

Conclusion. Veterans successfully collected their own specimens and had no strong preference for either device. The Tasso-SST combined with the InBios Spike IgG assay provided the highest combination of sensitivity and specificity. Limitations included one collection device per subject, varied timing of testing, unknown infection or vaccination status among some, and Tasso collection volume and Mitra whole blood dilution may have affected comparison across assays or performance.

Disclosures. All Authors: No reported disclosures

1006. Association of Development of Pneumonia and Virulence Gene Expression in *Acinetobacter baumannii* Isolated from Clinical Specimens

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Session: P-56. Microbial Pathogenesis

Background. Not all *Acinetobacter baumannii* isolated from respiratory specimens are true pathogens. Distinguishing between true pathogens and colonizers is important to initiate early treatment and to reduce the unnecessary prescription of antibiotics. To determine the microbiological factors contributing to the development of *A. baumannii* pneumonia, we investigated the association between the expression level of known *A. baumannii* virulence genes such as *ompA* and *hisF* and pneumonia.

Methods. Patients in whose respiratory specimens *A. baumannii* was identified between January 2018 and January 2019 in a tertiary university hospital were recruited into this study. Relevant radiologic findings and more than 5 days of susceptible antibiotic prescription started within 3 days of bacterial isolation were considered as having pneumonia. The absence of radiologic findings of pneumonia until 7 days after the isolation of *A. baumannii* was defined as colonization. The expression of *ompA* and *hisF* was determined with quantitative reverse-transcription polymerase chain reaction. Host factors known to be associated with pneumonia and expression levels of virulent genes were compared between the groups.

Results. Overall, 246 patients in whose respiratory specimens *A. baumannii* was identified were recruited into this study. Among them, 17 and 24 patients were assigned to the pneumonia and colonizer groups, respectively. In the univariable analysis, *ompA*, ICU stay, and mechanical ventilation were significantly associated with pneumonia ($p = 0.03$, < 0.01 , < 0.01 respectively). In the multivariable analysis, mechanical ventilation was significantly associated with pneumonia (OR = 9.75, $p = 0.03$). *ompA* expression was not significantly associated with pneumonia in the multivariable analysis (OR = 1.12, $p = 0.75$) (Table 1). *ompA* and *hisF* were significantly associated with the 30-day in-hospital mortality ($p = 0.02$, < 0.01).

Table 1. Univariable and multivariable analysis of factors related to pneumonia

	Univariable analysis		Multivariable analysis	
	pneumonia (n=17)	colonizer (n=24)	OR (95% CI)	P
Age, median (range), y	75 (66-81)	68 (59-78)	0.14	0.23
Female sex, No. (%)	4 (23.5)	5 (20.8)	1.00	
Charlson score, median (range)	5 (4-7)	4 (2-7)	1.32 (0.88-2.00)	0.18
Surgery, No. (%)	3 (17.6)	8 (33.3)	0.31	
ICU stay, No. (%)	16 (94.1)	13 (54.2)	< 0.01	9.05 (0.57-144.29)
Tracheostomy, No. (%)	3 (17.6)	10 (41.7%)	0.10	
Mechanical ventilation, No. (%)	13 (76.5)	6 (25.0)	< 0.01	9.75 (1.34-71.13)
Death, No. (%)	9 (52.9)	1 (4.2)	< 0.01	
<i>ompA</i> , median (range)	1.45 (0.88-2.24)	0.63 (0.13-1.39)	0.03	1.12 (0.57-2.19)
<i>hisF</i> , median (range)	0.86 (0.10-1.09)	0.12 (0.07-1.02)	0.16	

CI, confidence interval; OR, odds ratio; ICU, intensive care unit

Conclusion. The association between increased *ompA* expression in *A. baumannii* and the development of pneumonia was not statistically significant after adjusting for patient factors. However, the relatively high expression of *ompA* in pneumonia patients and their association with increased mortality suggests the need for larger-scale prospective studies to draw a conclusion.

Disclosures. All Authors: No reported disclosures

1007. Hypermutability in Clinical Strains of *Klebsiella pneumoniae*: Role of the V76G Mutation in *MutH*

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Session: P-56. Microbial Pathogenesis

Background. Hypermutator (HM) bacteria exhibit high spontaneous mutation rates due to DNA mismatch repair (MMR) gene mutations, which may facilitate antibiotic resistance. HM is best described for chronic infections or colonization,

in particular with *P. aeruginosa*. HM *K. pneumoniae* (KP) and carbapenem resistant Enterobacteriales (CRE) are rarely studied.

Methods. Longitudinal isolates from 5 patients (pts) with long-term ST258 CRKP infections (median: 1.4 yr, 0.5-4.1 yr) underwent Illumina HiSeq whole genome sequencing. Strains from 1 pt were tested for HM and resistance. Mutant strains were created by complementation and CRISPR.

