Epidemiology and Infection

Environmental effects on the survival and potential transmission of *Neisseria meningitidis* outside of the host

C. L. SWAIN, D. R. MARTIN, D. SIM, T. W. JORDAN andJ. K. MACKICHAN

**Supplementary Material**

Supplementary Figure S1. Survival of isolate NZ98/254 at ambient temperature and humidity, averaged by season. Later time points are in the inset box.



Supplementary Figure S2. Comparison of survival of C:2a:P1.7-2,4 with W:2a:P1.7-2,4 strain types at 30oC, 30% relative humidity. Error bars are standard deviations, three replicates for each of three strains per strain type. Results shown are for the first two days, results for later days are summarized in Supplementary Table S6.



Supplementary Table S1. Mean percent survival over time of four isolates dried onto glass and maintained under ambient conditions of temperature and humidity in the laboratory. Values in brackets are numbers of independent experiments.

|  |
| --- |
| **Percentage of meningococci surviving at each time** |
| **Time (h)** | **NZ98/254 (n=22)** | **NZ03/280** **(n=7)** | **NZ07/024** **(n=2)** | **Cu00162** **(n=2)** |
| 0 | 23.45 | 16.97 | 6.49 | 19.44 |
| 1 | 14.95 | 11.29 | 3.48 | 13.43 |
| 2 | 11.48 | 9.37 | 1.77 | 12.83 |
| 3 | 9.32 | 6.78 | 0.55 | 9.47 |
| 4 | 8.06 | 6.75 | 0.39 | 7.09 |
| 19 | 2.15 | 0.60 | 0.03 | 3.02 |
| 22 | 2.10 | 0.80 | 0.11 | 1.39 |
| 25 | 1.43 | 0.81 | 0.03 | 2.76 |
| 28 | 1.15 | 0.60 | 0.03 | 1.74 |
| 43 | 0.42 | 0.17 | 0.06 | 0.57 |
| 46 | 0.51 | 0.22 | 0.03 | 1.03 |
| 49 | 0.25 | 0.26 | 0.03 | 1.05 |
| 52 | 0.25 | 0.14 | 0.03 | 0.81 |
| 67 | 0.09 | 0.03 |  | 0.03 |
| 70 | 0.11 | 0.06 |  | 0.07 |
| 73 | 0.09 | 0.02 |  | 0 |
| 76 | 0.07 | 0.01 |  | 0 |
| 91 | 0.04 | 0.01 |  | 0 |
| 94 | 0.01 |  |  | 0.04 |
| 97 | 0.04 |  |  |  |
| 100 | 0.01 |  |  |  |

Surviving meningococci were measured at zero time immediately after drying, then at intervals until viable meningococci were not detected (0 values).

Supplementary Table S2

Analysis of survival of four strains under ambient conditions of temperature and humidity in the laboratory.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Strain**  | **Median**  | **n**  | **Mean**  | **SD**  | **SEM** | **Minimum**  | **Lower Quartile** **(25%)**  | **Upper Quartile** **(75%)**  | **Maximum** |
| NZ98/254 | -0.171 | 22 | -0.293 | 0.221 | 0.047 | -0.685 | -0.486 | -0.106 | -0.056 |
| NZ03/280 | -0.119 | 7 | -0.252 | 0.238 | 0.09 | -0.727 | -0.315 | -0.102 | -0.083 |
| NZ07/024 | -0.618 | 2 | -0.618 | 0.642 | 0.454 | -1.072 | -1.072 | -0.164 | -0.164 |
| Cu00162 | -0.113 | 2 | -0.113 | 0.079 | 0.056 | -0.168 | -0.168 | -0.057 | -0.057 |

Values shown include median, mean, and minimum and maximum values of for the independent biological replicates for each strain; n number of experiments, SD standard deviation, SEM standard error of the mean.

Supplementary Table S3

Mean percent survival at intervals for three strains in a warm room at 36oC and 20% relative humidity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (h)** | **NZ98/254**n=8 | **NZ03/280**n=5 | **Cu00162**n=2 |
| 0 | 26.03 | 15.77 | 19.44 |
| 1 | 15.27 | 9.46 | 13.18 |
| 2 | 11.52 | 8.71 | 8.78 |
| 3 | 8.26 | 6.4 | 8.12 |
| 4 | 8.84 | 3.92 | 6.63 |
| 19 | 2.50 | 1.48 | 4.14 |
| 22 | 2.03 | 1.3 | 3.72 |
| 25 | 1.22 | 1.26 | 3.31 |
| 28 | 1.45 | 2.23 | 2.99 |
| 43 | 0.49 | 0.64 | 2.35 |
| 46 | 0.65 | 0.54 | 2.83 |
| 49 | 0.64 | 0.78 | 2.17 |
| 52 | 0.46 | 0.52 | 2.57 |
| 67 | 0.38 | 0.35 | 1.34 |
| 70 | 0.38 | 0.32 | 0.94 |
| 73 | 0.30 | 0.20 | 0.72 |
| 76 | 0.46 | 0.45 | 0.53 |
| 91 | 0.2 | 0.09 | 0.27 |
| 94 | 0.35 | 0.17 | 0.37 |
| 97 | 0.16 | 0.09 | 0.08 |
| 100 | 0.1 | 0.05 | 0.12 |
| 115 | 0.06 | 0.03 | 0.21 |
| 118 | 0.07 | 0.02 | 0.11 |
| 121 | 0.11 | 0.04 | 0.12 |
| 124 | 0.06 | 0.04 | 0.11 |
| 139 | 0.04 | 0.01 | 0.06 |
| 142 | 0.08 | 0.01 | 0.16 |
| 145 | 0.04 | 0.06 | 0.22 |
| 163 | 0.01 | 0.01 | 0.04 |
| 172 | 0.01 | 0 | 0 |
| 187 | 0.02 | 0.02 | 0.04 |
| 196 | 0.01 | 0.01 | 0 |
| 211 | 0 | 0 | 0.04 |
| 220 | 0.02 | 0 | 0 |
| 235 | 0 | 0 | 0 |
| 244 | 0.01 |  |  |
| 259 | 0 |  |  |
| 268 | 0.02 |  |  |

