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Journal Title: PLoS Pathogens
Volume: Volume 15, Number 12
Publisher: PUBLIC LIBRARY SCIENCE | 2019-12-01, Pages e1008233-e1008233
Type of Work: Article
Publisher DOI: 10.1371/journal.ppat.1008233
Permanent URL: https://pid.emory.edu/ark:/25593/v7805

Final published version: http://dx.doi.org/10.1371/journal.ppat.1008233

Accessed February 13, 2020 3:41 PM EST
Transcriptional regulation of a gonococcal gene encoding a virulence factor (L-lactate permease)

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Abstract

GdhR is a GntR-type regulator of Neisseria gonorrhoeae encoded by a gene (gdhR) belonging to the MtrR regulon, which comprises multiple genes required for antibiotic resistance such as the mtrCDE efflux pump genes. In previous work we showed that loss of gdhR results in enhanced gonococcal fitness in a female mouse model of lower genital tract infection. Here, we used RNA-Seq to perform a transcriptional profiling study to determine the GdhR regulon. GdhR was found to regulate the expression of 2.3% of all the genes in gonococcal strain FA19, of which 39 were activated and 11 were repressed. Within the GdhR regulon we found that lctP, which encodes a unique L-lactate transporter and has been associated with gonococcal pathogenesis, was the highest of GdhR-repressed genes. By using in vitro transcription and DNase I footprinting assays we mapped the lctP transcriptional start site (TSS) and determined that GdhR directly inhibits transcription by binding to an inverted repeat sequence located 9 bases downstream of the lctP TSS. Epistasis analysis revealed that, while loss of lctP increased susceptibility of gonococci to hydrogen peroxide (H₂O₂) the loss of gdhR enhanced resistance; however, this GdhR-endowed property was reversed in a double gdhR lctP null mutant. We assessed the effect of different carbon sources on lctP expression and found that D-glucose, but not L-lactate or pyruvate, repressed lctP expression within a physiological concentration range but in a GdhR-independent manner. Moreover, we found that adding glucose to the medium enhanced susceptibility of gonococci to hydrogen peroxide. We propose a model for the role of lctP regulation via GdhR and glucose in the pathogenesis of N. gonorrhoeae.

Author summary

LctP is a unique lactate transporter encoded in the genome of N. gonorrhoeae and lactate is one of the few carbon energy sources that can be used by pathogenic Neisseria. Lactate acquisition has been shown to increase gonococcal resistance to complement-mediated killing by human serum and to enhance colonization and survival in a female mouse.
model of lower genital tract infection. Herein, we describe the transcriptional regulation of lctP in gonococci. We showed that the GdhR regulator and D-glucose act independently to dampen lctP expression and that such regulation has an impact on the resistance of gonococcal cells to killing by hydrogen peroxide. Thus, this study provides insights regarding the mechanism by which gonococci survive during infection and identify a new virulence phenotype linked to lctP gene expression.

Introduction

Gonorrhea is a sexual transmitted infection (STI) caused by the Gram-negative bacterium Neisseria gonorrhoeae. Gonorrhea is the second most common bacterial STI with a burden in the US alone of 468,514 reported cases [1] and an estimated 86.9 million cases worldwide in 2016 [2]. Since 1938 the gonococcus has developed resistance to all the antibiotics that have been introduced in the clinic to treat this STI, including recent cases of resistance to the currently used dual antibiotic treatment regimen of ceftriaxone and azithromycin [3]. Among the mechanisms developed by N. gonorrhoeae strains to acquire antimicrobial resistance are the re-modeling of the beta-lactam lethal target, penicillin-binding protein 2 (PBP2) due to acquisition of mutations within penA [4] or formation of a mosaic penA due to recombination of donated DNA sequences from commensal Neisseria [5,6]; amino acid replacements in the major porin protein encoded by porB, limiting the influx of antibiotics into the cell [7]; and the increased expression of the antimicrobial efflux pump MtrCDE due to transcriptional regulatory mutations impacting expression of the mtrR gene, which encodes the master repressor of mtrCDE [8].

MtrR is a global regulator of gonococci that belongs to the TetR-family and has a central role in pathogenesis and antibiotic resistance [9,10]. Its regulon extends beyond the mtr locus and comprises multiple genes required for pathogenesis and stress response such as the alternative sigma factor rpoH [9]. In this work we focused on one gene within the MtrR regulon, gdhR, encoding the GntR-type transcriptional regulator GdhR. The gene is located immediately downstream the mtrCDE locus and is subjected to MtrR transcriptional repression [11]. In previous work we showed that loss of GdhR results in enhanced gonococcal fitness in a female mouse model of lower genital tract infection [11]. However, the GdhR-regulated genes responsible for this in vivo phenotype remained unknown.

Most of the regulatory functions of GdhR have been studied in the closely related pathogen N. meningitidis that, unlike gonococci, can be carried commensally in many people [12]. In meningococci, GdhR mediates a growth phase- and carbon source-dependent positive regulation of gdhA encoding an L-glutamate dehydrogenase, a gene required for systemic infection in an infant rat model [13]; however in gonococci the corresponding gdhA homologue was not found to be regulated by GdhR [11]. This suggested that, despite the high degree of DNA sequence conservation in both gonococci and meningococci, their regulatory circuits do not share similar functions due to changes in non-coding regions such as promoter sequences [11]. In addition to gdhA, meningococcal GdhR was found to regulate several genes involved in glucose catabolism by the Entner-Doudoroff pathway and in L-glutamate import [14]. Similarly, genes within the GntR-family regulate different biological processes including the oxidation of substrates such as pyruvate (PdhR), lactate (LldR) or gluconate (GntR, the family founder) [15,16]. An important feature of these regulators is the presence of an N-terminal helix-turn-helix (HTH) DNA binding domain and a C-terminal metabolite-binding and oligomerization domain [15,17]. The HTH domain is highly conserved among the family members,
while the oligomerization domain is less conserved and can regulate the DNA-binding activity of the HTH domain by imposing steric constraints that influences protein mobility [18].

In this study we performed a transcriptomic analysis to determine the gonococcal GdhR regulon to help understand the mechanism by which loss of GdhR enhances the in vivo fitness and survival of gonococci during lower genital tract infection of female mice [11]. In this respect, we present evidence that GdhR is a direct repressor of lctP expression. The gene lctP encodes a unique L-lactate permease in the genome of gonococcal cells and has been linked to pathogenesis [19]. Previous work showed that gonococcal lctP null mutants had a growth defect in medium containing physiological concentrations of glucose and lactate, were more susceptible to killing by normal human serum and were significantly impaired for colonization and survival in the female mice model of infection [19]. In general, lactate derived from host cells enhances gonococcal metabolism and sialylation of the lipooligosaccharide (LOS), induces serum resistance and increases survival in human polymorphonuclear leukocytes and cervical epithelial cells [20–22]. Further, we show that GdhR regulation of lctP gene expression, as well as the presence of glucose in the medium, can independently influence the overall resistance of gonococci to hydrogen peroxide.

