Same-Day ID and Resistance Phenotyping Directly from Respiratory Specimens by Automated Microscopy

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AMENDED ABSTRACT

Background: Rapid, same-day, direct-from-specimen ID and AST of respiratory specimens could reduce clinical morbidity and mortality. A pilot study using respiratory specimens was performed to compare multiplexed automated digital microscopy (MADM) with cultures.

Methods: 281 de-identified remnant respiratory specimens were collected from hospital and commercial sources. Accompanying reports contained semi-quantitative ID and were used to select 92 bacteria-positive specimens for analysis by MADM. 25 endotracheal aspirates (ETA) of unknown age were obtained from a specimen vendor. 230 mini-bronchoalveolar lavage (mini-BAL) and 26 ETA specimens were obtained from Denver Health Medical Center (DHMC). DHMC specimens were 7-21 days old. AST results were available for all mini-BAL specimens but not for ETA. After culture re-test, 79 specimens were prepped and introduced into the MADM system. The system performed quantitative ID for *Staphylococcus aureus* (STAU), *Pseudomonas aeruginosa* (PSAE), and *Acinetobacter* spp. (ABCC). Concurrent quantitative culture (qCx) was performed on specimens. MADM performed resistance phenotype tests on STAU-containing specimens for cefoxitin (FOX) MRSA phenotyping and clindamycin (CLI) resistance. The system tested ABCC- and PSAE-containing specimens for amikacin (AN) and imipenem (IMP) resistance.

Results: By reported qCx 79 samples were above diagnostic threshold (1e4 for mini-BAL and 1e5 CFU/mL for ETA) and valid MADM tests were performed for 62 that met acceptance criteria. MADM was concordant with repeat qCx in 59/62. MADM identified 14/14 STAU, 3/3 PSAE, and 1/1 ABCC with one false positive from specimens. Two specimens yielded false positives for STAU and one yielded a STAU false negative. Overall ID performance was 95% sensitivity and 99% specificity. 2 specimens had STAU that expressed the MRSA phenotype by MADM (FOX) and Cx (OXA), and one by MADM only. None of the STAU expressed CLI-resistance. All PSAE and ABCC were susceptible to IMP and AN. MADM resistance detection was concordant with hospital AST results except with one STAU sample (MSSA by oxacillin MIC, MRSA by FOX in MADM). Complete MADM quantitative ID and phenotype data were available 4 hours after starting with a specimen.

Conclusions: MADM appears promising for same-day ID and resistance phenotyping directly from respiratory specimens with simultaneous multiple organisms and multiple resistance phenotypes. Expanded studies will more fully characterize MADM performance from ETA alone or in comparison with BAL or mini-BAL specimens.

INTRODUCTION

Nosocomial infections due to multiple drug resistant (MDR) bacteria are increasing in frequency and growing in complexity. For critically ill patients, resistance can render initial therapy ineffective, delaying the start of effective antimicrobial therapy. But standard diagnostic cultures introduce a 2-3 day delay to provide guidance. Multiplexed automated digital microscopy (MADM) has the potential to reduce turnaround time by rapidly analyzing bacteria extracted directly from a clinical specimen. The purpose of our study was to determine the speed and accuracy of a MADM system as an alternative to culturing with same-day quantitation, identification, and resistance phenotyping.

MATERIALS & METHODS

Customized MADM systems used commercial inverted microscopes with 12-bit monochrome cameras. A PC ran custom image analysis and experiment control software. 32-channel disposable cassettes (Fig. 1) enabled live microbial cell immobilization for microscopy and fluid exchanges for different test media and reagents.[1]

Figure 1: *32-channel flowcell cassette.*

Based on confirmed qCx, we tested 92/281 remnant clinical specimens labeled as BAL or mini-BAL and ETA from Denver Health, and ETA from a vendor. Targets included *Pseudomonas aeruginosa* (PSAE), *Acinetobacter baumannii* complex (ABCC), and *Staphylococcus aureus* (STAU). Controls used standard culturing methods (Cx).

Specimen preparation used a brief procedure to release bacteria, reduce imaging background, and suspend bacteria in a low ionic strength buffer. We rejected 13 specimens reported as positive but for which repeat qCx failed to confirm content. We rejected 10 samples with heavy interfering background when dilution to OD_{600}=0.3 yielded organism counts inadequate for analysis. We rejected 7 samples for other technical deficiencies. We tested the final 62 specimens.

20 μL samples were pipetted into independent flowcell channels (Fig. 2) and a low-voltage electrical field was applied for 5 minutes. The electrical field concentrated bacteria onto a poly-L-lysine film that immobilized the bacteria on the lower flowcell surface. Each flowcell channel received only one type of test reagent solution that contained a selective agent if required (only for channels used to test AB, sulbactam 32 μg/mL).

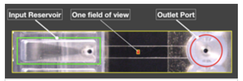

Figure 2: *One flowcell channel.*

The instrument acquired images at 10-minutes intervals for 180 minutes, using 10 fields of view in each flowcell channel through a 20x objective. Imaging used darkfield illumination. Identification variables included response to selective agents (AB with sulbactam), cell morphology, growth morphology, and growth rate.