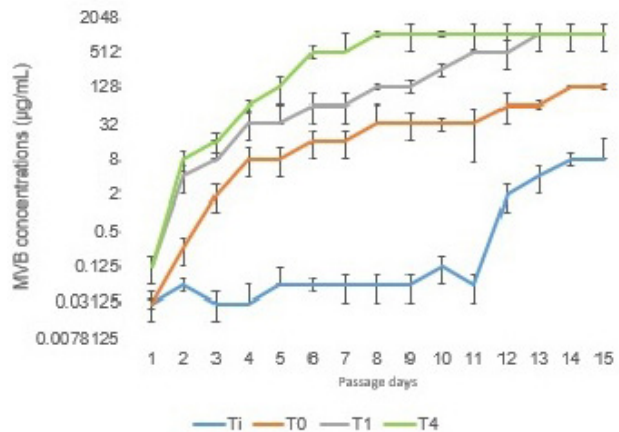
Results. In each pt, initial and recurrent isolates were genetically related (≤ 7 core genome (cg) SNP). In 1 pt, infection recurred 3.3 yrs after initial infection; baseline and recurrent (T0) isolates differed by 7-10 cgSNP. 4 ceftazidime-avibactam (CAZ) resistant isolates (T1-T4) were recovered ≥ 2 wks after treatment of T0 infection. Strains T1-T4 differed from T0 and each other by 109-214 and 58-137 cgSNP (Fig.1), respectively, and carried mutations in MMR genes (*mutS* -149ΔC (Δ*pmutS*); *mutH* V76G) and *blaKPC3* (D179Y). T1-T4 mutations were enriched for genes involved in metabolism (adjusted $p=1.46e^{-10}$), ABC transport ($p=4.1e^{-7}$), 2-component systems ($p=9.2e^{-5}$), signal transduction ($p=6e^{-4}$), and transcription regulation ($p=2.1e^{-4}$). *mutS* and *mutH* expression was 46-49% lower in T1-T4 than in T0. T1-T4 demonstrated rifampin mutational frequency $> 10^{-6.4}$, compared to $< 10^{-7.3}$ for earlier strains. Upon passage in meropenem-vaborbactam (MV), colistin and gentamicin, T1-T4 developed resistance faster and higher MICs than T0 (Fig 2). MV resistance was associated with *IS5 ompK36* promoter insertions or point, deletion or STOP mutations in *ompK36* coding region. Complementation of T1-T4 with wild-type (WT) *mutH* restored phenotypes. Introduction of V76G to WT *mutH* in T0 established HM and *in vitro* passage resistance phenotypes.

SNP matrix of 11 clinical isolates from a single patient with recurrent KPC-Kp infections

Months after initial infection	Sites	Rectal	Rectal	BAL	Neural fluid	Rectal	Rectal	Blood	BAL	Rectal	BAL	Abscess
	Samples	Tinitial1	Tinitial2	Tinitial3	Tinitial4	Tinitial5	Tinitial6	T0	T1	T2	T3	T4
	Tinitial1	2	3	3	2	3	10	101	125	137	206	
0.23	Tinitial2	2	5	3	4	5	10	101	125	137	206	
0.30	Tinitial3	3	5	6	5	4	13	104	128	140	209	
0.30	Tinitial4	3	3	6	5	6	11	102	126	138	207	
0.69	Tinitial5	2	4	5	5	5	12	103	127	139	208	
0.92	Tinitial6	3	5	4	6	5	13	104	128	140	207	
40.07	T0	10	10	13	11	12	13	109	133	145	214	
41.87	T1	101	101	104	102	103	104	109	58	68	137	
42.07	T2	125	125	128	126	127	128	133	58		90	159
43.05	T3	137	137	140	138	139	140	145	68	90		123
46.89	T4	206	206	209	207	208	207	214	137	159	123	

The first 6 isolates were recovered within 6 months of transplant (Tinitial). The later 5 isolates were recovered ~40 months after initial GI colonization. Number of SNPs for each pairwise comparison on isolates are shown. Gray highlighted boxes shown SNP differences between the 5 later strains.

Serial passages of 4 clinical isolates.



T1 and T4 harbored Δ*pmutS*. T1=Initial (baseline) isolates

Conclusion. MMR mutations emerged in longitudinal CRKP, which conferred HM phenotypes and were associated with CAZ and other anti-CRE antibiotic resistance. *mutH* V76 is crucial in MMR. Long-term colonization or recurrent infections in face of antibiotic exposure might predispose CRKP strains to HM.

