Antimicrobial resistance in group B streptococcus: the Australian experience

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Intrapartum chemoprophylaxis for pregnant group B streptococcus (GBS) carriers reduces vertical transmission, with a resultant decrease in neonatal as well as maternal morbidity from invasive GBS infection. Current Australian guidelines recommend penicillin for intrapartum prophylaxis of GBS carriers, with erythromycin or clindamycin for those with a β-lactam allergy. Recent reports globally suggest that resistance to erythromycin and clindamycin may be increasing; hence, a study was undertaken to promote an evidence base for local clinical guidelines. Samples collected for standardized susceptibility testing included 1160 invasive GBS isolates (264 isolates retrospectively from 1982 to 2001 and prospectively from 2002 to 2006, plus 896 prospectively collected colonizing GBS isolates gathered over a 12 month period from 2005 to 2006) from 16 laboratories around Australia. All isolates displaying phenotypic macrolide or lincosamide resistance were subsequently genotyped. No isolates showed reduced susceptibility to penicillin or vancomycin. Of the invasive isolates, 6.4 % demonstrated phenotypic erythromycin resistance and 4.2 % were clindamycin resistant. Of the erythromycin-resistant isolates, 53 % showed cross-resistance to clindamycin. Very similar results were found in colonizing specimens. There was no statistically significant change in macrolide-resistance rates over the two study periods 1982–2001 and 2002–2006. Genotyping for macrolide and lincosamide-resistant isolates was largely consistent with phenotype. These findings suggest that penicillin therapy remains an appropriate first-line antibiotic choice for intrapartum GBS chemoprophylaxis, with erythromycin and/or clindamycin resistance being low in the Australian population. It would, nevertheless, be appropriate for laboratories screening for GBS in obstetric patients to consider macrolide sensitivity testing, particularly for those with β-lactam allergy, to ensure appropriate chemoprophylaxis.

INTRODUCTION

Streptococcus agalactiae, or group B β-haemolytic streptococcus, is a common cause of neonatal sepsis and meningitis worldwide. The maternal genital tract is the usual source of group B streptococcus (GBS) causing early onset neonatal infection in the first week of life (Baker & Barrett, 1973). The prevalence of GBS carriage in the vagina at the time of delivery varies from 5 to 30 %, with peripartum transmission to the newborn resulting in colonization in 50–70 % of cases, if no action is taken to prevent transmission (Anthony, 1982).

Early onset GBS sepsis became a notable problem in many parts of the world in the late 1970s. At the Royal Women's Hospital in Melbourne, Australia, at its peak in 1979, the rate of early onset neonatal GBS sepsis was 3.2 per 1000 births, with a mortality rate of 40 % (Garland, 1991). With implementation of maternal screening and intrapartum

chemoprophylaxis during the 1980s, the rate of early onset GBS sepsis at the Royal Women's Hospital decreased to 0.5 per 1000 births (Garland & Fliegner, 1991). Similar improvements have been reported across Australia (where from 1992 to 2001 the rate of early onset GBS sepsis declined from a peak of 1.43 per 1000 live births in 1993 to 0.25 per 1000 in 2001; Daley & Garland, 2004; Daley & Isaacs, 2004), as well as worldwide (CDC, 2007; Wendel et al., 2002).

Over the past decade, the practice of universal screening at 35–37 weeks gestation, with intrapartum chemoprophylaxis for colonized mothers, has been endorsed by multiple organizations, including the United States Centers for Disease Control (Schrag et al., 2002), and by the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG, 2007).

The usual recommendation for the prevention of GBS transmission from colonized women to their infants during labour is to administer intravenous penicillin every 4 h for the duration of labour (AEG, 2010). Almost all GBS isolates are highly susceptible to penicillin; there have been only a few reported instances of penicillin-resistance worldwide (Moyo et al., 2001; Hsueh et al., 2001). Penicillin therefore remains the first-line treatment of choice (Schrag et al., 2002).

A number of women will, however, report a penicillin or cephalosporin allergy. Under these circumstances, the alternative antibiotic choices have traditionally been erythromycin or clindamycin. Current Australian antibiotic therapeutic guidelines (AEG, 2010) and Australasian Society for Infectious Diseases guidelines (Palasanthiran et al., 2002) recommend the use of clindamycin for second-line intrapartum prophylaxis in GBS-colonized mothers with β-lactam antibiotic allergy.

