Serotypes and antibiotic resistance in Group B streptococcus isolated from patients at the Maternity Hospital, Kuwait

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A total of 143 group B streptococcus (GBS) isolates collected from mothers at the Maternity Hospital in Kuwait were investigated for their serotypes and antibiotic resistance, and screened by PCR for the carriage of genes for resistance to tetracycline (*tetK*, *tetM*, *tetL*, *tetO*), erythromycin (*ermA*, *ermB*, *ermC*, *ermTR*, *ermM*, *mefA*, *mefE*, *msrA*) and aminoglycosides (*aph3*, *ant4*, *ant6*). All isolates were serotyped using a latex agglutination test. Most of the isolates belonged to serotypes V (38.5 %), III (20.9 %), Ia (7.7 %) and II (11.2 %). Sixteen isolates (11.2 %) were nontypable. All isolates were susceptible to penicillin, ampicillin and cefotaxime (MICs 0.016–0.094 µg ml⁻¹) but were resistant to trimethoprim (92.3 %), tetracycline (89.5 %), minocycline (89.5 %), high-level kanamycin (76.9 %), chloramphenicol (30.0 %), erythromycin (12.6 %), clindamycin (7.0 %), high-level streptomycin (3.5 %) and ciprofloxacin (0.7 %). The tetracycline-resistant isolates contained *tetM* (94.5 %), *tetO* (3.9 %), *tetL* (1.6 %) and *tetK* (0.8 %). The erythromycin-resistant isolates contained *ermB* (61.1 %), *ermTR* (38.9 %), *ermA* (5.5 %), *mefA* (5.5 %) and *mefE* (11 %). All high-level kanamycin-resistant isolates contained *aph3*. One of the high-level streptomycin-resistant isolates contained *ant6*. Partial DNA sequencing of *aph3* revealed sequences with 99 % similarity to *aph3* found in *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, suggesting that the GBS isolates could have acquired *aph3* from other Gram-positive cocci. The high proportion of isolates with resistance to tetracycline, high-level kanamycin and trimethoprim, and the increase in the prevalence of erythromycin resistance, represents an emerging public health concern that needs further surveillance.

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INTRODUCTION

Group B streptococcus (GBS), also known as *Streptococcus agalactiae*, is a leading cause of serious infections in neonates and immunocompromised adults (Truog *et al.*, 1976; Baker, 1979; High *et al.*, 2005).

GBS are encapsulated and have been classified into ten serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII and IX) on the basis of the capsular polysaccharide components of their cell wall (Victor & Rubens, 2000; Slotved *et al.*, 2007). The capsular polysaccharide, as a virulence factor, assists GBS survival *in vivo* by avoiding recognition by the host defence system (Rubens *et al.*, 1987). There are major differences between countries in the distribution of GBS serotypes. In the USA, Germany and Sweden, the most common serotypes isolated from infants and colonized mothers have been types Ia, III, and V (Persson *et al.*, 2004; Brimil *et al.*, 2006; Harrison *et al.*, 1998). Serotype V strains emerged in the 1990s as a frequent cause of neonatal sepsis and adult diseases in the USA. On the other hand,

serotypes VI and VIII represented 35.6 % and 24.7 %, respectively, of GBS isolated from pregnant women in Japan (Lachenauer *et al.*, 1999).

Penicillin G is the drug of choice for the intrapartum prophylaxis of GBS infections (Figueira-Coelho *et al.*, 2004). As GBS isolates remain uniformly susceptible to penicillin, its prophylactic use has been instrumental in significantly reducing the incidence of early-onset diseases in neonates (Pylipow *et al.*, 1994). However, GBS isolates with reduced susceptibility to penicillin have recently been reported (Chu *et al.*, 2007; Dahesh *et al.*, 2008; Kimura *et al.*, 2008; Nagano *et al.*, 2008).

Erythromycin and clindamycin are used as alternative treatments for patients who are allergic to penicillin. However, erythromycin and clindamycin resistance have emerged in several countries (Aracil *et al.*, 2002; Betriu *et al.*, 2003; de Azavedo *et al.*, 2001; De Mouy *et al.*, 2001). Erythromycin resistance in GBS isolates is due to target-site modification by rRNA methylase, encoded by the *erm* gene (Roberts *et al.*, 1999), and drug efflux encoded by the *mef* gene (macrolide efflux), which gives rise to the M

Abbreviation: GBS, group B streptococcus.

phenotype. Similarly, two tetracycline-resistance mechanisms, ribosomal protection and drug efflux, have also been observed in GBS. Ribosomal protection is encoded by *tetM* or *tetO* whereas the efflux pump is encoded by *tetK* or *tetL* (Nishimoto *et al.*, 2005).

