Identification of pathogens directly from respiratory specimens from patients with HAP within 40 minutes

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Introduction:

Hospital acquired pneumonia (HAP) is a leading cause of mortality, morbidity and increased hospital costs. Treatment of HAP mandates initiation of broad spectrum antibiotics after establishing the clinical diagnosis with deescalation of antibiotic therapy after pathogen identification. Classical microbiological culture and identification requires 24 to 48 hours. We have developed and tested a rapid method of identifying pathogens using a beacon-based fluorescent in situ hybridisation (bbFISH) method to identify the major Gram-negative pathogens causing HAP.

The often high viscosity of sputum has hitherto prevented the direct application of fluorescence-based assays however the method used here allows for a mucolytic step which both reduces background fluorescence and allows for accurate pathogen detection. Respiratory specimens obtained from an interdisciplinary surgical intensive care unit (SICU) were examined and high quality specimens as judged by the number of leukocytes were included in the study and subjected to additional bbFISH analysis. The results of bbFISH were compared to culture. A total of 303 specimens meeting the criteria were included. The investigator performing the bbFISH was blinded to the culture results of the specimens and these were retrospectively analysed. Specimens were examined routinely according to standard microbiological methods and in addition at the time of performing the bbFISH were cultured on standard agar plates in order to analyse possible discrepancies.

Results:

A total of 303 specimens (tracheal aspirate or bronchial lavage) from intubated patients with suspected ventilator associated pneumonia from a surgical intensive care unit were investigated. All the specimens were compared to culture results as the gold standard. As can be seen from table 1 the sensitivity for the different species of Gram-negative pathogens varied between 20 % (*Klebsiella pneumoniae*) and 100 % (*Serratia marcescens*, *Proteus* spp. and *P. aeruginosa*). In all cases where there was a discrepancy between bbFISH and culture the culture results were compared to cultures performed at the same time as the bbFISH (generally 24 h after routine cultures). There were no qualitative differences in the routine cultures compared to the cultures at the time of bbFISH.

In 131 cases the results of both culture and bbFISH were identical and in 24 cases the bbFISH was negative for a pathogen found in culture. This results in a total sensitivity of 84,5 %. Cultures were negative in 148 cases of which in 3 cases the bbFISH was positive for a pathogen. This results in a specificity of 98 %.

Table 2: Contingency table of total results

		FISH +	FISH -	Total
Culture	+	131	24	155
	-	3	145	148
Total		134	169	303

Sensitivity – 84,5 %

Specificity – 98 %

Conclusions:

The bbFISH is a very useful method to rapidly identify the major Gram-negative pathogens in respiratory specimens from patients with suspected pneumonia. The method provides an accurate diagnosis of the major „problem pathogens“ especially *Pseudomonas aeruginosa* and could thus lead to a more tailored empirical therapy. Particularly important is the possibility of excluding a significant finding of *P. aeruginosa* in patients that have been ventilated for longer than one week, which would possibly reduce the number of patients receiving dual-therapy thus reducing the antibiotic selection pressure. In no case was a specimen found free of *P. aeruginosa* by bbFISH and was positive in culture. Slightly disturbing is the poor performance of the bbFISH with respect to *Klebsiella* spp. A possible reason for this may have been a defective lot that was used initially as the results in the latter half of the study were decidedly improved. Further analysis will determine if this remains a problem. One advantage of the bbFISH is the ability to detect polymicrobial infections that are occasionally not detected by culture, either due to overgrowth of a dominant pathogen or because the colony morphology is not sufficiently different to allow clear identification. This may be of immediate importance for therapy and could also lead to an earlier detection of a possible “problem” pathogen such as multi-resistant *P. aeruginosa*.

The lack of a bbFISH for Gram-positive pathogens is for a surgical ICU of importance in institutions in which *Staphylococcus,* and especially MRSA, play a large role. In a sub-analysis it was found that the Gram-stain was sufficient to identify the patients with a significant growth of *S. aureus* in most cases and thus bbFISH may not have a great role in the SICU studied here.

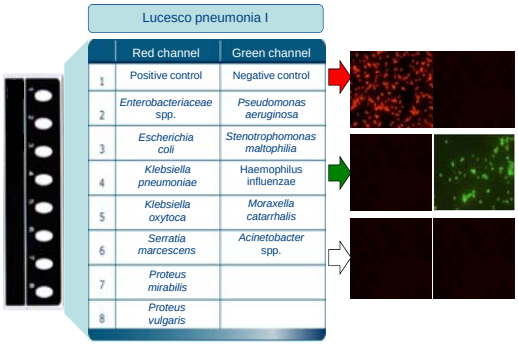

Fig.1: Beacon compilation and example of a typical read-out (upper panel: positive control; middle panel: positive identification of *H. influenzae*; lower panel: negative)

Table 1: Comparison of the results of bbFISH with culture in respiratory specimens from ventilated SICU patients.

Species	No. positive Culture	No. positive bbFISH	Sensitivity (%)
Enterobacteriaceae	77	71	92
E. coli	16	12	75
K. pneumoniae	10	2	20
K. oxytoca	8	5	63
S. marcescens	5	5	100
P. mirabilis	3	3	100
P. vulgaris	1	1	100
P. aeruginosa	21	21	100
Acinetobacter spp.	10	5	50
S. maltophilia	17	16	94
H. influenzae	10	8	80
M. catarrhalis	0	0	-

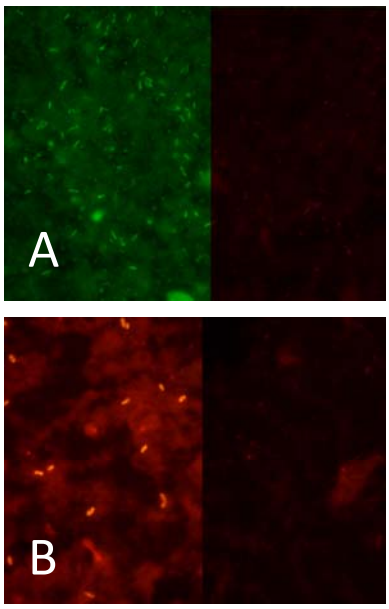

Fig. 1: *Pseudomonas aeruginosa* (A) and *Stenotrophomonas maltophilia* (B) in tracheal aspirates of patients with VAP. (In each picture the negative control is shown on the right.)