**Supplementary Material**

**Supplementary Table 1.** Weight of the larvae used in the experiment

|  |  |  |
| --- | --- | --- |
| **Condition**  | **Weight of the larvae used in experiment, mg** | **Average weight**  |
| PBS (ctrl)  | 329  | 315  | 371  | 352  | 346  | 351  | 363  | 382  | 350  | 356  | 310  | 348  | 371  | 352  | 375  | 351  | 364  | 305  | 354  | 357  | 350.1  |
| 1ADI EMA ERM  | 348  | 362  | 379  | 353  | 312  | 337  | 344 | 353  | 361  | 348  | 348  | 362  | 369  | 376  | 345  | 339  | 308  | 355  | 360  | 347  | 350.3  |
| 10ADI EMA ERM  | 367  | 355  | 360  | 314  | 360  | 373  | 352  | 349  | 351  | 357  | 368  | 357  | 305  | 355  | 361  | 375  | 345  | 304  | 365  | 340  | 350.65  |
| 0.1ADI WHO ERM  | 359  | 312  | 358  | 371  | 350  | 375  | 351  | 364  | 309  | 364  | 334  | 315  | 373  | 365  | 346  | 361  | 363  | 372  | 355  | 306  | 350.15  |
| 1ADI WHO ERM  | 346  | 323  | 362  | 359  | 371  | 345  | 339  | 308   | 352  | 356  | 370  | 360  | 357  | 310  | 361  | 374  | 352  | 348  | 351  | 353  | 349.85  |

**Supplementary Table 2.** Species identity on non-antibiotic plates confirmed by MALDI-TOF

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Condition**  | **Day**  | № of ***S. pneumoniae*** colonies  | № of **non- *S. pneumoniae*** colonies  | **Non-*S. pneumoniae* ID**  |
| PBS(ctrl)  | 1  | 3  | 2  | *E. casseliflavus, S. avidinii*  |
|    | 2  | 1  | 3  | *S. hominis, E. gallinarum*  |
|    | 3  | 2  | 2  | *E. gallinarum, S. avidinii*  |
|    | 4  | 2  | 1  | *E. casseliflavus*  |
|    | 5  | 1  | 2  | *L. agilis, S. sciuri*  |
|    | 6  | 2  | 2  | *E. gallinarum*  |
|    |    |    |    |   |
| 1ADI EMA ERM  | 1  | 1  | 2  | *E. casseliflavus*  |
|    | 2  | 3  | 1  | *S. klosii*  |
|    | 3  | 2  | 1  | *E. casseliflavus*  |
|    | 4  | 1  | 3  | *E. gallinarum, C. freundii*  |
|    | 5  | 2  | 1  | *E. casseliflavus*  |
|    | 6  | 1  | 2  | *S. klosii, E. mundtii*  |
|    |    |    |    |   |
| 10ADI EMA ERM  | 1  | 2  | 2  | *S.marcescens, E.casseliflavus*  |
|    | 2  | 1  | 2  | *E. gallinarum, E. mundtii*  |
|    | 3  | 1  | 3  | *B. thailandensis, E. casseliflavus*  |
|    | 4  | 2  | 1  | *S. klosii*  |
|    | 5  | 1  | 2  | *E. mundtii, E. gallinarum*  |
|    | 6  | 1  | 1  | *L. agilis*  |
|    |    |    |    |   |
| 0.1ADI WHO ERM  | 1  | 3  | 1  | *S. avidinii*  |
|    | 2  | 1  | 2  | *E. gallinarum,  S. klosii*  |
|    | 3  | 2  | 1  | *S. hominis*  |
|    | 4  | 2  | 3  | *E. casseliflavus,  E. gallinarum*  |
|    | 5  | 1  | 1  | *E. mundtii*  |
|    | 6  | 2  | 1  | *E. casseliflavus*  |
|    |    |    |    |   |
| 1ADI WHO ERM  | 1  | 2  | 1  | *E. mundtii*  |
|    | 2  | 3  | 2  | *S. hominis,  E. casseliflavus*  |
|    | 3  | 1  | 2  | *E. gallinarum*  |
|    | 4  | 1  | 3  | *E. casseliflavus,  E. mundtii*  |
|    | 5  | 2  | 1  | *S. avidinii*  |
|    | 6  | 1  | 2  | *A. encheleia,  E. mundtii*  |

**Supplementary Table 3.** Identification of **(a)** virulence genesusing Vfdb database and **(b)** antimicrobial resistance genes using card database

