O275

2-hour Oral Session

Effect of linezolid induced stringent response in *Staphylococcus aureus* on expression of virulence *in vitro* and *in vivo*

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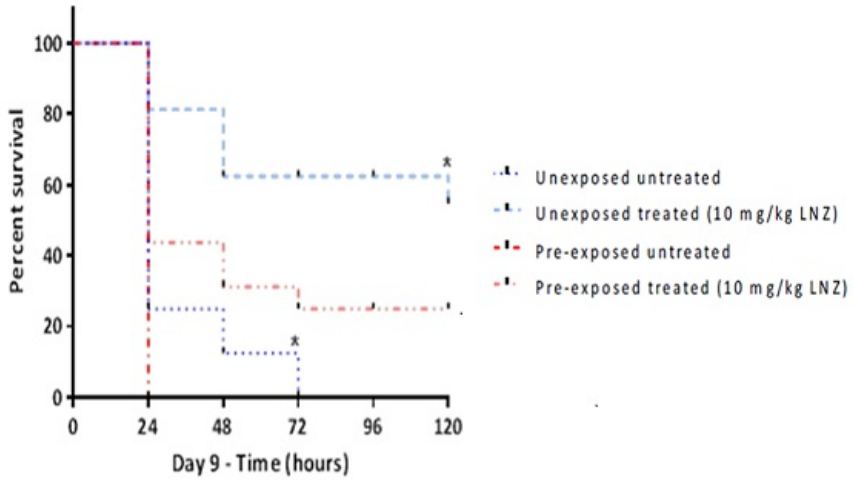
Objectives: Linezolid resistance remains infrequent; however, isolates which appear susceptible to the antibiotic may display tolerance *in vivo* leading to instances of treatment failure. The aim of this study was to determine the ability of linezolid to induce a stress-related survival mechanism termed the stringent response (SR) and to evaluate the impact of the SR on the expression of *Staphylococcus aureus* virulence factors and subsequent pathogenicity.

Methods: Six MSSA isolates (clinical and reference), were exposed to 10x the minimum inhibitory concentration (MIC) of linezolid during mid-log growth for 1h or serine hydroxamate, a known inducer of the SR. RNA was recovered from culture pellets while supernatant was retained for phenotypic studies to assess SR-related modulation of virulence genes. Real-time quantitative PCR (qPCR) confirmed induction of the SR (by an increase in *relA*) and determined changes in the expression of genes associated with adhesion *(clfB, fnbA*, *cna*), biofilm formation (*icaC*) and toxin production (*seb, sec, sed, see, tst, hla*, *lukE*). Secreted staphylococcal enterotoxin (SET) levels were measured in the supernatant by ELISA. To mimic linezolid therapy, *S.aureus* FRI326 and *S.aureus* NCTC10656 were exposed to linezolid for 1h daily over a 9-day period, and SR-related virulence changes assessed. To validate findings, cells harvested on days 1, 3, 6 and 9 of the repeat exposure experiment were used in a *Galleria mellonella* caterpillar *in vivo* infection assay.

Results: During a linezolid-induced SR the majority of isolates displayed a greater than 2-fold increase in expression of *icaC, fnbA, hla, sec, clfB, cna* and *see* while *seb, sed, tst* and *lukE* remained unaltered in comparison to the antibiotic-free control. Results from SET ELISA tests were in concordance with qPCR data. Repeated induction of the SR stimulated and progressively enhanced expression of virulence genes associated with adhesion and biofilm formation with each antibiotic exposure. *In vivo* analysis of FRI326 demonstrated enhanced virulence in the *G.mellonella* model after infection with cultures repeatedly induced into the SR over 9 days (figure). Linezolid therapy significantly improved survival of all caterpillars, though efficacy was reduced in the treatment of infections caused by repeated SR-induced FRI326 (50% survival after infection with wild-type FRI326 vs. 25% survival SR-induced at 5 days). The linezolid MIC of both strains also increased from 2-4mg/l over this same 9-day period.



Survival of *G. mellonella* caterpillars infected with *S. aureus* FRI326 after repeated induction of the SR with linezolid (LNZ). Bacterial inoculum 1.2±0.2 x 10^5 CFU/larva. Linezolid dose based on 600mg adult (60kg) dose. * P<0.05 untreated vs. treated.

Conclusion: This study demonstrated that *S.aureus* are able to mount a SR during exposure to linezolid leading to an increase in the expression of a range of virulence factors, which is further exacerbated with repeated antibiotic exposure. We hypothesise that patients receiving a prolonged course of linezolid for a complicated *S.aureus* infection are at risk of increased tissue pathology due to SR-enhanced virulence together with the distinct possibility of reduced susceptibility of the pathogen to the antibiotic.