Identification algorithms applied to each individual immobilized bacterial cell. The system measured the amount of change in mass over time to compute growth rates. Identification consisted of computing and combining probability scores for morphology, response to selective media, and growth rates to produce a receiver operating characteristic curve (ROC) to derive classification criteria.

RESULTS

Figure 3 shows organism growth after one hour to exemplify microscopy images used for time-lapse quantitative analysis. 80% of specimens had multiple species, but none had multiple target species.

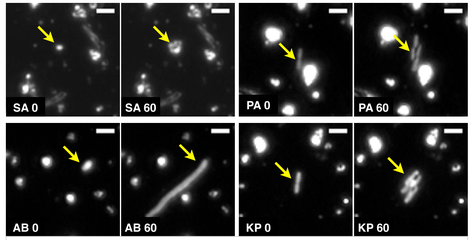

Figure 3. *Darkfield images, scale bars 5 µm. Time 0 and 60 minutes. AB in 32 µg/mL sulbactam. KP is Klebsiella pneumoniae (non-target). Non-growing pixel blobs are debris.*

Dark field illumination revealed specimen matrix residue pixel blobs with a broad range of size and morphology. The system distinguished live organisms by requiring measurable growth as well as morphologic criteria.

Identification scoring algorithms for STAU yielded 14/14 true positives (TP), 45/45 true negatives (TN), 2 false positives (FP), and 1 false negative (FN); for ABCC 1/1 TP, 60/61 TN, 1 FP; for PSAE 3/3 TP, 59/59 TN. For the 186 ID tests, Table 2 summarizes performance. Times to results were 1 hours for specimen prep and 3 hours to all analytical results for a total of 4 hours specimen-to-answer. Table 2 summarizes resistance phenotype results, with one discordant MRSA false positive.

TABLE 1: ID PERFORMANCE, MADM *vs.* Cx

Sensitivity	**95%**	CI95 = 73%-100%
Specificity	**98%**	94%-100%
Positive Predictive Value	**86%**	64%-96%
Negative Predictive Value	**99%**	96%-100%
Positive Likelihood Ratio	53	
Negative Likelihood Ratio	0.05	

DISCUSSION

2 false positive STAU IDs resulted from incorrect speciation (1 chained cocci, 1 *Enterococcus*). Test optimization or fastidious media could improve future versions. One ABCC false positive was *Enterobacter* sp. The false negative STAU had too few clones to meet the call criterion. Scanning more fields of view will resolve this problem. The MRSA discordance arose in Cx with oxacillin, which is no longer considered the most reliable phenotyping agent (FOX, as used for MADM). The small number of cells required for analysis is compatible with the bacterial concentration at BAL diagnostic threshold of 10^4 CFU/mL and ETA at 10^5 CFU/mL.

CONCLUSIONS

Multiplexed automated digital microscopy accurately analyzed live immobilized bacteria extracted directly from mini-BAL and ETA specimens. Total specimen-to-answer time was 4 hours. The method appears promising for rapid automated diagnostics with multi-species, multi-phenotype analyses from specimens that may include polymicrobial content.

1. Metzger S., et al. 2007. Direct Detection and Enumeration of Viable Bacteria in Human Bronchoalveolar Lavage Specimens Using Automated Growth Rate Analysis. 108th ASM General Meeting, Poster C-145.

TABLE 2: TARGET-POSITIVE SPECIMENS

SPECIMEN	TYPE	ID	Cx CLI	MADM CLI	Cx MRSA	MADM MRSA	Cx AN	MADM AN	Cx IMP	MADM IMP
DH 425	Mini-BAL	STAU	S	S	S	R				
DH 427	Mini-BAL	STAU	S	S	S	S				
DH 439	Mini-BAL	STAU	S	S	R	R				
DH 440	BAL	STAU	S	S	S	S				
T12	ETA	STAU	S	S	R	R				
ETA 280667	ETA	STAU	NA	NA	NA	NA				
DH 457	BAL	STAU	S	S	S	S				
DH 485	BAL	STAU	S	S	S	S				
DH 500	BAL	STAU	NA	NA	NA	NA				
DH 509	BAL	STAU	S	S	S	S				
DH 514	Mini-BAL	STAU	S	S	S	S				
DH 543	BAL	STAU	S	S	S	S				
DH 550	BAL	STAU	S	S	S	S				
DH 556	Mini-BAL	STAU	S	S	S	S				
DH 430	Mini-BAL	PSAE					S	S	S	S
DH 554	BAL	PSAE					S	S	S	S
DH 641	Mini-BAL	PSAE					S	S	S	S
		ABCC					NA	S	S	S

NA = not analyzed: STAU = *S. aureus*: PSAE = *P. aeruginosa*: ABCC = *Acinetobacter* sp.: CLI = clindamycin: AN = amikacin: IMP = imipenem: Cx = culture result