**Figure legends and statistics for a paper**

**Figure 1A.** The effect of varying inoculum dose of *N. gonorrhoeae* on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing inocula of gonococci (up to 6 × 108 CFU per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 8 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between control group of larvae injected with GC broth and others at each time point. \* p < 0.05.

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| **Time (h)** | **GC broth vs** **0.1 OD** | **GC broth vs** **0.4 OD** | **GC broth vs** **0.6 OD** | **GC broth vs** **0.8 OD** | **GC broth vs** **1.0 OD** |
| **19** | No (P=0.5201) | Yes (P=0.0009) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **20** | No (P=0.5201) | Yes (P=0.0010) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **22** | No (P=0.5201) | Yes (P=0.0010) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **43** | No (P=0.7742) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **44** | No (P=0.7742) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **46** | No (P=0.7742) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **48** | No (P=0.7742) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |

**Figure 1B.** Health index scores of *G. mellonella* injected with varying inoculum dose of *N. gonorrhoeae* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing inocula of gonococci (up to 6.7 × 108 CFU per larva), were examined with Health index scoring system over a period of 48 h. Data are mean health index score of 3 independent experiments. Error bars represent the standard deviation of the mean health index score. Unpaired t test was carried out between control larva health index scores and gonococci infected groups at each time point. \* p < 0.05.

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| **Time (h)** | **Control vs** **0.1 OD** | **Control vs** **0.4 OD** | **Control vs** **0.6 OD** | **Control vs** **0.8 OD** | **Control vs** **1.0 OD** |
| **16** | Yes (P=0.0142) | No (P=0.1033) | Yes (P=0.0431) | Yes (P=0.0170) | Yes (P=0.0289) |
| **24** | Yes (P=0.0284) | Yes (P=0.0058) | Yes (P=0.0016) | Yes (P=0.0070) | Yes (P=0.0003) |
| **40** | Yes (P=0.0307) | Yes (P=0.0124) | Yes (P<0.0001) | Yes (P=0.0005) | Yes (P=0.0003) |
| **48** | Yes (P=0.0346) | Yes (P=0.0068) | Yes (P<0.0001) | Yes (P=0.0005) | Yes (P=0.0003) |

**Figure 1C.** Recovery of *N. gonorrhoeae* (dose 8 × 107 CFU per larva) by plating haemolymph on VCNT supplemented plates over 48 h time-course from infected larvae. At least 30 larvae were infected with 10 µl of 8 × 107 CFU *N. gonorrhoeae* P9-17. The survival of *N. gonorrhoeae* within live and dead *G. mellonella* was determined by cutting the end of the larva, extracting haemolymph and plating it on to the VCNT supplemented GC agar plates at different time points (16, 24, 40, and 48 h) to assess the ability of the immune system of *G. mellonella* to control gonococci growth. Data are pooled from four independent experiments with 1 larva per time-point per experiment. Mean survival of larvae infected with gonococci (circles), Log10 CFU from live larvae over time (triangles), and Log10 CFU from dead larvae over time (squares). Error bars show standard deviation of the mean. Unpaired t test was carried out between live and dead CFU from larvae at each time point. \* p < 0.05.

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| **Live vs dead larvae CFU/****Time (h)** | **Unpaired t-test** |
| 16 | No (P=0.8389) |
| 24 | No (P=0.7839) |
| 40 | No (P=0.3232) |
| 48 | No (P=0.1356) |

**Figure 2.** Common lesion grading scheme criteria were separated and visualised separately. Mean total score (top left), melanisation (top right), adipose body necrosis (bottom left), and haemocyte reaction grades (bottom right) over time are shown. Symbols determine the mean and error bars standard deviation of the mean. Unpaired t test was carried out between infected and uninfected larvae scores at each time point. Ns, not significant, while \* is p < 0.05.

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| **Grading scheme criteria/****Time (h)** | **Total score** | **Melanisation** | **Adipose body necrosis** | **Haemocyte reaction** |
| 2 | Yes (P=0.0005) | Yes (P=0.0097) | No (P=0.3457) | Yes (P=0.0027) |
| 16 | Yes (P=0.0002) | Yes (P=0.0003) | No (P=0.5517) | Yes (P=0.0006) |
| 24 | Yes (P<0.0001) | Yes (P<0.0001) | No (P=0.9049) | Yes (P<0.0001) |

**Figure 3.** Histopathological analysis of*N. gonorrhoeae* infection of *G. mellonella* larvae (6.7 × 107 CFU per larva) over a time course of 24 h. Both *N. gonorrhoeae* infected and uninfected (control) larvae (n≤6 per group) were fixed by injecting 100 µL of neutral buffered formalin in to the last left pro-leg at each time-point (2, 16, and 24 h). Samples were embedded into molten paraffin wax, sectioned at 3 microns, and then stained with haematoxylin and eosin (H&E). Normal adipose body (AB), no haemocyte (arrows) reaction, monolayers formed by haemocytes in the subcuticular space next to cuticle (Cu) and individual haemocytes scattered within the adipose body (A, 2 h control larva). Locally extensive necrosis (Ne) of the adipose body with scattered melanin (arrows) is present between the crop (Cr) and tracheal apparatus (TA), (B, 2 h infected larva). 2 hour post-injection infected, larva 4. There are large numbers of haemocytes (H) forming a densely cellular aggregate adjacent to the adipose body, additional haemocytes are filling space between folds of the adipose body (C, 2 h infected larva). Normal adipose body, silk gland (SG), and intestinal tract (IT), (D, 16 h control larva). 16 hour post-injection infected, larva 3. Aggregates of haemocytes (H), occasionally with melanin, are present multifocally throughout the proleg. Skeletal muscle (SM) and melanised nodules (MN) can be seen as well (E, 16 h infected larva). There is proteinaceous fluid (PF) in the coelom adjacent to the crop (Cr) and intestinal tract (IT), (F, 24 h control larva). Multiple melanised nodules and clusters of haemocytes (arrows) within the coelom adjacent to the rectum (R), (G, 24 h infected larva). Diplococci (arrows) can be seen within *G. mellonella* tissue (H, infected larva). Images A, C, and G are at 40X, B and E at 20X, D and F at 10X, and H at 100X magnification.