**Results**

**Transcriptional modulation of a gonococcal virulence factor-encoding gene (lctP) by GdhR**

To determine the expression levels of gdhR (NGO1360 locus tag in the FA1090 reference strain) at different points of the growth curve we collected total RNA from *N. gonorrhoeae* strain FA19 grown in GC broth at different optical densities. The levels of gdhR mRNA were determined by qRT-PCR along with the levels of its direct repressor (MtrR) encoded by mtrR (NGO1366) and the MtrR-regulated gene mtrC (NGO1365). As a control we used rmpM (NGO1577) encoding a highly expressed and conserved outer membrane protein antigen [23]. The results showed that the expression levels of mtrR or two of its regulated genes (gdhR and mtrC) did not significantly change between the exponential and stationary phases of growth (S1 Fig).

To determine the GdhR regulon an RNA-Seq analysis was performed using total RNA samples collected from wild-type (WT) strain FA19 and its isogenic gdhR insertional mutant (FA19 gdhR::kan) grown to late-exponential phase in GC broth. The number of genes that were differentially regulated by GdhR represented 2.3% of all the genes in the FA19 strain genome (Fig 1A). Of the GdhR-regulated genes, 39 were activated and 11 were repressed (S1 Table). However, the list of differentially expressed genes in WT versus gdhR mutant compiled at a fold-change > 2 contained only 11 genes, of which only 8 corresponded to protein coding genes (Fig 1B). Anticipating that GdhR could be a cryptic regulator we also included a comparison of the transcriptomes of gdhR mutant cells and a complemented mutant in which gdhR expression signals are bypassed by overexpression from the inducible lac promoter in vector pGCC4 (strain JC01). This analysis showed that under conditions of overexpression, GdhR differentially regulated 46 genes (S2 Table), out which 12 were the same genes regulated in the WT background (S1 Table). These results suggested that GdhR is not a cryptic regulator in the GC broth-grown WT background, since it can regulate at least 12 identical genes under conditions of WT and artificial overexpression of GdhR levels. Accordingly, we focused on the WT background GdhR regulon, which included mostly genes annotated as fimbrial proteins and membrane transporters (Fig 1B and S1 Table). We also noted that lctP (NGO1449) was the highest of GdhR-repressed genes (Fig 1B and 1C).
Because of the recognized importance of lctP and lactate transport and utilization for gonococcal pathogenesis (reviewed in [24,25]), we focused on GdhR regulation of lctP. qRT-PCR analysis was performed to validate the RNA-Seq results. The results showed that lctP was repressed 7-fold by GdhR (Fig 2A). Similarly, under conditions of GdhR overexpression from an ectopic promoter lctP could be further repressed at 92-fold. To determine whether GdhR regulation of lctP is direct we conducted electrophoresis mobility shift assays (EMSA). We used the transcriptomic files of the RNA-Seq analysis to estimate the approximate location of the lctP TSS and promoter region (Fig 1C). We found that purified GdhR could bind a DNA fragment spanning the lctP promoter region (Fig 3). This binding was specific since an excess of unlabeled lctP promoter DNA completely competed with the labeled lctP DNA (Fig 3, lane j) while an excess of unlabeled DNAs encoding non-GdhR-regulated housekeeping genes could not (Fig 3 lanes k and l).

To test whether GdhR represses lctP in a different strain background we inactivated the gdhR gene in laboratory strain F62 and conducted qRT-PCR. The results revealed that GdhR also repressed lctP expression in F62 (S2 Fig).
**Fig 2. Regulation of the lctP allele by GdhR.** Relative levels of lctP mRNA were determined by qRT-PCR using recA (A) or 16S rRNA (B) as internal reference in total RNA samples from WT strain FA19 and its isogenic mutants gdhR::kan and gdhR::kan complemented strain JC02 (pMR33-gdhR). Cells were grown to late-logarithmic phase in GC broth supplemented with either glucose (grey bars) or lactate (black) at 22 mM each. Data are presented as the mean (bar) plus the standard error of the mean (error bar) of 3 biological samples. * represents significant statistical differences at p<0.05 as determined by a one-tailed non-parametric Mann Whitney U-test.

https://doi.org/10.1371/journal.ppat.1008233.g002

**Fig 3. Binding of GdhR to the lctP promoter region.** A DIG-labeled DNA fragment spanning nucleotides −313 to −23 of the lctP promoter relative to the start codon was incubated with increasing concentrations of purified GdhR. The mobility of free DNA and of the nucleoprotein complexes were determined by EMSA and are indicated at the right of the gel. Competitive EMSAs were prepared by adding a 100-fold excess of unlabeled DNA fragments encoding lctP and recA promoter regions (lane j and k respectively) and 16S ribosomal RNA (lane l).

https://doi.org/10.1371/journal.ppat.1008233.g003
intergenic region, containing the lctP promoter (Fig 1C), revealed identical sequences with only one nucleotide polymorphism among different laboratory strains and recent clinical isolates (S3 Fig). This and the high degree of conservation of the gdhR allele and its upstream region among different gonococcal strains (99.7%, S1 Appendix) suggests that GdhR regulation of lctP is likely to be a conserved trait among gonococci strains.

Molecular mechanism of lctP repression by GdhR

To reconstruct lctP transcription in vitro we used a PCR-amplified DNA template spanning the lctP promoter region, the 5′ untranslated region (5′ UTR) and part of the open reading frame (ORF), and purified E. coli Sigma-70-saturated RNA polymerase (RNAPσ70). We mapped the lctP TSS to a single transcription peak located at an adenine 121 bases upstream the start codon (Fig 4A). A primer extension assay using total RNA isolated in vivo from gdhR mutant cells revealed a TSS that maps to the same position as with the in vitro transcription system (Fig 4A). Since GdhR belongs to the FadR subfamily of the GntR-class of regulators [26] we used the FIMO (Find Individual Motif Occurrences) algorithm [27] and the reported consensus FadR DNA binding motif [28] to generate matches on the N.
gonorrhoeae genome. This analysis revealed an inverted repeat matching the FadR motif located 9 bases downstream of the lctP TSS (Fig 4B). DNase I footprinting analysis showed that GdhR protected a single region that included this inverted repeat and extended from +6 to +48 relative to the lctP TSS (Fig 5). The single region of GdhR binding at the lctP promoter and the two nucleoprotein complex species observed in the EMSA analysis (Fig 3 lanes d and e) suggest that a change in the oligomerization state of GdhR bound to lctP exists at different concentrations of GdhR.

