

Utilization of Target Enriched Multiplex-PCR for the detection of Pantan-Valentine leukocidin in necrotizing pneumonia complicating influenza A

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Introduction

Necrotizing pneumonia caused by *Staphylococcus aureus* (SA) is a complication of influenza A (INFA) related to the SA virulence factor, Pantan-Valentine leukocidin (PVL). PVL is a leukocidin produced by SA, and is expressed by both methicillin sensitive (MSSA) and methicillin resistant strains (MRSA). PVL-positive SA can complicate influenza infections in both healthy and immune suppressed individuals resulting in a severe necrotizing process often associated with neutropenia¹. PVL-negative SA can also cause pneumonia following influenza, but these infections are generally less fulminant. Thus, the timely accurate detection of PVL-positive SA in the presence of INFA increases the likelihood of correct diagnosis, targeted therapy, and patient recovery.

Technology

Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR™, Diatherix Laboratories Inc. Huntsville, AL) is a nucleic acid multiplex technology. The process begins with a target enrichment step where two pairs of nested gene-specific primers are designed for each target and used to amplify the target DNA. Next, during the exponential amplification step, a universal set of forward and biotin-labeled reverse SuperPrimers recognize unique tag sequences on the nested primers and provide bulk amplification. Target-specific fluorescent probes are then coupled to carboxyl groups on a bead-based substrate for detection².



Methods

Patients presenting to Kettering Health Network hospitals with influenza-like illness and severe respiratory distress underwent CT chest scans, bronchoscopy, and TEM-PCR testing using the Viral Respiratory Panel (VRP) and the Staphylococcus Differentiation Panel (SDP). The robustness of the VRP panel allows for the simultaneous detection of 10 respiratory pathogens including the detection of seasonal INFA, as well as, H1N1 specifically. The SDP detects the genetic targets for PVL and 5 types of *Staphylococcus* bacteria including NUC for SA and MeCA for MRSA. Specimens were obtained during bronchoscopy. Nucleic acid extraction for TEM-PCR panels was performed using a MagNA Pure96 system (Roche, Basel, Switzerland). The amplification products were completed using a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems®, Thermo Scientific). Probe fluorescence was read using a LumineX™ detection platform. VRP results were confirmed by the Ohio Department of Health and SDP results were confirmed via culture.

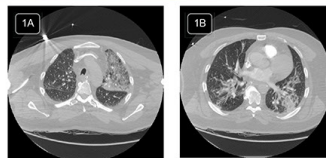
Results

Two patients with INFA complicated by SA PVL-associated necrotizing pneumonia are described.

Case Report, Patient 1: A young female presented with INFA, H3N2. The patient deteriorated within 24 hours exhibiting respiratory distress and toxic shock. Ten hours after admission, patient was intubated and became profoundly neutropenic (WBC 1.2x 10³). Bronchoscopy specimens were positive by TEM-PCR for H3N2 and PVL-positive SA. Bronchoscopy revealed severe, necrotizing, hemorrhagic pneumonia. Blood cultures confirmed the presence of MRSA. Patient expired.

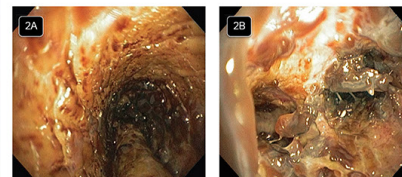
Case Report, Patient 2: A middle age male presented with progressive shortness of breath following influenza-like illness. Patient rapidly deteriorated with septic shock, multiorgan failure, and required mechanical ventilation and hemodialysis. CT chest scan demonstrated bilateral infiltrates indicative of pneumonia (Fig. 1A-B). Bronchoscopy showed severe necrotizing hemorrhagic tracheobronchitis (Fig 2A-B). TEM-PCR revealed INFA H1N109 and PVL-positive MRSA. Patient was successfully treated with oseltamivir, linezolid and clindamycin with resolution of bronchoscopic findings (Fig 3A-B, 4A-B). Shock, renal and respiratory failures have resolved and patient was eventually discharged home after prolonged hospitalization.

CT scan, Patient 2

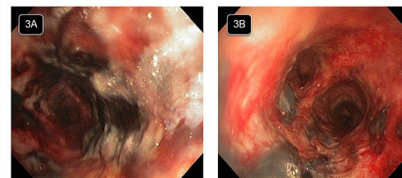


Figures 1A-B: CT scan with representative cuts of upper (1A) and lower (1B) lobes demonstrating bilateral infiltrates.

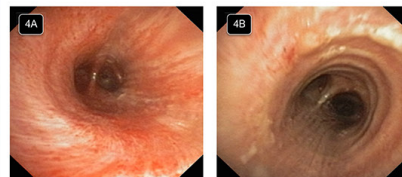
Bronchoscopy, Patient 2



Figures 2A-B: Initial bronchoscopy demonstrated severe necrotizing hemorrhagic tracheobronchitis (2A) with sloughing of mucosa obstructing the right mainstem bronchus (2B).



Figures 3A-B: Bronchoscopy Day 4 with partial improvement. Areas of mucosal hemorrhage are well visualized in the trachea (2A) and mainstem bronchus (2B).



Figures 4A-B: Bronchoscopy Day 10 with resolution of the trachea (3A) and the mainstem bronchus (3B).

Discussion

For patients infected with PVL-negative MRSA, the IDSA recommends various courses of antibiotic therapy which can include the use of linezolid, clindamycin, tetracyclines, or vancomycin. In patient 2, TEM-PCR accurately identified PVL-positive MRSA which allowed physicians to administer a more immediate and targeted combination therapy which included linezolid and clindamycin. Linezolid and clindamycin are both bacterial protein synthesis inhibitors, and are more effective in suppressing PVL toxin production than other antimicrobials, like vancomycin, which have a different mechanisms of action³. By identifying PVL-positive MRSA and H1N1, physicians avoided providing suboptimal empiric treatment which had a positive impact on antiviral and antimicrobial stewardship, as well as patient care.

Conclusion

PVL is a cytotoxin causing leukocyte destruction and tissue necrosis. Infection with PVL producing SA can result in toxic shock, respiratory failure and leukopenia, which are predictive of mortality. Bronchoscopy can reveal airway bleeding, frank hemorrhage, and acute inflammatory exudate. PVL-producing strains of SA have affinity for exposed basement membrane in the ciliary epithelium of the lung present after viral illness. TEM-PCR is unique technology which rapidly detects PVL-positive SA superinfecting INFA which promotes targeted antiviral and antimicrobial therapies resulting in improved patient outcomes.

References

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