

## Additional File 2

All the genes discussed in this article are all summarized below (Table S1).

**Table S1: The oligonucleotide primers used for amplifying various genes in GBS**

Primer Name	Name of Gene	Primer sequence (5' to 3')	Expected Product size	Reference
<i>atr</i>		F- CAA CGA TTC TCT CAG CTT TGT TAA R- TAA GAA ATC TCT TGT GCG GAT TTC	780	(28)
IS1548		F- TTG CGC AGT TGA ATT GGA TAG R- TTC TCT AAC TTC AAT CTG TCC CCT A	690	(7)
<i>bac</i>	beta/β antigens of the C protein	F- CTA TTT TTG ATA TTG ACA ATG CAA R- GTC GTT ACT TCC TTG AGA TGT AAC	592	(7)
<i>bca</i>	alpha/α antigens of the C protein	F- TAA CAG TTA TGA TAC TTC ACA GAC R- ACG ACT TTC TTC CGT CCA CTT AGG	535	(7)
<i>hly</i>	Hyaluronate lyase	F- TCC ATT TAA AGC CCT TGG TG R- GGC GCC AGT ATA AGC AAC AT	199	(7)
<i>rib</i>	Surface protein Rib	F- CAG GAA GTG CTG TTA CGT TAA AC R- CGT CCC ATT TAG GGT CTT CC	369	(7)
<i>scpB</i>	C5a peptidase	F- ACA ACG GAA GGC GCT ACT GTT C R- ACC TGG TGT TTG ACC TGA ACT A	255	(27)
<i>ermB</i>	Erythromycin Ribosomal Methylase	F- GAA AAG GTA CTC AAC CAA ATA R- AGT AAC GGT ACT TAA ATT GTT TAC	640	(12)
<i>ermTR</i>	Erythromycin Ribosomal Methylase	F- GAA GTT TAG CTT TCC TAA R- GCT TCA GCA CCT GTC TTA ATT GAT	400	(12)
<i>mefA</i>	Erythromycin resistance efflux pump	F- AGT ATC ATT AAT CAC TAG TGC R- TTC TTC TGG TAC TAA AAG TGG	348	(12)
<i>linB</i>	Lincosamide nucleotidyltransferases	F- CCT ACC TAT TGT TTG TGG AA R- ATA ACG TTA CTC TCC TAT TC	944	(12)
<i>tetM</i>	Tetracycline Resistance	F- GTG GAG TAC TAC ATT TAC GAG R- GAA GCG GAT CAC TAT CTG AG	359	(23)
<i>tetO</i>	Tetracycline Resistance	F- GCG GAA CAT TGC ATT TGA GGG R- CTC TAT GGA CAA CCC GAC AGA AG	538	(23)