**(a)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **VfdB**  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Genome Id  | *cbpD*  | *cbpG*  | *cps4A*  | *cps4B*  | *cps4C*  | *cps4D*  | *hysA*  | *lytA*  | *lytB*  | *lytC*  | *nanB*  | *pavA*  | *pce*  | *pfbA*  | *ply*  | *psaA*  |
| SP\_0603A1\_SC\_E14587\_lib691262\_10231  | 100  | 99.77  | 99.79  | 99.86  | 99.86  | 100  | 100  | 97.07;100.00  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| SP\_1403\_1B\_E14589\_lib691264\_10231  | 100  | 99.77  | 99.79  | 99.86  | 99.86  | 100  | 100  | 97.07;100.00  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| SP\_1403\_1C\_E14586\_lib691261\_10231  | 100  | 99.77  | 99.79  | 99.86  | 99.86  | 100  | 100  | 100.00;97.07  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| SP\_2802\_B\_E14588\_lib691263\_10231  | 100  | 99.77  | 99.79  | 99.86  | 99.86  | 100  | 100  | 100.00;97.07  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| SP\_ATCC\_YB\_lib707253\_10264  | 100  | 99.77  | 99.79  | 99.86  | 99.86  | 100  | 100  | 97.07;100.00  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| SP\_ctrl\_B2\_lib717407\_10294  | 100  | 99.77  | 99.79  | 99.86  | 99.86  | 100  | 100  | 97.07;100.00  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| SP\_ctrl\_B4\_lib717408\_10294  | 100  | 99.77  | 99.79  | 99.86  | 99.86  | 100  | 100  | 97.07;100.00  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |

**(b)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **CARD**  |    |    |    |    |    |
| Genome Id  | *RlmA(II)*  | *patA*  | *patB*  | *pmrA*  | *vanRC*  |
| NG-31926\_SP\_0603A1\_SC\_E14587\_lib691262\_10231.fna  | 100  | 100  | 100  | 100  | .  |
| NG-31926\_SP\_1403\_1B\_E14589\_lib691264\_10231.fna  | 100  | 100  | 100  | 100  | .  |
| NG-31926\_SP\_1403\_1C\_E14586\_lib691261\_10231.fna  | 100  | 100  | 100  | 100  | .  |
| NG-31926\_SP\_2802\_B\_E14588\_lib691263\_10231.fna  | 100  | 100  | 100  | 100  | 99.86  |
| NG-31926\_SP\_ATCC\_YB\_lib707253\_10264.fna  | 100  | 100  | 100  | 100  | .  |
| NG-34157\_SP\_ctrl\_B2\_lib717407\_10294.fna  | 100  | 100  | 100  | 100  | .  |
| NG-34157\_SP\_ctrl\_B4\_lib717408\_10294.fna  | 100  | 100  | 100  | 100  | .  |