High-level aminoglycoside resistance in GBS is mostly due to enzymic inactivation of the antibiotic by *N*-acetyltransferases, *O*-adenyltransferases and *O*-phosphotransferases, encoded by *aac*, *ant* and *aph*, respectively (Zeng *et al.*, 2006; Poyart *et al.*, 2003).

GBS is isolated regularly from mothers and babies at the Maternity Hospital in Kuwait, and an earlier study revealed that 14.6 % of pregnant women were colonized with GBS (Al-Sweih *et al.*, 2005a). A study conducted in 2003 documented the susceptibility of GBS isolates to β -lactams and other antibiotics (Al-Sweih *et al.*, 2005b). However, no data on the genetic mechanisms of antibiotic resistance were included. In the present study, we characterized GBS isolates obtained from mothers at the Maternity Hospital in Kuwait for their serotype distribution, susceptibility to antimicrobial agents and carriage of resistance genes.

METHODS

Setting. The Maternity Hospital in Kuwait is a 500 bed tertiary hospital with departments of Obstetrics and Gynaecology, Radiology, Neonatology, ICU, Genetics, Infection Control and Laboratory Medicine. It handles about 12 000 deliveries annually, which constitute 30 % of all deliveries in the state of Kuwait (Al-Sweih *et al.*, 2005a). Transmission of GBS from mother to baby has been reported at 35.5 %. However, neonatal sepsis caused by GBS is low, at 0.9 per 1000 live births (Al-Sweih *et al.*, 2005a).

GBS isolates. A total of 143 GBS isolates were obtained from 149 mothers and five neonates at the Maternity Hospital in Kuwait between 1 July 2007 and 31 October 2007. They were collected from 95 high vaginal swabs (66.4 %), 45 urine samples (31.5 %) and three low vaginal swabs (2.1 %). Isolation of GBS isolates was performed at the clinical microbiology laboratory at the Maternity Hospital using standard methods (Al-Sweih *et al.*, 2005a). Swabs were inoculated into tubes of selective Todd–Hewitt broth (Difco) and onto sheep blood agar plates (Oxoid). Both broth and agar media were incubated at 35 °C in 5 % CO₂ for 18 h. The broth culture was then subcultured onto non-selective 5 % sheep blood agar plates. Urine samples were plated directly onto selective streptococcal agar plates (Oxoid). Isolates were identified based on cultural characteristics, Gram stain, catalase test and CAMP test, and confirmed by a commercial latex agglutination test (Streptex kit, bioMérieux) and the Vitek 2 system (bioMérieux).

Serotyping. Serotyping was performed using a monospecific rabbit antiserum kit (Denka Seiken) according to the protocol provided by the manufacturer.

Antibiotic susceptibility testing. Susceptibility to ampicillin, cefazolin, cefotaxime, high-level gentamicin, linezolid, meropenem, penicillin, rifampicin, teicoplanin, vancomycin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, tetracycline and trimethoprim was determined by the disc diffusion method. The MICs of penicillin, ampicillin, erythromycin, clindamycin, cefotaxime, linezolid, vancomycin and ciprofloxacin were determined using the Etest

(AB Biodisk) according to the manufacturer's instructions. The agar dilution method was used to determine the MICs for tetracycline, minocycline, high-level gentamicin, high-level kanamycin and high-level streptomycin. The results were classified as susceptible, intermediate or resistant as recommended by the Clinical Laboratory Standards Institute (CLSI, 2005). The D-test was used to determine inducible clindamycin resistance (De Azavedo *et al.*, 2001).

DNA extraction for PCR. For DNA isolation, five single colonies of an overnight culture grown on 5 % sheep blood agar plates and incubated at 35 °C in CO₂ were suspended in 1 ml digestion buffer (1 M Tris, 45 % Sarkosyl) in 1.5 ml Eppendorf tubes. The tubes were heated at 95 °C in a heating block for 10 min and transferred to an ice bath for 2 min, then centrifuged at 16 000 *g* for 2 min to pellet the cell debris. The supernatant containing DNA was transferred to a fresh Eppendorf tube and stored at –20 °C until required for PCR.

