

Supplementary Table S1. Antimicrobial susceptibility testing results obtained by disc diffusion method and antimicrobial gradient diffusion method (E-test).

	E-test	Disc diffusion method												Resistance mechanisms
	Penicilin G	P* (1IU)		TE* (30µg)		TGC* (15 µg)		LEV* (5 µg)		E* (15 µg)		DA* (2 µg)		
Strains derived from pregnant women (n = 85)														
1	0.094	22	S	10	R	20	S	19	S	26	S	23	S	-
3	0.094	22	S	8	R	19	S	19	S	6	R	6	R	MLS _B c
4	0.064	25	S	8	R	20	S	20	S	27	S	25	S	-
7	0.094	21	S	9	R	19	S	19	S	25	S	22	S	-
8	0.094	22	S	13	R	21	S	23	S	24	S	22	S	-
9	0.094	23	S	8	R	19	S	20	S	25	S	22	S	-
10	0.064	22	S	11	R	22	S	23	S	28	S	22	S	-
11	0.125	23	S	8	R	20	S	20	S	27	S	24	S	-
12	0.064	23	S	14	R	19	S	20	S	24	S	21	S	-
13	0.094	24	S	8	R	19	S	20	S	12	R	19	R	MLS _B i
14	0.064	23	S	8	R	20	S	19	S	6	R	6	R	MLS _B c
17	0.094	21	S	8	R	21	S	20	S	25	S	22	S	-
19	0.064	22	S	8	R	19	S	19	S	25	S	23	S	-
20	0.094	23	S	12	R	21	S	21	S	28	S	24	S	-
21	0.064	25	S	26	S	21	S	22	S	26	S	22	S	-
22	0.094	23	S	8	R	19	S	19	S	13	R	12	R	MLS _B c
23	0.032	25	S	8	R	21	S	24	S	28	S	25	S	-
24	0.064	23	S	10	R	22	S	20	S	26	S	25	S	-
27	0.094	24	S	8	R	19	S	19	S	6	R	6	R	MLS _B c
30	0.125	25	S	15	R	21	S	24	S	24	S	22	S	-
31	0.094	23	S	8	R	19	S	20	S	25	S	22	S	-
35	0.064	22	S	10	R	20	S	20	S	25	S	22	S	-
36	0.094	24	S	15	R	21	S	20	S	6	R	6	R	MLS _B c
40	0.094	23	S	10	R	19	S	21	S	25	S	22	S	-
41	0.064	24	S	12	R	21	S	20	S	26	S	23	S	-
42	0.094	24	S	13	R	20	S	20	S	26	S	22	S	-
43	0.094	22	S	10	R	19	S	19	S	24	S	21	S	-
44	0.064	22	S	8	R	20	S	19	S	25	S	21	S	-
45	0.094	22	S	8	R	19	S	19	S	23	S	22	S	-
46	0.032	26	S	23	S	21	S	20	S	27	S	24	S	-
49	0.064	23	S	10	R	22	S	20	S	12	R	6	R	MLS _B c
51	0.094	21	S	10	R	19	S	20	S	25	S	22	S	-
53	0.094	22	S	12	R	20	S	19	S	25	S	23	S	-
54	0.064	23	S	10	R	19	S	20	S	6	R	6	R	MLS _B c
57	0.047	25	S	11	R	19	S	18	S	25	S	21	S	-
59	0.094	23	S	9	R	19	S	20	S	23	S	21	S	-
60	0.094	22	S	10	R	20	S	19	S	26	S	23	S	-
61	0.094	23	S	17	R	24	S	24	S	8	R	19	R	MLS _B i

