS to the macrolides in Taiwan is as critical as in the other regions of the world. By contrast, penicillin and cefepime showed high sensitivity, which is in accordance with other reports,[20,22,24] suggesting that penicillin is still among the best treatment choices for GBS infections.

PCR amplification and DNA sequencing of the 52 erythromycin-resistant GBS isolates (26 isolates each from Taiwan and China for comparison test) revealed that 20 (38.5%) carried the *ermB* gene (Table 5), and the sequence was highly similar to that of GenBank Accession number EF422360.1, which was isolated from cattle in Taiwan. Neither *mefA* nor *ermA* was detected from the isolates. This finding is in line with the study by Betriu et al,[25] in showing that *ermB* is the main mechanism of erythromycin resistance in GBS. However, none of these three genes were detected in 20 of the 26 isolates from Zhejiang nor in 12 of the 26 isolates from Taiwan that were resistant to

erythromycin. Remarkably, all the 94 erythromycin-resistant isolates we collected in this study at the Kuo General Hospital showed the *ermB* gene carried rate of 73.4%, which is higher than the rate mentioned based on a previous comparison test with 26 isolates (53.8%). This may be because the comparison test selects more erythromycin-resistant but CC-susceptible strains (9/26 vs. 20/94).

In this study, 73.4% of the isolates carried the *ermB* gene of the total GBS erythromycin-nonsusceptible strains, and the rate is significantly higher than that reported in USA and Spain (13.0% and 58.1%, respectively)[4,5]; however, our rate is close to that reported in Japan (87%) by Murayama et al.[6] In addition, the *ermA*, *ermB,* or *mefA* genes were not detected in 26.6% of the isolates. Dogan et al[4] reported the types of GBS that were unable to detect any of the erythromycin-resistant determinants examined (*ermA*, *ermB,* and *mefA*), and were highly resistant to erythromycin but susceptible to CC. As indicated in Table 5, in our comparison test, five isolates from Taiwan and China each showed results similar to that reported by Dogan et al[4]; nevertheless, the *ermB* gene was detected in two strains. In the 94 erythromycin-nonsusceptible GBS isolates surveyed for *ermA*, *ermB,* and *mefA*, 69 had the *ermB* gene but not *ermA* and *mefA*. Among them, 16 isolates were erythromycin resistant but susceptible to CC and TE, seven carried the *ermB* gene, and nine did not (Table 5). Although not currently reported in *S. agalactiae*, single-nucleotide changes in 23S ribosomal ribonucleic acid or in ribosomal proteins conferring macrolide resistance have been reported for many bacteria, including *Streptococcus pneumoniae*.[26,27] It is thus possible that a ribosomal mutation is responsible for the resistance in this specific isolate. This result implies that there might be additional mechanisms that are involved with erythromycin resistance, which merits further investigation.

Only 4.8% of the total GBS isolates were levofloxacin nonsusceptible. This rate is significantly lower than that reported in China (37.7% resistance),[23] and is even lower than that reported in Japan (18.4% resistance), as reported by Ueno et al.[24] Quinolones are not normally used in China for treating GBS infections in pregnant women and newborns. However, because quinolones are widely used in China for both clinical and agricultural purposes as well as in animal feedings, continuous and wide use of quinolones could have elevated the resistance of various pathogens including GBS to the quinolones. Research[28] indicated that there is a high rate of bacteria in the environment that carries quinolone-resistant genes, which might be relevant to the high resistance to quinolones. In addition to plasmid-mediated quinolone-resistant genes, there are other mechanisms such as the mutations in Subunits A and B of the DNA topoisomerases (*gyrA*, *gyrB*, *parC*, and *parE*) that are also involved in the high resistance against quinolones. No mutations were detected in the QRDRs of the *gyrB* and *parE* genes, which is similar to reports of other studies indicating that mutations for quinolone resistance among the GBS isolates are mainly due to the mutations of the *parC* and *gyrA* genes. The main mutation pattern observed was Ser79Phe and Ser79Tyr in the *parC* gene and Ser81Leu in the *gyrA* gene.[6,9,12] There are a few reports in Taiwan and China for such data. In our current study, we detected mutations from 14 of the 18 levofloxacin-nonsusceptible isolates; the mutation patterns of Ser79Tyr in *parC* and Ser81Leu in *gyrA* were the most frequent, followed by Ser79Phe in *parC* and Ser81Leu in *gyrA*.

