**Additional File 2: Table S2.** Number and toxigenicity of isolates collected between 1996 – 2014 in Belarus and those selected for genomic analyses in this study

| | Total isolates | | | Selected for this study | | |
|---|---|---|---|---|---|---|
| **Year** | **No. of isolates** | **tox+ (%)** | **tox- (%)** | **No. of isolates** | **tox+ (%)** | **tox- (%)** |
| 1996 | 486 | 47.12 | 52.88 | 6 | 50.00 | 50.00 |
| 1997 | 410 | 36.34 | 63.66 | 8 | 50.00 | 50.00 |
| 1998 | 362 | 19.61 | 80.39 | 8 | 37.50 | 62.50 |
| 1999 | 339 | 30.68 | 69.32 | 6 | 66.67 | 33.33 |
| 2000 | 403 | 29.78 | 70.22 | 3 | 33.33 | 66.67 |
| 2001 | 408 | 13.48 | 86.52 | 5 | 40.00 | 60.00 |
| 2002 | 310 | 8.06 | 91.94 | 5 | 20.00 | 80.00 |
| 2003 | 213 | 7.51 | 92.49 | 3 | 66.67 | 33.33 |
| 2004 | 290 | 19.31 | 80.69 | 8 | 37.50 | 62.50 |
| 2005 | 323 | 6.19 | 93.81 | 3 | 66.67 | 33.33 |
| 2006 | 201 | 3.48 | 96.52 | 9 | 55.56 | 44.44 |
| 2007 | 204 | 2.94 | 97.06 | 7 | 42.86 | 57.14 |
| 2008 | 158 | 5.70 | 94.30 | 5 | 40.00 | 60.00 |
| 2009 | 98 | 3.06 | 96.94 | 3 | 33.33 | 66.67 |
| 2010 | 77 | 5.19 | 94.81 | 4 | 50.00 | 50.00 |
| 2011 | 54 | 0.00 | 100.00 | 3 | 0.00 | 100.00 |
| 2012 | 21 | 0.00 | 100.00 | 2 | 0.00 | 100.00 |
| 2013 | 10 | 0.00 | 100.00 | 2 | 0.00 | 100.00 |
| 2014 | 15 | 0.00 | 100.00 | 2 | 0.00 | 100.00 |

**Additional File 2: Table S3.** Distribution of isolates from epidemic (≤ year 2000) and post-epidemic period (≥ year 2001) in major groups. All minor groups are pooled together for statistical analysis.

| Sampling period | Groups | | | Total |
|---|---|---|---|---|
| | ST5 | ST8 | Other | |
| Epidemic period | 10 | 13 | 9 | 32 |
| Post-epidemic period | 27 | 21 | 13 | 61 |
| Total | 37 | 34 | 22 | 93 |

**Chi square statistics on above distribution:**

| | Value | df | Asymptotic Significance (2-sided) | Monte Carlo Sig. (2-sided) | | |
|---|---|---|---|---|---|---|
| | | | | Significance | 99% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Pearson Chi-Square | 1.526[a] | 2 | 0.466 | .533[b] | 0.52 | 0.546 |
| Likelihood Ratio | 1.548 | 2 | 0.461 | .533[b] | 0.52 | 0.546 |
| Fisher's Exact Test | 1.579 | | | .533[b] | 0.52 | 0.546 |
| N of Valid Cases | 93 | | | | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.20.

**b.** b. Based on 10000 sampled tables with starting seed 1314643744.

**Additional File 2: Table S4.** Distribution of isolates from asymptomatic carriage, diphtheria and sore throat patients in major groups. All minor groups are pooled together for statistical analysis.

| Groups | Clinical information | | | Total |
|---|---|---|---|---|
| | Asymptomatic | Diphtheria | sore throat | |
| ST5 | 7 | 1 | 29 | 37 |
| ST8 | 10 | 18 | 6 | 34 |
| Other | 5 | 7 | 10 | 22 |
| Total | 22 | 26 | 45 | 93 |

**Chi square statistics on above distribution:**

| | Value | df | Asymptotic Significance (2-sided) | Monte Carlo Sig. (2-sided) | | |
|---|---|---|---|---|---|---|
| | | | | Significance | 99% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| Pearson Chi-Square | 30.542[a] | 4 | 0 | .000[b] | 0.000 | 0.000 |
| Likelihood Ratio | 35.572 | 4 | 0 | .000[b] | 0.000 | 0.000 |
| Fisher's Exact Test | 33.655 | | | .000[b] | 0.000 | 0.000 |
| N of Valid Cases | 93 | | | | | |

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.20.

