Rapid Microbiological Identification and Major Drug Resistance Phenotyping with Novel Multiplexed Automated Digital Microscopy (MADM) for Ventilator-associated Pneumonia (VAP) Surveillance

I. S. Douglas[1], C. Price[1], K. Overdier[1], K. Thompson[1], B. Wolken[1], S. Metzger[2], D. Howson[2].

[1]Denver Health Medical Center – Denver, CO, United States; [2]Accelr8 Technology Corp. Denver, CO, United States

Abstract

Introduction. Clinical diagnosis of VAP is imprecise. Cumbersome microbiological identification and antimicrobial sensitivity testing techniques delay treatment and are associated with increased morbidity, mortality and antimicrobial resistance. We hypothesized that rapid microbiological detection and sensitivity reporting from mini- BAL samples obtained during surveillance of at-risk mechanically ventilated (MV) adults would reduce time to initiation of targeted treatment for VAP compared with a clinical and quantitative-culture guided approach.

Methods. Adult MICU patients with identified surrogate were included within 72 hours of intubation and if anticipated to require MV for >48h. Moribund state or pregnancy were exclusions. Surveillance mini-BAL (Combicath, Plastimed) was performed on Day 1, 3, 5, 7 and 10 of MV. Samples were processed for both a) routine respiratory quantitative microbiological culture (QCx) and sensitivity assays (> 48h result availability) and b) rapid (<8 hour) flowcell/surface-capture and automated microscopy (MADM). Viable bacteria were identified using growth analysis enhanced by a focused VAP antibody panel (S. aureus, P. aeruginosa, A. baumannii). Untypable organisms were also reported. Sensitivity was assessed using growth analysis. Attending physicians were blinded to MADM results.

Results. 77 miniBALs (median 2; Range 1-7 per patient) were performed on 33 MV patients (Median age 55, range 26-84 years; 30% Female; 52% active smokers, Median APACHE II 21 (IQR 16-24). 20 (61%) patients had diffuse or patchy CXR infiltrates and 3 patients had no infiltrates on enrollment. 70 BAL samples were tested using MADM. 12 samples grew ≥ 1 bacterial type at >104 CFU/mL by QCx. 8 samples contained mixed respiratory bacteria. 7 samples contained VAP associated bacteria (4 S. aureus (incl 1 auxotroph and 3 MRSA), 2 S. maltophilia, 1 K. pneumoniae). MADM identified 3 of 4 target organisms accurately and antimicrobial response enabled identification of 2 of 2 S. maltophilia. A K. pneumoniae sample was reported untypable. Auxtrophic growth precluded testing for 1 S. aureus sample. Antimicrobial response matched in 5 samples (3 MRSA, 2 S. maltophilia). 14 samples grew ≥ 1 bacterial type at < 104 CFU/mL by QCx. 10 samples contained mixed respiratory bacteria, 3 samples yeast, 2 samples lactose fermenting GNB, 1 sample non lactose fermenting GNB, 1 sample H. influenzae, beta lactamase positive, 1 sample H. species, not influenzae, 1 sample Beta hemolytic Streptococcus. None of the patients having bacteria detected by QCx at <104 CFU/mL developed clinical VAP. In 98% of samples MADM was concordant with QCx-negative samples. MADM detected a enteric organism ($10^{5)}$) in one sample negative by QCx. One VAP was diagnosed by clinical criteria. MADM based ID would have resulted in important and earlier antibiotic change/addition in 63% of mini-BAL samples with above threshold target organisms by QCx.

Conclusions. MADM is 100% specific and had 85% identification consistency for high-risk panel organisms including MRSA in miniBAL surveillance samples from MV patients at risk for VAP. Highly specific rapid VAP surveillance with MADM is feasible and may inform antimicrobial stewardship.