We used the in vitro transcription system to study the effect of GdhR binding to the lctP promoter on transcription initiation. Purified GdhR was able to inhibit lctP transcription in a concentration-dependent manner with an inhibitory concentration 50% (IC50) of 0.4 μM (0.30–0.52 95% confidence intervals) as determined from the transcription inhibition curve as described in Materials and Methods (Fig 6). We used purified MtrR as a specificity control of the assay since it does not directly regulate lctP expression [9]. The transcription inhibition curve with MtrR did not show the same exponential decay shape as with GdhR, although it could repress significantly less probably due to nonspecific DNA binding (Fig 6A). To validate the function of the identified GdhR-binding site (Fig 5) on lctP transcription regulation, we deleted the 21 bp inverted repeat sequence located within the GdhR protected region and then

Fig 5. GdhR protects a DNA sequence near the lctP promoter. A. A DNA fragment spanning the lctP promoter region from nucleotide -192 to +381 (relative to the TSS) was fluorescently-labeled with 6-FAM (coding strand) and HEX (template strand) and incubated with BSA (control reaction) or GdhR prior to digestion with DNase I. The DNase I digestion products were analyzed by capillary electrophoresis. The fluorescence signal corresponding to the HEX probe is shown on the y axis of each electropherogram. Fragment coordinates (relative to the TSS) are shown along the top of the BSA electropherogram. Three electropherograms corresponding to 0.4, 1.5 and 3.0 μg of GdhR reactions are shown. The lctP promoter region protected by GdhR is boxed. Dideoxy sequencing reactions were manually-generated using the primer HEX-lctP-DNase and a PCR fragment encoding lctP promoter from -192 to +381 (bottom panel). B. DNA sequence of the GdhR-protected region on both strands (the electropherogram corresponding FAM-labeled coding strand is shown in S5 Fig). The consensus DNA-binding motif of the FadR-family of regulators is shown in bold font.

https://doi.org/10.1371/journal.ppat.1008233.g005
used this mutant promoter as template for in vitro transcription and EMSA. Transcription inhibition (Fig 6A) and binding (Fig 6C) of GdhR to the mutant lctP promoter was significantly reduced compared to the WT promoter, which indicates that GdhR requires binding to this inverted repeat sequence in order to repress lctP transcription.
Carbon source regulation of *lctP*

Lactate utilization operons encoding *lctP* and ortholog gene *lldP* can be induced by L-lactate in Gram-negative and -positive bacteria [29–31]. This is achieved by binding of L-lactate to the operon regulator, the GntR-type protein LldR, resulting in the modification of its DNA-binding activity [29–31]. Therefore, we compared the expression of *lctP* in gonococcal cells grown in GC broth supplemented with the standard concentration of D-glucose (22 mM) or with L-lactate (22 mM) (Fig 2). We found that *lctP* expression was enhanced up to 2.5-fold when glucose was replaced by lactate. This effect was, however, GdhR-independent since *gdhR* expression was not affected in the WT strain by the carbon source replacement (S4 Fig) and occurred approximately to same magnitude in the *gdhR* mutant background (Fig 2A). Moreover, we found in our EMSA analysis that neither L-lactate nor glucose impacted the binding of GhdR to the *lctP* promoter (Fig 3, lanes h and i).

The above results, however, did not discern between glucose repression and lactate induction of *lctP*. To study the effect of different carbon sources on *lctP* expression we constructed a *lacZ* translational fusion in vector pLES94 in which the *lacZ* gene was fused to the first codon of *lctP* and expressed from the *lctP* transcriptional and translational signals. WT or *gdhR* mutant gonococci bearing the *lctP*-*lacZ* fusion were grown to stationary phase in GC broth supplemented with either D-glucose, L-lactate or pyruvate in a concentration range of 1 to 6 mM each and β-galactosidase activity was determined as an indicator of LctP levels. This analysis showed that only glucose could repress *lctP* expression at physiological concentration levels, with 55% repression at 2.75 mM and 70% at 5.50 mM relative to 1.38 mM in the WT background (Fig 7, D-glucose titration bars). The glucose repression was again GdhR-independent since it occurred in the *gdhR* mutant background with approximately the same magnitude of effect. This analysis also showed that, different from *E. coli* [29], neither lactate nor pyruvate (its immediate oxidation product) can induce *lctP* expression.

Regulation of *lctP* by GdhR and D-glucose impacts gonococcal resistance to hydrogen peroxide

Lactate is one of the few carbon energy sources that can be used by pathogenic *Neisseria*, and phagocyte-derived lactate is available to gonococci and has been reported to enhance the rate of bacterial oxygen metabolism [32]. Exposure of gonococci to superoxide and hydrogen peroxide has been shown to increase bacterial metabolism, specifically L-lactate utilization and lactate dehydrogenase (LDH) activity. [33]. Moreover, expression of *lldD* (NGO0639), encoding a NAD-independent membrane-bound LDH, was enhanced in gonococci exposed to sublethal levels of hydrogen peroxide [34]. These results suggest that lactate metabolism is important for resistance to oxidative stress within phagocytes. Therefore, we tested the possibility that *lctP* regulation by GdhR or glucose could impact gonococcal resistance to hydrogen peroxide. For this purpose, we determined the effect of exposure to hydrogen peroxide in GC broth on the survival of WT strain FA19 as well as isogenic single and double *gdhR* or *lctP* mutants. We found that *gdhR* mutants were significantly more resistant to killing by hydrogen peroxide compared to WT cells (Fig 8A). This increased resistance could be reversed by genetic complementation of the *gdhR* allele. In contrast, deletion of *lctP* from the WT strain FA19 made gonococci more susceptible to hydrogen peroxide compared to the WT parent (Fig 8A). Interestingly, deletion of *lctP* in the *gdhR* background rendered the double mutant highly susceptible to hydrogen peroxide, which suggests that the *gdhR* mutation-associated resistant phenotype is epistatic to *lctP*. We also performed a *gdhR*-*lctP* epistasis analysis in the F62 background, which is the strain used to demonstrate the importance of *lctP* for colonization in vivo [19]. Because F62 was significantly more susceptible than FA19 using the hydrogen
peroxide susceptibility assay in GC broth (S2 Appendix), we assayed the F62 strains in GC-agar plates using a disk diffusion assay. This analysis confirmed the results shown in the FA19 background (Fig 8B). Further, the hydrogen peroxide susceptibility observed in the lctP mutant compared with WT F62 was genetically complemented using previously isolated lctP mutant (GP900) and complemented strain expressing lctP ectopically from the lac promoter (GP922) [19] (Fig 8C).