62	0.047	22	S	20	R	19	S	20	S	25	S	22	S	-
64	0.125	21	S	12	R	21	S	21	S	25	S	22	S	-
65	0.094	21	S	12	R	19	S	20	S	25	S	22	S	-
66	0.125	22	S	13	R	19	S	21	S	25	S	23	S	-
67	0.064	25	S	10	R	20	S	19	S	26	S	22	S	-
68	0.094	23	S	18	R	24	S	22	S	30	S	25	S	-
69	0.094	26	S	14	R	20	S	22	S	28	S	24	S	-
70	0.094	23	S	8	R	20	S	20	S	26	S	23	S	-
71	0.125	22	S	10	R	19	S	20	S	26	S	23	S	-
72	0.047	22	S	8	R	19	S	20	S	17	R	20	R	MLS _B i
75	0.064	26	S	26	S	22	S	23	S	27	S	22	S	-
78	0.064	30	S	12	R	21	S	24	S	6	R	6	R	MLS _B c
80	0.094	24	S	10	R	19	S	19	S	6	R	6	R	MLS _B c
81	0.047	22	S	10	R	19	S	20	S	26	S	23	S	-
82	0.064	25	S	10	R	22	S	22	S	28	S	26	S	-
83	0.094	23	S	9	R	19	S	19	S	25	S	21	S	-
86	0.125	22	S	17	R	21	S	19	S	24	S	21	S	-
88	0.094	18	S	12	R	20	S	22	S	26	S	23	S	-
90	0.094	23	S	10	R	19	S	21	S	25	S	22	S	-
91	0.094	22	S	20	R	19	S	20	S	25	S	22	S	-
94	0.064	23	S	10	R	19	S	20	S	26	S	23	S	-
96	0.064	24	S	10	R	19	S	21	S	14	R	6	R	MLS _B c
98	0.047	27	S	14	R	21	S	24	S	27	S	24	S	-
99	0.094	25	S	29	S	20	S	21	S	28	S	19	S	-
106	0.094	26	S	13	R	20	S	21	S	26	S	23	S	-
107	0.064	26	S	14	R	21	S	21	S	10	R	23	S	M
111	0.064	26	S	15	R	22	S	24	S	25	S	24	S	-
112	0.064	22	S	14	R	21	S	24	S	16	R	19	R	MLS _B i
113	0.064	28	S	14	R	22	S	24	S	29	S	24	S	-
114	0.094	23	S	14	R	20	S	21	S	25	S	22	S	-
115	0.064	25	S	25	S	21	S	22	S	25	S	22	S	-
117	0.064	25	S	18	R	22	S	25	S	6	R	6	R	MLS _B c
118	0.094	27	S	23	S	22	S	24	S	27	S	24	S	-
119	0.094	24	S	14	R	21	S	21	S	28	S	25	S	-
121	0.125	23	S	12	R	20	S	20	S	25	S	23	S	-
122	0.094	27	S	15	R	24	S	25	S	14	R	21	R	MLS _B i
123	0.064	28	S	14	R	21	S	22	S	15	R	22	R	MLS _B i
124	0.032	26	S	13	R	22	S	25	S	6	R	6	R	MLS _B c
125	0.094	25	S	14	R	21	S	22	S	20	S	23	S	-
127	0.064	30	S	17	R	22	S	21	S	6	R	6	R	MLS _B c
128	0.094	25	S	18	R	20	S	22	S	25	S	17	S	-
129	0.094	25	S	28	S	22	S	22	S	28	S	24	S	-
130	0.064	27	S	12	R	21	S	23	S	28	S	25	S	-
131	0.064	26	S	13	R	22	S	22	S	28	S	24	S	-
138	0.047	28	S	16	R	20	S	24	S	28	S	25	S	-

