Text S1. Supplementary materials and methods

Source of bacterial strains.

FA1090 was a gift from C. Genco.

28BL was a gift from S. Johnson.

H18-208 was a gift from D. Eyre.

MS11 was procured from ATCC (ATCC® BAA-133™).

The clinical isolates GCGS0402, GCGS0449, GCGS0759, and GCGS1095 were collected by the Centers for Disease Control and Prevention's Gonococcal Isolate Surveillance Project. [1]

The clinical isolate NY0195 was collected by the New York City Public Health Laboratory, Department of Health and Mental Hygeine. [2]

Preparation of compounds for bacteril killing assays.

In initial screening experiments, bile acids were screened at final concentrations of 1 mg/mL, 0.1 mg/mL, and 10 μg/mL. Fatty acids were screened at final concentrations of 1% (v/v), 0.1% (v/v), and 0.01% (v/v), except for lauric acid (C12:0) and palmitic acid (C16:0), which were screened at final concentrations of 0.1% (v/v), 0.01% (v/v), and 0.001% (v/v) due to solubility limitations.

Initial bile salt bacterial killing assays and assays of the bacterial strain panel were performed by preparing 2 mg/mL working stocks from deoxycholic acid or chenodeoxycholic acid solubilized to 50 mg/mL in pure ethanol and diluting these 1:1 with a bacterial suspension of OD_{600} 0.2. However, while both deoxycholic acid and chenodeoxycholic acid are soluble in GW at the final concentration of 1 mg/mL, precipitation (visible cloudiness) occurred in the 2 mg/mL working stock. To improve the quantitative accuracy of this process for dose-response experiments, we instead prepared stock solutions at 1 mg/mL and sonicated these in a bath sonicator at room temperature for 1 hour, after which solutions were visually clear. The 1 mg/mL

working stocks were then diluted with additional GW medium as appropriate, and mixed 1:1 with a bacterial suspension of OD_{600} 0.2 to test the efficacy of deoxycholic acid or chenodeoxycholic acid at concentrations of 0.5 mg/mL and below.

References.

1. Grad YH, Harris SR, Kirkcaldy RD et al. Genomic Epidemiology of Gonococcal Resistance to Extended-Spectrum Cephalosporins, Macrolides, and Fluoroquinolones in the United States, 2000-2013. *J Infect Dis* 2016; **214**: 1579-87.
2. Mortimer TD, Pathela P, Crawley A et al. The distribution and spread of susceptible and resistant Neisseria gonorrhoeae across demographic groups in a major metropolitan center. *Clin Infect Dis* 2020.