To test whether glucose regulation of lctP impacts the hydrogen peroxide susceptibility phenotype we assayed strains FA19 and F62 and their isogenic lctP mutants using the disk diffusion assay in GC-agar plates supplemented with different concentrations of D-glucose (Fig 8D). Glucose in the medium was shown to increase the killing by hydrogen peroxide of WT strains FA19 and F62 but not in the corresponding lctP mutants, which shows that the glucose effect depends on the presence of lctP.

To test whether the hydrogen peroxide resistant phenotype exhibited by gdhR mutant cells could be related to a differential expression of other key genes previously associated with such resistance, we determined their transcript levels in WT FA19 and F62 and their GdhR-negative strains by qRT-PCR (S3 Table). This analysis showed that among recA (NGO0741) [35], mpg (NGO1686) [36], NGO0554, katA (NGO1767) [37], ccp (NGO1769) [38], mntC (NGO0168) [39] and msrA (NGO2059) [40] only lctP was differentially regulated by GdhR. In addition, we

**Fig 7. Effect of different carbon sources on lctP expression.** FA19 reporter strain JC28 and isogenic mutant JC29 (gdhR::kan) containing an lctP-lacZ fusion in vector pLES94-lctP were grown to stationary phase on GC broth supplemented with a concentration range (1.38, 2.75 and 5.50 mM) for each carbon source. Titration of glucose supplemented cultures was done with a fix concentration of 3 mM L-lactate, and titration with L-lactate or pyruvate was done with a fix concentration of 1.5 mM glucose. β-galactosidase was expressed from the lctP transcriptional and translational signals and its activity was determined in Miller units. Data are presented as the mean (bar) plus the standard error of the mean (error bar) of 3 biological samples and two technical replicates each. **”** represents significant statistical differences at p<0.01 within each carbohydrate titration group as determined by a non-parametric Kruskal-Wallis test and a Dunn’s posttest.

https://doi.org/10.1371/journal.ppat.1008233.g007
found that compared to strain FA19, qRT-PCR analysis of RNA extracted from strain F62 showed a significantly lower expression level (3-fold) of ccp, which encodes a cytochrome-c peroxidase previously described as an antioxidant-encoding gene [38]. This differential expression could, in part, explain the higher hydrogen peroxide susceptibility of strain F62 compared to FA19.
Discussion

In this work we uncovered the GdhR regulon in order to follow up previous work that showed that loss of \textit{gdhR} enhanced the fitness of gonococci in a female mouse model of lower genital tract infection [11]. Herein, we showed that GdhR regulates 50 genes, encoding mostly membrane proteins. Interestingly, by comparing the GdhR regulon in gonococci with one reported in the meningococci strain H44/76 [14], we found no overlap between the regulons in these two genetically related \textit{Neisseria}. This confirmed our previous report that despite the high degree of sequence identity in the \textit{gdhR} locus (96%) between these two \textit{Neisseria} species, the regulatory activity of GdhR changes drastically due to differences in promoter sequences targeted by DNA-binding proteins [11].

Within the GdhR regulon we concentrated on \textit{lctP}, which encodes a unique L-lactate transporter and previously reported to be required for effective colonization in the experimental female mouse model of infection [19]. Using protein-DNA binding assays we found that GdhR binds to a 21 base inverted repeat sequence positioned very close and downstream to the \textit{lctP} TSS. This DNA sequence matched the reported consensus DNA-binding motif of FadR-type regulators, which was originally identified by aligning upstream regions corresponding to genes within the regulon of multiple members belonging to this GntR-subfamily [28]. HTH regulators generally bind as dimers to inverted repeat operators [15]. By EMSA, we found evidence for two different GdhR-\textit{lctP} nucleoprotein complexes that coexist and interconvert within a GdhR concentration range. These complexes most likely represent a dimer and tetramer of GdhR bound to DNA since we did not find a second binding site within the \textit{lctP} probe analyzed. Similarly, the lactate utilization operon repressor LldR of \textit{C. glutamicum} was found to form two nucleoprotein complexes likely corresponding to dimers and tetramers [30]; nonetheless, the oligomerization state of these regulators has to be further studied. We developed an \textit{in vitro} transcription system using purified components to demonstrate that GdhR binds to its operator sequence to effectively repress \textit{lctP} transcription. Transcription initiation can be inhibited at three different steps: promoter recognition by the RNAP, open complex formation or elongation. From our results it is not clear the precise step in which GdhR represses \textit{lctP} transcription initiation. However, it is likely that GdhR could impose a steric hindrance on the RNAP\textit{σ}70 recognition of the promoter elements given that RNAP\textit{σ}70 protects a region from -50 to +20 at \textit{σ}70 promoters [41,42], a region that overlaps the GdhR operator at \textit{lctP}.

A titration analysis of the \textit{lctP} promoter activity under different concentrations of L-lactate or pyruvate showed that these carbon sources do not have an effect on \textit{lctP} expression within physiological concentration levels. Further, L-lactate did not affect the DNA-binding activity of GdhR. These features deviate significantly from the paradigm in other lactate utilization operons in Gram-negative [29,31] and -positive [30] bacteria, where L,D-lactate can bind the GntR-type LldR repressor/activator to induce transcription of the operon. In \textit{N. meningitidis} 2-oxoglutarate can inhibit the binding of GdhR to the promoter of its regulated gene \textit{gdhA} [13]. We found that 2-oxoglutarate did not affect the DNA-binding activity of GdhR at the \textit{lctP} promoter (Fig 3, lane g). We do not discount, however, that an unidentified metabolite can bind to GdhR so as to regulate its binding to the \textit{lctP} promoter. The promoter titration analysis showed that D-glucose in the medium has a repressive effect on \textit{lctP} transcription that is not mediated through GdhR. Carbon catabolite repression (CCR) systems have been largely unexplored in \textit{N. gonorrhoeae}. Interestingly, a study to determine the transcriptomic response of \textit{N. meningitidis} to glucose in the medium showed that \textit{lctP} is subjected to glucose repression [43]. This group found that HexR, a regulator involved in CCR responses in different proteobacteria [44], controls 28% of the total glucose-regulated genes and \textit{lctP} is not among those.
The HexR DNA-binding motif is highly conserved among betaproteobacteria [45]. Using the FIMO algorithm, we scanned the gonococci genome to identify a possible match for the reported meningococcal HexR DNA-binding motif at the lctP locus [43]. This analysis revealed that the gonococcal genome possesses all the HexR motifs identified in meningococci but there is not a match in the lctP locus (S3 Appendix), which suggests that, equal to meningococci, glucose repression of lctP is not mediated by HexR in gonococci. Previously, an ortholog of ptsK (NGO0314) was identified among the few phosphotransferase (pts) annotated genes in the gonococci genome [46]. PtsK is a serine/threonine protein kinase (HPr(Ser) kinase) that controls CCR responses in G-positive bacteria and a ptsK mutant in B. subtilis is insensitive to transcriptional regulation by CCR [46]. Based on this, we constructed an insertional mutant of ptsK in strain FA19 harboring the lctP-lacZ fusion (JC41) but found that glucose could still repress lctP expression in the absence of the putative PtsK to the same extent as the WT parent (S4 Appendix). Thus, the mechanistic basis for glucose repression of lctP remains unknown and will be the subject of further study.