**Figure 4A.** The effect of different P9 variant on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with different P9 variants (6 × 107 CFU per larva), were examined for survival over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between GC broth infected control group and other P9 variants. As well, Unpaired t test was carried out between P9-17 infected group and other P9 variants at each time point. \* p < 0.05.

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| **Time (h)** | **GC broth vs** **P9-1 (Pil- Opa-)** | **GC broth vs** **P9-16 (Pil- Opa+)** | **GC broth vs** **P9-17 (Pil+ Opa+)[** | **GC broth vs** **P9-2 (Pil+ Opa-)** |
| **16** | Yes (P=0.0095) | Yes (P=0.0002) | Yes (P=0.0008) | Yes (P=0.0002) |
| **24** | Yes (P<0.0001) | Yes (P=0.0002) | Yes (P<0.0001) | Yes (P<0.0001) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |

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| **Time (h)** | **P9-17 (Pil+ Opa+)****vs P9-1 (Pil- Opa-)** | **P9-17 (Pil+ Opa+)****vs P9-16 (Pil- Opa+)** | **P9-17 (Pil+ Opa+)****vs P9-2 (Pil+ Opa-)** |
| **16** | No (P=0.2874) | No (P=0.6953) | No (P=0.8551) |
| **24** | No (P=0.5503) | No (P=0.7501) | No (P=0.5727) |
| **40** | No (P=0.1039) | No (P=0.6927) | No (P=0.6660) |
| **48** | No (P=0.1425) | No (P=0.8383) | No (P=0.6660) |

**Figure 4B.** The effect of different gonococci isolates on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with different gonococci isolates (6 × 107 CFU per larva), were examined for survival over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between GC broth infected control group and gonococci isolates. As well, Unpaired t test was carried out between P9-17 infected group and other P9 variants at each time point. \* p < 0.05.

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| **Time (h)** | **GC broth vs GC-26** | **GC broth vs GC-10** | **GC broth vs GC-30** | **GC broth vs GC-03** | **GC broth vs GC-17** | **GC broth vs GC-46** | **GC broth vs GC-15** | **GC broth vs GC-02** | **GC broth vs GC-38** | **GC broth vs P9-17** |
| **16** | Yes (P= 0.0008) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P= 0.0129) | No (P= 0.5599) | No (P=) 0.0993) | No (P= 0.0534) | No (P= 0.2971) | No (P= 0.9363) | Yes (P< 0.0001) |
| **24** | Yes (P= 0.0008) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P= 0.0008) | Yes (P= 0.0078) | Yes (P= 0.0006) | No (P= 0.0534) | No (P= 0.0852) | No (P= 0.5666) | Yes (P< 0.0001) |
| **40** | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P=0.0001) | Yes (P= 0.0097) | Yes (P= 0.0018) | No (P= 0.0852) | Yes (P< 0.0001) |
| **48** | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P< 0.0001) | Yes (P= 0.0097) | Yes (P= 0.0018) | No (P= 0.0852) | Yes (P< 0.0001) |

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| **Time (h)** | **P9-17 vs GC-26** | **P9-17 vs GC-10** | **P9-17 vs GC-30** | **P9-17 vs GC-03** | **P9-17 vs GC-17** | **P9-17 vs GC-46** | **P9-17 vs GC-15** | **P9-17 vs GC-02** | **P9-17 vs GC-38** |
| **16** | No (P=0.7824) | No (P=0.2790) | No (P=0.6340) | No (P=0.1269) | Yes (P= 0.0304) | No (P=) 0.1324 | No (P= 0.2000) | No (P= 0.1253) | Yes (P= 0.0357) |
| **24** | No (P= 0.8582) | No (P= 0.5632) | No (P= 0.8770) | No (P= 0.3905) | No (P= 0.1932) | No (P= 0.3918) | No (P= 0.0983) | No (P= 0.1013) | Yes (P= 0.0309) |
| **40** | No (P= 0.5591) | No (P= 0.4608) | No (P= 0.4516) | Yes (P= 0.0115) | No (P= 0.1662) | No (P= 0.0992) | Yes (P= 0.0027) | Yes (P= 0.0073) | Yes (P= 0.0003) |
| **48** | No (P= 0.9750) | No (P= 0.4992) | No (P= 0.7046) | No (P= 0.1120) | No (P= 0.0938) | No (P= 0.5759) | Yes (P= 0.0021) | Yes (P= 0.0054) | Yes (P= 0.0003) |

**Figure 5A**. The effect of heat-killed and live inoculum of *N. gonorrhoeae* on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with heat-killed and live gonococci (7.6 × 107 CFU per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between groups at each time point. \* p < 0.05. Final average survivals for heat-killed and live conditions were 63 and 23.