**Supplementary Table 4.** All SNPs found for *S. pneumoniae* isolates

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene Product** | **CDS** | **2802b** | **1403b** | **1403c** | **0303c** | **Ctrl\_B2** | **Ctrl\_B4** | **ATCC 49619** |
| Hypothetical Protein | CDS: OPMNIGBM\_00355, Gene: OPMNIGBM\_00355 | #N/A | #N/A | #N/A | X:OPMNIGBM\_00355:c.888A>C | #N/A | #N/A | #N/A |
| Hypothetical Protein | Gene: OPMNIGBM\_00536, CDS: OPMNIGBM\_00536 | #N/A | #N/A | #N/A | X:OPMNIGBM\_00536:p.Arg77Trp | #N/A | #N/A | #N/A |
| Tyrosine recombinase | CDS: xerS, Gene: xerS | #N/A | #N/A | #N/A | X:OPMNIGBM\_00645:p.Val324Leu | #N/A | #N/A | #N/A |
| 1,4-alpha-glucan branching enzyme | CDS: glgB, Gene: glgB | X:OPMNIGBM\_00661:p.Val194Gly | X:OPMNIGBM\_00661:p.Val194Gly | X:OPMNIGBM\_00661:p.Val194Gly | X:OPMNIGBM\_00661:p.Val194Gly | X:OPMNIGBM\_00661:p.Val194Gly | X:OPMNIGBM\_00661:p.Val194Gly | #N/A |
| ISL3 family transposase ISSpn14 | CDS: OPMNIGBM\_00680, Gene: OPMNIGBM\_00680 | X:OPMNIGBM\_00680:c.117A>G | X:OPMNIGBM\_00680:p.Thr45Ile | X:OPMNIGBM\_00680:c.183T>C | X:OPMNIGBM\_00680:c.183T>C | X:OPMNIGBM\_00680:c.117A>G | X:OPMNIGBM\_00680:c.117A>G | X:OPMNIGBM\_00680:p.Thr45Ile |
| IS630 family transposase ISSpn2 | CDS: OPMNIGBM\_00796, Gene: OPMNIGBM\_00796 | X:OPMNIGBM\_00796:p.Lys59fs | X:OPMNIGBM\_00796:p.Lys59fs | X:OPMNIGBM\_00796:p.Lys59fs | X:OPMNIGBM\_00796:p.Lys59fs | X:OPMNIGBM\_00796:p.Lys59fs | X:OPMNIGBM\_00796:p.Lys59fs | X:OPMNIGBM\_00796:p.Lys59fs |
| IS630 family transposase ISSpn2 | CDS: OPMNIGBM\_00797, Gene: OPMNIGBM\_00797 | X:OPMNIGBM\_00797:p.Val3Ala | X:OPMNIGBM\_00797:p.Val3Ala | X:OPMNIGBM\_00797:p.Val3Ala | X:OPMNIGBM\_00797:p.Val3Ala | X:OPMNIGBM\_00797:p.Val3Ala | X:OPMNIGBM\_00797:p.Val3Ala | X:OPMNIGBM\_00797:p.Val3Ala |
| Hypothetical Protein | CDS: OPMNIGBM\_00800, Gene: OPMNIGBM\_00800 | #N/A | #N/A | #N/A | X:OPMNIGBM\_00800:p.Ser121Gly | #N/A | #N/A | #N/A |
| Putative TrmH family tRNA/rRNA methyltransferase | CDS: OPMNIGBM\_00823, Gene: OPMNIGBM\_00823 | #N/A | #N/A | #N/A | X:OPMNIGBM\_00823:p.Ser203Arg | #N/A | #N/A | #N/A |
| Vitamin B12 import ATP-binding protein BtuD | CDS: btuD\_3, Gene: btuD\_3 | X:OPMNIGBM\_00839:c.708A>C | X:OPMNIGBM\_00839:c.708A>C | X:OPMNIGBM\_00839:c.708A>C | X:OPMNIGBM\_00839:c.708A>C | X:OPMNIGBM\_00839:c.708A>C | X:OPMNIGBM\_00839:c.708A>C | #N/A |
| Hypothetical Protein | CDS: OPMNIGBM\_00924, Gene: OPMNIGBM\_00924 | X:OPMNIGBM\_00924:c.123G>A | X:OPMNIGBM\_00924:c.123G>A | X:OPMNIGBM\_00924:c.123G>A | X:OPMNIGBM\_00924:c.123G>A | X:OPMNIGBM\_00924:c.123G>A | X:OPMNIGBM\_00924:c.123G>A |  |
| Hypothetical Protein | CDS: OPMNIGBM\_00925, Gene: OPMNIGBM\_00925 | #N/A | #N/A | #N/A | X:OPMNIGBM\_00925:p.Trp47Leu | #N/A | #N/A | #N/A |
| Ribulose-5-phosphate reductase | CDS: tarJ, Gene: tarJ | X:OPMNIGBM\_01124:p.Thr63Met | #N/A | X:OPMNIGBM\_01124:p.Thr63Met | X:OPMNIGBM\_01124:p.Thr63Met | X:OPMNIGBM\_01124:p.Thr63Met | X:OPMNIGBM\_01124:p.Thr63Met | #N/A |
| Hypothetical Protein | CDS: OPMNIGBM\_01263, Gene: OPMNIGBM\_01263 | #N/A | #N/A | #N/A | X:OPMNIGBM\_01263:p.Leu8Ser | #N/A | #N/A | #N/A |
