

Jordan Gibson, Justin Rice, Emily Young, and Deanna M. Schmitt, *Department of Biomedical Sciences, West Liberty University, West Liberty, WV, 26074*. The Effect of Efflux Pumps and Oxidative Stress on *Neisseria gonorrhoeae* Susceptibility to Resazomycins.

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.