Recent reports from around the world have, however, raised concerns about the rising rates of macrolide resistance in GBS (Andrews et al., 2000; Desjardins et al., 2004; Janapatla et al., 2008). Cross-resistance (either inducible or constitutive) to lincosamides such as clindamycin may exist (Castor et al., 2008). There are two major mechanisms of macrolide resistance and each genetic mechanism manifests a different resistance phenotype. In general, resistance to macrolides in GBS is conferred either by methylases encoded by erm genes (B, A/TR or C), giving rise to the macrolide–lincosamide–streptogramin (MLSB) resistance phenotype, or by membrane-bound pumps that cause efflux of macrolide antibiotics, encoded by mef genes (A or E), and giving rise to the M phenotype (Marimón et al., 2005). Erythromycin-resistant isolates with the MLSB phenotype will usually display cross-resistance to clindamycin, whereas isolates with the M phenotype usually display only erythromycin resistance.

Worldwide, resistance rates of up to 54 % for erythromycin (DiPersio & DiPersio, 2006) and 39 % for clindamycin

have been described (Janapatla et al., 2008). In light of the high levels of resistance reported worldwide, some experts have recommended that vancomycin (rather than macrolides or lincosamides) should constitute the second-line intrapartum chemoprophylaxis for GBS prevention (Peláez et al., 2009; Schrag et al., 2002).

To date, there have been no data to suggest that such a change to guidelines would be necessary in Australia. A small study performed on 250 colonizing GBS isolates at a single institution in Melbourne in 2001 showed that only 2.8 % of isolates were resistant to erythromycin (Stylianopoulos et al., 2002). This study was limited by its size, its inclusion of only colonizing isolates and the fact that it was not population based.

The purpose of this national survey was to obtain a more comprehensive understanding of rates of macrolide resistance in GBS around Australia. Another objective was to determine if there has been a change in resistance patterns amongst invasive GBS isolates over the past decade. This should provide a better evidence base for future antimicrobial recommendations for GBS intrapartum prophylaxis, particularly for women who report a β-lactam allergy.

METHODS

Study population. In 2004, an invitation to participate in a nationwide survey of GBS isolates was publicized through the Australasian Group for Antimicrobial Resistance. Sixteen public and private laboratories, servicing obstetric and neonatal patients across most of the States and Territories in Australia, participated in the study.

Both invasive (blood and cerebrospinal fluid specimens) and colonizing GBS isolates were requested and supplied. Participants were asked to submit the first ten colonizing anogenital isolates received by the laboratory each month over a period of 24 months from October 2004 to September 2006. In addition, stored invasive GBS isolates collected between 1982 and 2001 were requested, as well as prospectively collected invasive GBS isolates from 2002–2006. Specimens were submitted to a central bacteriology laboratory at the Royal Children's Hospital in Melbourne and stored at –70 °C until needed for further testing.

Confirmation of GBS identification and susceptibility testing. All submitted isolates had their identification confirmed by standardized methods at the central bacteriology laboratory. Test and control organisms were subcultured onto horse blood agar (Oxoid) and incubated at 35 °C in 5 % CO_2 for 24 h. To confirm identification of each isolate, a CAMP (Christie–Atkins–Munch-Petersen) test was performed (Gerhardt et al., 1994) on whole blood Mueller–Hinton agar (Oxoid). A positive control, Streptococcus agalactiae ATCC 13813, and two negative control organisms, Enterococcus faecalis ATCC 29212 and Streptococcus pyogenes ATCC 19615, were employed, along with Staphylococcus aureus ATCC 25923. Any referred isolates with a negative CAMP test had the test repeated. If the isolate failed the repeat test or produced equivocal results, a latex agglutination test was performed using the Streptex kit (Remel). If no agglutination occurred, the isolate was discarded.

All confirmed GBS isolates were tested for susceptibility to penicillin, erythromycin, clindamycin and vancomycin using a 0.5 McFarland

Table 1. Phenotypic resistance patterns amongst invasive GBS isolates over two study periods 1981–2001 and 2002–2006

Invasive	Total	1981–2001	2002–2006
Total no. of GBS isolates	264	117	147
Erythromycin resistance only	8	5	3
Clindamycin resistance only	2	0	2
Combined erythromycin and clindamycin resistance	9	1	8
Total no. of isolates with either erythromycin or clindamycin resistance	19	6	13

standard suspension in 2.0 ml Mueller–Hinton broth. Suspension turbidity was determined using a Vitek colorimeter (bioMérieux). Isolates were tested by agar disc diffusion, with manual reading of zone diameters analysed according to the interpretive criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (supplement M100-S16) (CLSI, 2006).

