The increasing prevalence of multidrug-resistant Neisseria gonorrhoeae strains underscores the need for novel antimicrobials against this pathogen. Resazomycins, derivatives of resazurin (Rz), have shown robust antimicrobial activity against N. gonorrhoeae (Ng) in vitro. In vivo, however, resazomycins exhibit limited efficacy in a mouse model of gonorrhea. Previous experiments have shown that N. gonorrhoeae is more resistant to resazurin at oxygen levels comparable to those seen in host tissue (2%). We hypothesized this difference in susceptibility at low oxygen compared to atmospheric oxygen (~20%) was due to altered activity of multi-drug efflux pumps. To test this, we screened a selection of N. gonorrhoeae mutants that do not express or overexpress either the MtrCDE or NorM efflux pumps for Rz susceptibility. Overexpression of MtrCDE resulted in increased resistance to Rz at both 2% and ~20% oxygen suggesting resazurin may be a substrate of this efflux pump. Loss of expression of either MtrCDE or NorM had no effect on the increased resistance of N. gonorrhoeae to resazurin at low oxygen. We next sought to determine whether the increased susceptibility of Rz at 20% oxygen is due to oxidative stress. To test this, we measured the susceptibility of N. gonorrhoeae to Rz in the presence and absence of the antioxidants, cysteine HCl and glutathione, at 20% oxygen. In the presence of cysteine HCl or glutathione, multiple N. gonorrhoeae strains had a higher Rz MIC at 20% oxygen. Here, we have shown oxygen concentration affects N. gonorrhoeae susceptibility to Rz due to increased oxidative stress.