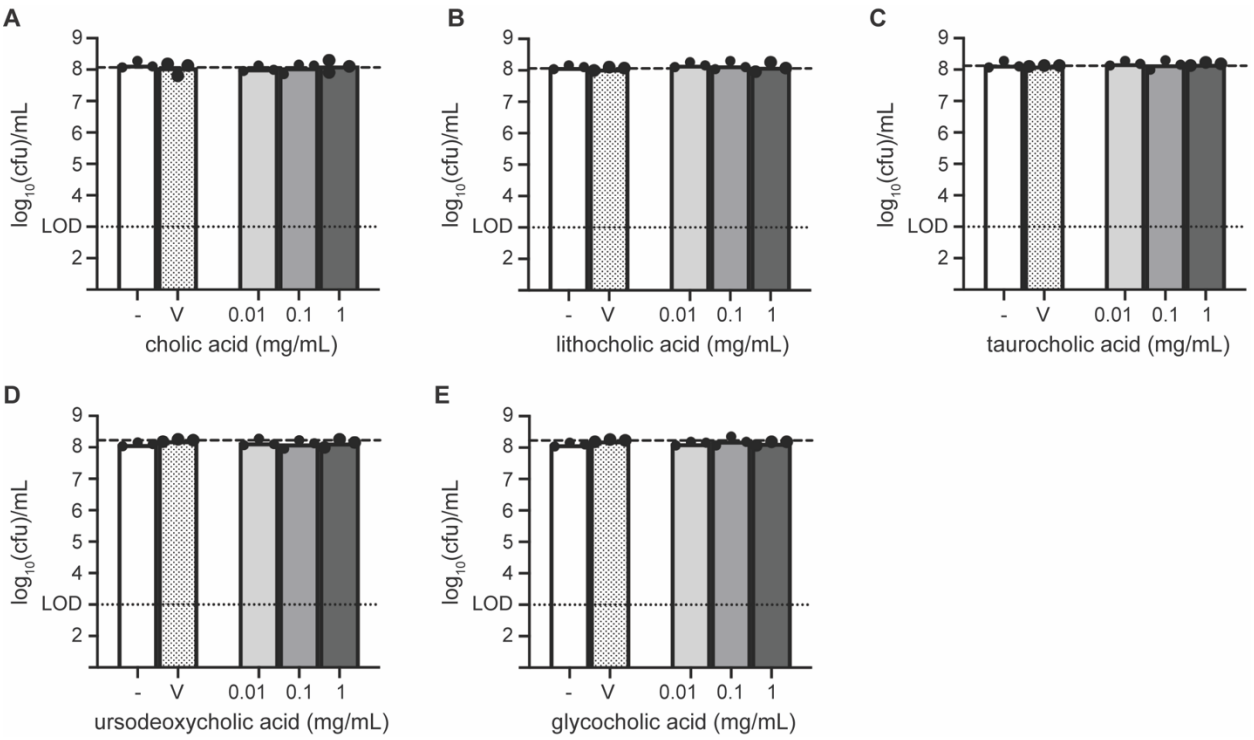

Figure S1. Survival of *N. gonorrhoeae* FA1090 in the presence of bile acids. Survival of FA1090 in Graver-Wade (GW) medium with no supplementation (-), GW with the corresponding vehicle control (V), or GW with varying concentrations of (**A**) cholic acid (vehicle control, 4% methanol); (**B**) lithocholic acid (vehicle control, 5% ethanol); (**C**) taurocholic acid (vehicle control, 2% water); (**D**) ursodeoxycholic acid (vehicle control, 2% ethanol); or (**E**) glycocholic acid (vehicle control, 2% ethanol). Samples were mixed gently and incubated at ambient temperature for one minute, after which exposure was halted by dilution and plating. Dotted line indicates the limit of detection (LOD, 10^3 cfu/mL). Dashed line indicates bacterial survival in the vehicle control condition. Where experiments shared control groups, these are reproduced in each panel for ease of reference.