Background

- **VAP diagnosis is imprecise**, treatment often delayed & associated with increased morbidity, mortality (28-d MR = 30%) and hospital costs.
- **Quantitative culture (QCx) of bronchoalveolar lavage (BAL)** is usually obtained only *AFTER* VAP is clinically diagnosed. **Surveillance** with multiple BALs is associated with significantly more antibiotic-free days & fewer deaths. However, surveillance QCx requires 48-72 hours for results from conventional labs. Susceptibility testing requires an additional day.
- Newer technologies could reduce diagnostic delays, antimicrobial failures & resistance, by providing rapid effective guidance (< 8h). This would also guide de-escalation therapy by specific resistance testing.
- **Gene based technologies** have **limited** capacity to differentiate live from dead cells, quantify organism concentration, and predict drug resistance.
- **MADM phenotyping (BACcel™)** extracts live bacteria directly from patient specimens, immobilizes them in fluidic chamber observed by an automated microscope, exchanges fluid media and reagents according to programmed analytical protocols, acquires time-lapse microscopy images, and analyzes the image sequences for each individual immobilized bacterial cell. Population models of the species are built to phenotype cells present in the sample, and applies expert rules to interpret results for clinical decision support. Analysis includes bacterial population profiling.

Hypothesis

Surveillance microbiological testing for **rapid bacterial identification** and antibiotic resistance testing, with MADM will 1) **sensitively identify** patients who subsequently develop VAP when compared to usual microbiological approaches using conventional culture methods of lower respiratory samples from patients at risk for VAP and 2) will **reduce time to initiation** of treatment and reduce failure rates of initial therapy.

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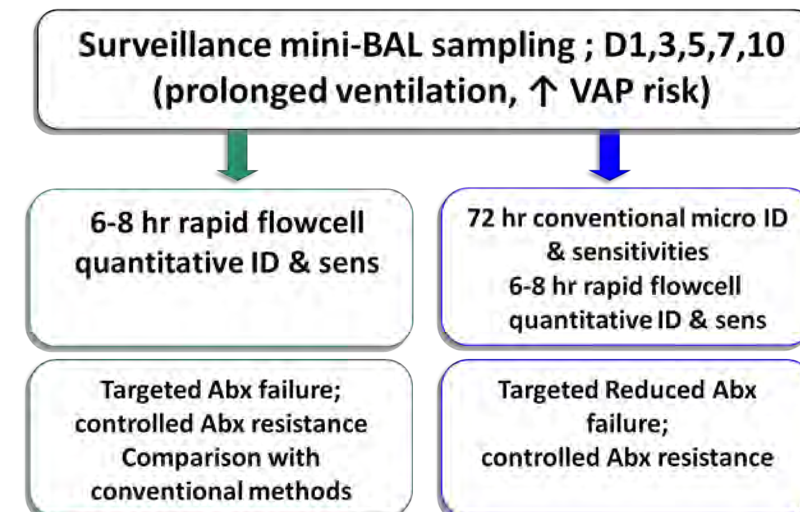
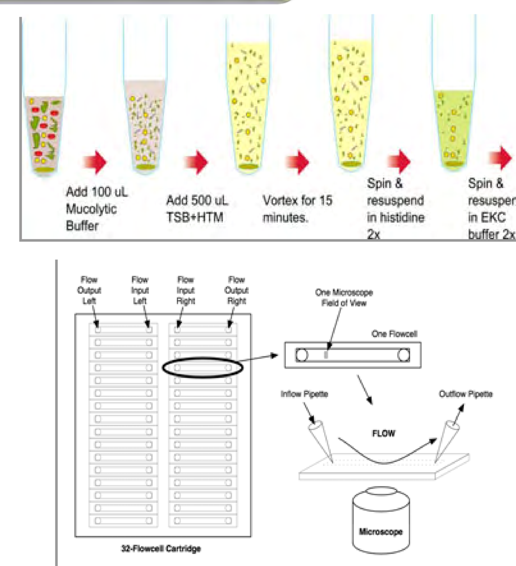
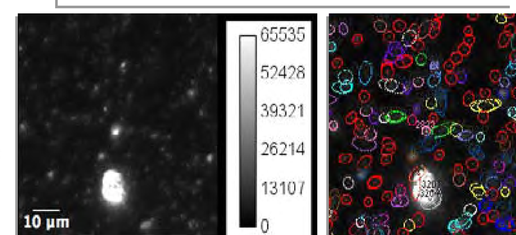
Methods

- **Prospective non-randomized clinical trial** – Medical ICU, academic community MC. Inclusions: adults within 72h of intubation and anticipated to require MV for >48h. Exclusions: Moribund state or pregnancy.
- Study procedures: CPIS score and demographics; Chest X-Ray; Surveillance mini-BAL (3x20mL NS), CombiCath (Plastimed) or AirLife™ (CareFusion) on Day 1, 3, 5, 7, 10 of MV. BAL split for conventional micro or BACcel rapid ID.



