Peptide Nucleic Acid (PNA) Probes to Define the Etiology of Ventilator Associated Pneumonia (VAP): Time for a new Gold Standard?


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ABSTRACT

The clinical diagnosis of ventilator associated pneumonia (VAP) is notoriously difficult because of the lack of specificity of clinical, radiologic and microbiologic findings. In theory, lung histopathology should provide the "gold standard" for the definition of pneumonia, however many factors influence histologic diagnosis such as the focal nature of VAP, time course in the evolution of the pathologic process, specific microbiologic involvement and even the interpretation by different pathologists. 1-3 Post-mortem cultures would seem logical enough to establish a bacterial etiology, but may be affected by airway (i.e. bronchoalveolar lavage, overgrowth after death, and sampling error. If multiple species are found, interpretation is even more difficult.

We studied post-mortem lung tissue from five patients with pneumonia using fluorescence labeled peptide nucleic acid (PNA) probes (AdvanDx, Woburn MA) specific for Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter aerogenes, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Stenotrophomonas maltophilia, Serratia marcescens, and Pseudomonas aeruginosa.

Five patients with VAP were identified from a previously reported study (Huff et al. abstract L-20, 101st ASM, 2001). A histologic diagnosis of VAP was defined by the presence of significant alveolar infiltration by polymorphonuclear leukocytes, 1 and further classified into gram positive, gram negative or mixed pathogen groups based on tissue gram's stain.

Two to four micron sections were cut from tissue blocks with areas of histologically proven pneumonia. The groups based on tissue gram's stain.

RESULTS

1. Figure 1 shows the specificity data for the S. aureus, K. pneumoniae, E. coli and P. aeruginosa PNA FISH probes.

2. Figure 2 shows the lung histology, gram's stain and S. aureus PNA probe from a 67 yo male with MRSA pneumonia at autopsy after 17 days mechanical ventilation.

3. Figure 3 shows the lung histology, gram's stain and K. pneumonia PNA probe from a 27 yo female with Klebsiella pneumonia at autopsy after 5 days mechanical ventilation.

4. Figure 4 shows the P. aeruginosa PNA probe for a 67 yo male in mechanical ventilation for 5 days. P. aeruginosa was isolated from blood and sputum.

5. Figure 5 shows a 75 yo female who had been on mechanical ventilation for 7 days with histologically proven pneumonia and P. aeruginosa grown from sputum and blood. PNA FISH probe for P. aeruginosa probes for E. coli and S. aureus were negative, 1000x oil. Inset showing PMNs and masses of gram positive bacteria. C – 1000x oil Gram 's stain showing intracellular gram negative rods; C  – 1000x oil PNA FISH probe specific for K. pneumoniae; P. aeruginosa probe negative. S. aureus was isolated from blood and sputum.

DISCUSSION AND CONCLUSIONS

This study demonstrates that common bacterial pathogens can be detected in situ in tissue sections, permitting the correlation of histopathology with definitive species identification.

In situ detection of bacteria together with their microscopic localization will allow a better interpretation of post-mortem culture results and ultimately lead to a better understanding of the pathogenesis of VAP. Histologic identification of VAP is not without complexity because it is typically multifocal and may be missed if the stain is not studied. Furthermore, the disease initially may show areas of bronchialization, hence progressing to more classic bronchopneumonia. However, if bacteria are of etiologic importance, they should be demonstrable from the early stages until well into the resolution phase, even with antibiotic treatment.

We propose that the demonstration of bacteria either by gram's stain or positivity by specific in situ methods that provide species information be included in the criteria for the histologic definition of ventilator associated bacterial pneumonia.

REFERENCES


3. Shiga M, Bridge A, Nishimura A, Sato S, Satoh T. Peptide nucleic acid (PNA) probes for E. coli, S. aureus, K. pneumoniae and P. aeruginosa were negative.

4. Figure 4 shows the P. aeruginosa PNA probe for a 47 yo male on mechanical ventilation for 7 days. P. aeruginosa was isolated from sputum and blood. PNA FISH probe for P. aeruginosa probes for E. coli and S. aureus were negative, 1000x oil. Inset showing PMNs and masses of gram positive bacteria. C – 1000x oil Gram 's stain showing intracellular gram negative rods; C  – 1000x oil PNA FISH probe specific for K. pneumoniae; P. aeruginosa probe negative.

5. Figure 5 shows a 75 yo female who had been on mechanical ventilation for 7 days with histologically proven pneumonia and P. aeruginosa grown from sputum and blood. PNA FISH probe for P. aeruginosa probes for E. coli and S. aureus were negative, 1000x oil. Inset showing PMNs and masses of gram positive bacteria. C – 1000x oil Gram 's stain showing intracellular gram negative rods; C  – 1000x oil PNA FISH probe specific for K. pneumoniae; P. aeruginosa probe negative.

MATERIALS AND METHODS

Five patients with VAP were identified from a previously reported study (Hull et al. abstract L-20, 101st ASM, 2001). A histologic diagnosis of VAP was defined by the presence of significant alveolar infiltration by polymorphonuclear leukocytes, 1 and further classified into gram positive, gram negative or mixed pathogen groups based on tissue gram's stain.

Two to four micron sections were cut from tissue blocks with areas of histologically proven pneumonia. The formalin fixed tissues were dehydrated, hybridized at 35°C for 1.5 h in a humidified chamber (Oncor, Gaithersburg, MD), dehydrated at 75°C for 1.5 h with each PNA probe in hybridization buffer and washed in wash buffer for 1/2 h. Positive controls were included in each batch as positive and negative controls. Sections were read with a Zeiss Axioskop Plus microscope using a dual band pass FITC/Trasfluor filter and photographed at 1000x under oil immersion. Specificity data for the S. aureus, K. pneumoniae, E. coli and P. aeruginosa PNA probes are shown in Figure 1.