All isolates had their penicillin MIC confirmed by Etest (bioMérieux) according to CLSI guidelines. In addition, all erythromycin and/or clindamycin-resistant isolates had their MICs for erythromycin and clindamycin determined by Etest.

For the detection of a 'D zone' indicating inducible clindamycin-resistant, erythromycin-resistant, clindamycin-sensitive strains were tested with erythromycin (15 µg) and clindamycin (2 µg) discs, with the inner edges 25 mm apart. Strains with D shaped (or flattened) clindamycin zones were classified as having inducible resistance to clindamycin (CLSI, 2006).

PCR and DNA sequencing. All erythromycin- and/or clindamycin-resistant strains were genotyped using a genotype specific PCR and/or sequencing of: a portion of the *cps* gene cluster; surface protein antigens, including Cα, Rib, α-like proteins 2-4 and Cβ; and 3–7 mobile genetic elements – IS*861*, IS*1548*, IS*1381* IS*Sa4*, IS*Sag1*, IS*Sag2* and GBSi1, as described by Zeng *et al.* (2006).

Statistical analysis. The Pearson's χ^2 test was used to assess the non-parametric data. The test of significance was two-tailed and a $P<0.05$ was considered significant. The test was performed with SPSS version 12 software (SPSS).

RESULTS AND DISCUSSION

In total, 1302 isolates were received from 16 independent laboratories around Australia. Of these, 142 were excluded for the following reasons: 98 were non-viable, 22 were not confirmed as GBS, 21 could not be identified as colonizing or invasive specimens by the submitting laboratory and 1

was sent in duplicate. Of the remaining 1160 isolates included in this survey, 264 were invasive isolates and 896 were colonizing isolates.

Antibiotic susceptibility

All isolates were susceptible to penicillin and vancomycin. There was no evidence of an increase in penicillin MICs over the study period (maximum penicillin MIC penicillin in this study 0.125 µg ml^{-1}).

This study found that rates of erythromycin and clindamycin resistance remain relatively low in GBS isolates across Australia, in both invasive and colonizing isolates. Of the invasive isolates, 17/264 (6.4 %) showed erythromycin resistance (with MICs between 6 and 12 µg ml^{-1}), whilst 11/264 (4.2 %) showed clindamycin resistance. Of the erythromycin-resistant isolates, 9/17 (53 %) showed cross-resistance to clindamycin. The macrolide and lincosamide resistance rates amongst invasive isolates are shown in Table 1.

Of note, there was no statistically significant ($P=0.43$) increase in the rate of macrolide resistance in invasive specimens between the two study periods 1982–2001 (5.1 %) and 2002–2006 (7.5 %), although there was a significant increase in the number of macrolide-resistant isolates that exhibited cross-resistance to clindamycin from 17 to 73 % ($P=0.03$). Interestingly, rates of macrolide resistance reported in this study are somewhat higher than those reported in a 2002 single centre study in Melbourne (Stylianopoulos *et al.*, 2002). No invasive isolates collected in the period 1981–2002 showed only clindamycin resistance, but from 2002 to 2006 there were two invasive isolates that demonstrated such resistance.

Table 2. Selected antimicrobial resistance patterns for GBS in published studies worldwide

Country	Reference	Year	Erythromycin resistance (%)	Clindamycin resistance (%)
United States	DiPersio & DiPersio (2006)		54	33
Taiwan	Janapatla *et al.* (2008)	2001–2007	44	39
Korea	Uh *et al.* (2007)	2003–2004	37	43
Norway	Bergseng *et al.* (2008)	2006	25	25
France	Fitoussi *et al.* (2001)	2000	18	12
Portugal	Figueira-Coelho *et al.* (2004)	1999–2002	11	10
Japan	Matsubara *et al.* (2001)	1999–2000	3	1

Table 3. Phenotypic resistance patterns amongst 19 invasive GBS isolates displaying phenotypic macrolide or lincosamide resistance, with the genotype correlates

