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SHORT REPORT
Genetic diversity of *Streptococcus agalactiae* strains colonizing the same pregnant woman

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SUMMARY

Pulsed-field gel electrophoresis (PFGE) of *Sma*I–DNA digests and serotyping was performed on 15 colonies of *Streptococcus agalactiae* (GBS) from each of 30 vaginal–rectal colonized women. Five distinct GBS serotypes were observed among the 30 specimens (Ia, Ib, II, III and V). In 29 of the 30 samples, the same serotype was observed among all 15 colonies; in the remaining specimen, the 15 colonies yielded two serotypes (II and V). The PFGE profiles of all colonies in 27 of the 30 subjects were indistinguishable within each subject. In the remaining women, different DNA profiles were identified among the colonies in each specimen, one of whom carried two different serotypes. Furthermore, strains of the same serotype belonging to different women were genetically heterogeneous.

Streptococcus agalactiae (group B streptococci, GBS) is the most important bacterial agent of early-onset neonatal sepsis and the incidence of GBS disease ranges from 0·7 to 3·7/1000 live neonates [1]. Neonates become colonized and infected by GBS mainly at the time of delivery and acquire the bacteria through the birth canal of GBS-colonized women. Vaginal or rectal GBS colonization ranges from 10 to 30% [1–3].

Nine different capsular polysaccharide GBS serotypes have been described (Ia, Ib, II, III, IV, V, VI, VII and VIII), five of which are responsible for most of the infections in neonates and adults (Ia, Ib, II, III and V) [1]. Although clinical isolates of GBS are usually characterized by serotyping, its discriminatory power is low and a small percentage of strains are untypable [4]. Recently, several molecular techniques have been applied to studying the genetic diversity of GBS, such as restriction fragment length polymorphism analysis (RFLP) [5], ribotyping [6], pulsed-field gel electrophoresis (PFGE) [4, 7, 8], multilocus enzyme electrophoresis (MLEE) [9], random amplification of polymorphic DNA (RAPD) [10] and multilocus sequence typing (MLST) [11]. These methodologies have revealed a high degree of genetic diversity of GBS strains within the same serotype and they also enable the differentiation between re-colonization by different strains and persistent colonization by the same strain. The clonal relationship among different GBS isolates belonging to the same serotype associated with invasive infection has also been demonstrated by genetic analysis [10].

The aim of this work was to characterize, by PFGE and serotyping, several GBS colonies of positive vaginal–rectal specimens from pregnant women in order to determine the heterogeneity of GBS colonization in this population.

GBS colonization was investigated in vaginal–rectal swabs taken from pregnant women (gestational weeks 35–37) who attended the Gynaecology Department at the University Hospital Virgen de las

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Nieves, Granada. Vaginal–rectal samples were collected by swabing the lower vagina (vaginal introitus), followed by the rectum (through the anal sphincter) using the same swab [12]. Samples were cultured in Granada medium in anaerobic conditions at 35 °C, and GBS colonies were identified by the specific orange pigment [13, 14].

Positive specimens were plated on Granada medium and 15 colonies from each sample were picked and subcultured onto blood agar. A total of 30 GBS-positive specimens from 30 pregnant women were collected between April 2001 and June 2002.

For PFGE analysis, the bacterial cells were incubated overnight in 5 ml of Todd–Hewitt broth at 35 °C, centrifuged at 2000 ***g*** for 15 min, and resuspended in 1 ml of Tris–NaCl buffer (1 M NaCl in 10 mM Tris–HCl; pH 7·6). The suspension was mixed with an equal volume of 1·2% (w/v) low-melting-temperature agarose (Sigma-Aldrich, St. Louis, MO, USA) in water and poured into plug moulds (Amersham Biosciences, Uppsala, Sweden). The bacteria were lysed with lysozyme, mutanolysin and proteinase K, and the bacterial DNA was digested with *Sma*I as described by Rolland et al. [8]. The plugs were subjected to electrophoresis in a 1·2% (w/v) agarose gel (Sigma-Aldrich) in TBE (44·5 mM Tris–HCl, 44·5 mM borate, 1·25 mM EDTA; pH 8) with a Gene System Navigator® (Amersham Biosciences). Lambda DNA concatemers were used as DNA size markers (Amersham Biosciences). Pulse times were programmed in three phases over 24 h at 200 V as follows: 5 s over 4 h, 25 s over 6 h and 45 s over 14 h. PFGE patterns were visualized by UV transillumination after ethidium bromide staining. PFGE was repeated twice for each specimen to ensure the reproducibility of the assay and DNA profiles were differentiated according to the criteria proposed by Tenover et al. [15].

