29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

The congress of ESCMID

P2803 Transcriptional response of vancomycin resistant *Enterococcus faecium* to a synergistic antibiotic-essential oil compound combination: a strategy to extend the utility of vancomycin?

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Background: New antimicrobials to treat Vancomycin Resistant *Enterococcus faecium* (VRE) infections are considered a high priority. Essential oil compounds have been shown to interact synergistically with antibiotics, and so could be used as adjuvants to preserve the antibiotic repertoire. A combination of carvacrol, cuminaldehyde and vancomycin was found to synergistically inhibit VRE, potentially extending the utility of vancomycin against VRE. This study aimed to investigate the mechanism of action of the carvacrol, cuminaldehyde and vancomycin combination against VRE using transcriptomic analysis.

Materials/methods: The antimicrobial activity of the combination in 1% DMSO was determined by a time-kill assay. Transcriptomic response of VRE to the combination was determined by microarray analysis. VRE was treated with either 0.031 mg/L vancomycin, 1.98 mM carvacrol, 4.20 mM cuminaldehyde or the ternary combination for 60 minutes. A control of 1% DMSO only was included. RNA was extracted, converted to cDNA labelled with Cy5 and hybridised onto a custom microarray and scanned. Significant ($p<0.05$) differences in gene expression were determined using a one-way Analysis of Variance (ANOVA) with Benjamini Hochberg FDR multiple testing correction and Tukey's post-hoc test. Expression changes of genes of interest were confirmed by real time quantitative PCR.

Results: The combination of carvacrol, cuminaldehyde and vancomycin reduced VRE by 3.96 $\log_{10}$ Colony Forming Units/mL over 24 hours. Expression of 14 genes were significantly altered by the combination ($p<0.05$, >2-fold change). Genes with the greatest change in expression mainly related to carbohydrate metabolism; the phosphotransferase system (PTS)-associated genes *mtlD, mtlF* and *agaC6* were downregulated 2.86-2.28-fold and *ulaA3* was upregulated 4.07-fold. Glutamine-fructose-6-phosphate aminotransferase (*glmS*), which is associated with amino sugar biosynthesis was downregulated 2.67-3.83-fold. Vancomycin resistance-associated genes were not altered by the combination or individual components.

Conclusions: Carvacrol and cuminaldehyde synergistically enhance the antimicrobial activity of vancomycin against VRE, and so could be useful to preserve the antibiotic repertoire. The combination affected carbohydrate metabolism and biosynthetic processes, indicating that the combination did not directly modulate antibiotic resistance genes. Further research will investigate significantly changed genes to enhance understanding of the synergistic mechanism of action of the combination.