**b.** Based on 10000 sampled tables with starting seed 299883525.

**Additional File 2: Table S5.** Allelic variation in the direct repeat and spacer sequences among the CRISPR loci of ST8 and ST5 isolates

| Strain | Year | Biovar | Clinical information | Country | DRA type | DRA locus | DRB type | DRB locus |
|---|---|---|---|---|---|---|---|---|
| 1480 | 1999 | Gravis | Diphtheria | Gomel | CT2-1 | D1-S111-S108 | CT1E-12 | D4-S97-S7-S65-S82-S8-S17-S58-S106-S54-S19-S62-S86-S42-S98-S46-S70-S64-S66-S91 |
| 4905 | 2008 | Gravis | Sore throat | Mogilev | CT2-2 | D1-S112-S27-S80-S103-S41-S111-S107 | CT1E-1 | D4-S97-S17-S58-S106-S54-S28-S2-S85-S71-S28-S2-S86-S85-S71-S61-S42-S98-S46-S70-S64-S5-S8 |
| 2637 | 2002 | Gravis | Asymptomatic | Minsk | CT2-3 | D1-S13-S27-S80-S103-S41-S111-S107 | CT1E-20 | D5-S95-S53-S57-S32-S39-S83-S48-S72-S14-S115-S78-S20-S74-S90-S94-S26-S18-S81-S24-S29-S43-S38 |
| 4601 | 2007 | Gravis | Asymptomatic | Vitebsk | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-4 | D4-S97-S65-S5-S8-S17-S58-S106-S54-S19-S28-S42-S98-S46-S70-S64-S66-S91-S7 |
| 300 | 1996 | Gravis | Diphtheria | Mogilev | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 1270 | 1998 | Gravis | Diphtheria | Gomel | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 1457 | 1999 | Gravis | Diphtheria | Minsk | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 1854 | 2000 | Gravis | Asymptomatic | Minsk | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 4031 | 2005 | Gravis | Diphtheria | Vitebsk | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-7 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 822 | 1997 | Gravis | Diphtheria | Minsk | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-9 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S86-S42-S98-S46-S70-S64-S66-S91 |
| 2173 | 2001 | Gravis | Diphtheria | Mogilev | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-10 | D4-S97-S7-S65-S82-S8-S17-S58-S106-S54-S19-S28-S86-S2-S85-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 3058 | 2004 | Gravis | Asymptomatic | Grodno | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-11 | D4-S97-S7-S65-S82-S8-S17-S58-S106-S54-S19-S28-S86-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 72 | 1996 | Gravis | Diphtheria | Mogilev | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-14 | D5-S26-S48-S72-S14-S115-S78-S74-S90-S94-S18-S81-S24-S29-S57-S32-S39-S83 |
| 1052 | 1998 | Gravis | Diphtheria | Gomel | CT2-4 | D1-S27-S80-S103-S41-S111-S107 | CT1E-20 | D5-S95-S53-S57-S32-S39-S83-S48-S72-S14-S115-S78-S20-S74-S90-S94-S26-S18-S81-S24-S29-S43-S38 |
| 2792 | 2003 | Gravis | Asymptomatic | Mogilev | CT2-5 | D1-S27-S80-S41-S111-S107 | CT1E-19 | D5-S60-S26-S18-S81-S24-S29-S43-S38-S53-S57-S32-S12-S39-S83-S48-S72-S14-S115-S78-S74-S90-S94-S6-S9-S95-S12-S9-S95-S20 |
| 4014 | 2005 | Gravis | Asymptomatic | Minsk | CT2-6 | D1-S34-S37-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 4938 | 2009 | Gravis | Diphtheria | Minsk | CT2-6 | D1-S34-S37-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 4728 | 2007 | Gravis | Asymptomatic | Minsk | CT2-7 | D1-S37-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 5073 | 2010 | Gravis | Diphtheria | Minsk | CT2-7 | D1-S37-S27-S80-S103-S41-S111-S107 | CT1E-18 | D5-S60-S26-S18-S53-S57-S32-S39-S83-S48-S72-S14-S12-S115-S78-S74-S90-S94-S6-S9-S95-S12-S9-S95-S20 |
| 5052 | 2010 | Gravis | Asymptomatic | Minsk | CT2-7 | D1-S37-S27-S80-S103-S41-S111-S107 | CT1E-6 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |

Additional File 2: Table S5 continues…

| Strain | Year | Biovar | Clinical information | Country | DRA type | DRA locus | DRB type | DRB locus |
|---|---|---|---|---|---|---|---|---|
| 4800 | 2008 | Gravis | Diphtheria | Minsk | CT2-7 | D1-S37-S27-S80-S103-S41-S111-S107 | CT1E-20 | D5-S95-S53-S57-S32-S39-S83-S48-S72-S14-S115-S78-S20-S74-S90-S94-S26-S18-S81-S24-S29-S43-S38 |
| 1072 | 1998 | Gravis | Diphtheria | Minsk | CT2-8 | D1-S44-S27-S80-S103-S41-S111-S107 | CT1E-13 | D4-S97-S7-S65-S82-S8-S17-S58-S106-S54-S19-S86-S42-S98-S46-S70-S64-S66-S91 |
| NCTC 13129 | 1997 | Gravis | Diphtheria | UK | CT2-9 | D1-S89-S27-S80-S103-S41-S111-S107 | CT1E-5 | D4-S97-S65-S82-S8-S17-S58-S106-S54-S19-S28-S2-S86-S85-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S91-S91-S7 |
| 933a | 1997 | Gravis | Sore throat | Minsk | CT2-10 | D1-S45-S113-S110 | CT1E-19 | D5-S60-S26-S18-S81-S24-S29-S43-S38-S53-S57-S32-S12-S39-S83-S48-S72-S14-S115-S78-S74-S90-S94-S6-S9-S95-S12-S9-S95-S20 |
| 2789 | 2003 | Gravis | Sore throat | Grodno | CT2-11 | D3-S33-S3-S21-S63-S92-S55 | CT1E-10 | D4-S97-S7-S65-S82-S8-S17-S58-S106-S54-S19-S28-S86-S2-S85-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 4299 | 2006 | Gravis | Diphtheria | Vitebsk | CT2-11 | D3-S33-S3-S21-S63-S92-S55 | CT1E-16 | D5-S60-S24-S29-S43-S38-S53-S57-S32-S39-S83-S48-S12-S72-S14-S115-S78-S74-S90-S94-S6-S9-S95-S20-S26-S18-S81 |
| 2225 | 2001 | Gravis | Diphtheria | Brest | CT2-11 | D3-S33-S3-S21-S63-S92-S55 | CT1E-17 | D5-S60-S24-S29-S43-S38-S53-S57-S32-S39-S83-S48-S95-S72-S14-S115-S94-S12-S9-S95-S20-S26-S18-S81 |
| 4525 | 2006 | Gravis | Diphtheria | Minsk | CT2-12 | D3-S33-S3-S21-S63-S92-S55-S114 | CT1E-7 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 4720 | 2007 | Gravis | Diphtheria | Minsk | CT2-12 | D3-S33-S3-S21-S63-S92-S55-S114 | CT1E-8 | D4-S97-S7-S65-S82-S51-S36-S5-S8-S17-S58-S106-S86-S54-S19-S28-S42-S98-S46-S70-S64-S66-S91 |
| 2278a | 2001 | Gravis | Sore throat | Minsk | CT2-13 | D3-S33-S3-S21-S63-S92-S55-S35-S30 | CT1E-2 | D4-S97-S19-S28-S86-S42-S98-S91-S17-S58-S106-S54 |