All the primers used in this study were from Inqaba biotech, South Africa

**Table S2: Presence or absence of the expected amplicons in the 43 GBS isolates**

No.	Sample #	Housekeeping gene	MGE		Virulence Genes					Antibiotic Resistance Genes				
			<i>atr</i>	IS1548	<i>rib</i>	<i>hly</i>	<i>scpB</i>	<i>bca</i>	<i>bac</i>	<i>tetM</i>	<i>tetO</i>	<i>ermB</i>	<i>ermTR</i>	<i>mefA</i>
1	014	+	-	-	+	+	+	-	+	-	-	-	-	-
2	19	+	-	+	+	+	+	-	+	-	-	+	-	-
3	22	+	-	+	+	-	+	-	+	-	-	-	-	-
4	27	+	-	+	+	+	+	-	+	-	-	-	-	-
5	31	+	-	+	+	+	+	-	+	-	+	-	-	-
6	36	+	-	+	+	+	+	-	+	-	-	-	-	-
7	55	+	+	+	-	+	+	-	+	-	-	-	-	-
8	64	+	-	+	+	+	+	-	+	-	-	-	-	-
9	66	+	-	+	+	+	+	-	+	-	-	-	-	-
10	124	+	-	+	+	+	+	-	+	+	-	-	-	-
11	127	+	-	+	+	+	-	-	+	-	-	-	-	-
12	131	+	-	-	+	+	+	-	+	-	+	-	-	-
13	132	+	-	+	+	-	+	-	+	-	-	-	-	-
14	133	+	-	-	+	-	+	-	+	-	-	-	-	-
15	140	+	+	+	+	+	+	-	-	-	-	-	-	-
16	157	+	-	-	+	+	+	+	+	-	-	-	-	-
17	164	+	-	-	+	+	+	+	+	-	+	-	-	-
18	173	+	+	+	+	+	+	-	+	-	-	-	-	-
19	182	+	-	+	+	+	+	-	+	-	-	-	-	-
20	192	+	-	+	+	+	-	-	+	-	+	-	-	-
21	205	+	-	+	+	+	-	-	+	-	-	+	-	-
22	209	+	-	+	+	+	-	-	+	-	-	-	-	-
23	210	+	-	+	+	+	+	-	+	-	-	-	-	-
24	220	+	-	+	+	+	+	-	+	-	-	-	-	-
25	231	+	-	+	+	+	+	-	+	-	-	-	-	-
26	241	+	-	+	+	+	+	-	+	-	-	-	-	-
27	243	+	-	+	+	+	-	-	+	-	-	+	-	-
28	267	+	-	-	+	+	+	+	+	-	-	-	-	-
29	282	+	-	+	+	+	+	-	+	-	-	-	-	-
30	288	+	-	-	+	+	+	-	+	-	-	-	-	-
31	298	+	-	+	+	+	+	-	+	-	-	-	-	-
32	300	+	-	+	+	-	+	-	+	-	-	-	-	-
33	307	+	-	+	+	+	+	-	+	-	-	-	-	-
34	315	+	+	+	+	+	+	-	+	-	+	-	+	-
35	322	+	-	+	+	+	+	+	+	-	-	-	+	-
36	325	+	-	-	+	+	+	+	+	-	+	-	-	-
37	328	+	-	-	+	+	+	-	+	-	-	-	-	-
38	363	+	-	+	+	+	-	-	+	-	+	-	-	-
39	368	+	-	-	+	+	+	-	+	-	-	-	-	-
40	375	+	-	+	+	+	+	-	+	-	+	-	-	-
41	398	+	-	-	+	+	+	-	+	-	+	-	-	-
42	407	+	-	-	+	+	+	-	+	-	+	-	-	-
43	411	+	-	-	+	+	+	-	+	-	-	+	-	-

Presence (+) or absence (-) of the expected amplicon during the PCRs.

**Table S3: Presence or absence of antibiotic resistance genes in the resistant and intermediate isolates**

Number	Sample #	CLN (2µg)	<i>linB</i>	ERY (15µg)	<i>ermB</i>	<i>ermTR</i>	<i>mefA</i>	TET (30µg)	<i>tetM</i>	<i>tetO</i>
1	014	R	-	I	-	-	-	R	+	-
2	19			R	-	+	-	R	+	-
3	22	R	-					R	+	-
4	27	R	-	I	-	-	-	R	+	-
5	31	R	-	R	+	-	-	R	+	-
6	36	R	-	R	-	-	-	R	+	-
7	55	R	-	I	-	-	-	R	+	-
8	64	R	-	R	-	-	-	R	+	-
9	66	I	-	I	-	-	-	R	+	-
10	124	R	-	I	-	-	-	R	+	+
11	127	I	-							
12	131	R	-	R	+	-	-	R	+	-
13	132							R	+	-
14	133							R	+	-
15	140	I	-	I	-	-	-	R	-	-
16	157	I	-					R	+	-
17	164	R	-	R	+	-	-	R	+	-
18	173	R	-	R	-	-	-	R	+	-
19	182							R	+	-
20	192	R	-	R	+	-	-	R	+	-
21	205	I	-	R	-	+	-	R	+	-
22	209	I	-	I	-	-	-	R	+	-
23	210	R	-					R	+	-
24	220	I	-					R	+	-
25	231	R	-	I	-	-	-	R	+	-
26	241	R	-	I	-	-	-	R	+	-
27	243							R	+	-
28	267							R	+	-
29	282	I	-					R	+	-
30	288	R	-					R	+	-
31	298			I	-	-	-	R	+	-
32	300	R	-	R	-	-	-	R	+	-
33	307	R	-	I	-	-	-	R	+	-
34	315	R	-	R	+	-	+	R	+	-
35	322							R	+	-
36	325	R	-	I	+	-	-	R	+	-
37	328	I	-					R	+	-
38	363	R	-	R	+	-	-	R	+	-
39	368	I	-	I	-	-	-	R	+	-
40	375	R	-	R	+	-	-	R	+	-
41	398	R	-	I	+	-	-	R	+	-
42	407	I	-	I	+	-	-	R	+	-
43	411	R	-	I	-	+	-	R	+	-