When exposed to sub-lethal concentrations of hydrogen peroxide N. gonorrhoeae programs its transcriptome resulting in phenotypic adaptation to oxidative stress of greater magnitude [33,34]. This adaptation requires new protein synthesis, especially those proteins related to an increase in lactate metabolism such as lactate dehydrogenase [33,34]. In addition, it was shown that gonococci can use phagocyte-derived lactate to enhance its metabolism which stimulates oxygen consumption [32,47]. Thus, gonococci can compete with neutrophils for oxygen resulting in a decreased neutrophil production of reactive oxygen species [48].

Herein, we have shown for the first time that a mutant unable to utilize lactate is more susceptible than its WT parent to killing by hydrogen peroxide. We also showed that regulation of lctP by the transcriptional repressor GdhR and by glucose in the medium independently have an impact in the overall resistance of gonococci to hydrogen peroxide. While we do not yet understand why loss of LctP or the presence of glucose increases gonococcal susceptibility to hydrogen peroxide we emphasize that this regulation could be relevant during infection. Thus, gonococci surviving within phagolysosomes will face a glucose-limited environment and may rely on lactate as a carbon source [49] [50]. The level of hydrogen peroxide in the neutrophil phagosome can reach up to 100 mM [51]. To test whether GdhR regulation of lctP could be relevant under this hydrogen peroxide levels, we performed the killing assay in GC broth shown in Fig 8A but with a higher gonococci cellular concentration. We found that gdhR mutants survived hydrogen peroxide concentrations up to 150 mM. In contrast, the WT strain failed to survive at a concentration of > 50 mM and the complemented mutant, which overexpresses lctP (Fig 2), > 13 mM (S6 Fig). To test whether hydrogen peroxide could diminish GdhR binding to the lctP promoter, we conducted an EMSA in the presence of hydrogen peroxide with or without ferrous iron that catalyzes the Fenton reaction known to result in highly damaging hydroxyl radicals (S7 Fig). From this analysis we concluded that while hydrogen peroxide has little influence on its own on the GdhR-lctP nucleoprotein complex, resulting hydroxyl radicals from the Fenton reaction have a negative impact in the formation of the nucleoprotein complex at lethal hydrogen peroxide concentrations (i.e. higher than 15 mM and defined as a concentration that kills more than 10% of the cells as per [34] and S6 Fig). This finding and conclusion are consistent with the increased fitness of the gdhR mutant in the female mouse model of lower genital tract infection at days 3 and 5 [11], when the influx of polymorphonuclear leukocytes would be elevated compared to earlier stages of infection (A. E. Jerse et al., personal communication). This leukocyte influx would potentially increase levels of hydrogen peroxide. Under aerobic conditions, which would facilitate hydrogen peroxide production by leukocytes [52], lctp and lldD expression is elevated compared to anaerobic conditions [53]. The switch to anaerobic growth has been proposed to occur when gonococci...
grow within biofilms attached to cervical cells using host-derived nitrite as a terminal electron acceptor [54–56] and is of likely importance when gonococci ascend to the upper female reproductive tract where anaerobes are predominant.

Taken together, our results and previously published data ([11,19,32–34,47,48,53,55]), we propose a model (Fig 9) in which under conditions of low D-glucose concentration and oxygen availability (i.e. those within neutrophils where oxygen is required for hydrogen peroxide production and glucose is excluded from the phagosome) the lctP promoter is released from glucose and GdhR repression to reach maximal expression. This increases L-lactate transport and metabolism which results in increased resistance to hydrogen peroxide and survival in vivo. Under this condition (oxygen availability or oxidative stress such as hydrogen peroxide treatment), expression of lldD, encoding L-lactate dehydrogenase, is also increased.

Materials and methods
Strains and media

*N. gonorrhoeae* strains used in this study are derived from the laboratory strains FA19 and F62 and are described in S4 Table. Gonococcal strains were grown overnight at 37°C under 5%
(v/v) CO₂ on GC agar plates containing Kellogg’s supplements I and II [57]. When indicated glucose in supplement I was replaced by L-lactate or pyruvate at indicated concentrations. Growth in liquid medium was at 37˚C with agitation (225 r.p.m.) in GC broth containing Kellogg’s supplements I and II and 0.042% (w/v) sodium bicarbonate. When necessary, culture media were supplemented with ampicillin (Amp; 100 μg/mL), chloramphenicol (Cm; 0.5–1.0 μg/mL), kanamycin (Km; 50 μg/mL), erythromycin (Erm; 1 μg/mL), isopropyl-β-D-thiogalactopyranoside (IPTG; 0.5 to 1.0 mM as indicated) or 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; 20 μg/mL). E. coli TOP10 (Life Technologies, Carlsbad, CA) and ER2566 (New England BioLabs, NEB) were used for cloning and protein expression purposes respectively and grown on LB medium.

Construction of mutant strains and lctP-lacZ translational reporters

Plasmids and oligonucleotide primers used throughout this work are described in S4 and S5 Tables respectively. To construct complemented strains of mutant FA19 gdhR::kan, the gdhR allele was placed under the lac promoter in vectors pGCC4 and pMR33. Briefly, a DNA fragment encoding gdhR ORF was amplified by PCR with primers pac1gepR3 and pme1gepR4 from FA19 genomic DNA (gDNA), digested with PacI-PmeI and ligated into similarly digested pGCC4 and pMR33 to create pGCC4-gdhR and pMR33-gdhR respectively. The inserted gdhR allele was confirmed by sequencing with primer pMR33Fw. Vectors pGCC4-gdhR and pMR33-gdhR (linearized with NheI) were used to transform strain FA19 gdhR::kan by homologous recombination to generate strains JC01 and JC02 respectively. Transformants were selected on GC agar plates containing Erm. Primer pairs NGO1450-F/pac1gepR 3 and pMR33Fw/igaRv were used to confirm by PCR the correct integration of vectors pGCC4-gdhR and pMR33-gdhR into their respective chromosomal loci. To construct a gdhR insertional mutant of strain F62, plasmid pUC18us-gdhR::kan [11] (linearized with NheI) was used to transform F62 by electroporation using the method described by Dillard J.P. [58] and generating strain JC16. Transformant strains were selected on GC-agar plates containing Km and disruption of the gdhR allele was confirmed by PCR with primers gdhR-pTXF and gdhR-pTXR.