141	0.094	28	S	18	R	23	S	25	S	17	R	25	S	M
146	0.094	25	S	11	R	19	S	22	S	6	R	6	R	MLS _B c
Strains derived from other adults (n = 44)														
50	0.094	25	S	14	R	20	S	26	S	27	S	25	S	-
63	0.064	27	S	19	R	22	S	24	S	21	S	19	S	-
76	0.032	28	S	17	R	23	S	25	S	28	S	27	S	-
93	0.064	26	S	13	R	22	S	19	S	27	S	25	S	-
97	0.064	27	S	16	R	22	S	23	S	30	S	25	S	-
100	0.094	25	S	13	R	22	S	23	S	15	R	22	R	MLS _B i
101	0.094	25	S	11	R	21	S	23	S	26	S	25	S	-
105	0.047	31	S	17	R	23	S	22	S	28	S	24	S	-
116	0.094	25	S	15	R	21	S	24	S	25	S	23	S	-
142	0.064	26	S	25	S	21	S	23	S	26	S	24	S	-
148	0.125	23	S	13	R	22	S	20	S	24	S	22	S	-
149	0.047	27	S	17	R	20	S	20	S	27	S	22	S	-
153	0.125	23	S	17	R	20	S	21	S	25	S	24	S	-
154	0.064	25	S	15	R	22	S	22	S	27	S	24	S	-
157	0.047	24	S	13	R	20	S	21	S	14	R	21	R	MLS _B i
159	0.047	28	S	12	R	22	S	24	S	6	R	6	R	MLS _B c
163	0.094	28	S	13	R	19	S	21	S	6	R	6	R	MLS _B c
165	0.064	26	S	26	S	24	S	22	S	28	S	25	S	-
168	0.094	25	S	11	R	20	S	21	S	14	R	13	R	MLS _B c
170	0,064	24	S	12	R	21	S	21	S	6	R	6	R	MLS _B c
171	0.064	26	S	14	R	21	S	24	S	26	S	23	S	-
172	0.064	27	S	13	R	27	S	24	S	6	R	6	R	MLS _B c
173	0.094	26	S	14	R	21	S	22	S	28	S	25	S	-
174	0.094	24	S	14	R	21	S	25	S	27	S	24	S	-
175	0.064	26	S	12	R	21	S	23	S	6	R	6	R	MLS _B c
177	0.094	26	S	13	R	22	S	24	S	29	S	26	S	-
179	0.064	26	S	14	R	19	S	23	S	14	R	21	R	MLS _B i
180	0.094	25	S	17	R	23	S	22	S	28	S	24	S	-
181	0.064	25	S	15	R	21	S	24	S	26	S	24	S	-
182	0.047	29	S	16	R	20	S	23	S	24	S	22	S	-
183	0.094	26	S	17	R	22	S	24	S	17	R	23	S	M
184	0.032	26	S	16	R	25	S	23	S	26	S	23	S	-
185	0.064	26	S	17	R	24	S	23	S	29	S	24	S	-
186	0.047	26	S	13	R	21	S	21	S	26	S	24	S	-
188	0.064	27	S	16	R	21	S	22	S	28	S	25	S	-
189	0.094	25	S	17	R	21	S	23	S	28	S	24	S	-
18	0.064	27	S	25	S	22	S	21	S	28	S	23	S	-
58	0.125	24	S	18	R	21	S	22	S	16	R	6	R	MLS _B c
77	0.064	26	S	12	R	21	S	22	S	6	R	6	R	MLS _B c
133	0.047	29	S	16	R	24	S	23	S	29	S	25	S	-
143	0.064	26	S	17	R	27	S	24	S	16	R	26	S	M
147	0.064	25	S	14	R	21	S	23	W	14	R	21	R	MLS _B i

161	0.064	27	S	19	R	22	S	26	S	31	S	25	S	-
164	0.094	25	S	15	R	22	S	26	S	26	S	24	S	-
Strains derived from newborn (n = 36)														
2	0.094	25	S	14	R	21	S	20	S	26	S	22	S	-
5	0.064	25	S	13	R	22	S	23	S	17	R	21	R	MLS _B c
16	0.125	25	S	15	R	22	S	22	S	15	R	24	S	M
25	0.047	29	S	16	R	22	S	24	S	30	S	26	S	-
26	0.094	25	S	18	R	25	S	24	S	28	S	24	S	-
28	0.064	26	S	18	R	21	S	24	S	29	S	24	S	-
34	0.094	24	S	16	R	20	S	21	S	25	S	22	S	-
38	0.125	22	S	8	R	19	S	20	S	25	S	22	S	-
55	0.064	25	S	16	R	21	S	23	S	25	S	24	S	-
56	0.047	22	S	15	R	19	S	20	S	21	S	22	S	-
79	0.064	26	S	13	R	22	S	21	S	22	S	25	S	-
85	0.064	26	S	14	R	22	S	20	S	12	R	6	R	MLS _B c
89	0.094	25	S	13	R	21	S	20	S	24	S	23	S	-
95	0.094	25	S	16	R	19	S	19	S	16	R	20	R	MLS _B i
102	0.064	25	S	27	S	25	S	25	S	28	S	25	S	-
120	0.094	24	S	13	R	20	S	20	S	6	R	6	R	MLS _B c
134	0.047	26	S	15	R	22	S	26	S	21	S	19	S	-
135	0.064	25	S	16	R	20	S	21	S	27	S	24	S	-
136	0.032	27	S	14	R	23	S	25	S	18	I	27	S	-
137	0.094	24	S	14	R	22	S	25	S	16	R	26	S	M
139	0.064	25	S	13	R	22	S	24	S	21	S	25	S	-
140	0.125	25	S	16	R	22	S	24	S	12	R	25	S	M
145	0.047	27	S	20	R	25	S	27	S	28	S	24	S	-
151	0.064	24	S	17	R	21	S	23	S	16	R	20	S	M
152	0.064	25	S	13	R	22	S	21	S	6	R	6	R	MLS _B c
155	0.047	24	S	16	R	19	S	22	S	25	S	22	S	-
156	0.064	25	S	12	R	21	S	23	S	27	S	24	S	-
160	0.094	26	S	13	R	20	S	19	S	28	S	24	S	-
167	0.047	23	S	15	R	20	S	21	S	24	S	21	S	-
187	0.125	23	S	12	R	19	S	20	S	6	R	6	R	MLS _B c
15	0.094	25	S	27	S	24	S	23	S	29	S	23	S	-
33	0.064	25	S	14	R	20	S	21	S	6	R	6	R	MLS _B c
39	0.064	25	S	16	R	22	S	24	S	28	S	26	S	-
84	0.047	27	S	14	R	23	S	20	S	10	R	6	R	MLS _B c
144	0.094	25	S	13	R	22	S	23	S	17	R	25	S	M
162	0.064	26	S	13	R	19	S	23	S	6	R	6	R	MLS _B c

