2604. Changes in a Fatty Acid Kinase Associated with Daptomycin (DAP) Resistance Lead to Increased Collagen Binding and Biofilm Formation in Enterococcus faecalis

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**Session:** 269. Pathogenesis and Host-Response Interactions

**Background:** Enterococci are a major cause of healthcare-associated infections with limited treatment options. We previously identified that mutations in dak (a gene encoding a putative fatty acid kinase), ace (a collagen adhesin) and the Yxdk stress response system are associated with DAP resistance (DAP-R) in E. faecalis (Efs) in the absence of a functional LiaFSR system. Here, we examined the role of DAK in pathogenesis by examining the ability of the mutants to produce biofilm and bind to collagen, an important protein of the extracellular matrix.

**Methods:** Previously, the Efs strain OG1RFΔliaR (inactive LiaFSR system, DAP susceptible) was adapted to make a DAP-R derivative (mutations in yxdk, dak, and ace), and the mutant OG1RFΔliaRΔc-dak, lacking the C-terminal domain of dak, and its complement OG1RFΔliaRΔc-dak::c-dak were constructed to study the dak mutation in isolation. Biofilm formation (BF) for the above strains was assayed after growth in tryptic soy broth with glucose in 96-well plates at 37°C for 24 hours. Bacteria were fixed with Bouin’s solution OG1RFΔliaRΔc-dak showing increased binding to collagen when compared with OG1RFΔliaRΔc-dak::c-dak (7.9 vs. 1.2, P < 0.001), a phenotype which reverted on complementation (7.9 vs. 1.2, P < 0.001). This enhanced biofilm phenotype was also seen in the setting of DAP-R.

**Conclusion:** Changes in an enzyme involved in DAP adaptation lead to biofilm formation and adherence to extracellular matrix proteins, potentially enhancing virulence in the setting of DAP-R.

Disclosures. All authors: No reported disclosures.
Table 1. Clinical and microbiological characteristics, and treatment outcomes of patients infected by MRSA with mixed hematic phenotype

| Variable          | 1 | 2 | 3 | 4 | 5 | 6 |
|-------------------|---|---|---|---|---|---|
| Age (y)/gender    | 66M | 65F | 66M | 69Y | 66M | 55F |
| Underlying disease| diabetes | heart failure | cancer | chronic kidney failure | chronic obstructive pulmonary disease | kidney transplant |
| Previous use of antibiotics | No | No | No | No | No | Yes |
| Acquisitions      | hospital-inpatient | hospital-inpatient | hospital-inpatient | hospital-inpatient | hospital-inpatient | hospital-inpatient |
| Type of infection | mixed | mixed | mixed | mixed | mixed | mixed |
| Microbiological data | mixed | mixed | mixed | mixed | mixed | mixed |

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**Background:** *Pseudomonas aeruginosa* is an aerobic Gram-negative bacterium that causes life-threatening acute and chronic infections in humans. Genotypic mutations and phenotypic variations are key features of its antimicrobial resistance and adaptation to the host environment. Pyoverdine associated genes and divergent receptors play a key role in acute *Pseudomonas* infections. This study seeks to address the heterogeneity of ferrichrome-iron receptor (fpv) expression, its effect on pathogenicity and its propensity to cause acute infections clinically. Genetic and phenotypic variation of a clinical isolates of *P. aeruginosa* (PA097 and PA115) were identified by complete genome sequencing method.

**Methods:** An IRB-approved prospective study collected 38 *P. aeruginosa* clinical isolates and stored at Carilion Medical Center. Two genetically unrelated clinical strains were selected from tracheal aspirates: PA097 and PA115. These isolates were characterized by pyoverdin (pvd) quantification in planktonic culture filtrate at OD660 nm. Multiplex PCR was carried out using primers for fpv receptors. Quantification of iron acquisition was done on chrome azurol S (CAS) agar. Genomes for PA115 and PA097 were sequenced by Illumina Next-generation DNA sequencing.