To construct lctP insertional mutant strains a DNA fragment encoding lctP disrupted with a Cm acetyltransferase cassette (cat) was amplified by PCR with primers F1-lctP and R1-lctP using gDNA from strain GP900 as a template [19]. The PCR fragment was used to transform FA19, FA19 gdhR::kan, F62 and JC16 to generate strains JC03, JC04, JC05 and JC24 respectively. Transformant strains were selected on GC-agar plates containing Cm and disruption of the lctP allele was confirmed by PCR with primers lctP-check and lctP-R2.

To create lctP reporter strains a transcriptional/translational lctP-lacZ fusion was created in vector pLES94 [59]. Briefly, a DNA fragment encompassing the lctP promoter, the 5’ UTR and the first codon was amplified by PCR using primers lctPlacZ-F and lctPlacZ-R and FA19 gDNA. The resulting PCR fragment was ligated into BamHI-digested pLES94 to create pLES94-lctP. Vector pLES94-lctP was linearized with HindIII and used to transform FA19 and FA19 gdhR::kan to generate strains JC28 and JC29 respectively. Transformants were selected on GC-agar plates containing Cm and the integration of the lacZ fusion at the proAB locus was confirmed by PCR with primers proABFw and lacZRv.

Extraction of total RNA and qRT-PCR

N. gonorrhoeae cultures were grown in GC broth at 37˚C with agitation to late exponential phase before being used for RNA purification. One mL samples were centrifuged, resuspended in 200 μL RNAlater solution (Ambion) and incubated 10 min on ice. Total RNA extraction was conducted using the RNeasy mini kit (Qiagen) following the manufacturer’s protocol.
Contamination with gDNA was removed using the Turbo DNA-free Kit (Invitrogen). For qRT-PCR the DNase I-digested total RNA samples were reverse transcribed using the QuantiTect Reverse Transcription Kit (QIAGEN). For primer extension assay and RNA-Seq, DNase I-digested total RNA samples were further concentrated and cleaned-up using the RNeasy MinElute Cleanup Kit (QIAGEN). The integrity of the purified RNA samples was determined by formaldehyde gel electrophoresis as described before [60].

qRT-PCR was conducted using the IQ SYBR Green Supermix and a CFX Connect
Real Time System (Bio-Rad Laboratories). Relative expression values were calculated as $2^{(C_{T\text{reference}} - C_{T\text{target}})}$, where CT is the fractional threshold cycle. The level of recA mRNA and 16S rRNA were used as internal reference. The following primer pairs were used to quantify relative mRNA levels: recA_qFw/recA_qRv for recA, 16Sma-RTF/16Sma-RTF for 16S rRNA, lctP_qFw/lctP_qRv for lctP, gepR_qRT_F/gdhR_qRT_R2 for gdhR, rmpM_qRT_F/rmpM_qRT_R for mtrR, 1686qRT-F/1686qRT-R for mpg, 0554qRT-F/0554qRT-R for NGO0554, katA_qRT_F/katA_qRT_R for katA, ccp_qRT_F1/ccp_qRT_R1 for ccp, mntCqRT-F/mntCqRT-R for mntC, and msrA_qRT_F/msrA_qRT-R for msrA.

RNA-Seq and bioinformatics analysis
RNA-sequencing was performed on the Illumina NextSeq 500 as described by the manufacturer (Illumina Inc., San Diego, CA). Briefly, the quality of the total RNA was assessed using the Agilent 2100 Bioanalyzer. RNA with a RNA Integrity Number (RIN) of 7.0 or above was used for sequencing library preparation. Library preparation was done using the Agilent SureSelect Strand Specific mRNA library kit as per the manufacturer’s instructions (Agilent, Santa Clara, CA). Library construction began with ribosome reduction using the RiboMinus rRNA depletion kit for bacteria (Invitrogen). The resulting RNA was randomly fragmented with cations and heat, which was followed by first strand synthesis using random primers with inclusion of Actinomycin D (2.4ng/μL final concentration). Second strand cDNA production was done with standard techniques, the ends of the resulting cDNA were made blunt, A-tailed and adaptors ligated for amplification and indexing to allow for multiplexing during sequencing. The cDNA libraries were quantitated using qPCR in a Roche LightCycler 480 with the Kapa Biosystems kit for Illumina library quantitation (Kapa Biosystems, Woburn, MA) prior to cluster generation. Cluster generation was performed according to the manufacturer’s recommendations for onboard clustering (Illumina).

For the RNA-Seq bioinformatics analysis, softwares deposited in the public ABIMS Galaxy tool shed (Station Biologique de Roscoff-CNRS-Sorbonne University) were used. Briefly, paired-end fastq files generated by the sequencing platform were aligned to the N. gonorrhoeae FA19 genome (GenBank assembly accession: GCA_000273665.1) using the TopHat2 algorithm to generate BAM files. Transcript differential expression between samples (n = 2) was determined using the aligned BAM files and the Cuffdiff algorithm. Finally, the BAM files, the TopHat2 alignment rates and the RNA-Seq laboratory and bioinformatics methods were deposited in the Gene Expression Omnibus (GEO) [61] with GEO series accession number GSE134959.

Protein purification
MtrR was purified as described before [62]. To purify GdhR, the gene was amplified with primers gdhR-pTXF and gdhR-pTXR from FA19 gDNA and cloned into NdeI/SapI-digested pTXB1 (NEB) to generate pTXB1-gdhR. The GdhR-intein-CBD encoding fusion in pTXB1-gdhR was confirmed by DNA sequencing using T7 universal and Mxe Intein II reverse primers.
(NEB). GdhR encoding gene was expressed from the T7 promoter and the protein purified from *E. coli* French press-generated lysates using the NEB IMPACT protein purification system following the company protocol.