Detection of antibiotic resistance determinants. Genes for resistance to kanamycin, streptomycin, erythromycin and tetracycline were detected by PCR. All amplifications were performed in an Eppendorf Mastercycler gradient thermocycler. The amplification of genes for aminoglycoside-modifying enzymes, *aph3*, *ant4* and *ant6*, were performed using primers and conditions published previously (Udo *et al.*, 2004). The amplification of tetracycline-resistance determinants, *tetM*, *tetO*, *tetK* and *tetL*, employed primers and conditions published by Nishimoto *et al.* (2005). The erythromycin-resistant isolates were investigated for the presence of *ermA*, *ermB*, *ermC*, *ermM*, *mefA*, *mefE* and *msrA* using the primers and conditions described by Lopardo *et al.* (2003). Amplification of the *ermTR* gene was performed as described by Marimón *et al.* (2005). PCR products were analysed by agarose gel electrophoresis using 2 % (w/v) agarose gels.

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNA of GBS isolates was prepared in agarose plugs as previously described (Benson & Ferrieri, 2001) and digested with 40 U *Sma*I (New England Biolabs) for 4 h at 25 °C. The fragments were separated by PFGE in 1.2 % pulsed-field-grade agarose (Bio-Rad) in the CHEF-DRIII system (Bio-Rad) with pulse times of 10–45 s for 20 h at 12 °C at 6 V cm^{–1}. PFGE patterns were interpreted according to the criteria of Tenover *et al.* (1995).

Partial DNA sequencing of *aph3*. The amplified products for *aph3* were purified using a PCR purification kit (Qiagen) following instructions provided by the manufacturer, and sequenced using the Big Dye terminator method in an automated ABI PRISM 377 DNA sequencer (Applied Biosystems). The resultant nucleotide sequences were compared with *aph3* from other bacterial species using the BLAST software available at the National Center for Biotechnology Information website <http://www.ncbi.nlm.nih.gov/blast>.

Statistics. Differences in the distribution of erythromycin resistance between groups were assessed by chi-squared test. A *P*-value of ≤ 0.05 was considered significant.

RESULTS

Distribution of serotypes

Fifty-five (38.5 %) of the 143 GBS isolates belonged to serotype V, which was the most prevalent serotype, followed by serotypes III (30 isolates, 20.9 %), Ia (11 isolates, 7.7 %) and II (16 isolates, 11.2 %). Five isolates (3.5 %) belonged to serotype Ib, and five (3.5 %) to serotype IV. Serotypes VI (2.8 %) and VII (0.6 %) were less common. Sixteen isolates

(11.2 %) did not react with any of the nine GBS antisera after repeated testing and were considered nontypable.

Antimicrobial susceptibility of GBS isolates

All of the isolates were susceptible to ampicillin, cefazolin, cefotaxime, high-level gentamicin, linezolid, penicillin, rifampicin, teicoplanin and vancomycin. However, they were resistant to trimethoprim (132, 92.3 %), tetracycline (128, 89.5 %), minocycline (128, 89.5 %), high-level kanamycin (110, 76.9 %), chloramphenicol (43, 30.0 %), erythromycin (18, 12.6 %), clindamycin (10, 7.0 %), high-level streptomycin (5, 3.5 %), and ciprofloxacin (1, 0.7 %).

Table 1 shows the MIC distribution for the β -lactam antibiotics tested in this study. Although all of the GBS isolates were susceptible to the β -lactam antibiotics that were tested, the MICs of penicillin for 119 (83.2 %) isolates were relatively high, 0.047–0.094 $\mu\text{g ml}^{-1}$. Likewise, the MICs of ampicillin and cefotaxime were 0.047–0.094 $\mu\text{g ml}^{-1}$ for 127 (88.8 %) and 122 (85.3 %) of the isolates, respectively.

Detection of antibiotic-resistance genes

The *tetM* gene was detected in 121 (94.5 %) of the 128 tetracycline- and minocycline-resistant isolates. Five isolates (3.9 %) carried *tetO*. Two isolates carried *tetL* and one carried *tetK*. One isolate carried both *tetO* and *tetK* and one isolate carried *tetO* and *tetL*.

Table 2 shows the distribution of *erm* genes among the erythromycin-resistant isolates. None of the isolates was positive for *ermC*, *ermM* or *msrA*.

All of the 110 high-level kanamycin-resistant isolates were positive for *aph3*. None of them was positive for *ant4*, and only one of the five high-level streptomycin-resistant isolates was positive for *ant6*.

