Genotypes and Virulence Genes in Group B Streptococcus Isolated in the Maternity Hospital, Kuwait

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**Key Words**
Group B streptococcus · Genotypes · Pulsed-field gel electrophoresis · Virulence genes

**Abstract**

**Objective:** To characterize group B streptococcus (GBS) isolates obtained from patients at the Maternity Hospital in Kuwait for their genotypes and carriage of virulence genes.

**Materials and Methods:** A total of 154 GBS isolates were obtained from July 1 to October 31, 2007, from vaginal swabs (n = 95), urine (n = 46), blood (n = 4) and miscellaneous sources (n = 9). Genotypes were obtained by pulsed-field gel electrophoresis (PFGE), following digestion with **Sma** or **Eag** restriction enzymes. PCR was used to screen for the carriage of virulence genes including: surface protein of group B streptococcus (**spb1**), secreted fibrinogen-binding protein (**fbsB**), C5a peptidase (**scpB**), laminin-binding protein (**lmb**), α- (bca) and β-subunits of the C protein (**bac**), resistance to protease immunity protein (**rib**), and phage-associated gene (**pag**); regulatory protein (**dltR**), and toxins CAMP factor (**cfb**), hyaluronidase (**hylB**) and superoxide dismutase (**sodA**).

**Results:** PFGE defined 14 genotypes differentiating isolates with the same serotypes into different genetic backgrounds. All isolates contained genes for virulence factors. However, **cfb** (99.4%), **scpB** (88.3%), **lmb** (88.3%), **bac** (57.8%), **sodA** (55.8%) and **dltR** (53.9%) were the common virulence genes. In total, 144 (90.3%) of the isolates contained 3 or more virulence genes. However, while **cfb**, **lmb** and **scpB** occurred in all genotypes, others occurred in some but not in all genotypes.

**Conclusions:** GBS isolates obtained at the Maternity Hospital, Kuwait, belonged to diverse genetic backgrounds with the majority carrying multiple virulence genes.

**Introduction**

Group B streptococcus (GBS) or *Streptococcus agalactiae* is an important cause of serious infections in newborn babies and pregnant women [1]. GBS has also been reported to cause a wide range of infections such as bacteremia, skin and soft tissue infection, bone infection, pneumonia, arthritis, endocarditis and meningitis in adult males and nonpregnant women [2, 3].

GBS has a variety of virulence factors that facilitate its ability to cause disease. Some of these virulence factors have been identified and characterized, and include capsular polysaccharides, regulatory proteins, surface-localized proteins and toxins [1, 4–7]. A number of the surface
proteins act as adhesins and may also be involved in the evasion of the immune system [6, 7]. They include Bca (α-subunit of C protein), Bac (β-subunit of C protein), Rib (resistant to proteases immunity), Alp2 (Ca-like protein 2), C5α peptidase (ScpB), laminin-binding surface protein (Lmb), fibrinogen-binding protein (FbsA), secreted fibrinogen-binding protein (FbsB), cell surface protease (Csp), and surface protein of GBS (Sbp1). GBS also produces a range of toxins such as hemolysins, hyaluronidase (hylB) and superoxide dismutase (SodA) and CAMP factor (cfb) that promote pathogen entry into host cells and facilitate its intracellular survival and spread [8].

Molecular typing of GBS isolates obtained from different sources has revealed extensive variation in genetic backgrounds [9, 10]. These studies have been important in documenting clonal spread of GBS isolates and furthered the appreciation of the roles that different genotypes expressing different virulence factors play in GBS diseases [8]. GBS isolated in Australia and New Zealand [11], Norway [12] and the USA [13, 14] have been extensively studied for virulence genes. However, there are no data on genotype distribution and carriage of genes for virulence factors among GBS isolates obtained from clinical sources in Kuwait. In previous studies, GBS isolates obtained from patients at the Maternity Hospital in Kuwait were investigated for their serotypes and susceptibility to antibiotics [15, 16]. In this study, the isolates were investigated further for their genotypes and carriage of virulence genes.