Extraction & ID of live bacteria:
- BAL sample is split & sample is prepared **BACcel MADM analysis**
- BAL sample is prepared, removing debris, and bacteria are extracted from sample. Sample is introduced into a multichannel fluidic cassette. Bacteria are concentrated and retained on the lower surface of all flowcells using low-voltage electrical field (5 min). Antibody labeling of bacteria in flowcells assists identification.
- Automated digital microscopy (MADM, BACcel; Accelr8, Denver CO) performs dark field imaging of 10 fields of view every 10 minutes for 180 minutes in each flowcell channel. Initial epifluorescence imaging is also performed for antibody detection. The BACcel uses identification algorithms on each individual immobilized cell that exhibits growth and measures growth rates of progeny for the duration of the test. Identification consists of probability scores for clone morphology, growth rates, and antibody labeling, then classifying as STAU, PSAE, or non-target. Antibiotic responses are used to aid identification when appropriate. Quantitation is performed by counting identified clones and computing original specimen CFU/mL.





Computer-imposed colored ellipses indicate potential organisms (pixel blobs) tracked for growth during analysis.

Species	Resistance Mechanism
S. aureus (STAU)	MRSA. phenotype
	Clinda resistance (any)
P. aeruginosa (PSAE)	Amikacin resistance
	Piperacillin/tazobactam resistance
A. baumannii (ABCC)	Imipenem resistance
	Cefepime resistance

VAP target organism panel for identification & susceptibility >10^4 CFU/ml

Conventional Micro identification
- Performed by DHMC micro lab (CLSI)

Clinical micro data provided to ICU clinicians for medical decision making
BACCel micro ID was prospectively performed but not available for clinical decision making

Study Power and Sample Size: Primary outcome assumptions
- 10% target incidence
- 40h difference in clinically reportable VAP target: QCx BAL ID (48h) + resistance (18h) vs. BACcel quant BAL ID (4h) + resistance (2h)
- 80% power, two-tailed α ≤0.01 requires 35 patients, assuming a median of 2 mini-BAL per patient (~8 unique isolates)

Results

Patient Demographics; n=34

Age; Median (IQR)		55 (41-60)
Gender		21M: 13F
Ethnicity	Hispanic	14 (42%)
	Native American	1 (3%)
	Caucasian	14 (42%)
	African American	4 (12%)
Smoking	Ever	27 (82%)
	Current	17 (52%)
Alcohol Use AUDIT Score	Median (IQR)	7 (0-18)
APACHE II	Median (IQR)	21 (16-24)
Mech. Vent (days)	Median (IQR)	4 (6-10)
ICU LOS (days)	Median (IQR)	10.5 (6.5 - 18.2)
ICU D/C Status	Deceased	11 (33%)
	Home	18 (55%)
	SNF	3 (9%)
	T/F -acute hospital	1 (3%)

BAL Surveillance & Safety

Patients enrolled	34	
Surveillance mini-BAL performed	77	
Combicath (Plastimed)		66
AirLife™ Catheter (Carefusion)		11
BAL per patient; Median (IQR, range)	2 (1-4, 1-7)	
BAL return; Average (SEM)	5.2±0.5 mL	

Surveillance BAL Adverse Events (Total BALs=77)	n	%
Desaturation requiring increase Fi02	2	3%
Tachycardia	1	1%
Agitation post mini BAL (60min)	2	3%
Bloody return	4	5%
Total	**9**	**12%**