P (1IU) - penicilin; TE (30 µg) - tetracycline; TGC (15 µg) - tigecycline; LEV (5 µg) - levofloxacin; E (15 µg) - erythromycin; DA (2 µg) – clindamycin; MLS_B c - constitutive mechanism of resistance to macrolides, lincosamides and streptogramin B; MLS_B i - inductive mechanism of resistance to macrolides, lincosamides and streptogramin B; M - M phenotype; (-) - no resistance mechanism;

*Diameter of the inhibition zone [mm] and interpretation of the results of susceptibility testing (accordance with the EUCAST and KORDL recommendations [61, 62]). S - susceptible ; R –resistant.

Supplementary Table S2. Results of serotyping and detection of virulence factors performed in the assessed strains

Serotype		The presence of genes encoding fimbriae				The presence of genes encoding surface proteins					Ability to form biofilm		
n		PI-1 +PI-2a	PI-1 +PI-2b	PI-2a	PI-2b	<i>epsilon</i>	<i>rib</i>	<i>alp2/3</i>	<i>bca</i>	<i>alp4**</i>	Weak	Moderate	Strong
Strains derived from pregnant women (n = 85)													
Ia	15	0	0	15	0	12	3	0	0	0	6	8	1
Ib	7	4	1	1	1	3	1	3	0	0	1	6	0
II	9	8	1	0	0	0	5	1	3	0	3	6	0
III	31	14	15	2	0	2	23	6	0	0	14	16	1
IV	1	0	1	0	0	1	0	0	0	0	1	0	0
V	19	18	0	1	0	0	3	16	0	0	9	10	0
VI	3	3	0	0	0	0	2	1	0	0	1	2	0
VII	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	85	47	18	19	1	18	37	27	3	0	35	48	2
Strains derived from other adults (n = 44)													
Ia	6	1	0	5	0	5	1	0	0	0	5	1	0
Ib	4	4	0	0	0	0	3	0	1	0	2	2	0
II	6	5	0	0	1	1	2	1	2	0	3	2	1
III	12	6	4	2	0	0	8	4	0	0	4	6	2
IV	1	0	1	0	0	1	0	0	0	0	1	0	0
V	14	10	2	2	0	0	5	9	0	0	5	8	1
VI	0	0	0	0	0	0	0	0	0	0	0	0	0
VII	1	0	1	0	0	0	0	1	0	0	1	0	0
Total	44	26	8	9	1	7	19	15	3	0	21	19	4
Strains derived from newborn (n = 36)													
Ia	9	1	0	8	0	9	0	0	0	0	6	3	0
Ib	1	1	0	0	0	0	0	0	1	0	0	1	0
II	4	3	0	0	1	1	2	0	1	0	2	2	0
III	11	2	8	0	1	0	9	2	0	0	5	6	0
IV	2	1	1	0	0	1	1	0	0	0	1	1	0
V	9	7	0	2	0	1	4	4	0	0	2	6	1
VI	0	0	0	0	0	0	0	0	0	0	0	0	0
VII	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	36	15	9	10	2	12	16	6	2	0	16	19	1

*The *alp4* gene was not found in the tested strains

Supplementary Table S3. Percentage of *Streptococcus agalactiae* strains with different biofilm forming capacity

Ability to form biofilm*	Isolates [n]	Isolates [%]
Not a biofilm producer	0	0.0
Weak biofilm producer	72	43.6
Moderate biofilm producer	86	52.1
Strong biofilm producer	7	4.2

*The tested strains were classified on the basis of the following criteria: not a biofilm producer: $OD \leq OD_c$, (all strains with OD values below 0,13); weak biofilm producer $OD_c < OD \leq 2 \times OD_c$, (all strains with OD values above 0,13 and below 0,26); moderate biofilm producer: $2 \times OD_c < OD \leq 4 \times OD_c$ (all strains with OD values above 0,26 and below 0,52); strong biofilm producer: $OD > 4 \times OD_c$ (all strains with OD values above 0,52). The mean optical density (OD) of the negative control +3 standard deviations of negative control was considered the cut-off ($OD_c = 0,13$).