DNA sequencing of *aph3*

PFGE analysis of the high-level kanamycin-resistant isolates revealed 12 PFGE patterns, with 74.7 % of them distributed among four PFGE patterns. The *aph3* PCR products of four isolates chosen to represent the four major genetic backgrounds defined by PFGE were sequenced to determine their relatedness. The nucleotide sequences of

the four PCR products were identical to each other, and had 99 % similarity to *aph3* found in genomic DNA of *Enterococcus faecium* and *Enterococcus faecalis* and on plasmid DNA of *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Distribution of antibiotic-resistance genes among GBS serotypes

The distribution of resistance genes among the different GBS serotypes is summarized in Table 3. The *tetM* gene was detected in all of the GBS serotypes. Most of the 121 *tetM*-positive isolates were distributed among four serotypes (V, III, Ia and II) and the nontypable group. The *aph3* gene was also widely distributed among the different serotypes, whereas *ermB* and *ermTR* were found in four and three serotypes, respectively.

DISCUSSION

This study has demonstrated changes in the serotype distribution and antibiotic resistance in GBS isolated from patients at the Maternity Hospital in Kuwait, namely a shift from serotype III to serotype V as the dominant GBS serotype among colonized women. As serotype V GBS isolates have become the predominant GBS serotype causing invasive infections in adult patients and neonates in the USA and Canada (Castor *et al.*, 2008; Dogan *et al.*, 2005), Australia and New Zealand (Zhao *et al.*, 2008), the predominance of serotype V among Kuwaiti women may be considered an emerging public health concern. Curiously, our results differed from those reported for pregnant women in neighbouring countries such as the United Arab Emirates, where serotype IV (26.3 %) was the most common serotype among GBS isolates (Amin *et al.*, 2002), or Iran, where serotype III (41.8 %) was the most common serotype (Mansouri *et al.*, 2008).

A high proportion of the isolates were resistant to tetracycline, trimethoprim, high-level kanamycin and chloramphenicol. Quinolone resistance has recently been described in GBS (Biedenbach *et al.*, 2006; Wu *et al.*, 2008), and we detected one ciprofloxacin-resistant isolate, which could indicate an emerging public health problem. The high prevalence of chloramphenicol resistance (30.0 %) in this study is difficult to explain since chloramphenicol is

Table 1. Distribution of MICs of β -lactam antibiotics for GBS isolates from Kuwait (July–October, 2007)

PG, penicillin; Am, ampicillin; CTX, cefotaxime.

β -Lactam	No. of strains (and % of total) for which the MIC value ($\mu\text{g ml}^{-1}$) was:						
	0.016	0.023	0.032	0.047	0.064	0.094	0.125
PG	1 (0.7)	3 (2.1)	20 (13.8)	55 (38.5)	60 (41.9)	4 (2.8)	–
Am	0	0	18 (12.6)	32 (22.4)	57 (39.9)	36 (24.0)	–
CTX	1 (0.7)	1 (0.7)	20 (13.9)	35 (24.5)	59 (41.2)	28 (19.6)	–

Table 2. Distribution of *erm* genes among erythromycin-resistant GBS isolates from Kuwait (July–October 2007)

Em, erythromycin; CC, clindamycin.

No. of resistant isolates (total=18)	MIC ($\mu\text{g ml}^{-1}$)		Resistance genotype(s)
	Em	CC	
8	≥ 8	>256	<i>ermB</i>
7	1.5	0.125	<i>ermTR</i> + <i>ermA</i>
	1.5	0.094	<i>ermTR</i>
	1.5	0.19	<i>ermTR</i>
	1	0.094	<i>ermB</i> + <i>ermTR</i>
	1	0.094	<i>ermTR</i>
	4	0.064	<i>ermB</i> + <i>mefE</i>
	4	0.125	<i>ermTR</i>
1	12	0.094	<i>ermB</i> + <i>mefA</i>
2	2	2	<i>ermTR</i> + <i>mefE</i>

rarely used in Kuwait. However, high prevalence of chloramphenicol resistance in GBS (44.2 %) was also observed in Turkey (Acikgoz *et al.*, 2004).

Although the isolates were susceptible to the β -lactam antibiotics tested, the MICs of penicillin, ampicillin and cefotaxime for the majority of them were in the range 0.047–0.094 $\mu\text{g ml}^{-1}$, similar to the results of a previous study on GBS isolated in the same hospital in 2003 (Al-Sweih *et al.*, 2005b). However, in view of recent reports of reduced susceptibility to penicillin in some GBS isolates (Chu *et al.*, 2007; Dahesh *et al.*, 2008; Kimura *et al.*, 2008; Nagano *et al.*, 2008), these data suggest that further regular surveillance studies should be carried out on β -lactam susceptibility in GBS isolates.

