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| **Mouse precision-cut lung slices as a novel model of early bacterial infection with Methicillin-resistant Staphylococcus aureus** |
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| **Introduction/Aim:**  Methicillin-resistant Staphylococcus aureus (MRSA) is a feature of both hospital- and community-acquired pneumonia, but translatable models to investigate early pathogenesis and potential treatments are limited. Precision-cut lung slices (PCLS) may provide a novel approach for dynamic time-course studies of MRSA infection with higher throughput than *in vivo* mouse models.  **Methods:**  To establish infection, PCLS from agarose-inflated lungs of male C57BL/6 mice (8 -10 weeks old) were incubated for 2h with MRSA (dsRED-labelled, 105 colony-forming units (CFU)) and transferred to fresh media. Up to 48h post-infection (hpi), MRSA were visualised under fluorescent microscopy or quantitated as CFU in intact or homogenised PCLS. MRSA association with alveolar macrophages (AMs) and dendritic cells (DCs) was detected by flow cytometry. Media was assayed for lactate dehydrogenase activity (viability measure) or cytokines by BioLegend LEGENDplex™ (iinflammation).  **Results:**  Viability was similar in uninfected and infected PCLS. Diffuse MRSA infection was evident across all structures (airways, arteries, parenchyma) in PCLS, increasing >100,000 fold by 48hpi (n=6, P<0.001). The increased proportion of CD45+ immune cells associated with MRSA over time (24hpi 9±2%; 48hpi 74±10%, n=4, P<0.05) was evident in AMs and DCs but not non-phagocytic B cells. TNF-α, GM-CSF and IL-6 were increased in MRSA-infected PCLS within 24hpi (n=6, P<0.05).  **Conclusion:**  Mouse PCLS provide an *ex vivo* model of MRSA infection involving intact resident immune cells and inflammation. Our findings support the future application of human PCLS for the assessment of drugs to prevent infection or limit replication of resistant bacterial strains.        **Grant Support:** Monash BDI Seeding Grant |