Invasive GBS	Total	ermA	ermB	mefA
Erythromycin resistance only	8	6	–	8
Clindamycin resistance only	2	2	–	–
Both erythromycin and clindamycin resistance	9	4	8	9

Amongst the colonizing isolates, 55/896 (6.1 %) were resistant to erythromycin (MICs 4–16 µg ml^{-1}, except for 2 isolates with MICs >256 µg ml^{-1}) and 60/896 (6.7 %) were clindamycin resistant. Of the erythromycin-resistant isolates 38/55 (69 %) showed cross-resistance to clindamycin; of these 19 demonstrated inducible resistance (based on a positive D test) and 19 demonstrated constitutive resistance (based on CLSI zone of inhibition interpretation). Of note, rates of erythromycin resistance did not vary significantly between invasive and colonizing isolates (6.4 % versus 6.1 %; $P=0.86$).

This situation in Australia differs from other parts of the world, where rates of erythromycin resistance appear to be rising rapidly. Resistant rates of up to 54 % for erythromycin and 43 % for clindamycin have been described in international studies (DiPersio & DiPersio, 2006) (Table 2). In one series, 71 % of erythromycin-resistant strains were also resistant to clindamycin (Bergseng et al., 2008).

Correlation of phenotype and genotype

All isolates with phenotypic macrolide or lincosamide resistance were further investigated by genotyping, comprising a total of 19 invasive and 77 colonizing isolates. In general, genotypes were largely consistent with phenotype.

Amongst the invasive isolates, all 8 of the erythromycin-only resistant isolates contained the mefA gene and 6/8 (75 %) additionally carried the ermA gene. The two clindamycin-only resistant isolates carried only the mefA gene. Amongst the 9 isolates with a phenotype of combined macrolide and lincosamide resistance, all 9 carried the mefA gene and 8/9 (89 %) additionally carried the ermB gene (Table 3), as might be expected for the MLSB phenotype.

Amongst the colonizing isolates, 16/17 (94 %) of the erythromycin-only resistant isolates carried the mefA gene.

Amongst those that demonstrated phenotypic macrolide-lincosamide cross-resistance, 18/38 (47 %) carried the ermB gene and 22/38 (58 %) carried the ermA gene; 15/38 (39 %) additionally carried the mefA gene. Of note, in 16 isolates (14 of which demonstrated constitutive clindamycin resistance only), no genotypic resistance mechanism could be determined. Most of these isolates displayed only constitutive lincosamide resistance, similar to the LSA phenotype reported in New Zealand (Malbruny et al., 2004). The macrolide and lincosamide resistance rates amongst colonizing isolates, with associated genotyping, are shown in Table 4.

Limitations

This study was limited by the non-randomized nature of laboratory participation and may therefore not adequately represent the true geographical spread of GBS phenotypes across all of Australia. In addition, samples prior to 2002 were collected based upon available stored samples, which may not have been randomly chosen for storage and may potentially bias results.

Conclusion

In the Australian context, penicillin remains the antibiotic of choice for intrapartum GBS chemoprophylaxis, and erythromycin and clindamycin remain appropriate alternatives for β-lactam allergic patients requiring prophylaxis. In light of the possibility of macrolide and lincosamide resistance, however, it is advisable that laboratories should perform macrolide and lincosamide susceptibility testing, particularly for those women with β-lactam allergy, to ensure appropriate chemoprophylaxis. If GBS susceptibility is unknown at the time of delivery, consideration should be given to the use of vancomycin for second-line chemo-prophylaxis (Schrag et al., 2002).

Table 4. Phenotypic resistance patterns amongst colonizing GBS isolates, with genotype correlates

Colonizing GBS	Total	Inducible	Constitutive	ermA	ermB	mef	None
Erythromycin resistance only	17	–	–	–	–	16	1
Clindamycin resistance only	22	3	19	7	1	–	14
Both erythromycin and clindamycin resistance	38	19	19	22	18	15	1
Total	77	22	38	29	19	31	16

In view of the global situation, where macrolide and lincosamide resistance is rising sharply in some regions, together with concerns about the clonal spread of resistance, rates of GBS resistance in Australia require ongoing close surveillance to ensure that antibiotic recommendations for GBS prophylaxis remain appropriate and safe.

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