Materials and Methods

Bacterial Isolates

In total, 154 GBS isolates were obtained between July 1 and October 31, 2007, from clinical samples of mothers and babies at the Maternity Hospital, Kuwait. They were obtained from vaginal swabs (n = 95), urine (n = 46), blood (n = 4), and nonspecified sources (n = 9). Isolation and identification of the isolates have been described previously [16].

Serotyping

Serotyping of the isolates was performed using a monospecific rabbit antisera kit (Denka, Seiken, Japan) according to the protocol provided by the manufacturer and reported previously [16].

Genotyping

Genotyping was performed by pulsed-field gel electrophoresis (PFGE) as described previously by Benson and Ferriero [17]. The blocks were digested with 50 units Smal enzyme for 3 h at 25°C in a water bath or with 50 units Eagl at 37°C for 3 h. Electrophoresis was performed in 1.2% (w/v) pulsed-field grade agarose (Bio-Rad, USA) in 0.5× TBE using a CHEF-DR III system (Bio-Rad). The run parameters were 6.0 V/cm with an initial switch time of 10 s and a final switch time of 45 s at 12°C in 0.5× TBE running buffer for 20 h. A phage λ DNA molecular weight marker (Sigma-Aldrich, USA) was used. The gel was stained with 0.5 mg/ml ethidium bromide (Sigma-Aldrich) photographed under ultraviolet illumination, and imaged by using the Gene Genius Bio Imaging system (Syngene Corp., USA). PFGE patterns were interpreted according to recommendations by Tenover et al. [18]. Genetic relatedness of the isolates was assessed by cluster analysis with the unweighted pair group method with an arithmetic averages (unweighted pair group method with arithmetic mean) algorithm using Gene Tool and Gene Directory software (Syngene, UK). Band position tolerance was set at 0.5%.

Determination of Virulence Genes

The isolates were investigated for the following genes that encode surface-localized proteins: (1) surface protein of GBS (spb1), (2) secreted fibrinogen-binding protein (fbsB), (3) C5α peptidase (scpB), (4) laminin-binding protein (lmb), (5) α- (bca) and (6) β-subunits (bac) of C protein, (7) resistance to protease immunity (rib), and (8) phage-associated gene (pag), as well as (9) regulatory protein (dltR), and toxins (10) CAMP factor (cfb), (11) hyaluronidase (hylB) and (12) superoxide dismutase (sodA) in PCR assays using primers and conditions published previously [11, 19–21].

Results

PFGE Analysis of GBS Isolates

Of the 154 isolates, 148 (96.1%) were digested with Smal restriction enzyme. Six isolates could not be digested with Smal after repeated testing. However, further PFGE analysis with an alternative rare cutting restriction enzyme, Eagl, successfully digested all 6 isolates. The 148 Smal-digested isolates generated 11 different PFGE patterns and subtypes (genotypes) with genotype 1 in 53 cases (34.4%) as the dominant genotype. This was followed by genotypes 3 in 23 (14.9%), 5 in 22 (14.3%), 10 in 19 (12.3%) and 11 in 11 cases (7.1%). The other genotypes occurred less frequently. Digestion of the 6 isolates that were not digested with Smal but with Eagl produced 3 additional genotypes designated genotypes A (2), B (3) and C (1) resulting in a total of 14 genotypes.

