28th ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Madrid, Spain
21 – 24 April 2018

P0555 Development of a loop-mediated isothermal amplification (LAMP) assay for the rapid detection of pathogenic bacteria in bronchoalveolar lavage specimens

Andrea Vergara Gomez*[1], Adrià Cruells[2], Míriam López[1], Jorge Puig de la Bellacasa[1], Francesc Marco Reverte[1], Jordi Vila[1 3]

[1]Department of Clinical Microbiology, CDB, Hospital Clínic of Barcelona – University of Barcelona – Barcelona, Spain, Barcelona, , [2]University of Barcelona, Spain, Barcelona, Spain, [3]Institute for Global Health (ISGlobal), Barcelona, Spain., Barcelona, Spain

Background: Ventilator-associated pneumonia (VAP) is a frequent infection in patients in Intensive Care Units (ICUs) with high associated morbidity and mortality. Proper and early treatment is crucial, but diagnosis is difficult. The objective of this study was to evaluate a loop-mediated isothermal amplification (LAMP) assay to diagnose VAP.

Materials/methods: Positive and negative bronchoalveolar lavage (BAL) samples from 58 patients were included. Quantitative cultures were the gold standard. Samples were concentrated by centrifugation, DireCtQuant 100W buffer (directquant) was added and boiled. Previously described primers were used for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Acinetobacter baumannii* and new primers were designed for *Klebsiella pneumonie* (PrimerExplorer4 software). The LAMP was performed in 25 µL of reaction mixture: 5 µL primers (0.2µM outer, 1.6µM inner and 0.4µM loop primers), 15µL Isothermal Master Mix (Optigene) and 5.0µL of DNA. The reaction was conducted in a Versant kPCR (Siemens) at 65°C for 40 minutes. Different concentrations of each microorganism were inoculated to negative samples (sensitivity) and each sample was tested for each microorganism (specificity).

Results: The LAMP sensitivity was 10^2CFU/mL for *S. aureus*, *E. coli*, *P. aeruginosa and K. pneumoniae* and 10^4CFU/mL for *S. maltophilia* and *A. baumannii*. Concordance among culture and LAMP results are showed in Table 1.

Table 1.

Microorganism identified by culture	Nº of samples tested by LAMP and culture	Concordance	Minor errors	Major errors	Comments
S.aureus	19	15	2[A,B]	2[C,D]	[A]LAMP: *S.aureus*+*S.maltophilia* detected [B]LAMP: *S.aureus*+*P.aeruginosa* detected (Gram-negative bacilli in GRAM) [C,D]Very few colonies, LAMP:negative
P.aeruginosa	11	9	-		[E]<1000CFU *P.aeruginosa*, LAMP:

				2[E,F]	*S.aureus* [F]<1000CFU *P.aeruginosa*, LAMP:negative
S.maltophilia	6	6	-	-	-
K.pneumoniae	4	2	-	2[G]	[G]<1000CFU K.pneumoniae
E.coli	3	3	-	-	-
A.baumannii	2	1	-	1[H]	[H]<100000CFU *A.baumannii*
Negative	7	6	1[H]	-	[I]LAMP: *K.pneumoniae* detected (Gram-negative bacilli in GRAM)
Mixed flora	6	3	3[J,K,L]	-	[J]LAMP: *P.aeruginosa* detected [K]LAMP: *S.aureus* detected [L]LAMP: *K.pneumoniae* detected

Conclusions: LAMP method could be used to detect the more frequent bacteria causing VAP. It is a simple, cheap, sensitive, specific and rapid assay. More samples are needed before implementing it as a routine test.