Although the resistance pattern of the 30 selected quinolone- and/or macrolide-resistant GBS from Taiwan had highly similar resistant profiles to those from China, the gene analysis indicated that the *ermB*-positive rate (53.8%, 14/26) for GBS from Taiwan was much higher than that of the isolates from China (23.1, 6/26). Among the 18 levofloxacin-nonsusceptible GBS isolates, nine were from Taiwan and nine were from China. Although the mutation patterns in the *parC* and *gyrA* genes from both Taiwan and China were dominated by the Ser79Tyr and Ser81Leu patterns, other mutations were also detected in the isolates from China: six isolates had the predominant Ser79Tyr and Ser81Leu mutations, one had Ser79Phe and Ser81Leu mutations, one had only the Ser79Tyr mutation, and one had only the Ser81Leu mutation. By contrast, only four of nine isolates in Taiwan had the Ser79Tyr and Ser81Leu mutations and one had the Ser79Phe and Ser81Leu mutations. No mutations were detected in the *parC* and *gyrA* genes from the four nonsusceptible isolates in Taiwan, which is similar to the results of Ki et al,[14] although these test strains were intermediate resistant to levofloxacin (Table 4). Detection of the active efflux pump using reserpine as the inhibitor was unable to identify any isolates that demonstrated a greater than fourfold decrease in the MIC, indicating that the reserpine inhibiting efflux pump is not functioning in those isolates. Other mechanisms, including plasmid-encoded quinolone resistance, have been reported for Gram-negative organisms,[29] but their association with quinolone resistance in Gram-positive organisms has not been demonstrated.[30] Mutations outside the QRDRs may also contribute to the expression of resistance in streptococci. In a laboratory-generated quinolone-resistant strain, mutations in the promoter region of the *parE* gene(s) decreased transcript levels of *parC* and increased ciprofloxacin resistance even when no mutations were present in the QRDR.[31]

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

We are grateful to Dr Zhu Jian Jun from the Women's Healthcare Hospital (Jiaxin, Zhejiang) and Dr Rong Zhang from the Second Affiliated Hospital of Zhejiang University for providing raw data and conducting the analysis of the GBS genes from their respective hospitals. The study was approved by the Institutional Review Board of the Kuo General Hospital in Tainan, Taiwan (KGH IRB No. 100-O-23/HR-11-K010).

References

1. Regan JA, Klebanoff MA, Nugent RP. The epidemiology of group B streptococcal colonization in pregnancy. Vaginal Infections