Surviving meningococci were measured at zero time immediately after drying, then at intervals until viable meningococci were not detected (0 values).

Supplementary Table S4

Survival of three strains in an incubator at 36oC and 95% relative humidity.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolate** | **Inoculation****(CFU)** | **Survival, 0 min****%** | **Survival, 30 min****%** | **Survival, 60 min****%** |
| **NZ98/254** |  |  |  |  |
| Trial 1 | 934 | 26.8 | 0 | 0 |
| Trial 2 | 647 | 25.5 | 0 | 0 |
| **NZ03/280** |  |  |  |  |
| Trial 1 | 804 | 18.7 | nd | 0 |
| Trial 2 | 885 | 13.2 | 0 | 0 |
| Trial 3 | 777 | 17.8 | nd | 0 |
| **Cu00162** |  |  |  |  |
| Trial 1 | 135 | 42.9 | 1.9 | 0 |
| Trial 2 | 610 | 20.6 | 1.0 | 0 |
| Trial 3 | 610 | 24.6 | nd | 0 |

nd = not done; measurements were not made at these time points

Supplementary Table S5

Analysis of survival at 30oC, 30% relative humidity.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Strain type and isolate**  | **Median** | **Mean**  | **SD** | **SEM** | **Minimum** | **Lower Quartile****(25%)** | **Upper Quartile****(75%)** | **Maximum** |
| **B:4:P1.7-2,4** |  |  |  |  |  |  |  |  |
| NZ94/167  | -0.153 | -0.145 | 0.035 | 0.020 | -0.175 | -0.164 | -0.130 | -0.107 |
| NZ98/254  | -0.118 | -0.132 | 0.080 | 0.027 | -0.335 | -0.133 | -0.089 | -0.067 |
| NZ03/280  | -0.191 | -0.233 | 0.106 | 0.061 | -0.354 | -0.273 | -0.173 | -0.154 |
| NZ06/007  | -0.126 | -0.128 | 0.013 | 0.007 | -0.141 | -0.134 | -0.121 | -0.116 |
| NZA2-085-1\* | -0.075 | -0.074 | 0.004 | 0.002 | -0.078 | -0.077 | -0.073 | -0.070 |
| NZA2-362-1\* | -0.090 | -0.086 | 0.021 | 0.012 | -0.105 | -0.098 | -0.077 | -0.064 |
| NZA2-367-1\* | -0.094 | -0.102 | 0.013 | 0.008 | -0.117 | -0.106 | -0.094 | -0.094 |
| **C:2a:P1.7-2,4** |  |  |  |  |  |  |  |  |
| NZ96/059 | -0.131 | -0.126 | 0.021 | 0.012 | -0.144 | -0.138 | -0.117 | -0.103 |
| NZ03/236 | -0.113 | -0.110 | 0.015 | 0.009 | -0.124 | -0.119 | -0.104 | -0.094 |
| NZ04/154 | -0.098 | -0.098 | 0.013 | 0.007 | -0.110 | -0.104 | -0.092 | -0.085 |
| **W:2a:P1.7-2,4** |  |  |  |  |  |  |  |  |
| NZ04/102 | -0.129 | -0.236 | 0.199 | 0.115 | -0.465 | -0.297 | -0.122 | -0.114 |
| NZ04/117 | -0.180 | -0.177 | 0.043 | 0.025 | -0.218 | -0.199 | -0.157 | -0.133 |
| NZ05/050 | -0.101 | -0.136 | 0.074 | 0.043 | -0.221 | -0.161 | -0.094 | -0.087 |
| **W:nt:P1.18-1,3** |  |  |  |  |  |  |  |  |
| NZ04/140 | -0.518 | -0.757 | 0.628 | 0.363 | -1.47 | -0.994 | -0.401 | -0.824 |
| NZ06/105 | -0.243 | -0.235 | 0.124 | 0.071 | -0.355 | -0.299 | -0.176 | -0.108 |
| NZ06/113 | -0.267 | -0.266 | 0.069 | 0.04 | -0.334 | -0.301 | -0.232 | -0.196 |
| NZ07/024 | -0.228 | -0.286 | 0.188 | 0.109 | -0.497 | -0.363 | -0.181 | -0.134 |
| NZA1-153-1\* | -0.304 | -0.315 | 0.061 | 0.035 | -0.381 | -0.343 | -0.282 | -0.26 |
| NZA1-478-1\* | -0.169 | -0.45 | 0.518 | 0.30 | -1.048 | -0.609 | -0.151 | -0.330 |
| **B:4:P1.19,15** |  |  |  |  |  |  |  |  |
| Cu00612 | -0.113 | -0.125 | 0.036 | 0.018 | -0.176 | -0.149 | -0.1 | -0.097 |
| **B:4:P1.7,16** |  |  |  |  |  |  |  |  |
| H44/76-SL | -0.1 | -0.114 | 0.032 | 0.015 | -0.163 | -0.129 | -0.09 | -0.086 |

