**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** |
| *atr* |  | F- CAA CGA TTC TCT CAG CTT TGT TAA  R- TAA GAA ATC TCT TGT GCG GAT TTC | 780 | (30) |
| IS1548 |  | F- TTG CGC AGT TGA ATT GGA TAG  R- TTC TCT AAC TTC AAT CTG TCC CCT A | 690 | (9) |
| *bac* | 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 | (9) |
| *bca* | 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 | (9) |
| *hly* | Hyaluronate lyase | F- TCC ATT TAA AGC CCT TGG TG  R- GGC GCC AGT ATA AGC AAC AT | 199 | (9) |
| *rib* | Surface protein Rib | F- CAG GAA GTG CTG TTA CGT TAA AC  R- CGT CCC ATT TAG GGT CTT CC | 369 | (9) |
| *scpB* | C5a peptidase | F- ACA ACG GAA GGC GCT ACT GTT C  R- ACC TGG TGT TTG ACC TGA ACT A | 255 | (29) |
| *ermB* | Erythromycin Ribosomal Methylase | F- GAA AAG GTA CTC AAC CAA ATA  R- AGT AAC GGT ACT TAA ATT GTT TAC | 640 | (14) |
| *ermTR* | Erythromycin Ribosomal Methylase | F- GAA GTT TAG CTT TCC TAA  R- GCT TCA GCA CCT GTC TTA ATT GAT | 400 | (14) |
| *mefA* | Erythromycin resistance efflux pump | F- AGT ATC ATT AAT CAC TAG TGC  R- TTC TTC TGG TAC TAA AAG TGG | 348 | (14) |
| *linB* | Lincosamide nucleotidyltransferases | F- CCT ACC TAT TGT TTG TGG AA  R- ATA ACG TTA CTC TCC TAT TC | 944 | (14) |
| *tetM* | Tetracycline Resistance | F- GTG GAG TAC TAC ATT TAC GAG  R- GAA GCG GAT CAC TAT CTG AG | 359 | (25) |
| *tetO* | Tetracycline Resistance | F- GCG GAA CAT TGC ATT TGA GGG  R- CTC TAT GGA CAA CCC GAC AGA AG | 538 | (25) |

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 | | | | | |
|  |  | ***atr*** | **IS1548** | ***rib*** | ***hly*** | ***scpB*** | ***bca*** | ***bac*** | ***tetM*** | ***tetO*** | ***ermB*** | ***ermTR*** | ***mefA*** | ***linB*** |
| 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  (2 µg) | *linB* | ERY (15 µg) | *ermB* | *ermTR* | *mefA* | TET (30 µg) | *tetM* | *tetO* |
| 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**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Disk Diffusion (mm) | | | | | | | | | E-test  0.006-256 (μg/ml) | | |
| Sample # | **10 µg AMP** | **10 Units PEN** | **30 µg CEFA** | **30 µg VAN** | **15 µg ERY** | **2 µg CLN** | **30 µg CHL** | **30 µg CRO** | **30 µg 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) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| CLSI 2017 ranges | ≥24 (S) | ≥24 (S) | ≥24 (S) | ≥17 (S) | ≥21 (S),  16-20 (I),  ≤15 (R) | ≥19 (S),  16-18 (I),  ≤15 (R) | ≥21 (S),  18-20 (I),  ≤17 (R) | ≥24 (S) | ≥23 (S),  19-22 (I),  ≤18 (R) | ≤0.25 (S) | ≤1 (S) | ≤4 (S),  8 (I), ≥16 (R) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| *S.pneumonia* ATCC 49619 | 30 | 30 | 30 | 20 | 28 | 22 | 22 | 35 | 22 | 0.025 | 0.25 | 2 |
| Control Ranges and Comment | 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**

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