**Cover letter**

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**Title page**

**Characterization of *streptococcus agalactiae* using Multiple-Locus Variable-number tandem-repeat Analysis**

Running title: Efficiency of MLVA typing method for *S. agalactiae*

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**Table 1.** MLVA primers, copy number, number of alleles and index of diversity (D)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| No | Sequence (5'->3') | PeriodSize | CopyNumber | No. of alleles | K-value4 | Hunter-Gaston diversity index3 | Simpson’s diversity index1 |
| CL2 | HGDI | CL | SDI |
| TR1 | F: TCATTATGTAAATGGTGGTGTTGAR: TGGGTTTTATGTCCCTCTTCA | 18 | 3.8 | 4 | 11 | 0.857-0.900 | 0.878 | 0.847-0.890 | 0.869 |
| TR2 | F: TGACTGTTTGTTAGAGTCACCTTGAR: TTTGGCTTTATATGGGAGTGC | 48 | 13.2 | 10 | 4 | 0.560-0.670 | 0.615 | 0.553-0.663 | 0.608 |
| TR3 | F: TTTGAAAAGTGTAACACTAGCTCCAR: GGAGCATTCGTAGCTCTTGG | 159 | 4.7 | 3 | 8 | 0.760-0.841 | 0.800 | 0.751-0.832 | 0.791 |
| TR4 | F: TTTTTAACCGCCAAGTTTCCR: CCACTGATCAAGCAAATCAA | 12 | 22.6 | 7 | 6 | 0.999-1.000 | 0.683 | 0.619-0.732 | 0.676 |
| TR5 | F: GTTGATAAAGTTGATGTTCCGR: AGCCTTCTTCAACTATAGGTG | 18 | 5.8 | 6 | 91 | 0.999-1.000 | 1.000 | 0.949-0.950 | 0.989 |

1. SID: Simpson's diversity index

2. CI: Confidence Intervals

3. HGDI: Hunter-Gaston diversity index

4. K-value: Number of different repeats present at each locus in this sample set

**Table 2:** Presents the relationships between Characterization of GBS using Multiple-Locus Variable-number tandem-repeat Analysis

|  |  |  |  |
| --- | --- | --- | --- |
| **Origin of isolates (No. Of isolates)** | MLVA genotypes | Serotyes | Erythromycin resistance phenotype |
| Blood(N=13) | Vaginal carriage(N=34) | Urinarytract infections in women(N=13) | Spermatic fluid(N=4) | Pregnant women(N=26) |
|  |  | 1 |  |  | 2 | III | cMLSB1 |
|  | 1 |  |  |  | 4 | III | M3  |
|  |  |  |  | 1 | 5 | III | L4 |
|  | 1 |  |  |  | 6 | V | L |
| 1 |  |  |  |  | 6 | V | M |
| 1 |  |  |  |  | 6 | III | M |
|  |  |  |  | 1 | 6 | V | cMLSB |
|  |  | 1 |  |  | 6 | V | cMLSB |
| 1 |  |  |  |  | 8 | V | M |
|  |  | 1 |  |  | 8 | V | cMLSB |
|  | 1 |  |  |  | 10 | III | cMLSB |
|  | 1 |  |  |  | 13 | III | cMLSB |
|  |  |  |  | 1 | 14 | II | L |
|  | 1 |  |  |  | 15 | III | cMLSB |
|  | 1 |  |  |  | 16 | III | cMLSB |
|  | 1 |  |  |  | 16 | III | cMLSB |
|  | 1 |  |  |  | 16 | III | cMLSB |
| 1 |  |  |  |  | 16 | III | cMLSB |
|  | 1 |  |  |  | 16 | III | L |
| 1 |  |  |  |  | 20 | III | M |
| 1 |  |  |  |  | 21 | III | iMLSB2 |
|  |  |  |  | 1 | 26 | III | L |
| 1 |  |  |  |  | 28 | III | L |
|  | 1 |  |  |  | 28 | III | M |
|  |  |  |  | 1 | 30 | III | M |
|  |  |  |  | 1 | 31 | III | M |
|  |  |  |  | 1 | 34 | III | M |
| 1 |  |  |  |  | 34 | III | L |
|  | 1 |  |  |  | 38 | V | iMLSB |
|  | 1 |  |  |  | 40 | III | cMLSB |
|  |  | 1 |  |  | 43 | II | cMLSB |

1. cMLSB phenotype: resistance to both erythromycin and clindamycin
2. iMLSB phenotype: resistance to erythromycin, susceptibility to clindamycin (positive D test)
3. M phenotype: resistance to erythromycin, susceptibility to clindamycin (negative D test)
4. L phenotype: susceptibility to erythromycin, resistance to clindamycin



**Figure 1:** The polymorphism of Five VNTRs is shown by agarose gel electrophoresis of PCR products.



**Figure2:** Cluster analysis of 90 *S. agalactiae* isolates using the UPGMA (unweighted-pair group method using average linkages) algorithm, generated with Bionumerics 6.0 software**.** Clusters are classified on the basis of the level of genetic relatedness, serotypes and pilus island genes.