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| **Time (h)** | **GC broth vs live** | **GC broth vs heat-killed** | **Heat killed against live** |
| 4 | Yes (P= 0.0006) | No (P=0.0816) | No (P=0.3524) |
| 20 | Yes (P<0.0001) | Yes (P=0.0066) | No (P=0.0671) |
| 24 | Yes (P<0.0001) | Yes (P=0.0066) | Yes (P=0.0421) |
| 28 | Yes (P<0.0001) | Yes (P=0.0066) | Yes (P=0.0337) |
| 48 | Yes (P<0.0001) | Yes (P=0.0012) | Yes (P=0.0352) |

**Figure 5B**. The effect of varying inoculum dose of *N. gonorrhoeae* P9-17 outer membrane (OM) on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with varying doses of OM (up to 80,000 ng per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between GC broth infected control group and OM groups at each time point. \* p < 0.05.

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| **Time (h)** | **GC broth vs 80,000 ng/larva** | **GC broth vs 8,000 ng/larva** | **GC broth vs 800 ng/larva** | **GC broth vs 80 ng/larva** | **GC broth vs 6.5 ng/larva** | **GC broth vs 0.65 ng/larva** | **GC broth vs 0.0065 to 0.065 ng/larva** |
| **16** | Yes (P<0.0001) | Yes (P=0.0006) | Yes (P=0.0022) | Yes (P=0.0055) | No (P=0.0817) | No (P=0.8632) | No (P=0.2446) |
| **24** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P=0.0014) | Yes (P=0.0001) | No (P=0.0579) | No (P=0.8632) | No (P=0.2446) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P=0.0014) | Yes (P<0.0001) | Yes (P=0.0143) | No (P=0.8632) | No (P=0.2446) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P=0.0014) | Yes (P<0.0001) | Yes (P=0.0143) | No (P=0.8632) | No (P=0.2446) |

**Figure 6.** The effect of varying inoculum dose of various *Neisseria* organisms on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing inocula of *N. gonorrhoeae, N. meningitidis*, and *N. lactamica* (up to 108 CFU per larva) were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between GC broth infected control group and *Neisiriae* groups at each time point. As well, Unpaired t test was carried out between *G. mellonella* infected with *N. gonorrhoeae* survival and other *Neisseriae* survivals at each time point. \* p < 0.05.

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| **OD 0.1 (105 CFU per larva)** |
| **Time (h)** | **GC broth vs *N. gonorrhoeae***  | **GC broth vs *N. meningitidis*** | **GC broth vs *N. lactamica*** | ***N. gonorrhoeae* vs *N. meningitidis*** | ***N. gonorrhoeae* vs *N. lactamica*** |
| **16** | No (P=0.5201) | No (P=0.7269) | No (P=0.3156) | No (P=0.3140) | No (P=0.2897) |
| **24** | No (P=0.5201) | No (P=0.4105) | No (P=0.6964) | No (P=0.0929) | No (P >0.9999) |
| **40** | No (P=0.7742) | No (P=0.4105) | No (P=0.8632) | No (P=0.2446) | No (P=0.6964) |
| **48** | No (P=0.7742) | No (P=0.4105) | No (P=0.0724) | No (P=0.2446) | Yes (P= 0.0255) |

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| **OD 0.4 (~0.5 ×** **105 CFU per larva)** |
| **Time (h)** | **GC broth vs *N. gonorrhoeae***  | **GC broth vs *N. meningitidis*** | **GC broth vs *N. lactamica*** | ***N. gonorrhoeae* vs *N. meningitidis*** | ***N. gonorrhoeae* vs *N. lactamica*** |
| **16** | Yes (P=0.0009) | Yes (P=0.0003) | No (P=0.2897) | No (P= 0.1972) | No (P=0.1387) |
| **24** | Yes (P=0.0010) | Yes (P<0.0001) | Yes (P=0.0179) | Yes (P=0.0412) | No (P=0.4691) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P= 0.0179) | Yes (P= 0.0395) | Yes (P= 0.0302) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P= 0.0395) | No (P=0.1171) |

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| **OD 0.6 (106 CFU per larva)** |
| **Time (h)** | **GC broth vs *N. gonorrhoeae***  | **GC broth vs *N. meningitidis*** | **GC broth vs *N. lactamica*** | ***N. gonorrhoeae* vs *N. meningitidis*** | ***N. gonorrhoeae* vs *N. lactamica*** |
| **16** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P=0.0003) | Yes (P=0.0139) | No (P=0.8325) |
| **24** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P= 0.0010) | No (P=0.1881) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P= 0.0187) | No (P=0.2384) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P= 0.0114) | Yes (P= 0.0146) |

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| **OD 0.8 (107 CFU per larva)** |
| **Time (h)** | **GC broth vs *N. gonorrhoeae***  | **GC broth vs *N. meningitidis*** | **GC broth vs *N. lactamica*** | ***N. gonorrhoeae* vs *N. meningitidis*** | ***N. gonorrhoeae* vs *N. lactamica*** |
| **16** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | No (P= 0.1387) | No (P= 0.4708) |
| **24** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | No (P= 0.0838) | No (P= 0.0614) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | No (P=0.1454) | No (P=0.1322) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | No (P=0.1075) | No (P=0.0698) |

