ABSTRACT

DISSERTATION/THESIS/RESEARCH PAPER/CREATIVE PROJECT: EFFECTS OF SUBINHIBITORY CARVACROL LEVELS ON *BACILLUS CEREUS* VIRULENCE DURING ENDOPHTHALMITIS IN RETINAL PIGMENTED EPITHELIAL CELLS (ARPE-19)

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Bacillus cereus can cause irreversible eye damage in humans as early as nine hours after initial infection through production of exotoxins, including hemolysin BL (Hbl) and nonhemolytic entertoxin (Nhe) during endophthalmitis. Carvacrol is an extract from oregano oil that may be able to treat *B. cereus* endophthalmitis, due to well-known antimicrobial and anti-inflammatory qualities. However, sublethal levels of carvacrol may increase *B. cereus* virulence. The goal of this study was to measure relative levels of regulator and virulence gene expression in subinhibitory concentration (SIC) of carvacrol-stressed *Bacillus cereus* earlier shown to elevate virulence when sublehtally-injured. Subinhibitory levels of carvacrol increase relative expression of *hblc*, *nheA*, and *plcR* to *16s* expression in *B. cereus* infected human retinal pigment epithelium, ARPE-19 cells. The cytotoxic levels of carvacrol in ARPE-19 cells were determined using a lactate dehydrogenase (LDH) cytotoxicity assay. **Methods** ARPE-19 cells were cultured in DMEM/F12 supplemented with 10% fetal bovine serum (FBS). ARPE-19 cells were treated with 10^5 CFU/ml. *B.*

cereus alone, or *B. cereus* + SIC (1 mM) of carvacrol. Relative differences in expression of *plcR, hblC*, and *nheA* regulator and virulence gene expression in *B. cereus* treated with SIC of carvacrol, and untreated *B. cereus* was quantified by mean cycle threshold (CT) value comparisons using real-time PCR, and statistically analyzed using one-way ANOVA in Minitab17, . DAPI staining was performed to visualize *B.cereus* internalization in ARPE-19 cells 0, 24 and 48 hours post infection. **Results** By RT-PCR, relative *plcR*, *hblC,* and *nheA* mRNA expression were not different between SIC carvacrol-treated *B. cereus* levels compared to untreated cultures at 0, 24 or 48 h post infection. DAPI staining revealed no detectable invasion by *B. cereus* at 0, 24 and 48 h post infection. Cytotoxicity in ARPE-19 cells was detected in response to carvacrol compared to the negative control. Percentage of cytotoxicity in ARPE-19 cells infected with *B. cereus* and treated with SIC levels of carvacrol was not significantly different. **Conclusion** These data indicate that sublethal chemical stress by carvacrol did not change the potential virulence of this pathogen *via* an up/down regulation in the global regulator (*plcR*) production and virulence factors such as HblC, NheA and/or the global effector PlcR. Also, DAPI staining findings showed that *B. cereus* does not detectably attach or invade ARPE-19 cells through 48 hours after infection. However, carvacrol is cytotoxic to ARPE-19 cells. Further investigation is needed to fully understand mechanism of effects of carvacrol on ARPE-19 layer as an *in vitro* model for the blood retinal barrier.