**Electrophoresis mobility shift assay (EMSA)**

EMSAs were conducted using the second-generation digoxigenin (DIG) gel shift kit (Roche Applied Sciences, Madison, WI) as previously described [63]. Briefly, 12 fmol of DIG-labeled DNA fragments were incubated with increasing concentrations of purified GdhR protein for 25 min at 30˚C before being separated by electrophoresis in 5% Mini-Protean TBE Precast Gels (Bio-Rad) and transferred to nylon membranes. The protein-DNA binding reactions were carried out in 20 μL of 20 mM Hepes, pH 7.6, 1 mM EDTA, 10 mM (NH₄)₂SO₄, 1 mM DTT, 0.2% (v/v) Tween-20, 30 mM KCl and 1.25 ng/μL Type XV calf thymus DNA. Gel images were developed using an anti-DIG Fab fragment-AP conjugate and chemiluminescence detection, acquired with the Gel Doc XR Molecular Imager (Bio-Rad) and processed with the Image Lab software (Bio-Rad). Specificity of GdhR binding to the DIG-labeled *lctP* promoter was tested by adding to the binding reactions a 100-fold excess of unlabeled DNA fragments encoding the house-keeping gene *recA* promoter region or 16S ribosomal RNA. DNA fragments were generated by PCR from *N. gonorrhoeae* FA19 gDNA with primer pairs GdhR-EMSA-F/GdhR-EMSA-R for *lctP* promoter, recAP-F/recAP-R for *recA* promoter and 16Smai-RTF/16Smai-RTR for 16S ribosomal RNA.

**In vitro transcription and primer extension reactions**

*In vitro* transcription assays were carried out as described before [64]. Briefly, a DNA fragment encoding *lctP* promoter, 5’ UTR and part of the coding sequence was amplified from FA19 gDNA with primers GdhR-EMSA-F and lctPqRv and used as a template. One μg of template DNA was incubated with either purified GdhR or MtrR proteins before being transcribed with 1.0 unit of *E. coli* RNAP σ₇₀ (NEB) and nucleotide triphosphates. The transcription reactions were digested with 3 units of RQ1 DNase I (Promega). The RNA transcripts were purified with the QIAGEN RNeasy MinElute cleanup kit before being reverse-transcribed using HEX-labeled primer HEX-*lctP*-IvT (complementary to the *lctP* ORF) and the SuperScript II Reverse Transcriptase system (Invitrogen) following the company protocol. A 369 bp HEX-labeled DNA standard generated with primers GdhR-EMSA-F and HEX-*lctP*-IvT was added to each sample to a final concentration of 0.75 ng/μL. For primer extension assay 16.4 μg of total RNA were isolated from strain FA19 *gdhR::kan* as described in the above section (*Extraction of total RNA*), annealed with primer HEX-*lctP*-IvT and extended similarly to the *in vitro* transcription RNA products.

To create a mutant *lctP* promoter template lacking the identified FadR DNA binding motif (*lctPΔFadR*), overlap extension PCRs were carried out with primer pairs GdhR-EMSA-F/MotifDel-R and MotifDel-F/lctPqRv. Primers MotifDel-R and MotifDel-F are complementary to one another, for which in the second-round of PCR, aliquots of both first-round PCRs were mixed and used as template with primers GdhR-EMSA-F and lctPqRv to generate *lctPΔFadR*.

**Quantitative analysis of in vitro transcription and primer extension reactions**

The resulting fluorescently-labeled cDNA fragments were analyzed in a 3730 capillary sequencer (Applied Biosystems) and the sequencing files were visualized with the GeneMapper software v.4.0 (Applied Biosystems). To accurately assign a nucleotide base to *in vitro* transcription or primer extension peaks, a sequencing ladder was generated using the above *lctP* template DNA, primer HEX-*lctP*-IvT and the Thermo Sequenase Dye Primer Manual cycle.
sequencing kit (USB Corporation) as described before [65]. The GeneMapper software was used to generate alignments between the electropherograms of the transcription reactions and dideoxy sequencing reactions to determine the size and start nucleotide of the lctP transcripts. The effect of GdhR and MtrR on lctP in vitro transcription was estimated from the height of lctP transcript peaks present in the electropherograms. Transcription inhibition curves were generated with the aid of GraphPad Prism 5.0 (GraphPad, San Diego, CA) using the log (inhibitor) vs. response-variable slope nonlinear regression analysis.

DNase I footprinting
A PCR fragment spanning the lctP promoter was amplified using the 6-carboxyfluorescein (FAM)- and 6-carboxy- 2',4,4',5,7,7'- hexachlorofluorescein (HEX)-labeled primers FAM-lctP-DNase and HEX-lctP-DNase. GdhR protein binding to the labeled DNA probe and DNase I digestion reactions were performed as described previously [65]. Detection of the DNase I digestion peaks was carried out in a 3730 capillary sequencer (Applied Biosystems) and the alignment of the corresponding electropherograms was generated using GeneMapper software v.4.0 (Applied Biosystems). Negative control reactions were done using BSA at the same mass concentration used for GdhR. A PCR DNA template was amplified with primers GdhR-EMSA-F and lctPqRv to generate a sequence ladder for each strand with primers FAM-lctP-DNase (coding strand) and HEX-lctP-DNase (template strand) as described for the In vitro transcription section and as previously described [65].

β-galactosidase activity
β-galactosidase enzymatic activity was determined using the substrate o-nitro phenyl-β-D-galactopyranoside (ONPG) as described by Miller J.H. before [66]. β-galactosidase activities are given in Miller units using the formula [1,000 × OD_{420nm} / (t × v × OD_{600nm})], where t is the reaction time in min and v is the volume of cell lysates in mL per reaction.

Hydrogen peroxide susceptibility assays
H₂O₂ susceptibility assays were performed in liquid medium with gonococcal cells collected from overnight growth on GC-agar plates. The bacteria were then resuspended to 5·10⁷ CFU/mL in 200 μL of GC liquid medium containing Kellogg’s supplement I and II, 0.042% NaHCO₃, 3 mM L-lactate and 9 mM H₂O₂. After overnight incubation at 37˚C cell viability was assessed by dilution in GC broth and spot plating on GC-agar plates. To test susceptibility of gonococci to different H₂O₂ concentrations a variation of the liquid assay was made consisting of overnight incubation of 5·10⁷ CFU/mL gonococcal cells in 96-well flat-bottom sterile plates with lid at 37˚C under 5% (v/v) CO₂. Cell viability was assessed this time with the AlamBlu dye (Bio-Rad) and fluorescence reading at 560/590 nm (excitation/emission), considering 100% survival the cellular growth in wells without H₂O₂. For the hydrogen peroxide susceptibility using the disk diffusion assay, a 10⁹ CFU/mL suspension of gonococcal cells was spread onto GC-agar plates containing Kellogg’s supplement I and II and 3 mM L-lactate and incubated 30 min at 37˚C under 5% (v/vl) CO₂. Then, Whatman filter disks (1 cm) presoaked in 3% H₂O₂ were placed on top of the plates and further incubated overnight before growth inhibition zones were measured in mm from the edge of the disk.