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| **OD 1.0 (108 CFU per larva)** |
| **Time (h)** | **GC broth vs *N. gonorrhoeae***  | **GC broth vs *N. meningitidis*** | **GC broth vs *N. lactamica*** | ***N. gonorrhoeae* vs *N. meningitidis*** | ***N. gonorrhoeae* vs *N. lactamica*** |
| **16** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | No (P=0.5896) | No (P=0.2534) |
| **24** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P= 0.0439) | No (P=0.7947) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | No (P=0.0812) | No (P=0.8916) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | No (P=0.1039) | No (P=0.3400) |

**Figure 7**. The effect of different *Lactobacillus* isolates and co-infection on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing inocula of *L. brevis, L. crispatus,* and *L. gasseri* (up to 108 CFU per larva), were examined for survival over a period of 48 h. Data are mean survival from a minimum of 4 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between lowest inocula (105 CFU per larva) and other inocula doses for each isolate at each time point. \* p < 0.05. For co-infection, healthy *G. mellonella* larvae (n = 10 per group) infected with differing inocula of *L. gasseri* (0.1, 0.6, and 0.8 OD) 16 h before secondary infection with *N. gonorrhoeae* (0.8 OD), were examined for survival over a period of 48 h. Data are mean survival from 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between the survival of GC broth infected control group and the survival of larval infected with different *Lactobacillus* group at each time point. As well, Unpaired t test was carried out between survival of larval infected with *N. gonorrhoeae* alone and co-infected groups at each time point. \* p < 0.05.

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| ***L. brevis*** |
| **Time (h)** | **GC broth vs 0.1 OD** | **GC broth vs 0.6 OD** | **GC broth vs 0.8 OD** | **GC broth vs 1.0 OD** |
| **20** | Yes (P=0.0142) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **24** | Yes (P=0.0002) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **48** | Yes (P=0.0002) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |

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| ***L. crispatus*** |
| **Time (h)** | **GC broth vs 0.1 OD** | **GC broth vs 0.6 OD** | **GC broth vs 0.8 OD** | **GC broth vs 1.0 OD** |
| **16** | No (P=0.0534) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **24** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |

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| ***L. gasseri*** |
| **Time (h)** | **GC broth vs 0.1 OD** | **GC broth vs 0.6 OD** | **GC broth vs 0.8 OD** | **GC broth vs 1.0 OD** |
| **16** | No (P=0.1516) | No (P=0.6311) | No (P=0.4446) | No (P=0.2860) |
| **24** | No (P=0.3348) | No (P=0.6325) | Yes (P=0.0196) | Yes (P=0.0463) |
| **40** | No (P=0.9049) | No (P=0.4889) | Yes (P=0.0113) | Yes (P=0.0377) |
| **48** | No (P=0.9049) | No (P=0.1890) | Yes (P=0.0057) | Yes (P=0.0066) |

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| **Time (h)** | **GC brothvs****P9-17 + *L. gasseri* (0.1 OD)** | **GC brothvs****P9-17 + *L. gasseri* (0.6 OD)** | **GC brothvs****P9-17 + *L. gasseri* (0.8 OD)** |
| **24** | No (P= 0.6932) | No (P= 0.2796) | No (P= 0.6272) |
| **48** | No (P= 0.4766) | No (P= 0.2791) | No (P= 0.1926) |

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| **Time (h)** | ***N. gonorrhoeae* vs****P9-17 + *L. gasseri* (0.1 OD)** | ***N. gonorrhoeae* vs****P9-17 + *L. gasseri* (0.6 OD)** | ***N. gonorrhoeae* vs****P9-17 + *L. gasseri* (0.8 OD)** |
| **24** | No (P= 0.6932) | No (P= 0.2796) | No (P= 0.6272) |
| **48** | No (P= 0.4766) | No (P= 0.2791) | No (P= 0.1926) |

**Figure 8**. The effect of priming with *N. gonorrhoeae* non-killing dose (105 CFU per larva) on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) pre-treated with non-killing dose 16 h before subsequent infection with low dose of *N. gonorrhoeae* (A, 0.1 OD), medium dose of *N. gonorrhoeae* (B, 0.4 OD), high dose of *N. gonorrhoeae* (C, 0.8 OD), high dose of *L. gasseri* (D, 0.8 OD), heat-inactivated (HI) high dose of *N. gonorrhoeae* and *L. gasseri* (E, 0.8 OD), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between primed group and control groups at each time point. \* p < 0.05.