Disclosures. Cornelius J. Clancy, MD, Merck (Grant/Research Support)

1008. Presence of Antibody Dependent Cell Cytotoxicity (ADCC) Functional Antibodies that Target a Complex Gp41 Epitope Correlates with Long-term Non-progression and ADCC is Maintained with Mutants Using Germline Heavy Chain Variable Gene Sequence of VH1-02 Gene

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Session: P-56. Microbial Pathogenesis

Background. Recent data supports that improved qualitative antibody responses correlate with elite controllers (EC) of HIV. As ADCC has been associated with protection in vaccine studies, thorough exploration of antibodies that facilitate ADCC is warranted. In studies on monoclonal antibodies from long-term non-progressors (LTNPs), our laboratory has previously described highly mutated antibodies against

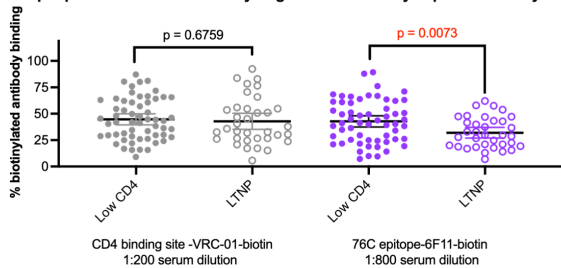
a complex conformational epitope with contributions from both gp41 heptad repeat regions. Despite using the VH1-02 gene segment, known to contribute to some of the broadest neutralizing antibodies against HIV, members of these antibodies, termed group 76C antibodies, did not exhibit broad neutralization.

Methods. Our goal was to characterize the non-neutralizing functions of antibodies of group 76C, to assess targeting of the epitope in various clinical presentations, and to assess the development of these antibodies by comparison to their predicted common ancestor. Serum samples were obtained from HIV+ clinical groups: EC, LTNP, stable CD4 counts on therapy, and those off therapy.

Results. In antibody/serum competition assays, comparison to VRC01 which also uses VH1-02, showed that antibodies targeting the 76C group epitope were enriched in LTNPs. We then show recombinant antibodies of 76C members 6F5 and 6F11 both have robust ADCC activity, despite their sequence disparity. Sequence analysis predicted the common ancestor of this clonal group would utilize the germline non-mutated variable gene. We produced a recombinant ancestor Ab (76Canc) with a heavy chain utilizing the germline variable gene sequence paired to the 6F5 light chain. 76Canc binds HIV envelope constructs near the original group C epitope. 76Canc also shows comparable ADCC to 6F5 and 6F11 on both clade B and C constructs.

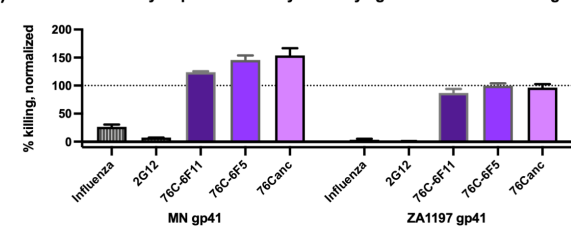
Common ancestor antibodies maintain function and these types of antibodies correlate to a non-progressive clinical state.

A) Serum from LTNPs is enriched for antibodies targeting a complex conformational epitope that can be functionally targeted for antibody dependent cell cytotoxicity



Serum from LTNPs compared to serum from a group of HIV infected with lower CD4 levels as a control for viral load were used to compete against biotinylated CD4 binding site (VRC01) and 76C Gp41 conformational epitope (6F11) targeting antibodies. Serum dilutions were chosen to align means near 50%. Means with 95% confidence intervals are shown.

B) Common ancestor antibody (76Canc) with germline VH1-02 sequence maintains robust Antibody Dependent Cell Cytotoxicity against Clade B and C targets



(A) Serum from long-term non-progressors (LTNPs) compared to serum from a group of HIV infected with lower CD4 levels as a control for viral load were used to compete against biotinylated CD4 binding site (VRC01) and 76C Gp41 conformational epitope (6F11) targeting antibodies. Serum dilutions were chosen to align means near 50%. Means with 95% confidence intervals are shown. (B) Monoclonal antibody 76Canc was created using the germline sequence of the heavy chain variable region with the CDR3 and light chain of 76C member. Antibody dependent cell cytotoxicity flow cytometric based assays were performed using gp41 proteins from clade B (MN) and clade C (ZA1197).

Conclusion. Certain antibodies present early on in infection may contribute to overall clinical course. Variable gene germline sequences that support functional activity against HIV could be targeted in vaccine regimens.

