

JORDAN GIBSON, JUSTIN RICE, EMILY YOUNG, and DEANNA M. SCHMITT,
Department of Biomedical Sciences, West Liberty University, West Liberty, WV, 26074.
Hypoxia increases *Neisseria gonorrhoeae* resistance to Resazomycins: Role of Efflux
Pumps and Oxidative Stress.

Neisseria gonorrhoeae (*Ng*) has developed resistance to all antibiotics previously and currently recommended for treatment of gonorrhea, underscoring the need for novel antimicrobials against this pathogen. Resazomycins are highly effective antimicrobials against *Ng* in vitro. However, when these compounds were tested in a mouse model of gonorrhea, only one resazomycin, resorufin pentyl ether (RPE), exhibited any therapeutic effect. One reason for this difference between in vitro and in vivo therapeutic efficacy is increased resistance to resazomycins at low levels of oxygen (2%). We hypothesized this difference in susceptibility was due to altered activity of multi-drug efflux pumps. To test this, we screened a selection of *Ng* 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 *Ng* 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 *Ng* 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 *Ng* strains had a higher Rz MIC at 20% oxygen. Here, we have shown oxygen concentration affects *Ng* susceptibility to Rz due to increased oxidative stress.