Micro ID; Clinical Correlations

		MADM Micro ID 4-6 hours (BACcel™)			Conventional Micro ID 48-72 hours			
Spec #	**CPIS**	**BACcel ID**	**Conc (CFU/mL)**	**Phenotype, sensitivity**	**Isolate**	**Concordance**	**Abx at time of mini-BAL**	**DC Status**
003-D1	4	Fastidious Organism	$1.07x10^4$	Phenotype not assessed	10^4–10^5 MSSA	no	None	SNF
005-D7	3	Enteric	$1.28x10^5$	AN, IMP – no growth, CAZ, CLI, FOX, TZP - all growth	No isolate	no	CTX D5 not on day	Died
006-D1	6	Steno	$7.68x10^5$	AN, CAZ, CLI, FOX, IMP -all growth, TZP-antimicrobial effect,	>10^5 *S. maltophilia*	yes	Vanco/ Icaspo/ imipenem	Home
006-D3	9	Steno	$1.60x10^4$	AN, CAZ, CLI, FOX, IMP -all growth, TZP-antimicrobial effect,	10^4–10^5 *S. maltophilia*	yes	TMP/ Levaquin/ Casp/Vanco	Home
008-D7	9	STAU	$1.11x10^6$	FOX - R (MRSA) CLI – R	>10^5 MRSA	yes	Metronid azole only	Died
008-D10	9	STAU	$1.42x10^5$	Technical failure, no phenotype	10^4–10^5 MRSA	yes	Metronid azole only	Died
017-D1	7	UNK/enteric	$1.87x10^4$	ID UNK, no phenotype available	10^4–10^5 *K. pneumo.*	yes	Vanco, HIV,	SNF
022-D3	8	STAU	$4.00x10^4$	MRSA	10^4–10^5 MRSA	yes	Vanco; (Zosyn DC d2)	Home
033-D7	6	STAU	$6.64x10^4$	MRSA, CLI-R	10^4–10^5 *Candida* spp.	no	Cefepime, Vanco flouc;	Home

Microbiology Performance

Performance Characteristic	Rate	Comments
BAL Samples with target organism micro ID	12 (15.6%)	9 patients
Concordance Conventional vs. CPIS >=6	7 of 8	
Concordance BACcel vs. CPIS >=6	8 of 9	
BACcel call prior to routine change care	9 of 9	Change of Abx in 6; Abx stopped in 2
VAP diagnosis by CDC NIS criteria	1	Enteric organism*

*Organism not speciated. BACcel positive on D7. No antibiotic for 2 days at time of miniBAL; Patient died.

		Clin Micro Presence/Absence ≥1x10^4 CFU/mL: *Positive*	*Negative*	STAU, PSAE, ABCC, Steno, Enteric
BACcel MADM	*Positive*	True Positive (N=6)	False Positive (N=2 *‡)	→ Positive predictive value 75% (6/8)
	Negative	False Negative (N=1†)	True Negative (N=61)	→ Negative predictive value 98% (61/62)
		↓ Sensitivity = 86% (6/7)	↓ Specificity = 97% (61/63)	

* Patient with diffuse infiltrates + clinical pneumonia CPIS score (≥ 6)
‡ BACcel isolate: Gr +ve clustered cocci. Speciation pending *S. aureus vs.* CNS
† STAU grew in fastidious growth media flow cell but STAU ID test was not activated in that flow cell. STAU was called correctly after activation.

POSITIVE diagnostic likelihood ratio (+DLR) = 27: 95% CI [6.7 - 109]
NEGATIVE diagnostic likelihood ratio (-DLR) = 0.15: 95% CI [0.02 - 0.91]

Conclusions

- Mini-BAL based surveillance for VAP is both feasible and safe in ventilated at-risk patients
- MADM-based microbiological surveillance for VAP is sensitive (86%) and specific (97%), and is associated with a significant reduction in time to clinically available bacterial ID and resistance (approx 40-66h lead time) for multiple organisms and resistance types.
- In 5 of 7 (63%) mini-BAL samples with a target organism above threshold by QCx, MADM-based ID would have resulted in important and earlier antibiotic changes/additions
- MADM is a promising approach for rapid surveillance in patients at risk for VAP