| Hypothetical Protein | CDS: OPMNIGBM\_01320, Gene: OPMNIGBM\_01320 | X:OPMNIGBM\_01320:p.Gln1702Pro | X:OPMNIGBM\_01320:p.Gln1702Pro | X:OPMNIGBM\_01320:p.Gln1702Pro | X:OPMNIGBM\_01320:p.Gln1702Pro | X:OPMNIGBM\_01320:p.Gln1702Pro | X:OPMNIGBM\_01320:p.Gln1702Pro | #N/A |
| Sugar phosphatase YidA | Gene: yidA\_2, CDS: yidA\_2 | X:OPMNIGBM\_01507:p.Pro60Leu | #N/A | X:OPMNIGBM\_01507:p.Pro60Leu | X:OPMNIGBM\_01507:p.Pro60Leu | X:OPMNIGBM\_01507:p.Pro60Leu | X:OPMNIGBM\_01507:p.Pro60Leu | #N/A |
| IS3 family transposase ISSpn11 | CDS: OPMNIGBM\_01705, Gene: OPMNIGBM\_01705 | X:OPMNIGBM\_01705:p.Tyr91fs | X:OPMNIGBM\_01705:p.His57Tyr | X:OPMNIGBM\_01705:p.Ala52Gly | X:OPMNIGBM\_01705:p.Thr30Pro | X:OPMNIGBM\_01705:p.Thr30Pro | X:OPMNIGBM\_01705:p.Thr30Pro | X:OPMNIGBM\_01705:p.Thr30Pro |
| ISL3 family transposase IS1167 | CDS: OPMNIGBM\_01721, Gene: OPMNIGBM\_01721 | X:OPMNIGBM\_01721:p.Lys65Glu | X:OPMNIGBM\_01721:p.Lys65Glu | X:OPMNIGBM\_01721:p.Lys65Glu | X:OPMNIGBM\_01721:p.Lys65Glu | X:OPMNIGBM\_01721:p.Lys65Glu | X:OPMNIGBM\_01721:p.Lys65Glu | X:OPMNIGBM\_01721:p.Lys65Glu |
| Hypothetical Protein | Gene: OPMNIGBM\_00216, CDS: OPMNIGBM\_00216 | #N/A | X:OPMNIGBM\_00216:p.Glu159\* | #N/A | #N/A | #N/A | #N/A | #N/A |
| IS5 family transposase ISSpn7 | CDS: OPMNIGBM\_00292, Gene: OPMNIGBM\_00292 | X:OPMNIGBM\_00292:p.Ala3Thr | X:OPMNIGBM\_00292:p.Ala3Thr | #N/A | #N/A | X:OPMNIGBM\_00292:p.Ala3Thr | X:OPMNIGBM\_00292:p.Ala3Thr | X:OPMNIGBM\_00292:p.Ala3Thr |
| ISL3 family transposase IS1167 | CDS: OPMNIGBM\_00678, Gene: OPMNIGBM\_00678 | X:OPMNIGBM\_00678:p.Asn25Lys | X:OPMNIGBM\_00678:c.189T>C | #N/A | #N/A | X:OPMNIGBM\_00678:p.Asn25Lys | X:OPMNIGBM\_00678:p.Asn25Lys | X:OPMNIGBM\_00678:p.Asn25Lys |
| Hypothetical Protein | CDS: OPMNIGBM\_00913, Gene: OPMNIGBM\_00913 | #N/A | X:OPMNIGBM\_00913:p.His44Asn | #N/A | #N/A | #N/A | #N/A | #N/A |
| Arylsulfatase | CDS: OPMNIGBM\_00351, Gene: OPMNIGBM\_00351 | X:OPMNIGBM\_00351:c.750T>C | #N/A | X:OPMNIGBM\_00351:c.750T>C | #N/A | #N/A | #N/A | #N/A |
| Heat-inducible transcription repressor | Gene: hrcA, CDS: hrcA | X:OPMNIGBM\_00988:c.954C>A | #N/A | X:OPMNIGBM\_00988:c.954C>A | #N/A | #N/A | #N/A | #N/A |
| 50S ribosomal protein L1 | Gene: rplA, CDS: rplA | X:OPMNIGBM\_01084:p.Asn34Lys | #N/A | X:OPMNIGBM\_01084:p.Asn34Lys | #N/A | #N/A | #N/A | #N/A |
| Hypothetical Protein | Gene: OPMNIGBM\_02076, CDS: OPMNIGBM\_02076 | X:OPMNIGBM\_02076:p.Arg6Lys | X:OPMNIGBM\_02076:p.Arg6Lys | X:OPMNIGBM\_02076:p.Arg6Lys | X:OPMNIGBM\_02076:p.Arg6Lys | X:OPMNIGBM\_02076:p.Arg6Lys | X:OPMNIGBM\_02076:p.Arg6Lys | X:OPMNIGBM\_02076:p.Arg6Lys |

**Supplementary Figure** **1**. Mortality of *G. mellonella* larvae between 1-6 days after the injections of *S. pneumoniae* followed by administration of various concentrations of erythromycin or PBS (control). **(A)**. Injection of erythromycin ADI doses defined by EMA:1.75 ng (1ADI EMA) and 17.5 ng (10ADI EMA). **(B)**. Injection of erythromycin ADI doses defined by WHO: 0.025 ng (0.1ADI WHO) and 0.25 ng (1ADI WHO).