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| **A** |
| **Time (h)** | **Primed vs control (0.1 OD)** | **Primed vs control (GC broth)** |
| **20** | No (P=0.5185) | No (P= 0.8449) |
| **24** | Yes (P=0.0352) | Yes (P=0.0073) |
| **28** | Yes (P=0.0352) | Yes (P=0.0073) |
| **48** | Yes (P=0.0053) | Yes (P=0.0002) |

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| **B** |
| **Time (h)** | **Primed vs control (0.1 OD)** | **Primed vs control (0.4 OD)** | **Primed vs control (GC broth)** |
| **-3** | No (P= 0.6779) | No (P= 0.3739) | No (P= 0.0816) |
| **0** | No (P= 0.6779) | No (P= 0.3739) | No (P= 0.0816) |
| **2** | No (P= 0.2028) | No (P= 0.1194) | Yes (P=0.0033) |
| **4** | Yes (P=0.0053) | Yes (P=0.0315) | Yes (P<0.0001) |
| **12** | Yes (P=0.0007) | Yes (P=0.0075) | Yes (P<0.0001) |
| **24** | Yes (P=0.0007) | Yes (P=0.0075) | Yes (P<0.0001) |
| **40** | Yes (P=0.0007) | Yes (P=0.0075) | Yes (P<0.0001) |
| **48** | Yes (P=0.0002) | Yes (P=0.0145) | Yes (P<0.0001) |

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| **C** |
| **Time (h)** | **Primed vs control (0.1 OD)** | **Primed vs control (0.8 OD)** | **Primed vs control (GC broth)** |
| **4** | Yes (P=0.0002) | Yes (P=0.0002) | Yes (P <0.0001) |
| **20** | Yes (P <0.0001) | Yes (P=0.0002) | Yes (P <0.0001) |
| **24** | Yes (P <0.0001) | Yes (P=0.0023) | Yes (P <0.0001) |
| **28** | Yes (P <0.0001) | Yes (P=0.0309) | Yes (P <0.0001) |
| **48** | Yes (P <0.0001) | Yes (P=0.0399) | Yes (P <0.0001) |

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| **D** |
| **Time (h)** | **Primed vs control (0.1 OD)** | **Primed vs control (0.8 OD)** | **Primed vs control (GC broth)** |
| **4** | No (P=0.1191) | Yes (P=0.0498) | Yes (P=0.0025) |
| **20** | Yes (P=0.0062) | Yes (P=0.0009) | Yes (P <0.0001) |
| **24** | Yes (P=0.0071) | Yes (P=0.0021) | Yes (P <0.0001) |
| **28** | Yes (P=0.0071) | Yes (P=0.0025) | Yes (P <0.0001) |
| **48** | Yes (P=0.0012) | Yes (P=0.0007) | Yes (P <0.0001) |

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| **E** |
| ***N. gonorrhoeae*** |
| **Time (h)** | **Primed vs control P9-17 (0.8 OD and HI)**  | **Primed vs control P9-17 (0.8 OD)** | **Primed vs control P9-17 (0.1 OD)** | **Primed vs control (GC broth)** |
| **4** | Yes (P=0.0013) | Yes (P=0.0003) | Yes (P=0.0008) | Yes (P<0.0001) |
| **20** | Yes (P=0.0285) | No (P=0.1036) | Yes (P=0.0006) | Yes (P<0.0001) |
| **24** | Yes (P=0.0285) | No (P=0.1103) | Yes (P=0.0008) | Yes (P<0.0001) |
| **28** | Yes (P=0.0132) | No (P=0.2428) | Yes (P=0.0001) | Yes (P<0.0001) |
| **48** | Yes (P=0.0031) | No (P=0.1861) | Yes (P<0.0001) | Yes (P<0.0001) |
| ***L. gasseri*** |
| **Time (h)** | **Primed vs *L. gasseri* (0.8 OD, HI)** | **Primed vs *L. gasseri* (0.8 OD)** | **Primed vs control *L. gasseri* (0.1 OD)** | **Primed vs control (GC broth)** |
| **4** | No (P=0.1583) | No (P=0.1583) | No (P=0.2428) | Yes (P=0.0073) |
| **20** | No (P=0.8298) | No (P=0.6433) | No (P=0.0784) | No (P=0.4791) |
| **24** | No (P=0.8298) | No (P=0.6433) | No (P=0.0522) | No (P=0.4791) |
| **28** | No (P=0.3739) | No (P=0.3739) | No (P=0.0522) | No (P=0.4791) |
| **48** | No (P=0.2302) | No (P=0.3739) | Yes (P=0.0457) | No (P=0.1901) |

**Figure 9**. The effect of depleting haemocytes with clodronate liposomes and subsequent infection with varying doses of *N. gonorrhoeae* on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) pre-treated with either empty liposomes or clodronate liposomes 16 h before subsequent infection with low dose of *N. gonorrhoeae* (A, 0.1 OD), medium dose (B, 0.4 OD), and high dose (C, 0.8 OD), were examined for survival by response to touch over a period of 48 h. Tested liposome concentrations were neat and 1/5. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between empty liposome groups and clodronate groups at each time point. \* p < 0.05.

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| **A** |
| **Time (h)** | **Neat empty vs neat clodronate** | **1/5 empty vs 1/5 clodronate** |
| **4** | No (P>0.9999) | Yes (P= 0.0016) |
| **20** |  | Yes (P= 0.0072) |
| **24** | No (P=0.0550) | Yes (P= 0.0072) |
| **28** | No (P=0.0550) | Yes (P= 0.0097) |
| **48** | No (P=0.0890) | Yes (P= 0.0215) |

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| **B** |
| **Time (h)** | **Neat empty vs neat clodronate** | **1/5 empty vs 1/5 clodronate** |
| **2** | No (P=0.5237) | No (P=0.2309) |
| **20** | No (P=0.3195) | No (P=0.2242) |
| **24** | No (P=0.3195) | No (P=0.2242) |
| **28** | No (P=0.3195) | No (P=0.1967) |
| **48** | No (P=0.2111) | No (P=0.1967) |