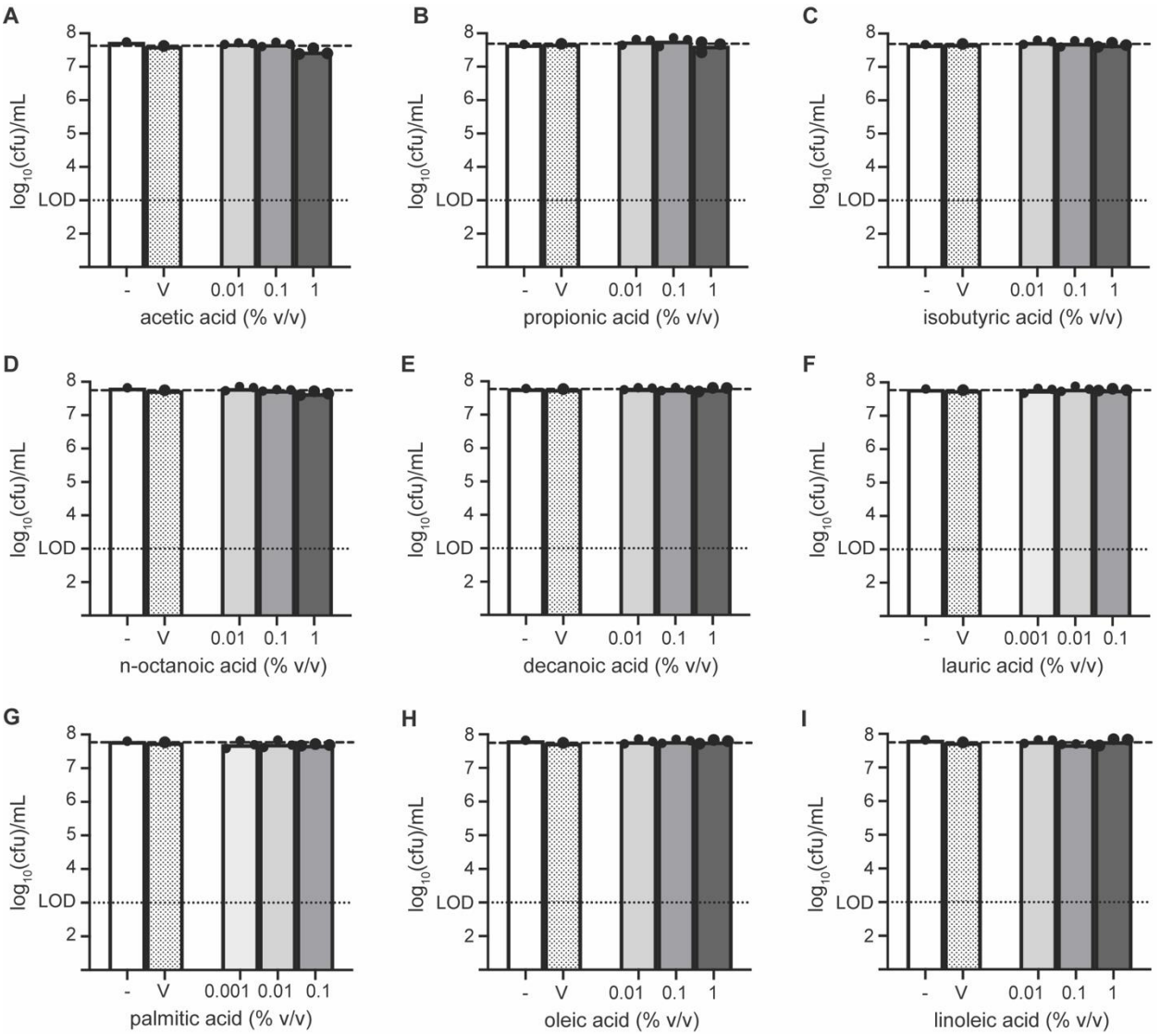

Figure S2. Survival of *N. gonorrhoeae* FA1090 in the presence of fatty acids. Survival of FA1090 in Graver-Wade (GW) medium with no supplementation (-), GW with vehicle only (V), or GW with varying concentrations of (**A**) acetic acid (vehicle control, 1% ethanol), (**B**) proprionic acid (vehicle control, 1% ethanol), (**C**) isobutyric acid (vehicle control, 1% ethanol), (**D**) n-octanoic acid (vehicle control, 1% ethanol), (**E**) decanoic acid (vehicle control, 1% ethanol), (**F**) lauric acid (vehicle control, 0.1% ethanol), (**G**) palmitic acid (vehicle control, 0.1% ethanol), (**H**) oleic acid (vehicle control, 1% ethanol), or (**I**) linoleic acid (vehicle control, 1% ethanol). Samples were mixed gently and incubated at ambient temperature for one minute, after which exposure was halted by dilution and plating. Dotted line indicates the limit of detection (LOD, 10^3 cfu/mL). Dashed line indicates bacterial survival in the vehicle control condition. Where experiments shared control groups, these are reproduced in each panel for ease of reference.