Supplementary Table S4. Primer sets used for identification, serotyping and detection of virulence genes in *S. agalactiae*

	Starter	Primer sequence (5'→3')	Amplicon size (bp)	Annealing temp. (°C)	Reference
Identification					
GBS cAMP	F	TTTTCAACCAGCTGTATTAGAAGTA	155	48.0	[60]
	R	GTTCCCTGAACATTATCTTTGAT			
Serotyping					
Serotyp Ia <i>AB028896</i>	F	GGTCAGACTGGATTAATGGTATGC	521	57.0	[65]
	R	GTAGAAATAGCCTATATACGTTGAATGC			
Serotyp Ib <i>AB050723</i>	F	TAAACGAGAATGGAATATCACAAACC	770	57.0	[65]
	R	GAATTAACCTCAATCCCTAAACAATATCG			
Serotyp II <i>AY375362</i>	F	GAGAAGCCCAAGGATGGATT	188	49.2	DGFM
	R	TCCACTATGCTTTTGTA AAAATIGG			
Serotyp III <i>AF163833</i>	F	CCACATATGAGAATAAGACTTGC	187	57.0	[60]
	R	CCAACATACAGACCAATAGTATTC			
Serotyp IV <i>AF355776.1</i>	F	TTTGCTTGTGAGCTACCTGGA	314	52.0	DGFM
	R	TCTCCTCCCAATTTTCGTC			
Serotyp V <i>AF349539</i>	F	AACAGAGGCCAATCAGTTGC	247	57.0	DGFM
	R	CTTCCAACGCCACCTCTAAA			
Serotyp VI <i>AF337958.1</i>	F	GGACTTGAGATGGCAGAAGGT	523	54.0	DGFM
	R	CTCGTACAACTTCGCTTCCAC			
Serotyp VII <i>AY376403</i>	F	GGAGAGAACAATGTCCAGATTACG	369	54.0	DGFM
	R	AGCTGGTCGTGATTTCTACACA			
Virulence genes					
PI-1 <i>EU929540.1</i>	F	AACCACTAGCAGGCGTTGTCTTTG	298	60.0	This work
	R	TGAGCCCGGAAATTCTGATATGCC			
PI-2a <i>EU929374.1</i>	F	GCCGTTAGATGTTGTCTTCGTA CT	575	60.0	This work
	R	TTTACTGCGGTCCCAAGAGCTTC			
PI-2b <i>EU929426.1</i>	F	AAGTCTTGACCAAGGATACGACGC	314	60.0	This work
	R	ATCGTGTTACTTGCCCTGCGTA			
Universal	F	TGATACTTCACAGACGAAACAACG	-	58.0	[22]
Alpha-C	R	TACATGTGGTAGTCCATCTTCACC	398	58.0	[22]
Rib	R	CATACTGAGCTTTTAAATCAGGTGA	295	58.0	[22]
Epsilon	R	CCAGATACATTTTTACTAAAGCGG	200	58.0	[22]
Alp2/3	R	CACTCGGATTACTATAATTTTAGCAC	334	58.0	[22]

DGFM - primers from a set of primers designed at the Department of Genetics and Pharmaceutical Microbiology.

The primers used in PCR reactions were designed using the Primer3 program ([www.http://bioinfo.ut.ee/primer3-0.4.0/](http://bioinfo.ut.ee/primer3-0.4.0/)) and checked for the relevant parameters using the Primer-BLAST function (Basic Local Alignment Search Tool) available from the NCBI (National Center for Biotechnology Information). The specificity of the primers was checked in the BLAST Assembled Ref Genomes program <https://blast.ncbi.nlm.nih.gov/Blast.cgi>.

To identify serotypes II, IV, VI and VII, single PCR reactions were carried out, whereas PCR multiplexes were optimized for serotypes Ia, Ib, III and V. Genes encoding various types of fimbriae (PI-1, PI-2a, PI-2b) were detected using single standard PCR reactions. In contrast, the identification of genes encoding surface proteins from the Alp family was carried out in the multiplex PCR reaction using a modified methodology described by Creti R et al. [22]. Also to identify genes encoding fibrinogen-binding proteins, a multiplex PCR reaction was used.