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SHORT REPORT
Emergence of group B *Streptococcus* serotype IV in women of child-bearing age in Ireland

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SUMMARY

This study determined the carriage rate and serotype distribution of group B *Streptococcus* (GBS) in women of child-bearing age in the southern region of Ireland. A total of 2000 vaginal swabs collected in two periods in 2004 and 2006 were examined and revealed a GBS carriage rate of 16·1%. Serotyping of isolates showed that serotypes Ia, II, III, IV, and V were the most prevalent. A high prevalence of serotype IV was found, increasing from 7·6% to 15·2% between 2004 and 2006. Random amplified polymorphic DNA analysis demonstrated considerable genetic heterogeneity in the serotype IV isolates. This serotype should be considered for inclusion in potential vaccines for use in Ireland.

Key words: Colonization, group B *Streptococcus* (GBS), serotype, *Streptococcus agalactiae*.

Streptococcus agalactiae, also known as group B *Streptococcus* (GBS) is the leading cause of meningitis, pneumonia and bacterial sepsis in neonates in the USA and Europe [1]. Maternal intrapartum GBS colonization is a risk factor for early onset GBS disease and this has been linked with vertical transmission of GBS in over 95% of neonatal carriers or cases, the remainder being accounted for by rare cases of transmission through human milk and by nursery personnel [2].

Studies aimed at developing a GBS vaccine are ongoing worldwide and attention has focused on the contribution to immunoprotection of the capsular polysaccharide antigen (CPS) and its potential as a vaccine target [3]. Nine serologically distinct capsular polysaccharide serotypes had been identified until relatively recently in GBS, namely Ia, Ib, II, III, IV, V, VI, VII, and VIII; a further serotype has latterly been added, designated serotype IX [4]. It has been shown previously that predominating GBS serotypes change over time, vary with ethnic origin and can be associated with different diseases [3, 5]. Knowledge of local GBS serotype distribution is therefore important for the development of an effective vaccine tailored for a target population. To this end the current study assessed the prevalence and serotype distribution of GBS in a large population of women of child-bearing age in southern Ireland.

Two thousand (1000 each between October and November 2004, and between October and November 2006, respectively) non-duplicate vaginal swabs from females aged between 15 and 54 years at Cork University Hospital were screened for the presence of GBS. All swabs were anonymized and assigned a unique identifying number for the purpose of the study. Swabs were cultured on Islam agar (Oxoid®, UK) and incubated anaerobically at 37 °C for 48 h.

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Orange-red colonies were subcultured on Columbia blood agar base (Oxoid) and incubated in 5% CO_2 atmosphere at 37 °C for 18–24 h. Presumptive GBS colonies were assigned to Lancefield groups using the Remel Streptex® kit (Launch Diagnostics, UK) according to the manufacturer's instructions. Isolates were stored on cryobeads at −70 °C (Technical Service Consultants Ltd, UK).

A total of 323 GBS isolates were serotyped by latex agglutination with a serotyping kit (Essum AB, Sweden) consisting of nine antisera to serotypes Ia–VIII. Non-reactive isolates were further tested with the 10-serum kit (Ia–IX) [Statens Serum Institut (SSI), Denmark]. Isolates that failed to type by either serotyping kit were deemed serologically non-typable. All serotype IV isolates initially identified using the Essum Probiotics kit, except for three unrecoverable isolates, were re-tested using the SSI kit, as were a set of 15 isolates that included a variety of serotypes (but excluding serotype IV). All of these isolates gave concordant results for the two serotyping kits.

The genetic relatedness of serotype IV isolates was investigated using random amplified polymorphic DNA analysis (RAPD). Purified DNA was extracted from a 10 μl loopful of agar culture emulsified in 400 μl of phosphate buffered saline; 10 μl of 10 mg/ml lysozyme (Sigma Aldrich, Ireland) was added to this suspension and the solution was incubated at 37 °C for 15 min, and DNA was extracted using the MagNA Pure Compact Nucleic Acid Isolation kit according to the manufacturer's instructions (Roche Diagnostics, Ireland). The concentration of DNA from each sample was quantified spectrophotometrically and adjusted to 100 ng/μl. RAPD fingerprinting was performed according to a previously published method [6] (and using the primer OPB18), in duplicate to ensure reproducibility. A dendrogram of the relatedness of the RAPD profiles was constructed using the DendroUPGMA program (http://genomes.urv.cat/UPGMA).

The GBS colonization rate was 16·1% (323/2000) which is comparable with the rates reported from Western Europe (11–26%) [7, 8] but below rates from Eastern Europe (20–29%) and Scandinavia (24–36%) [8]. We employed a GBS selective agar as a screening tool because it has been suggested that along with enrichment broths this improves the sensitivity of GBS screening tests [9]. The addition of an enrichment broth might well have increased the detection rate further. The pregnancy status of women in this study was not established as it was previously shown that there is no difference in colonization rates between pregnant and non-pregnant women of child-bearing age [10].

Table 1. *Serotype distribution in 323 GBS isolates collected in 2004 and 2006 from women of child-bearing age*

	No. of isolates	
Serotype	2004 (%)	2006 (%)
I(a)	32 (18·6)	43 (28·5)
I(b)	12 (7)	14 (9·3)
II	30 (17·4)	21 (13·9)
III	35 (20·3)	28 (18·5)
IV	13 (7·6)	23 (15·2)
V	36 (20·9)	18 (11·9)
VI	1 (0·6)	1 (0·7)
VII	1 (0·6)	0
VIII	4 (2·3)	1 (0·7)
Non-typable	6 (3·5)	0
Not available	2 (1·2)	2 (1·3)
Total	172	151

In the current study all serotypes except IX were detected with Ia, II, III, IV, and V predominating as shown in Table 1. The latter types in addition to serotype Ib accounted for 305/323 isolates (94·4%) with Ib being the least frequent. Serotype Ia increased in prevalence from 18·6% to 28·5% between the two sampling periods, while serotype V decreased from 20·9% to 11·9%. However, the most dramatic finding in this study lay in the prevalence of serotype IV, at 11·2% (7·6% of the 2004 isolates and 15·2% of the 2006 isolates) which contrasts with an earlier Dublin study where serotype IV prevalence was 1·9% [11]. The emergence of this serotype also contrasts with global epidemiological studies, where it had only been previously reported in significant numbers in colonizing and invasive GBS strains isolated in the United Arab Emirates (26·3%) and in other countries of which Turkey shows the highest prevalence at 8·3% [12, 13]. Fewer than 2% of isolates in the current study were serologically non-typable.

RAPD analysis of 33/36 serotype IV isolates (data not shown) revealed seven distinct clusters which contained two or more isolates suggesting considerable genetic heterogeneity within the serotype.

Serotypes Ia, Ib, II, III, and V are associated with neonatal invasive disease [14]. Concurrently with this study GBS was isolated by our department in 13 cases of neonatal invasive disease with the following serotypes identified: Ia ($n=4$), Ib ($n=2$), II ($n=1$), III

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($n=6$). It is noteworthy that serotype Ib, which accounted for 8·1% of colonizing strains, was represented by two of the 13 invasive isolates, while serotype V (16·7% of the colonizing strains) was not identified in the invasive isolates.

In conclusion, although the GBS colonization rates are relatively low at 16·1% in the current study, we have highlighted the unexpected emergence of serotype IV. This serotype may need to be included in any GBS vaccine developed for use in Ireland. To date, no vaccine incorporating serotype IV has reached clinical trial, but it has been included in at least one vaccine under consideration [15].

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DECLARATION OF INTEREST

None.

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