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| **C** |
| **Time (h)** | **Neat empty vs neat clodronate** | **1/5 empty vs 1/5 clodronate** |
| **18** | No (P=0.0591) | No (P=0.2978) |
| **24** | Yes (P= 0.0106) | Yes (P= 0.0468) |
| **42** | Yes (P= 0.0240) | Yes (P= 0.0468) |
| **48** | Yes (P= 0.0054) | Yes (P= 0.0141) |

**Figure 10**. The effect of varying dosing of ceftriaxone and azithromycin on the survival of *N. gonorrhoeae* infected *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing doses of antibiotic and same dose of different gonococci isolates (108 CFU per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between the survival of gonococci infected *G. mellonella* group without antibiotic and the survival of *G. mellonella* group injected with gonococci and antibiotic at each time point. \* p < 0.05.

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| **P9-17 (CEF)** |
| **Time (h)** | **0 vs 0.625** | **0 vs 1** | **0 vs 2.5** | **0 vs 5** |
| **16** | No (P=0.3727) | No (P=0.1818) | Yes (P= 0.0345) | No (P=0.0505) |
| **24** | No (P= 0.6533) | No (P=0.1446) | Yes (P= 0.0211) | Yes (P=0.0354) |
| **40** | No (P=0.1357) | No (P=0.0976) | Yes (P=0.0001) | Yes (P=0.0001) |
| **48** | No (P=0.1945) | Yes (P=0.0442) | Yes (P<0.0001) | Yes (P<0.0001) |

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| **GC-03 (CEF)** |
| **Time (h)** | **0 vs 0.008** | **0 vs 0.1** | **0 vs 1** | **0 vs 8** | **0 vs 16** |
| **16** | No (P=0.3918) | No (P=0.2754) | No (P=0.3384) | No (P=0.4134) | No (P=0.7196) |
| **24** | No (P= 0.1508) | Yes (P=0.0330) | No (P=0.1476) | No (P=0.2250) | No (P=0.5955) |
| **40** | No (P= 0.3108) | Yes (P=0.0347) | No (P=0.7351) | No (P=0.3768) | No (P=0.3768) |
| **48** | No (P= 0.5078) | Yes (P=0.0173) | No (P=0.6174) | No (P=0.6852) | No (P=0.3749) |

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| **GC-10 (CEF)** |
| **Time (h)** | **0 vs 0.125** | **0 vs 0.625** | **0 vs 3.125** |
| **16** | Yes (P=0.0454) | No (P=0.1265) | Yes (P=0.0027) |
| **24** | Yes (P=0.0338) | No (P=0.1591) | Yes (P=0.0026) |
| **40** | Yes (P=0.0443) | Yes (P=0.0380) | Yes (P=0.0001) |
| **48** | Yes (P=0.0240) | Yes (P=0.0215) | Yes (P<0.0001) |

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| **GC-17 (CEF)** |
| **Time (h)** | **0 vs 0.03** | **0 vs 0.125** | **0 vs 0.625** | **0 vs 3.125** | **0 vs 30** | **0 vs 60** |
| **16** | No (P=0.0698) | No (P=0.0919) | No (P=0.5261) | No (P=0.1287) | No (P=0.7689) | No (P=0.0703) |
| **24** | No (P= 0.1803) | Yes (P=0.0291) | No (P=0.2451) | No (P=0.0631) | No (P=0.9164) | Yes (P=0.0412) |
| **40** | No (P=0.4797) | No (P=0.0876) | No (P=0.6845) | No (P=0.4248) | No (P>0.9999) | No (P=0.0716) |
| **48** | No (P=0.4328) | No (P=0.0775) | No (P=0.6169) | No (P=0.3776) | No (P=0.9188) | No (P=0.0919) |

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| **GC-26 (CEF)** |
| **Time (h)** | **0 vs 0.125** | **0 vs 0.625** | **0 vs 3.125** |
| **16** | No (P=0.3371) | No (P=0.0782) | No (P=0.0571) |
| **24** | No (P=0.5605) | No (P=0.1725) | No (P=0.1407) |
| **40** | No (P=0.3866) | No (P=0.2154) | Yes (P=0.0297) |
| **48** | No (P=0.1880) | No (P=0.0706) | Yes (P=0.0076) |

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| **GC-30 (CEF)** |
| **Time (h)** | **0 vs 0.05** | **0 vs 1** | **0 vs 5** | **0 vs 25** | **0 vs 50** | **0 vs 500** | **0 vs 1000** |
| **16** | No (P=0.0802) | No (P=0.2197) | No (P=0.5384) | Yes (P=0.0433) | Yes (P=0.0048) | No (P=0.0872) | No (P=0.0763) |
| **24** | No (P= 0.3091) | Yes (P=0.0493) | No (P=0.2372) | Yes (P=0.0484) | Yes (P=0.0010) | Yes (P=0.0245) | No (P=0.0649) |
| **40** | No (P=0.5966) | No (P=0.4629) | No (P=0.5944) | No (P=0.1213) | No (P=0.1572) | Yes (P=0.0287) | No (P=0.1130) |
| **48** | No (P=0.4061) | No (P=0.5765) | No (P=0.3601) | No (P=0.1363) | No (P=0.2100) | Yes (P=0.0218) | No (P=0.0629) |

**Supplementary figures**

**Figure 1S**. The effect of growth supplements on *G. mellonella* survival following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with different growth supplements were examined for survival by response to touch over a period of 72 h. Data are mean survival from a minimum of 6 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between the survival of untreated group against remaining groups at each time point. \* p < 0.05.