Presence (+) or absence (-) of the expected amplicon during the PCRs.

**Table S4: Disk diffusion zone diameters and E-test results for the 43 GBS isolates**

Sample #	Disk Diffusion (mm)									E-test 0.006-256 (µg/ml)		
	10µg AMP	10 Units PEN	30µg CEFA	30µg VAN	15µg ERY	2µg CLN	30µg CHL	30µg CRO	30µg TET	E- AMP	E- VAN	E-CHL
14	R (21)	S (28)	R (12)	S (17)	I (17)	R (12)	I (19)	R (23)	R (0)	R (0.75)	R (3)	S (1.5)
19	S (26)	S (28)	S (30)	S (18)	R (0)	S (19)	I (20)	S (27)	R (0)	S (0.064)	S (0.75)	S (2)
22	R (22)	R (20)	R (12)	S (17)	S (22)	R (12)	S (22)	S (26)	R (0)	R (0.75)	R (4)	S (1.5)
27	R (20)	R (17)	R (12)	R 14	I (20)	R (0)	I (18)	R (0)	R (0)	R (0.75)	R (3)	I (12)
31	R (18)	R (15)	R (12)	S (20)	R (0)	R (0)	S (22)	R (16)	R (0)	R (0.50)	S (1.0)	S (2)
36	R (20)	R (22)	R (11)	R (16)	R (8)	R (0)	S (22)	S (25)	R (0)	R (0.50)	R (4)	S (4)
55	R (21)	R (16)	R (12)	S (17)	I (16)	R (0)	S (23)	S (30)	R (0)	R (0.50)	S (1.0)	S (0.38)
64	R (20)	R (17)	R (10)	S (17)	R (13)	R (0)	R (0)	R (0)	R (0)	R (0.50)	R (4)	S (1.5)
66	R (18)	R (17)	R (0)	R (16)	I (16)	I (16)	R (16)	R (21)	R (0)	R (0.50)	S (1.0)	S (1.0)
124	S (25)	R (18)	R (12)	S (19)	I (16)	R (0)	I (19)	R (14)	R (5)	S (0.25)	R (2)	S (1.5)
127	S (26)	S (24)	S (27)	S (18)	S (21)	I (18)	S (22)	S (30)	S (28)	S (0.064)	S (0.38)	S (1.5)
131	R (23)	R (17)	R (11)	R (15)	R (0)	R (0)	I (18)	S (24)	R (0)	R (0.75)	R (3)	S (0.38)
132	S (26)	R (21)	S (25)	S (20)	S (25)	S (24)	S (25)	S (30)	R (0)	S (0.094)	S (1.0)	S (0.50)
133	R (0)	R (0)	R (10)	R (16)	S (23)	S (20)	S (24)	S (27)	R (13)	S (0.25)	R (1.5)	S (1.0)
140	S (26)	S (27)	S (27)	S (17)	I (17)	I (17)	R (17)	S (25)	R (12)	S (0.023)	S (0.75)	S (2)
157	S (25)	S (27)	S (27)	S (17)	S (22)	I (18)	I (18)	R (22)	R (0)	S (0.032)	S (1.0)	S (1.0)
164	R (18)	R (17)	R (12)	R (16)	R (0)	R (0)	I (18)	S (30)	R (10)	R (0.50)	R (2)	S (1.5)
173	R (21)	R (20)	R (13)	S (18)	R (15)	R (15)	R (17)	R 23	R (7)	R (0.75)	S (0.25)	S (0.50)
182	S (29)	S (30)	S (24)	S (19)	S (26)	S (20)	I (20)	S (24)	R (0)	S (0.016)	S (0.38)	S (4)
192	R (23)	R (17)	R (12)	R (16)	R (8)	R (0)	R (8)	S (26)	R (0)	R (0.75)	R (1.5)	S (2)
205	R (22)	R (16)	R (11)	S (18)	R (10)	I (17)	I (19)	R (23)	R (9)	S (0.064)	S (1.0)	S (1.5)
209	R (22)	R (18)	R (10)	S (18)	I (20)	I (18)	I (20)	S (30)	R (0)	R (0.75)	S (1.0)	S (1.5)
210	S (29)	S (29)	S (28)	S (17)	S (21)	R (15)	S (26)	S (26)	R (6)	S (0.023)	S (0.38)	S (1.5)
220	R (20)	R (15)	R (11)	S (17)	S (24)	I (18)	R (16)	S (27)	R (0)	R (0.75)	S (1.0)	S (0)
231	R (16)	R (15)	R (8)	R (14)	I (17)	R (0)	R (13)	R (0)	R (0)	R (0.75)	R (4)	I (12)