Serotyping was carried out on all 15 colonies selected from each sample with type-specific antisera against Ia, Ib, II, III, IV and V (Denka Seiken, Tokyo, Japan), following the manufacturer's instructions.

A total of 450 GBS colonies (15 colonies from each of 30 selected specimens) were analysed by serotyping and PFGE. Five different GBS serotypes were identified (Ia, Ib, II, III and V). A single serotype was found among all colonies tested from 29 of the 30 specimens, and two serotypes, II and V, coexisted in one woman (see Table).

This study demonstrates that a minority of GBS-colonized women may harbour different serotypes of the microorganism. Serotyping may not allow the characterization of all GBS strains as a small percentage of the strains are untypable [4]. Here, 3 of the 15 colonies from one specimen were untypable with the latex agglutination method used, with the remaining colonies belonging to serotype II (data not shown). However, all 15 colonies from this specimen, including the three untypable colonies, could be characterized by PFGE and gave the same PFGE profile. The untypable colonies probably belong to serotype II and could not be typed because other factors may have affected the serotyping procedure.

Table. *Distribution of GBS serotypes among 30 vaginal–rectal specimens from 30 women*

GBS serotype	No. women	No. PFGE patterns
Ia	9*	8
Ib	4	4
II	3	3
III	12*	11
V	1	1
II and V	1	3

* Two different PFGE profiles were observed in one specimen.

Twenty-seven of the 30 subjects harboured GBS with indistinguishable PFGE profiles within each subject [Fig. (*a*)]. In the remaining three specimens, different DNA profiles were found. One specimen harboured strains with three different DNA profiles representing two serotypes; 3 colonies of serotype II with the same DNA profile and 12 colonies of serotype V comprising two DNA profiles. In each of the other two specimens, two different PFGE patterns were detected but the serotype was homogeneous within each patient. Two, or less, band differences in DNA patterns were found among colonies within the same specimen [Table, Fig. (*b*)].

Most reports on typing studies of GBS have employed a single colony from each positive sample [4, 8, 10, 16]. To our knowledge, this is the first report in which several colonies from individual subjects have been subjected to phenotypic and genotypic characterization.

Molecular techniques have proven to be more discriminatory than serotyping for strain characterization of GBS [4]. In this study three women harboured GBS with minor differences ($\leqslant 2$ bands) in DNA patterns. This may indicate colonization by closely related variants within a predominant parental clone, which are derived from the latter as a result of single