and Prematurity Study Group. *Obstet Gynecol* 1991;**77**:604—10.
2. Yancey MK, Schuchat A, Brown LK, Ventura VL, Markenson GR. The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery. *Obstet Gynecol* 1996;**88**:811—5.
3. Campbell JR, Hillier SL, Krohn MA, Ferrieri P, Zaleznik DF, Baker CJ. Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. *Obstet Gynecol* 2000;**96**:498—503.
4. Dogan B, Schukken YH, Santisteban C, Boor KJ. Distribution of serotypes and antimicrobial resistance genes among *Streptococcus agalactiae* isolates from bovine and human hosts. *J Clin Microbiol* 2005;**43**:5899—906.
5. Marimón JM, Valiente A, Ercibengoa M, García-Arenzana JM, Pérez-Trallero E. Erythromycin resistance and genetic elements carrying macrolide efflux genes in *Streptococcus agalactiae*. *Antimicrob Agents Chemother* 2005;**49**:5069—74.
6. Murayama SY, Seki C, Sakata H, Sunaoshi K, Nakayama E, Iwata S, et al. Capsular type and antibiotic resistance in *Streptococcus agalactiae* isolates from patients, ranging from newborns to the elderly, with invasive infections. *Antimicrob Agents Chemother* 2009;**53**:2650—3.
7. Back EE, O'Grady EJ, Back JD. High rates of perinatal group B *Streptococcus clindamycin* and erythromycin resistance in an upstate New York hospital. *Antimicrob Agents Chemother* 2012;**56**:739—42.
8. Chohan L, Hollier LM, Bishop K, Kilpatrick CC. Patterns of antibiotic resistance among group B streptococcus isolates: 2001—2004. *Infect Dis Obstet Gynecol* 2006;**2006**:57492.
9. Kawamura Y, Fujiwara H, Mishima N, Tanaka Y, Tanimoto A, Ikawa S, et al. First *Streptococcus agalactiae* isolates highly resistant to quinolones, with point mutations in *gyrA* and *parC*. *Antimicrob Agents Chemother* 2003;**47**:3605—9.
10. Barros RR, Kegele FC, Paula GR, Brito MA, Duarte RS. Molecular characterization of the first fluoroquinolone resistant strains of *Streptococcus agalactiae* isolated in Brazil. *Braz J Infect Dis* 2012;**16**:476—8.
11. Miró E, Rebollo M, Rivera A, Alvarez MT, Navarro F, Mirelis B, et al. *Streptococcus agalactiae* highly resistant to fluoroquinolones. *Enferm Infecc Microbiol Clin* 2006;**24**:562—3 [Article in Spanish].
12. Wehbeh W, Rojas-Diaz R, Li X, Mariano N, Grenner L, Segal-Maurer S, et al. Fluoroquinolone-resistant *Streptococcus agalactiae*: epidemiology and mechanism of resistance. *Antimicrob Agents Chemother* 2005;**49**:2495—7.
13. Nagano N, Nagano Y, Toyama M, Kimura K, Tamura T, Shibayama K, et al. Nosocomial spread of multidrug-resistant group B streptococci with reduced penicillin susceptibility belonging to clonal complex 1. *J Antimicrob Chemother* 2012;**67**:849—56.
14. Ki M, Srinivasan U, Oh KY, Kim MY, Shin JH, Hong HL, et al. Emerging fluoroquinolone resistance in *Streptococcus agalactiae* in South Korea. *Eur J Clin Microbiol Infect Dis* 2012;**31**:3199—205.
15. Cherkaoui A, Emonet S, Fernandez J, Schorderet D, Schrenzel J. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of beta-hemolytic streptococci. *J Clin Microbiol* 2011;**49**:3004—5.
16. Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Böttger EC, Hombach M. Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. *J Clin Microbiol* 2013;**51**:1834—40.
17. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing, Document M100-S22*. Wayne, PA: CLSI; 2012.
18. Bast DJ, Low DE, Duncan CL, Kilburn L, Mandell LA, Davidson RJ, et al. Fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*: contributions of type II topoisomerase mutations and efflux to levels of resistance. *Antimicrob Agents Chemother* 2000;**44**:3049—54.
19. Baker CJ. Group B streptococcal infections. In: Stevens DL, Kaplan EL, editors. *Streptococcal infections. Clinical aspects, microbiology, and molecular pathogenesis*. Oxford, UK: Oxford University Press; 2000. pp. 222—37.
20. Lopardo HA, Vidal P, Jeric P, Centron D, Paganini H, Facklam RR, et al. Six-month multicenter study on invasive infections due to group B streptococci in Argentina. *J Clin Microbiol* 2003;**41**:4688—94.
21. Corvec S, Illiaquer M, Touchais S, Boutoille D, van der Mee-Marquet N, Quentin R, et al. Clinical features of group B streptococcus prosthetic joint infections and molecular characterization of isolates. *J Clin Microbiol* 2011;**49**:380—2.
22. Hraoui M, Boutiba-Ben Boubaker I, Rachdi M, Slim A, Ben Redjeb S. Macrolide and tetracycline resistance in clinical strains of *Streptococcus agalactiae* isolated in Tunisia. *J Med Microbiol* 2012;**61**:1109—13.
23. Wang H, Zhao C, He W, Zhang F, Zhang L, Cao B, et al. High prevalence of fluoroquinolone-resistant group B streptococci among clinical isolates in China and predominance of sequence type 19 with serotype III. *Antimicrob Agents Chemother* 2013;**57**:1538—41.
24. Ueno H, Yamamoto Y, Yamamichi A, Kikuchi K, Kobori S, Miyazaki M. Characterization of group B streptococcus isolated from women in Saitama city, Japan. *Jpn J Infect Dis* 2012;**65**:516—21.
25. Betriu C, Culebras E, Rodríguez-Avial I, Gómez M, Sánchez BA, Picazo JJ. *In vitro* activities of tigecycline against erythromycin-resistant *Streptococcus pyogenes* and *Streptococcus agalactiae*: mechanisms of macrolide and tetracycline resistance. *Antimicrob Agents Chemother* 2004;**48**:323—5.
26. Tait-Kamradt A, Davies T, Cronan M, Jacobs MR, Appelbaum PC, Sutcliffe J. Mutations in 23S rRNA and ribosomal protein L4 account for resistance in pneumococcal strains selected *in vitro* by macrolide passage. *Antimicrob Agents Chemother* 2000;**44**:2118—25.
27. Vester B, Douthwaite S. Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob Agents Chemother* 2001;**45**:1—12.
28. Zhang R, Ichijo T, Huang YL, Cai JC, Zhou HW, Yamaguchi N, et al. High prevalence of qnr and aac(6′)-Ib-cr genes in both water-borne environmental bacteria and clinical isolates of *Citrobacter freundii* in China. *Microbes Environ* 2012;**27**:158—63.
29. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. *Antimicrob Agents Chemother* 2005;**49**:118—25.
30. Rodríguez-Martínez JM, Velasco C, Briales A, García I, Conejo MC, Pascual A. Qnr-like pentapeptide repeat proteins in Gram-positive bacteria. *J Antimicrob Chemother* 2008;**61**:1240—3.
31. Ince D, Hooper DC. Quinolone resistance due to reduced target enzyme expression. *J Bacteriol* 2003;**185**:6883—92.