**Results:** Genome assembly shows a 6.3 Mb genome size in PA115 with G+C content of 66.4%. Seven insertion sequence elements were located. We found a 101 kb locus for pvd and a highly diversified fpv associated with an insertional element (IS3). PA115, exhibits rich green pigment of pvd followed by PA097 in LB media (Figure 1A) and also in Planktonic culture filtrate (Fig 1B) for quantitative estimation of pvd (Figure 1C). On CAS agar, PA115 showed high uptake of iron by orange pigment compared with lower pigmentation in PAO1 and PA097 (Fig 1D). We confirmed the ferrichrome-iron receptor as fpvAlb in PA115 by Multiplex PCR seen in sequencing of PA115 (Figure 2).

**Conclusion:** We found high genetic and phenotypic variation in clinical isolate of *P. aeruginosa* (PA115) from an acute pneumonia patient. The novel IS element found in its receptor gene locus suggests an increased role in pvd expression and iron uptake from the host. Increased pvd expression and diversified fpvAlb association with an IS3 element may indicate higher virulence in the PA115 strain.

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**Figures:**

**Figure 1.** Mixed hematic pattern observed in single colony evaluation. *A.* The mixed hematic phenotypes of ST72-MRSA strain. *B.* The mixed hematic phenotypes of ST5-MRSA strain. All isolates were inoculated onto sheep blood agar plates containing RN4220 supernatant of β-hemolysin, and hematic phenotype was evaluated in each single colony after overnight culture. Arrows indicate hematic (black) and non-hematic (white) colony.

**Figure 2.** Comparison of the transcriptional expression of genes encoding hemolysins and *saeR/saeS*, two-component regulatory system between hematic (H) and non-hematic (NH) colonies in four ST72 isolates showing mixed hematic pattern. *P* < 0.05 by Mann-Whitney U-test.

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**2606. A Divergent Ferrichrome Receptor Associated with an Insertional Element (IS3) Identified on Novel Locus in Clinical Strain of Pseudomonas aeruginosa**

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**Session:** 269. Pathogenesis and Host-Response Interactions

**Saturday, October 5, 2019: 12:15 PM**

**Background:** Efficient animal models are needed in order to investigate fungal pathogenicity and antifungal therapy in the context of epithelial injury, e.g., due to antineoplastic chemotherapy. Using a Gal4 enhancer trap (GET) zebrafish line facilitating metronidazole (MTZ)-inducible ablation of epithelial (periderm) cells, we aimed to establish a mucositis model predisposing larvae for fungal invasion.

**Methods:** An IRB-approved prospective study collected 38 *P. aeruginosa* clinical isolates and stored at Carilion Medical Center. Two genetically unrelated clinical strains were selected from tracheal aspirates: PA097 and PA115. These isolates were characterized by pyoverdin (pvd) quantification in planktonic culture filtrate at OD660 nm. Multiplex PCR was carried out using primers for fpv receptors. Quantification of iron acquisition was done on chrome azurol S (CAS) agar. Genomes for PA115 and PA097 were sequenced by Illumina Next-generation DNA sequencing.

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**2607. Establishment of a Novel High-Throughput Fungal Infection Model in Zebrafish Larvae by Controlled Ablation of Epithelial Cells**

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**Methods:** An IRB-approved prospective study collected 38 *P. aeruginosa* clinical isolates and stored at Carilion Medical Center. Two genetically unrelated clinical strains were selected from tracheal aspirates: PA097 and PA115. These isolates were characterized by pyoverdin (pvd) quantification in planktonic culture filtrate at OD660 nm. Multiplex PCR was carried out using primers for fpv receptors. Quantification of iron acquisition was done on chrome azurol S (CAS) agar. Genomes for PA115 and PA097 were sequenced by Illumina Next-generation DNA sequencing.

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**Conclusion:** We found high genetic and phenotypic variation in clinical isolate of *P. aeruginosa* (PA115) from an acute pneumonia patient. The novel IS element found in its receptor gene locus suggests an increased role in pvd expression and iron uptake from the host. Increased pvd expression and diversified fpvAlb association with an IS3 element may indicate higher virulence in the PA115 strain.

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