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| **Growth supplement/****Time (h)** | **Trauma** | **Supplemented GC broth** | **PBS** | **PBS + GC supplements** | **PBS + ferric nitrate** | **PBS + ferric citrate** |
| **24** | No (P=0.0810) | Yes (P=0.0214) | No (P=0.0713) | No (P=0.1159) | Yes (P=0.0007) | Yes (P<0.0001) |
| **48** | No (P=0.0530) | No (P=0.1100) | No (P=0.0789) | No (P=0.1143) | Yes (P=0.0011) | Yes (P<0.0001) |
| **72** | No (P=0.7105) | No (P=0.7569) | No (P=0.6970) | No (P=0.3499) | Yes (P=0.0083) | Yes (P=0.0004) |

**Figure 2S**. The effect of varying inoculum dose of sialylated *N. gonorrhoeae* on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing inocula of sialylated gonococci (up to 5 × 108 CFU per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 4 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between sialylated and not groups at each time point. \* p < 0.05.

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| **Time (h)** | **0.1 OD vs 0.1 OD (CMP)** | **0.6 OD vs 0.6 OD (CMP)** | **0.8 OD vs 0.8 OD (CMP)** | **1.0 OD vs 1.0 OD (CMP)** |
| **14** | No (P=0.1778) | Yes (P=0.0351) | No (P=0.0714) | No (P=0.6328) |
| **19** | No (P>0.9999) | No (P=0.0690) | No (P=0.5896) | No (P=0.7132) |
| **24** | No (P=0.3784) | No (P=0.9448) | No (P=0.6332) | No (P=0.9373) |
| **40** | No (P=0.4211) | No (P=0.4697) | No (P=0.5432) | No (P=0.8217) |
| **48** | No (P=0.3870) | No (P=0.8023) | No (P=0.5292) | No (P=0.8217) |

**Figure 3S.** The effect of varying inoculum dose of *P. aeruginosa* on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing inocula of *P. aeruginosa* (up to 250,000 CFU per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 6 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between the survival of GC broth infected control group and the survival of *P. aeruginosa* infected groups at each time point. \* p < 0.05.

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| **Time (h)** | **GC broth vs 150** | **GC broth vs 240** | **GC broth vs 2400** | **GC broth vs 25,000** | **GC broth vs 250,000** |
| **2** | No (P=0.2337) | No | No | No | No (P=0.2337) |
| **3** | No (P=0.2337) | No | No | No | No (P=0.2337) |
| **4** | No (P=0.2337) | No | No | No | No (P=0.2337) |
| **6** | No (P=0.2337) | No | No | No | No (P=0.2337) |
| **7** | No (P=0.2337) | No | No | No | Yes (P=0.0090) |
| **8** | No (P=0.2337) | No | No | No | Yes (P<0.0001) |
| **9** | No | No | Yes (P=0.0031) | No (P=0.0861) | Yes (P<0.0001) |
| **10** | No | No | Yes (P=0.0031) | Yes (P=0.0041) | Yes |
| **11** | No  | No (P=0.0861) | Yes (P<0.0001) | Yes (P=0.0011) | Yes |
| **16** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **24** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **40** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |
| **48** | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) | Yes (P<0.0001) |

**Figure 4S**. The effect of ceftriaxone and azithromycin on the survival of *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing doses of ceftriaxone (up to 1000 µg/ml) and azithromycin (up to 1024 µg/ml), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 2 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between the survival of GC broth infected control group and the survival of antibioticinfected groups at each time point. \* p < 0.05.

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| **Time (h)** | **GC broth vs 50 cef** | **GC broth vs 500 cef** | **GC broth vs 1000 cef** | **GC broth vs 256 azi** | **GC broth vs 512 azi** | **GC broth vs 1024 azi** |
| **16** | No (P=0.2446) | No (P=0.2446) | No (P=0.2446) | No (P=0.5666) | No (P=0.1588) | No (P=0.3156) |
| **24** | No (P=0.2446) | No (P=0.2446) | No (P=0.2446) | No (P=0.5666) | No (P=0.1588) | No (P=0.3156) |
| **40** | No (P=0.2446) | No (P=0.2446) | No (P=0.2446) | No (P=0.5666) | No (P=0.1588) | No (P=0.3156) |
| **48** | No (P=0.5361) | No (P=0.2446) | No (P=0.2446) | No (P=0.5666) | No (P=0.1588) | No (P=0.3156) |

**Figure 5S.** The effect of varying dosing of ceftriaxone and azithromycin combined treatment on the survival of *N. gonorrhoeae* infected *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with differing doses of antibiotic and same dose of different gonococci isolates (108 CFU per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from a minimum of 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between the survival of gonococci infected *G. mellonella* group without antibiotic and the survival of *G. mellonella* group injected with gonococci and antibiotic at each time point. \* p < 0.05.