| 1006 | 1998 | Gravis | Sore throat | Minsk | CT2-13 | D3-S33-S3-S21-S63-S92-S55-S35-S30 | CT1E-15 | D5-S60-S18-S81-S24-S29-S43-S38-S53-S57-S32-S39-S12-S83-S78-S74-S90-S94-S6-S12-S6-S9-S95-S20-S26 |
| 700a | 1997 | Gravis | Diphtheria | Minsk | CT2-14 | D3-S33-S3-S21-S63-S92-S55-S68 | CT1E-10 | D4-S97-S7-S65-S82-S8-S17-S58-S106-S54-S19-S28-S86-S2-S85-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S91 |
| 2803 | 2004 | Gravis | Asymptomatic | Minsk | CT2-15 | D3-S33-S3-S21-S63-S92-S92-S55-S10 | CT1E-3 | D4-S97-S5-S8-S17-S58-S106-S54-S19-S28-S2-S85-S86-S71-S28-S2-S85-S71-S61-S42-S98-S46-S70-S64-S66-S36 |
| 2393 | 2002 | Gravis | Sore throat | Minsk | CT2-16 | D3-S33-S3-S92-S55-S52-S50 | CT1E-15 | D5-S60-S18-S81-S24-S29-S43-S38-S53-S57-S32-S39-S12-S83-S78-S74-S90-S94-S6-S12-S6-S9-S95-S20-S26 |
| 1438 | 1999 | Mitis | Asymptomatic | Grodno | - | - | CT1E-19 | D5-S60-S26-S18-S81-S24-S29-S43-S38-S53-S57-S32-S12-S39-S83-S48-S72-S14-S115-S78-S74-S90-S94-S6-S9-S95-S12-S9-S95-S20 |
| 4502 | 2006 | Gravis | Asymptomatic | Gomel | CT2-17 | D2-S116-S25-S77-S31-S16-S93-S93-S16 | | |
| 209 | 1996 | Gravis | Asymptomatic | Minsk | CT2-18 | D2-S40-S67-S49-S15-S16 | | |
| 2379 | 2002 | Belfanti | Sore throat | Mogilev | CT2-19 | D2-S75-S15-S101 | | |
| 4499 | 2006 | Belfanti | Sore throat | Gomel | CT2-20 | D2-S75-S40-S101-S15-S101 | | |
| 5171 | 2012 | Mitis | Sore throat | Minsk | CT2-21 | D2-S75-S40-S16 | | |
| 1137 | 1998 | Belfanti | Sore throat | Minsk | CT2-22 | D2-S75-S40-S16-S15-S101 | | |
| 1556 | 1999 | Belfanti | Sore throat | Minsk | CT2-22 | D2-S75-S40-S16-S15-S101 | | |
| 1734 | 2000 | Belfanti | Sore throat | Mogilev | CT2-22 | D2-S75-S40-S16-S15-S101 | | |
| 4069 | 2005 | Belfanti | Asymptomatic | Vitebsk | CT2-22 | D2-S75-S40-S16-S15-S101 | | |
| 5162 | 2012 | Gravis | Sore throat | Brest | CT2-23 | D2-S75-S76-S67-S79-S49-S15-S16-S16-S22-S22 | | |
| 5191 | 2013 | Gravis | Sore throat | Minsk | CT2-24 | D2-S79-S116-S11-S16 | | |