Values shown include median, mean, and minimum and maximum values of for the independent biological replicates for each strain; n number of experiments, SD standard deviation, SEM standard error of the mean.

n=3 for all strains except NZ98/254 (n=9), Cu00162 (n=4), H44/76-SL (n=5).

\* Carriage strains. All other strains are invasive isolates.

Supplementary Table S6

Comparison of survival data at 30°C, 30% relative humidity for C:2a:P1.7-2,4 and its W:2a:P1.7-2,4 capsule switch variant. Three isolates from each strain type were analysed in triplicate.

|  |  |  |
| --- | --- | --- |
|  | **C:2a:P1.7-2,4** | **W:2a:P1.7-2,4** |
| Initial survival % (range) | 12.98 (7.7-19.6) | 4.6 (1.57-9.32) |
| Decrease in survival first 4 h (mean %) | 4.7 | 9.6 |
| Survival range Day 2 (%) | 0.35-0.61 | 0.09-0.14 |
| Survival range Day 3 (%) | 0.06-0.18 | 0.02-0.05 |
| Survival range Day 4 (%) | 0.01-0.06 | 0-0.01 |
| Survival range Day 5 (%) | 0.007-0.01 |  |
| Survival range Day 6 (%) | 0-0.06 |  |

Supplementary Table S7

Analysis of survival at 30°C, 22% relative humidity. All strains were examined in triplicate.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Strain type and isolate**  | **Median** | **Mean**  | **SD** | **SEM** | **Minimum** | **Lower Quartile****(25%)** | **Upper Quartile****(75%)** | **Maximum** |
| **B:4:P1.7-2,4** |  |  |  |  |  |  |  |  |
| NZ94/167  | -0.086 | -0.086 | 0.018 | 0.01 | -0.103 | -0.095 | -0.077 | -0.67 |
| NZ98/254  | -0.04 | -0.052 | 0.025 | 0.014 | -0.080 | -0.060 | -0.038 | -0.038 |
| NZA2-367-1  | -0.066 | -0.08 | 0.037 | 0.021 | -0.122 | -0.094 | -0.059 | -0.052 |
| **W:nt:P1.18-1,3** |  |  |  |  |  |  |  |  |
| NZ04/140 | -0.128 | -0.113 | 0.039 | 0.023 | -0.142 | -0.135 | -0.098 | -0.068 |
| NZ07/024 | -0.212 | -0.241 | 0.076 | 0.044 | -0.327 | -0.270 | -0.198 | -0.183 |
| NZA1-478-1 | -0.891 | -1.037 | 0.643 | 0.371 | -1.74 | -1.316 | -0.685 | -0.479 |
| **B:4:P1.19,15** |  |  |  |  |  |  |  |  |
| Cu00612 | -0.05 | -0.05 | 0.009 | 0.005 | -0.059 | -0.055 | -0.046 | -0.041 |
| **B:4:P1.7,16** |  |  |  |  |  |  |  |  |
| H44/76-SL | -0.05 | -0.049 | 0.005 | 0.003 | -0.053 | -0.052 | -0.047 | -0.044 |

Values shown include median, mean, and minimum and maximum values of for the independent biological replicates for each strain; n number of experiments, SD standard deviation, SEM standard error of the mean.

Supplementary Table S8

 Mann-Whitney U statistical comparison of survival at 30°C, 30% relative humidity with 30°C, 22% relative humidity.

|  |  |  |  |
| --- | --- | --- | --- |
| Strain type | Number of experiments | Test statistic (U) | Probability (*P*) |
|  | 30% RH | 22% RH |  |  |
| B:4:P1.7-2,41 | 27 | 9 | 41.0 | 0.002 |
| W:nt:P1.18-1,32 | 18 | 9 | 72.0 | 0.668 |
| Cuban and Norwegian strains3 | 9 | 6 | 0.00 | <0.001 |

1,2. Results for New Zealand carriage and invasive isolates have been combined for this analysis.

3. Results are combined for the Cuban and Norwegian epidemic isolates.