Supporting information
S1 Fig. Kinetics of expression of gdhR in gonococcal cells. Relative levels of gdhR, mtrR, mtrC and rmpM mRNA were determined by qRT-PCR using recA (A) and 16S rRNA (B) as
internal reference genes. Total RNA samples were collected from WT strain FA19 at different optical density (OD) points of its growth in GC broth. Data are presented as the mean (bar) plus the standard deviation (error bar) of 3 biological samples. ‘*’ represents significant statistical differences at p<0.05 as determined by a non-parametric Kruskal-Wallis test and Dunn posttest.

(TIF)

S2 Fig. GdhR repression of lctP in the F62 background. Relative levels of lctP mRNA were determined by qRT-PCR using recA (A) and 16S rRNA (B) as internal reference genes. Total RNA samples were collected from WT strain F62 and its isogenic mutant JC16 (gdhR::kan) grown in GC broth to late-logarithmic phase. Data are presented as the mean (bar) plus the standard deviation (error bar) of 3 biological samples. Significant statistical differences (p<0.01) were determined by a T-test.

(TIF)

S3 Fig. Alignment of the lpxH-lctP intergenic region among different N. gonorrhoeae strains. The promoter elements (-35 and -10), the TSS (+1) and Shine-Delgarno (SD) regions are underlined. A G-C polymorphism is highlighted in yellow. The end and start of ORFs corresponding to lpxH and lctP are indicated under the sequence.

(TIF)

S4 Fig. Expression profile of gdhR under growth on different carbon sources. Relative levels of gdhR mRNA were determined by qRT-PCR using recA (A) and 16S rRNA (B) as internal reference genes. Total RNA samples were collected from WT strain FA19 grown to late-logarithmic phase in GC broth supplemented either with D-glucose (22 mM) or L-lactate (22 mM). Data are presented as the mean (bar) plus the standard deviation (error bar) of 4 biological samples.

(TIF)

S5 Fig. GdhR DNase I footprint of the lctP promoter coding strand. A DNA fragment spanning the lctP promoter region from nucleotide -192 to +381 (relative to the TSS) was fluorescently-labeled with 6-FAM (coding strand) and HEX (template strand) and incubated with BSA (control reaction) or GdhR prior to digestion with DNase I. The DNase I digestion products were analyzed by capillary electrophoresis. The fluorescence signal corresponding to the 6-FAM probe is shown on the y axis of each electropherogram. Fragment coordinates (relative to the TSS) are shown along the top of the BSA electropherogram. Three electropherograms corresponding to 0.4, 3.0 and 6.0 μg of GdhR reactions are shown. The lctP promoter region protected by GdhR is boxed. Dideoxy sequencing reactions were manually-generated using the primer FAM-lctP-DNase and a PCR fragment encoding lctP promoter from -192 to +381 (bottom panel).

(TIF)

S6 Fig. Mutants lacking gdhR are highly resistant to hydrogen peroxide. (A) Gonococcal cells (5·10^8 CFU/mL) of the WT FA19 strain, its isogenic mutant gdhR::kan and gdhR-complemented mutant JC02 (pMR33-gdhR + 1 mM IPTG) were exposed to different concentration of hydrogen peroxide in GC broth and grown overnight in 96-wells plates. Cell viability was determined with the Alamar blue dye. (B) Graphical representation of killing by H_2O_2 from the fluorescence reading data considering the 0 mM point as 100% growth. Representative experiment of at least two.

(TIF)

S7 Fig. Effect of hydrogen peroxide and ferrous iron on the GdhR-lctP nucleoprotein complex formation. (A) Shown are results from an EMSA experiment that used purified GdhR
(120 nanomolar) and a digoxigenin-labeled DNA encompassing the lctP promoter (−313 to −23 relative to the start codon). Binding reactions were performed in the presence of increasing concentrations of either H₂O₂ alone or H₂O₂ and 12 μM FeSO₄ (Fe²⁺) that catalyzes the Fenton reaction. The mobility of free DNA and of the nucleoprotein complexes are indicated at the right of the gel. (B) The effect of H₂O₂ and Fe²⁺ on the top nucleoprotein complex formation (red arrow) was examined by densitometry of the EMSA gel using the ImageLab 6.0 software.

**S1 Table. Differential gene expression between WT and gdhR mutant.** Excel file containing the list of GdhR-regulated genes generated by a transcript differential expression analysis of the WT vs. gdhR:kan mutant sequencing files. Tab-1 shows differentially expressed genes. Tab-2 shows differentially expressed genes at a fold-change >2. Tab-3 shows the expression levels (in FPKM) of all sequenced genes.

**S2 Table. Differential gene expression between gdhR mutant and complemented strains.** Excel file containing the list of GdhR-regulated genes generated by a transcript differential expression analysis of the gdhR:kan mutant vs. pGCC4-gdhR complemented mutant (JC01) sequencing files. Tab-1 shows differentially expressed genes. Tab-2 shows differentially expressed genes at a fold-change >2. Tab-3 shows the expression levels (in FPKM) of all sequenced genes.

**S3 Table. Relative expression of genes required for the hydrogen peroxide oxidative damage response in N. gonorrhoeae.**

**S4 Table. Strains and plasmids used in this study.**

**S5 Table. Oligonucleotide primers used in this study.**

**S1 Appendix. Alignment of the gdhR allele and upstream sequence from different N. gonorrhoeae strains.**

**S2 Appendix. Sensitivity of N. gonorrhoeae strains to hydrogen peroxide in GC broth.**

**S3 Appendix. Bioinformatic detection of the N. meningitidis HexR DNA-binding motif within the N. gonorrhoeae FA1090 genome using the FIMO algorithm.**

**S4 Appendix. Effect of the ptsK allele deletion on the glucose-mediated repression of lctP.**

**Acknowledgments**

We thank Ann E. Jerse (F.E. Hébert School of Medicine, Uniformed Services University) and Joseph P. Dillard (University of Wisconsin School of Medicine) for providing plasmid vectors and strains used in this study. We further thank C. Rouquette-Loughlin (Emory University School of Medicine) for critical review of this paper before submission. We are grateful to
Michael Crowley and Caitlin M. Cox (University of Alabama at Birmingham, Heflin Center for Genomic Sciences) for their assistance in the high throughput sequencing and capillary electrophoresis analyses.

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