241	S (25)	R (20)	R (12)	S (17)	I (18)	R (0)	R (17)	R (0)	R (0)	S (0.064)	S (0.50)	S (2)
243	S (30)	S (29)	S (29)	S (19)	S (22)	S (19)	I (20)	S (30)	R (0)	S (0.047)	S (0.38)	S (1.5)
267	S (30)	S (28)	S (27)	S (25)	S (22)	S (24)	S (23)	S (24)	R (6)	S (0.016)	S (0.25)	S (1.5)
282	R (17)	R (17)	R (14)	R (16)	S (25)	I (18)	I (20)	S (26)	R (0)	R (1.0)	R (4)	S (4)
288	R (20)	R (15)	R (11)	S (18)	S (25)	R (9)	S (23)	R (22)	R (12)	S (0.12)	S (0.25)	S (0.38)
298	S (25)	R (23)	R (22)	S (18)	I (20)	S (22)	I (20)	S (27)	R (0)	R (0.5)	S (0.38)	S (1.0)
300	S (24)	R (23)	S (29)	S (18)	R (15)	R (15)	R (15)	R (23)	R (0)	S (0.064)	S (0.75)	S (4)
307	R (22)	R (16)	R (10)	R (16)	I (20)	R (15)	R (17)	R (21)	R (0)	R (0.75)	R (8)	S (2)
315	S (24)	S (25)	R (21)	S (18)	R (12)	R (15)	R (15)	R (17)	R (0)	S (0.064)	S (0.38)	S (2)
322	S (27)	S (26)	R (14)	S (17)	S (21)	S (20)	S (22)	S (30)	R (12)	S (0.19)	S (0.75)	S (0.75)
325	R (21)	R (17)	R (12)	S (17)	I (16)	R (0)	R (17)	R (0)	R (0)	S (0.064)	S (0.75)	S (1.0)
328	S (27)	S (25)	S (27)	S (18)	S (22)	I (17)	S (23)	R (22)	R (14)	S (0.047)	S (1.0)	S (1.5)
363	R (22)	R (17)	R (8)	R (16)	R (0)	R (0)	R (0)	R (15)	R (0)	S (0.125)	R (2)	S (0.125)
368	R (20)	R (18)	R (12)	R (16)	I (20)	I (17)	I (20)	S (25)	R (0)	R (0.75)	R (2)	S (1.5)
375	S (30)	S (28)	R (18)	S (18)	R (0)	R (0)	R (0)	S (24)	R (0)	S (0.064)	S (0.75)	S (3)
398	R (21)	R (16)	R (8)	R (10)	I (16)	R (0)	R (17)	R (0)	R (0)	R (4)	R (1.5)	S (1.5)
407	S (25)	R (14)	R (8)	S (17)	I (20)	I (18)	I (18)	S (24)	R (0)	S (0.19)	S (1.0)	S (0.25)
411	R (20)	R (23)	S (24)	S (18)	I (16)	R (14)	I (18)	R (22)	R (0)	S (0.04)	S (0.75)	S (4)
<b>CLSI 2017 ranges</b>	$\geq 24$ (S)	$\geq 24$ (S)	$\geq 24$ (S)	$\geq 17$ (S)	$\geq 21$ (S), 16-20 (I), $\leq 15$ (R)	$\geq 19$ (S), 16-18 (I), $\leq 15$ (R)	$\geq 21$ (S), 18-20 (I), $\leq 17$ (R)	$\geq 24$ (S)	$\geq 23$ (S), 19-22 (I), $\leq 18$ (R)	$\leq 0.25$ (S)	$\leq 1$ (S)	$\leq 4$ (S), 8 (I), $\geq 16$ (R)
<b><i>S.pneumonia</i> ATCC 49619</b>	30	30	30	20	28	22	22	35	22	0.025	0.25	2
<b>Control Ranges and Comment</b>	30-36 Pass	24-30 Pass		20-27 Pass	25-30 Pass	19-25 Pass	23-27 Pass	30-35 Pass	27-31 Pass	0.06- 0.25 Pass	0.12- 0.5 Pass	2-8 Pass