Correlation between Serotypes and Genotypes of GBS Isolates

The GBS isolates belonged to the following serotypes: V, 59 (38.3%); III, 30 (19.5%); Ia, 16 (10.4%); II, 16 (10.4%); Ib, 5 (3.3%); IV, 5 (3.3%); VI, 4 (2.6%); VII, 1 (0.6%); VIII, 1 (0.6%), and nontypeable, 17 (11.0%). Analysis of the PFGE patterns against serotypes showed that each serotype included isolates with diverse genotypes.
Genotypes and Virulence Genes in Group B Streptococcus

Table 1. Correlation between genotypes and serotypes among GBS isolates

| PFGE patterns | Serotypes | Total |
|---------------|-----------|-------|
|               | Ia | Ib | II | III | IV | V | VI | VII | VIII | NT |
| 1             | 8  | 1  | 37 | 1   | –  | – | –  | –   | –    | 5  |
| 2             | 2  | 1  | –  | –   | –  | – | –  | –   | –    | 3  |
| 3             | 1  | 3  | –  | 12  | –  | – | –  | –   | –    | 6  |
| 4             | –  | –  | 3  | –   | –  | – | –  | –   | –    | 3  |
| 5             | 11 | 7  | 1  | –   | –  | – | –  | –   | –    | 22 |
| 6             | –  | 2  | 1  | 1   | 2  | – | –  | –   | –    | 6  |
| 7             | 1  | –  | 3  | –   | 1  | 2 | –  | –   | –    | 4  |
| 8\(^1\)       | –  | –  | –  | 1   | –  | – | –  | –   | –    | 1  |
| 9             | 1  | –  | –  | 1   | –  | – | –  | –   | –    | 3  |
| 10            | 3  | 4  | 3  | –   | 2  | 1 | 1  | 5   | –    | 19 |
| 11            | –  | 2  | 5  | 4   | –  | – | –  | –   | –    | 11 |
| A\(^1\)       | –  | –  | 2  | –   | –  | – | –  | –   | –    | 2  |
| B\(^1\)       | –  | –  | –  | 1   | 2  | – | –  | –   | –    | 3  |
| C\(^1\)       | –  | 1  | –  | –   | –  | – | –  | –   | –    | 1  |
| Total         | 16 | 5  | 16 | 30  | 5  | 59| 4  | 1   | 1    | 17 |

NT = Nontypeable; total = total numbers of the PFGE patterns and their subtypes. \(^1\) No subtypes.

Detection of Virulence Genes in GBS Isolates

In total, 153 (99.4%) of the isolates were positive for cfb, and 136 (88.3%) were positive for scpB and lmb making them the dominant virulence genes in these isolates. The other common virulence genes were bca (89; 57.8%), sodA (86; 55.8%) and dltR (83; 53.9%). The 154 isolates were grouped into 8 clusters according to the number of virulence genes in them (table 2). One hundred and forty-four isolates (90.3%) contained 3 or more virulence genes. Five (3.2%) isolates contained 2 virulence genes and 1 isolate was positive for 8 virulence genes. Only 5 (3.2%) isolates were positive for a single virulence gene (cfb).

Distribution of Virulence Genes among Genotypes and Clinical Sources

The distribution of virulence genes among the different GBS genotypes is summarized in table 3. It shows that lmb, scpB, sodA, dltR, cfb and bca were widely distributed among the different genotypes. Interestingly bac and spb1 were not detected in any genotype 1 isolates. Genotype 1 isolates contained the highest numbers of virulence genes while the lowest number was detected in genotype 8 isolates. The distribution of virulence genes according to clinical sources of GBS isolates shows that GBS isolated from vaginal swabs were positive for all of the virulence genes tested while the urinary isolates carried all tested genes except spb1. Isolates obtained from Bartholin gland, perianal, vulval and nasal samples contained fewer virulence genes. Whereas lmb, scpB, bca, cfb and sodA were widely distributed among isolates from different clinical samples, fbsA, rib and bac were obtained only from vaginal and urinary isolates.