Disclosures. All Authors: No reported disclosures

1009. Biofilm Formation in *Acinetobacter Baumannii* Clinical Isolates

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Session: P-56. Microbial Pathogenesis

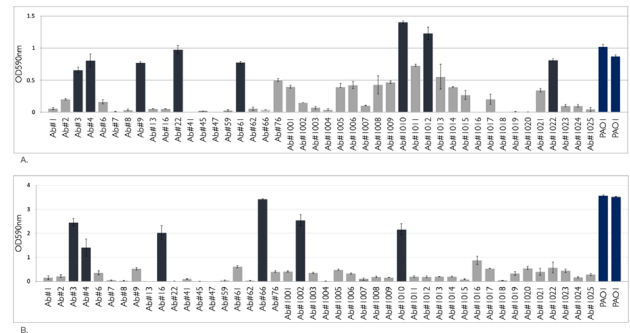
Background. Multidrug resistant *Acinetobacter baumannii* (MDR-Ab) is a Gram-negative bacterium known for causing severe nosocomial infections, attributed in part to its formation of biofilm. Siderophore is a virulence factor known to support biofilm formation by regulating iron availability. In this study, we screened 44

isolates of MDR-Ab from our Gram-negative repository to determine the strains that phenotypically form biofilm and produce siderophore. The results were compared to *Pseudomonas aeruginosa* PAO1, which produces both biofilm and siderophore.

Methods. Isolates were grown overnight in minimal M9 medium supplemented with casamino acids and hydroxyquinones at 37°C. Bacterial cells were normalized (to OD 600=0.01) and a standard diluted 10⁻³ tube was used in the study. A 96-well plate was inoculated with 100 microliters of each isolate in quadruplicates. This process was repeated in Tygon tubes with 50 microliters of each isolate in triplicates. The plate and Tygon tubes were incubated statically for 48 hours at 30°C and then stained with crystal violet. The contents were dissolved in 33% glacial acetic acid and analyzed by spectrophotometry to measure biofilm formation. Siderophore secretion was measured in supernatants with Chrome Azurol S (CAS) reagent and production was observed on CAS agar plates.

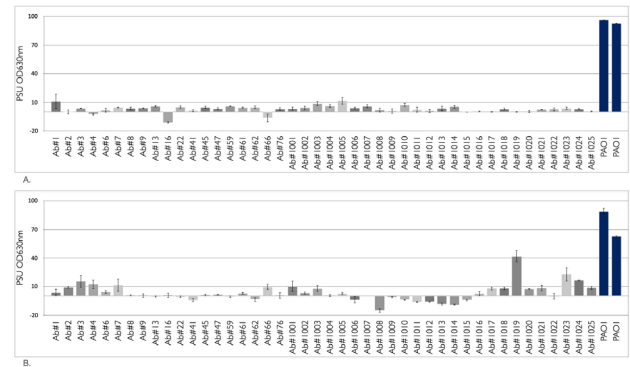
Results. High levels of biofilm formation were observed in 8 strains of MDR-Ab in the 96-well plate (3, 4, 9, 22, 61, 1010, 1012, 1022) and 6 strains in Tygon tubes (3, 4, 16, 66, 1002, 1010) (Fig. 1). There was minimal siderophore production in MDR-Ab isolates compared to PAO1 in both the 96-well plate and Tygon tubes (Fig. 2). Only 4 strains lacked siderophore production on CAS agar and were inversely negative for the secretion in medium.

Figure 1 Biofilm formation in a 96-well plate and Tygon tubes



(A) High levels of biofilm formation were observed in MDR-Ab strain numbers 3, 4, 9, 22, 61, 1010, 1012, 1022 in the 96-well plate. (B) High levels of biofilm formation were observed in MDR-Ab strain numbers 3, 4, 16, 66, 1002, 1010 in Tygon tubes.

Figure 2 Degree of siderophore production in a 96-well plate and Tygon tubes



Siderophore production of MDR-Ab was limited compared to PAO1 after inoculation in a 96-well plate (A) and in Tygon tubes (B).

Conclusion. Many strains of MDR-Ab readily form biofilm. Overall siderophore production is lower in MDR-Ab compared to consistent production by PAO1, but this does not appear to affect MDR-Ab's ability to form biofilm. Unlike in PAO1, biofilm formation in MDR-Ab may occur independently of siderophore production. This research serves as a basis for understanding future MDR-Ab biofilm elimination in patient catheters and indwelling devices.

Disclosures. All Authors: No reported disclosures

1010. Cross-Species Translation of Correlates of Protection for COVID-19 Vaccine Candidates Using Quantitative Tools

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Session: P-56. Microbial Pathogenesis

Background. Several COVID-19 vaccines have been authorized, and the need for rapid, further modification is anticipated. This work uses a Model-Based Meta-Analysis (MBMA) to relate, across species, immunogenicity to peak viral load (VL) after challenge and to clinical efficacy. Together with non-clinical and/or early clinical immunogenicity data (ECID), this enables prediction of a candidate vaccine's clinical efficacy. The goal of this work was to enable the accelerated development of vaccine candidates by supporting