## Spearman Rank Correlations

Table S4 below shows that there was a negative correlation between *tetO* and all the macrolide resistant determinants; between *ermB* and *ermTR* and also between *ermTR* and *mefA*. A positive correlation was observed between the *tetM* and all the macrolide resistant determinants (such as *ermB*); between *tetM* and *tetO* and also between *ermB* and *mefA*. However, the P values for all these correlations are greater than the level of significance implying that correlations are not statistically significant, meaning they have occurred by chance. Hence, they cannot be generalized into the general GBS population. The *linB* gene could not be computed because it was a constant. Table 3 also shows that there is a negative correlation between *hly* and IS1548 as well as between *bac* and *rib* and this is statistically significant at ( $p < 0.01$ ). These relationships can be generalized into the pregnant women GBS population. However, some non-statistically significant positive correlations were also observed between IS1548 and (*rib/scpB/bca*) as well as *bac* and (*hly/scpB/bca*). The remainder of the genes had a negative correlation which was also not statistically significant.

**Table S5: GBS Virulence and Antibiotic Resistance Genes Spearman Rank Correlations**

<b>Virulence Genes</b>	<b>IS1548</b>	<b><i>rib</i></b>	<b><i>hly</i></b>	<b><i>scpB</i></b>	<b><i>bca</i></b>	<b><i>bac</i></b>
<b>IS1548</b>	1	0.18	-.563**	0.088	0.11	-0.099
<b><i>rib</i></b>	0.18	1	-0.102	-0.036	-0.265	-.393**
<b><i>hly</i></b>	-.563**	-0.102	1	-0.049	-0.062	0.056
<b><i>scpB</i></b>	0.088	-0.036	-0.049	1	-0.129	0.116
<b><i>bca</i></b>	0.11	-0.265	-0.062	-0.129	1	0.146
<b><i>bac</i></b>	-0.099	-.393**	0.056	0.116	0.146	1
<b>Antibiotic Resistance Genes</b>	<b><i>tetM</i></b>	<b><i>tetO</i></b>	<b><i>ermB</i></b>	<b><i>ermTR</i></b>	<b><i>mefA</i></b>	<b><i>linB</i></b>
<b><i>tetM</i></b>	1	0.024	0.085	0.049	0.034	.a
<b><i>tetO</i></b>	0.024	1	-0.085	-0.049	-0.034	.a
<b><i>ermB</i></b>	0.085	-0.085	1	-0.176	0.14	.a
<b><i>ermTR</i></b>	0.049	-0.049	-0.176	1	-0.071	.a
<b><i>mefA</i></b>	0.034	-0.034	0.14	-0.071	1	.a
<b><i>linB</i></b>	.a	.a	.a	.a	.a	.a

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

a Cannot be computed because at least one of the variables is const