Discussion

This study has provided initial data on the genotypes and prevalence of virulence genes in GBS isolates obtained at the Maternity Hospital, Kuwait. PFGE analysis revealed that the isolates belonged to diverse genetic backgrounds with 3 dominant genotypes obtained mostly from vaginal swabs and urine samples. PFGE analysis also revealed the presence of 6 GBS isolates that were not digested with Smal, a restriction enzyme which is widely used to type Gram-positive cocci, but were digested with Eagl. To the best of our knowledge this is the first report of this phenomenon in GBS isolates. However, Staphylococcus aureus strains that were not digested with Smal but digested with Cfr91 or ApaI enzymes have been reported among livestock isolates [22]. These strains defined a novel S. aureus multilocus sequence type, ST398 [22]. Significantly, PFGE analysis demonstrated genetic diversity in isolates belonging to the same serotypes, including the serologically nontypeable isolates. This observation is in agreement with the study of Ramaswamy et al. [14] that also used PFGE to characterize nontypeable GBS isolated in the USA and showed that PFGE typing has a higher discriminating power than serotyping for epidemiological typing of GBS isolates.

Our study revealed a high prevalence of virulence genes among the GBS isolates with 90.3% of the isolates harboring 3–6 virulence genes that code for surface-localized proteins, regulatory proteins and toxins. We observed differences in the distribution of genes for the surface-localized proteins. While lmb, scpB and bca were distributed in all
genotypes, \(fbsA\) and \(fbsB\) were restricted to fewer isolates belonging to few genotypes. In contrast to their low prevalence (3.2%) in this study, \(fbsA\) and \(fbsB\) were reported in 49.5% of GBS isolated in France [19].

This study showed that the most common gene cluster detected in 13.6% of the GBS isolates was \(\text{lmb, scpB, dltR, cfb, sodA and bca}\). Similarly, Duarte et al. [23] reported the presence of a cluster of genes consisting of \(\text{bca, lmb and scpB}\) in 66.9% of GBS isolates of human origin and \(\text{scpB}\) in 44.7% of GBS isolates of bovine origin highlighting the importance of multiple virulence factors to the success of GBS isolates as pathogens.

The \(\alpha\)- and \(\beta\)-antigens of protein C, encoded by \(\text{bca and bac}\), and the Rib protein encoded by \(\text{rib}\), have been

Table 2. Distribution of multiple virulence genes in GBS isolates (numbers of isolates in parentheses)

| Number of genes | Gene clusters |
|-----------------|---------------|
| Two genes       | \(\text{cfb, sodA (2); cfb, bca (1); cfb, pag (1); cfb, dltR (1)}\) |
| Three genes     | \(\text{lmb, scpB, cfb, pag, dltR (2); lmb, scpB, sodA (1); lmb, scpB, bca (1); cfb, sodA, bca (1); cfb, sodA, pag (1); cfb, fbsA, bca (1); cfb, dltR, pag (1); cfb, dltR, sodA (1)}\) |
| Four genes      | \(\text{lmb, scpB, cfb, bca (15); lmb, scpB, cfb, sodA (5); lmb, scpB, cfb, dltR (5); lmb, scpB, sodA, hylB (1); cfb, sodA, bca, bac (1)}\) |
| Five genes      | \(\text{lmb, scpB, dltR, cfb, sodA, bca (12); lmb, scpB, dltR, cfb, bca (10); lmb, scpB, cfb, sodA, bca (8); lmb, scpB, dltR, cfb, rib (2); lmb, scpB, cfb, pag, bca (2); lmb, scpB, dltR, cfb, pag, cfb (2); lmb, scpB, cfb, fbsA, bca (1); lmb, scpB, cfb, sodA, dltR, sopB (1); lmb, scpB, cfb, bac, hylB (1)}\) |
| Six genes       | \(\text{lmb, scpB, dltR, cfb, sodA, bca (21); lmb, scpB, dltR, cfb, sodA, rib (7); lmb, scpB, dltR, cfb, pag, bca (2); lmb, scpB, cfb, sodA, bca, bac (1); lmb, scpB, dltR, cfb, sodA, dltR, sopB (1); lmb, scpB, cfb, sodA, bac, bca (1)}\) |
| Seven genes     | \(\text{lmb, scpB, dltR, cfb, sodA, bca (21); lmb, scpB, dltR, cfb, sodA, rib (7); lmb, scpB, dltR, cfb, pag, bca (2); lmb, scpB, dltR, cfb, sodA, hylB (1); lmb, scpB, cfb, sodA, pag, bca (1); lmb, scpB, cfb, sodA, dltR, sopB (1); lmb, scpB, cfb, sodA, bac, bca (1)}\) |
| Eight genes     | \(\text{lmb, scpB, dltR, cfb, sodA, pag, bac, hybB (1)}\) |