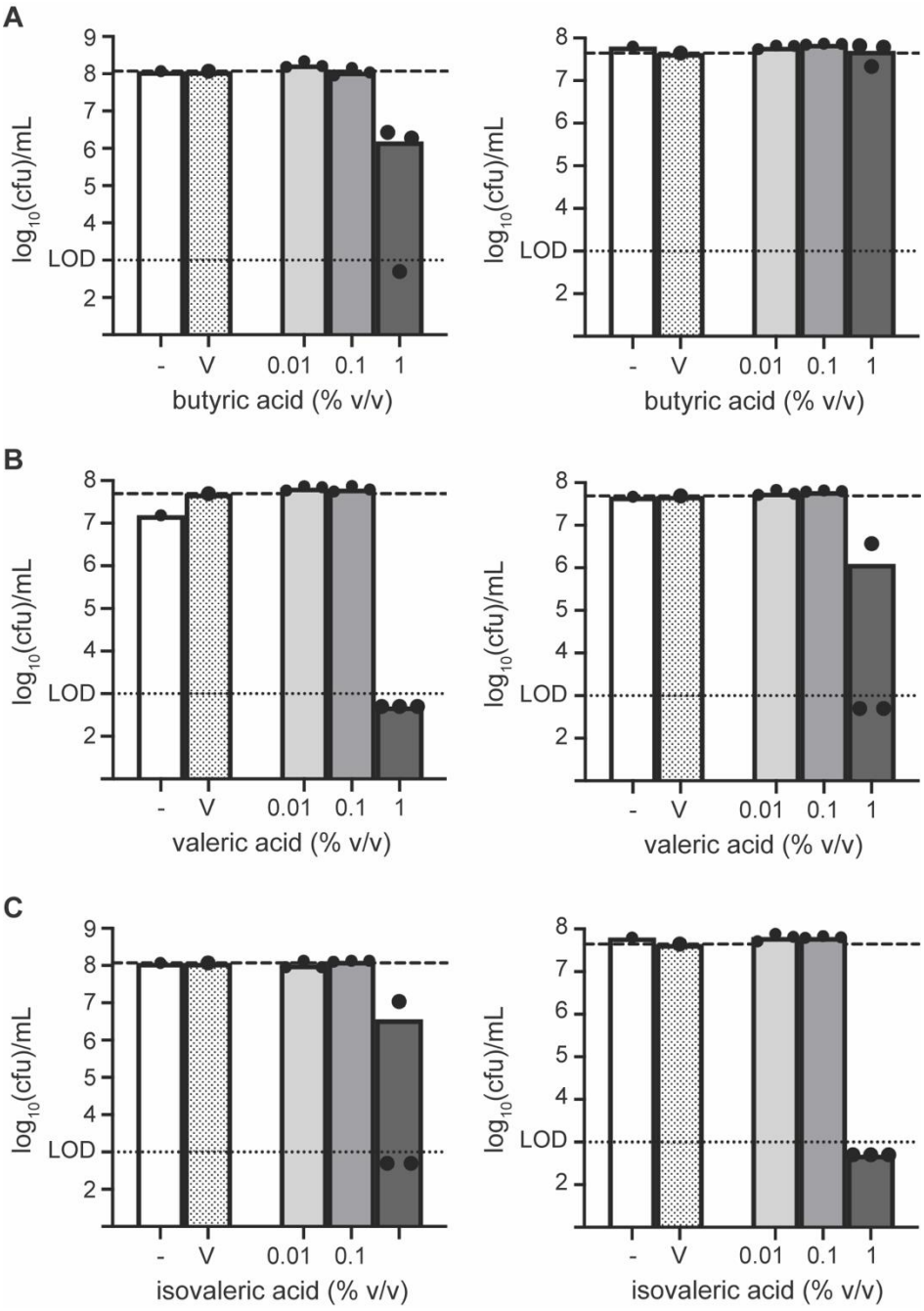

Figure S3. Fatty acids with variable killing efficacy against *N. gonorrhoeae* FA1090. Survival of FA1090 in Graver-Wade (GW) medium with no supplementation (-), GW containing 1% ethanol (vehicle control, V), or GW with varying concentrations of (**A**) butyric acid, (**B**) valeric acid, or (**C**) isovaleric acid. Samples were mixed gently and incubated at ambient temperature for one minute, after which exposure was halted by dilution and plating. Dotted line indicates the limit of detection (LOD, 10^3 cfu/mL). Dashed line indicates bacterial survival in the vehicle control condition. Results of two independent experiments shown for each fatty acid. Where experiments shared control groups, these are reproduced in each panel for ease of reference.