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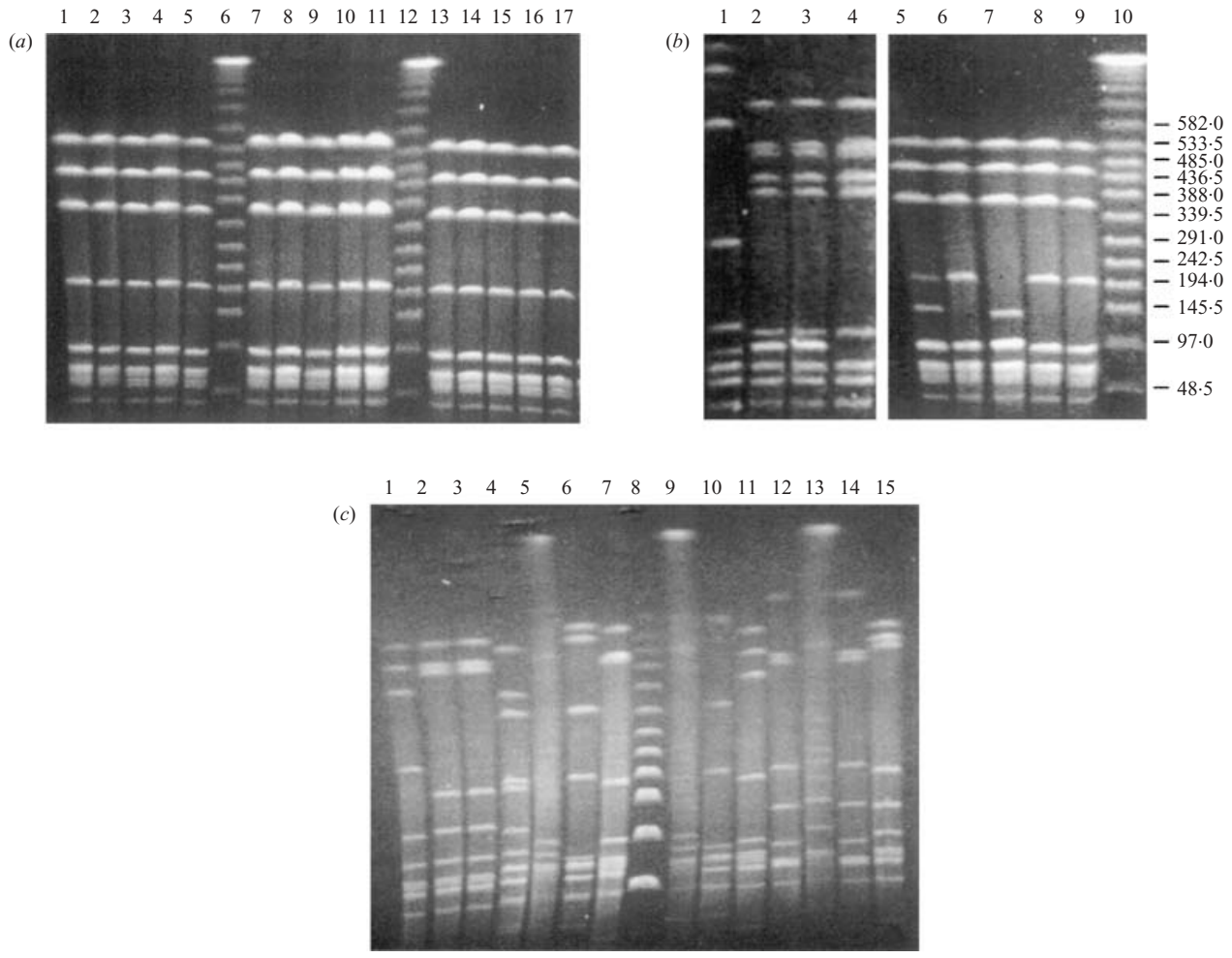

Fig. (*a*) PFGE of 15 colonies (lanes 1–5, 7–11 and 13–17) from a sample with indistinguishable DNA profiles; lanes 6 and 12, lambda DNA marker (Amersham Biosciences). (*b*) Lanes 1–4, PFGE of four colonies from the sample with three different DNA profiles and two serotypes; serotype II GBS colony (lane 1), and serotype V GBS colonies (lanes 2–4). Lanes 5–10, PFGE from a sample with a unique GBS serotype (serotype III) and different DNA profiles among 5 of the 15 colonies analysed (lanes 5–9), and lambda DNA marker (lane 10). (*c*) PFGE from all colonies and/or strains with serotype III (lanes 1–7 and 9–15); lane 8, lambda DNA marker.

or a few mutations. Further studies would be necessary to prove this. The third specimen with different DNA patterns corresponded to a woman colonized by two serotypes and both serotypes represented genetically distinct populations by PFGE [Fig. (*b*), left panel]. Similar results have been reported for *Streptococcus pneumoniae* strains recovered from nasopharyngeal samples of colonized children [17].

In a further experiment, we found that strains of the same serotype recovered from different women were heterogeneous in DNA profiles, which supports the finding of genetic variability of GBS strains as previously reported [16]. Among women harbouring serotype III strains, there were two pairs of women who shared indistinguishable PFGE profiles [Fig. (*c*), lanes 2/3 and lanes 12/14]. Among women with serotype Ia, indistinguishable DNA profiles were observed in three samples and these were quite different from the other Ia strains. Heterogeneous DNA profiles were observed within serotypes Ib, II and V strains (data not shown). Thus, a very low correlation between serotyping and genotyping was found among GBS strains colonizing different women, supporting previously reported data [6].

In summary, we have shown that most of the women studied were colonized by a single GBS strain, although closely related strains may coexist in the vagina and/or rectum of pregnant women.

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Furthermore, different GBS serotypes can simultaneously colonize an individual and the same GBS serotype, colonizing different women, is most often heterogeneous.

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