Table 3. Distribution of virulence genes in GBS genotypes

| Genotypes | \(\text{lmb}\) | \(\text{scpB}\) | \(\text{dltR}\) | \(\text{cfb}\) | \(\text{fbsA}\) | \(\text{fbsB}\) | \(\text{sodA}\) | \(\text{pag}\) | \(\text{rib}\) | \(\text{bca}\) | \(\text{bac}\) | \(\text{hylB}\) | \(\text{spb1}\) | Total |
|-----------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------|
| 1         | 50            | 50            | 39            | 53            | 1             | 1             | 37            | 6             | 5             | 41            | –             | 5             | –             | 53       |
| 2         | 3             | 3             | 2             | 3             | 8             | 3             | 3             | 2             | 1             | 1             | 9             | 1             | 2             | 13       |
| 3         | 15            | 15            | 3             | 23            | 9             | 2             | 1             | 11            | –             | 1             | 1             | 22            | –             | 23       |
| 4         | 3             | 3             | 2             | 3             | 2             | 1             | 3             | 2             | –             | 3             | –             | –             | 3             | 3         |
| 5         | 19            | 19            | 11            | 22            | 3             | 1             | 1             | 9             | 2             | 1             | 11            | –             | 1             | 12       |
| 6         | 5             | 5             | 1             | 6             | –             | –             | 2             | 2             | –             | 5             | 1             | 1             | –             | 6        |
| 7         | 3             | 3             | 4             | 4             | –             | –             | 2             | 2             | 1             | 1             | –             | –             | –             | 4        |
| 8\(^1\)  | 1             | –             | –             | –             | –             | –             | –             | –             | –             | –             | –             | –             | –             | 1        |
| 9         | 3             | 3             | 2             | 3             | –             | 1             | 2             | –             | 3             | –             | 1             | –             | –             | 3        |
| 10        | 17            | 17            | 5             | 19            | –             | 1             | 9             | 2             | 1             | 13            | 2             | 3             | –             | 19       |
| 11        | 11            | 11            | 8             | 11            | –             | –             | 7             | –             | 3             | 5             | –             | –             | –             | 11       |
| 12        | 2             | 2             | 2             | 2             | –             | –             | 2             | –             | –             | –             | –             | –             | –             | 2        |
| 13        | 3             | 3             | 3             | 3             | –             | –             | 3             | –             | 1             | 1             | –             | –             | –             | 3        |
| 14        | 1             | 1             | 1             | 1             | –             | –             | 1             | –             | 1             | –             | –             | –             | –             | 1        |
| Total     | 136           | 136           | 83            | 153           | 5             | 5             | 86            | 17            | 13            | 89            | 5             | 12            | 2             | 154      |

\(^1\)No subtypes.
investigated as possible vaccine candidates because of their ability to elicit protective immunity against GBS infections [19, 24]. However, the detection of bca, bac and rib only in 57.8, 3.2 and 8.4%, respectively, of the isolates in this study suggests that a GBS vaccine containing these proteins would be less effective against our population. These findings have enriched our understanding of the molecular epidemiology of GBS isolates in Kuwait and contributed to the body of knowledge on the distribution of virulence-associated genes in GBS in general. Further studies will be required to monitor any changes in these genotypes over time.

Conclusion

This study has shown that GBS isolates obtained in a Kuwait hospital belonged to diverse genetic backgrounds with the majority carrying multiple virulence genes.

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