TABLE S1

Strain	poly-T sequence in <i>norM</i> promoter	Source
H041	6	R. Nicholas ^a
F89	6	R. Nicholas
NYC-201409-05	6	C. del Rio ^b
NYC-201411-12	6	C . del Rio
NYC-201411-06	6	C . del Rio
AZM LLR1	6	M. Unemo ^c
AZM LLR2	6	M. Unemo
AZM LLR3	6	M. Unemo
SK33414	6	J. Dillon ^d
SK1902	7	J. Dillon

^a University of North Carolina-Chapel School of Medicine, Chapel Hill, NC; ^b Emory University School of Medicine, Atlanta, GA; ^c Orebro Hospital, Orebro, Sweden; ^d University of Saskatchewan, Saskatoon, Canada

TABLE S2. Susceptibility of gonococcal strain HO41 to NorM substrates

MIC $(\mu g/ml)^a$

Strains	BE^b	EB	SOM
HO41	>40	20	0.15
HO41norM::kan	10	1.25	0.0375

^aAll results are representative from 3 or more independent determinations

^bBE: berberine; EB: ethidium bromide; SOM: solithromycin

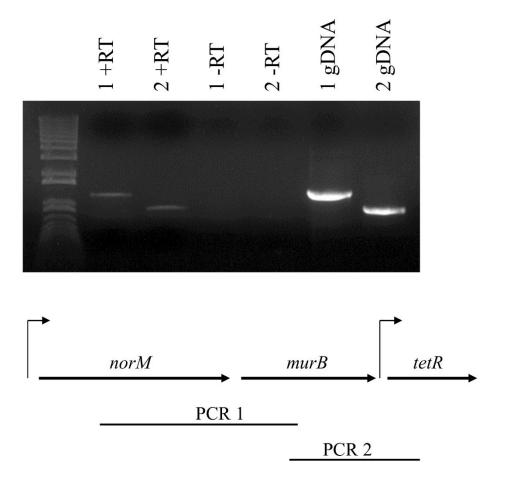


FIG S1: RT-PCR was performed on RNA from strain FA19Str^R. Reverse transcription was done with random hexamers while PCRs were done with primers tetRSma1/murB_qRTF for PCR 1 (1 +RT) and N8/murB_qRTR for PCR 2 (2 +RT). Controls without reverse transcriptase (-RT) and on genomic DNA (gDNA) were done with the same primers.

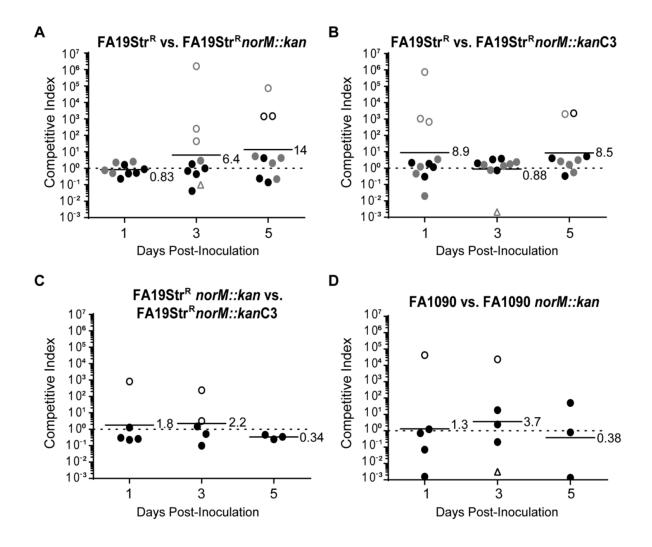


FIG S2 Mutation of *norM* did not alter the *in vivo* fitness of *N. gonorrhoeae* strain FA19 or FA1090 in the female mouse model of gonorrhea infection. Mice were inoculated vaginally with similar numbers of wild-type bacteria and the mutant or complemented mutant strains. Results are expressed as the competitive index (CI) for each mouse on each culture day (CI =1, equal competitition; CI <1, mutant strain attenuated; CI >1, mutant strain out-competed the wild-type strain). The geometric mean of the CI values is shown and is represented by the bars. Open circles indicate that only the mutant strain was recovered from the vaginal swabs at the indicated time point. Open triangles indicate that only the wild-type strain was recovered from the vaginal

swabs at the indicated time point. A. The FA19Str^R *norM::kan* mutant and B. FA19Str^R *norM::kan*C3 complemented mutant exhibited similar fitness as wild-type FA19Str^R bacteria in vivo. Pairwise analyses of competitive indices from days 1, 3, and 5 of the FA19Str^R vs.

FA19Str^R *norM::kan* and the FA19Str^R vs. FA19Str^R *norM::kan*C3 competitions did not show a statistical difference by the Mann-Whitney test, indicating that all strains competed similarly *in vivo*. C. No difference in the ability of the FA19Str^R *norM::kan* mutant and the FA19Str^R *norM::kan*C3 complemented mutant to compete *in vivo* was observed. D. The FA1090 vs.

FA1090*norM::kan* competitive experiment also did not show a difference in *in vivo* fitness between the tested strains.