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| **P9-17** |
| **Time (h)** | **0 vs 256A and 0.625C** | **0 vs 256A and 3.125C** |
| **16** | No (P=0.0511) | No (P=0.0511) |
| **24** | Yes (P=0.0248) | No (P=0.0996) |
| **40** | Yes (P=0.0001) | Yes (P=0.0010) |
| **48** | Yes (P<0.0001) | Yes (P=0.0004) |

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| **GC-10** |
| **Time (h)** | **0 vs 256A and 0.625C** | **0 vs 256A and 3.125C** |
| **16** | Yes (P=0.0258) | Yes (P=0.0075) |
| **24** | Yes (P=0.0219) | Yes (P=0.0067) |
| **40** | Yes (P=0.0052) | Yes (P=0.0016) |
| **48** | Yes (P=0.0030) | Yes (P=0.0009) |

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| **GC-26** |
| **Time (h)** | **0 vs 256A and 0.625C** | **0 vs 256A and 3.125C** |
| **16** | No (P=0.8073) | No (P=0.2371) |
| **24** | No (P=0.8371) | No (P=0.2310) |
| **40** | No (P=0.2958) | Yes (P=0.0471) |
| **48** | No (P=0.0844) | Yes (P=0.0155) |

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| **GC-30** |
| **Time (h)** | **0 vs 256A and 0.625C** | **0 vs 256A and 3.125C** |
| **16** | No (P=0.0994) | No (P=0.8669) |
| **24** | No (P=0.1964) | No (P=0.6960) |
| **40** | No (P=0.0764) | No (P=0.5122) |
| **48** | No (P=0.1010) | No (P=0.2721) |

**Figure 6S.** The effect of varying dosing of topical treatments of gonococci on the survival of *N. gonorrhoeae* infected *G. mellonella* following incubation at 37°C for 48 h. Healthy larva (n = 10 per group) infected with *N. gonorrhoeae* (107 CFU per larva) and differing doses of monocaprin (up to 50 mM per larva) and silver nanoparticles (up to 58.44 µM per larva), were examined for survival by response to touch over a period of 48 h. Data are mean survival from 3 independent experiments. Error bars represent the standard deviation of the mean. Unpaired t test was carried out between the survival of gonococci alone infected *G. mellonella* group without and the survival of *G. mellonella* group injected with gonococci and either monocaprin or silver nanoparticles at each time point. As well, Unpaired t test was carried out between the survival of GC broth infected control group and the survival of monocaprin and silver nanoparticlesinjected groups at each time point. \* p < 0.05.

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| **Monocaprin** |
| **Time (h)** | **P9-17 vs P9-17 (0.1 mM)** | **P9-17 vs P9-17 (0.5 mM)** | **P9-17 vs P9-17 (1.0 mM)** | **P9-17 vs P9-17 (10mM)** | **P9-17 vs P9-17 (50 mM)** |
| **16** | No (P=0.1568) | No (P=0.8817) | No (P=0.8026) | No (P=0.2096) | No (P=0.0881) |
| **24** | No (P=0.1518) | No (P=0.5415) | No (P>0.9999) | No (P=0.2904) | No (P=0.0917) |
| **40** | No (P=0.2311) | No (P=0.4713) | No (P=0.2284) | No (P=0.6253) | No (P=0.0826) |
| **48** | No (P=0.2311) | No (P=0.4713) | No (P=0.2284) | No (P=0.6253) | No (P=0.0826) |

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| **Time (h)** | **GC broth vs 0.1 mM** | **GC broth vs 0.5 mM** | **GC broth vs 1.0 mM** | **GC broth vs 10mM** | **GC broth vs 50 mM** |
| **16** | No (P=0.5666) | No (P=0.5666) | No (P=0.5666) | No (P=0.6454) | Yes (P<0.0001) |
| **24** | No (P=0.5666) | No (P=0.5666) | No (P=0.5666) | No (P=0.6454) | Yes (P<0.0001) |
| **40** | No (P=0.5666) | No (P=0.5666) | No (P=0.5666) | No (P=0.6454) | Yes (P<0.0001) |
| **48** | No (P=0.5666) | No (P=0.5666) | No (P=0.5666) | No (P=0.6454) | Yes (P<0.0001) |

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| **Silver nanoparticles** |
| **Time (h)** | **P9-17 vs P9-17 (0.467 µM)** | **P9-17 vs P9-17 (2.338 µM)** | **P9-17 vs P9-17 (11.688 µM)** | **P9-17 vs P9-17 (58.44 µM)** |
| **16** | No (P=0.7676) | No (P=0.0890) | No (P=0.1012) | No (P=0.1161) |
| **24** | No (P=0.5391) | No (P=0.0734) | No (P=0.1012) | No (P=0.2746) |
| **40** | No (P=0.5790) | No (P=0.5614) | No (P=0.8340) | No (P=0.3453) |
| **48** | No (P=0.7247) | No (P>0.9999) | No (P=0.8298) | No (P=0.6433) |

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| **Time (h)** | **GC broth vs 0.467 µM** | **GC broth vs 2.338 µM** | **GC broth vs 11.688 µM** | **GC broth vs 58.44 µM** |
| **16** | No (P=0.2446) | No (P=0.2446) | No (P=0.8449) | No (P=0.2446) |
| **24** | No (P=0.2446) | No (P=0.2446) | No (P=0.8449) | No (P=0.2446) |
| **40** | No (P=0.9112) | No (P=0.2446) | No (P=0.1901) | No (P=0.5361) |
| **48** | No (P=0.4591) | No (P=0.2446) | No (P=0.1901) | No (P=0.5361) |