Additional File 2: Table S5 continues…

| Strain | Year | Biovar | Clinical information | Country | DRA type | DRA locus | DRB type | DRB locus |
|---|---|---|---|---|---|---|---|---|
| 728 | 1997 | Mitis | Sore throat | Minsk | CT2-25 | D6-S100-S1-S117-S87-S4 | | |
| 1054 | 1998 | Gravis | Asymptomatic | Gomel | CT2-26 | D6-S117-S102-S59-S88-S87-S4 | | |
| 768a | 1997 | Gravis | Sore throat | Brest | CT2-27 | D6-S117-S1-S88-S87-S4 | | |
| 2399 | 2002 | Gravis | Sore throat | Minsk | CT2-28 | D6-S117-S84-S104 | | |
| 4727 | 2007 | Gravis | Sore throat | Minsk | CT2-28 | D6-S117-S84-S104 | | |
| 4994 | 2009 | Gravis | Sore throat | Brest | CT2-28 | D6-S117-S84-S104 | | |
| 2163 | 2001 | Gravis | Asymptomatic | Minsk | CT2-28 | D6-S117-S84-S104 | | |
| 2650 | 2002 | Mitis | Sore throat | Minsk | CT2-29 | D6-S117-S87-S4-S104 | | |
| 2926 | 2004 | Gravis | Asymptomatic | Gomel | CT2-30 | D6-S23-S109-S56-S88-S87-S4 | | |
| 74 | 1996 | Mitis | Asymptomatic | Mogilev | CT2-31 | D6-S47-S1-S117-S87-S4 | | |
| 631 | 1997 | Belfanti | Sore throat | Vitebsk | CT2-31 | D6-S47-S1-S117-S87-S4 | | |
| 5130 | 2011 | Belfanti | Sore throat | Vitebsk | CT2-31 | D6-S47-S1-S117-S87-S4 | | |
| 4766 | 2008 | Belfanti | Sore throat | Gomel | CT2-32 | D6-S47-S1-S47-S87-S4 | | |
| 5201 | 2014 | Mitis | Sore throat | Minsk | CT2-33 | D6-S47-S1-S87-S4 | | |
| 4663 | 2007 | Belfanti | Sore throat | Gomel | CT2-34 | D6-S47-S87-S4-S105 | | |
| 987 | 1998 | Mitis | Sore throat | Gomel | CT2-35 | D6-S4-S104 | | |
| 2370 | 2001 | Mitis | Sore throat | Minsk | CT2-35 | D6-S4-S104 | | |
| 2686 | 2003 | Mitis | Sore throat | Minsk | CT2-35 | D6-S4-S104 | | |
| 2915 | 2004 | Mitis | Sore throat | Vitebsk | CT2-35 | D6-S4-S104 | | |
| 4288 | 2006 | Mitis | Diphtheria | Minsk | CT2-35 | D6-S4-S104 | | |
| 4552 | 2006 | Mitis | Sore throat | Vitebsk | CT2-35 | D6-S4-S104 | | |
| 4726 | 2007 | Mitis | Sore throat | Minsk | CT2-35 | D6-S4-S104 | | |
| 4774 | 2008 | Mitis | Sore throat | Minsk | CT2-35 | D6-S4-S104 | | |
| 5005 | 2009 | Mitis | Sore throat | Gomel | CT2-35 | D6-S4-S104 | | |
| 5085 | 2010 | Mitis | Sore throat | Mogilev | CT2-35 | D6-S4-S104 | | |
| 5138 | 2011 | Gravis | Sore throat | Brest | CT2-36 | D6-S69-S4-S73-S73-S117-S117-S1-S96-S56-S88 | | |
| ISS 4060 | 1999 | Gravis | Pharyngitis/tonsilitis | Italy | CT2-37 | D6-S99-S117-S1-S109-S56-S88-S87-S87-S4 | | |

Note: Direct-repeat and spacer sequences from CRISPR loci were extracted using CRISPRFinder [1]. All direct-repeat sequences and spacer sequences were separately aligned using T-Coffee [2] at the Ressource Parisienne en BioInformatique Structurale server (http://mobyle.rpbs.univ-paris-diderot.fr/). ML trees were calculated from the alignment of direct-repeat sequences with JC substitution model and from the alignment of spacer sequences with HKY model, each with 100,000 SH-like approximate likelihood ratio tests (SH-aLRT) and 100,000 ultrafast bootstrap iterations using IQ-Tree [3]. An allele number was assigned to each group of direct-repeats and spacers in the phylogenetic tree with a phylogenetic distance of zero.

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3. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ: **IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies**. *Mol Biol Evol* 2015, **32**(1):268-274.