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Pathogen surveillance by mNGS for intubated patients: A reflex to culture model

04. Diagnostic microbiology

4h. Clinical metagenomics

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Background Diagnosis of bacterial and fungal infections still relies on traditional culture despite limited sensitivity and complex, slow workflows, because of lacking molecular alternatives and need for antibiotic susceptibility testing (AST). While culture setup can be automated, positive cultures require time-consuming workup by specialized staff straining laboratories that are faced with consolidation, a shrinking workforce, and other priorities such as COVID-19. Broad pathogen detection by next generation sequencing (NGS) can improve sensitivity, time-to-result (<24h), and scalability. Given decreasing costs, automated data analysis, and increasing ability to predict AST, NGS + culture with selective reflex to AST, can optimize performance and time-to-result. We demonstrated proof-of-concept using banked tracheal aspirates (TA) from intubated patients during the first COVID-19 wave.

Methods Culture was performed using routine protocols. Total nucleic acid was extracted, NGS libraries prepared and enriched using the Respiratory Pathogen ID/AMR Panel (RPIP; IDbyDNA, Illumina), sequenced (5M reads/sample, NextSeq

2000, Illumina) and analyzed with Explify (IDbyDNA). RPIP detects 187 bacterial, 53 fungal, and 42 viral pathogens plus 2108 antimicrobial resistance (AMR) markers. No growth/normal flora and RPIP detection of Candida species other than C. auris were considered negative.

Results Bacterial or fungal pathogens were detected in 67 of 104 samples (64.4%) from 55 patients by culture, in 75 (72.1%) by RPIP, and in 80 (76.9%) by culture + RPIP. RPIP-based ID/AMR results from 72 samples (69.2%) could have been reported after 24h; 66 samples (63.4%) would have been reflexed to AST, eliminating workup of 38 cultures (36.5%). In 5 of 34 patients (14.7%) with serial samples, pathogens were detected by RPIP an average of 5.4 days prior to culture (Table 1). RPIP detected additional bacteria (n=28) and fungi (n=1) in 25 samples (24%) and viruses in 41 samples (39.4%, 29 patients, Table 2).

Conclusions Culture plus RPIP provided improved and earlier detection of relevant respiratory pathogens, including cases where management would have changed (e.g., for C. auris, Pseudomonas aeruginosa, Staphylococcus aureus). Combining culture with RPIP enables selective reflexing to AST where phenotype prediction is not possible yet, while streamlining laboratory workflows and minimizing time to result.

Table 1. Earlier pathogens detections by RPIP in patients with serial samples

Patient	Pathogen Detected Earlier by RPIP	Time Between Detection by RPIP and Culture (Days)
L9	*Staphylococcus aureus*	11
L19	*Burkholderia cepacia* complex	3
L25	*Pseudomonas aeruginosa*	2
L33	*Staphylococcus aureus*	2
L34	*Candida auris*	9

Table 2. Additional pathogens detected by RPIP

Pathogen Class	Pathogen	Patients
Bacteria	*Staphylococcus aureus*	9
	Burkholderia cepacia complex	4
	Pseudomonas aeruginosa	3
	Serratia marcescens	3
	Escherichia coli	2
	Stenotrophomonas maltophila	1
	Achromobacter xylosoxidans	1
	Other *Enterobacterales*	5
Fungi	*Candida auris*	1
Viruses	SARS-COV-2	15
	HSV-1	19
	EBV	6
	CMV	3
	HHV-6	1

Keyword 1
clinical metagenomics

Keyword 2
hospital acquired infections
Keyword 3
SARS-CoV-2

Conflicts of interest

Do you have any conflicts of interest to declare?
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