In contrast to the results for β -lactams, the 12.6 % erythromycin-resistance rate in the present study represents a significant increase ($P < 0.001$) in the proportion of erythromycin-resistant isolates from the 0.7 % prevalence

reported in GBS isolates obtained from patients in the same hospital in 2003 (Al-Sweih *et al.*, 2005b). This increase may be related to the increased use of macrolides in the hospital. A higher prevalence of erythromycin resistance has been reported in other studies, namely from Korea (20 %) (Uh *et al.*, 2001), Brazil (53 %) (Betriu *et al.*, 2003) and Taiwan (46 %) (Hsueh *et al.*, 2001). Most of the erythromycin-resistant GBS isolated in our study (Table 3) contained *ermB* or *ermTR*. Other studies have also reported high prevalence of *ermB* among macrolide-resistant GBS isolates (Betriu *et al.*, 2003; Diekema *et al.*, 2003; Zhao *et al.*, 2008), and *ermTR* was the most common macrolide-resistance determinant in the USA and Canada, followed by *ermB* (de Azavedo *et al.*, 2001; Dogan *et al.*, 2005). The low prevalence of *mef* genes in the Kuwaiti GBS isolates is similar to those reported by others (Aracil *et al.*, 2002; de Azavedo *et al.*, 2001).

The carriage of *tetM* by 94.5 % of the tetracycline- and minocycline-resistant isolates indicated that ribosomal protection was the most important mechanism of tetracycline resistance in our isolates. Similarly, *tetM* was the most common tetracycline-resistance determinant in GBS isolates in studies done elsewhere (Dogan *et al.*, 2005; Duarte *et al.*, 2005; Zhao *et al.*, 2008), apparently indicating the wide dissemination of *tetM* among GBS isolates.

In comparison to what was previously reported for GBS isolates (Poyart *et al.*, 2003; Zeng *et al.*, 2006; Zhao *et al.*, 2008), we detected a higher proportion of isolates (76.9 %) resistant to high-level kanamycin. It is uncertain whether this observation represents a new development in Kuwait or a new awareness of a previously existing problem, since high-level kanamycin resistance was not assessed in our previous evaluation (Al-Sweih *et al.*, 2005a). Interestingly, our kanamycin-resistant GBS isolates carried *aph3* with nucleotide sequences that were 99 % similar to *aph3* found in *E. faecium*, *E. faecalis*, *S. aureus* and *S. epidermidis*, suggesting that GBS could have acquired *aph3* from other Gram-positive bacteria. However, unlike kanamycin

Table 3. Distribution of antibiotic-resistance genes among GBS isolates from Kuwait (July–October, 2007)

GBS serotype* (n)	No. of isolates with genes indicated										
	<i>tetK</i>	<i>tetO</i>	<i>tetL</i>	<i>tetM</i>	<i>aph3</i>	<i>ermA</i>	<i>ermB</i>	<i>ermTR</i>	<i>mefA</i>	<i>mefE</i>	<i>ant6'</i>
Ia (11)		1		8	4		1		1	1	
Ib (5)				2	4	1		1			
II (16)		1		13	13						
III (30)		2	1	27	24		6	4		1	
IV (5)				4	4						1
V (55)	1	1	1	50	44		3	2			
VI (4)				3	3						
VII (1)				1							
NT (16)				13	14		1				
Total (143)	1	5	2	121	110	1	11	7	1	2	1

*NT, nontypable.

resistance, high-level streptomycin resistance was detected in only 3.5 % of the isolates in the present study, and only one of these isolates was positive for *ant6*, suggesting that streptomycin resistance in the *ant6*-negative isolates was due to other resistance mechanisms. Similarly, Poyart *et al.* (2003) reported low prevalence of high-level streptomycin resistance in GBS isolates.

With regard to the distribution of resistance determinants among GBS serotypes, the common resistance genotypes, *tetM* and *aph3*, were widely distributed across serotypes, with the majority of the isolates belonging to serotype V, in agreement with observations reported by Zhao *et al.* (2008). In contrast to the findings in our study, Zeng *et al.* (2006) reported *aph3* mostly in serotype III but not in serotype V isolates. This apparent discrepancy may simply be a consequence of the fact that the majority of our isolates belonged to serotype V.

In conclusion, this study has demonstrated changes over time in serotype distribution and antibiotic resistance in GBS isolated from patients at the Maternity Hospital in Kuwait. The results show a shift from serotype III to serotype V as the dominant GBS serotype among colonized women at the Maternity Hospital. Although all isolates were susceptible to penicillin, ampicillin and cefotaxime, the majority of them were susceptible at higher MIC values (0.047–0.094 µg ml⁻¹). This finding, together with the observation that a high proportion of the isolates were resistant to tetracycline, high-level kanamycin and trimethoprim, and the increase in the prevalence of erythromycin resistance, represents an emerging public health concern that needs further surveillance.

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