

Design, development and validation of a real-time PCR assay for detection of Klebsiella pneumoniae complex in environmental matrixes

Elodie Barbier, C. Rodrigues, Géraldine Depret, V. Passet, Laurent Gal,

Pascal Piveteau, S. Brisse

▶ To cite this version:

Elodie Barbier, C. Rodrigues, Géraldine Depret, V. Passet, Laurent Gal, et al.. Design, development and validation of a real-time PCR assay for detection of Klebsiella pneumoniae complex in environmental matrixes. 1. One Health European Joint Programme Annual Scientific Meeting, May 2019, Dublin, Ireland. , 2019. hal-02787597

HAL Id: hal-02787597 https://hal.inrae.fr/hal-02787597

Submitted on 18 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Design, development and validation of a real-time PCR assay for detection of the *Klebsiella pneumoniae* complex in environmental matrixes

<u>E. Barbier¹, C. Rodrigues², G. Depret¹, V. Passet², L. Gal¹, P. Piveteau¹, S. Brisse²</u>

INRA, AgroSup Dijon, Univ. Bourgogne Franche-Comté Dijon, Agroecology, France
Institut Pasteur, Biodiversity and Epidemiology of Bacterial Pathogens, Paris, France

Klebsiella pneumoniae (Kp) represents a growing public

Specificity

health concern due to the emergence of multidrug resistance and hypervirulence.

- Seven closely related phylogroups constitute the Kp species complex: *Klebsiella pneumoniae* (phylogroup Kp1), *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2) and subsp. *similipneumoniae* (Kp4), *K. variicola* subsp. *variicola* (Kp3) and subsp. *tropicalensis* (Kp5), *K.quasivariicola*' (Kp6) and *K. africanensis* (Kp7).
- Kp is described as ubiquitous in nature. Water, sewage, soil and vegetation could be environmental sources of Kp but their exact contribution in its transmission routes is still unknown.

Objective: To develop a specific and sensitive real time PCR assay for the detection of all phylogroups (Kp1 to Kp7) of the Kp complex from environmental sources.

→ 100 % positives with Kp complex members (melting T°: 78,5 to 80,1)

 \rightarrow No false positive with non Kp (Ct>35, melting T° \neq).



Figure 2 Melting curve peaks of positive (red arrow) and negative (black arrow) sample

- Sensitivity on pure DNA of Kp1 ATCC13883T
 - \rightarrow Detection down to 45 pg (15 genome copies)

METHODS

- In silico identification of a conserved region in all phylogroups for specific primers design
- Validation of a Sybergreen Real-time PCR assay
 - Specificity was tested on 40 Kp complex members (Kp1 to Kp7) and 90 non-Kp complex bacteria
 - Sensitivity was tested on 5g-soil microcosms spiked with Kp1 ATCC13883T after a 24h-enrichment step in LB + ampicillin (10 mg/l) and a short treatment (washing, boiling, dilution)
- Implementation on 84 environmental samples (soil, roots, leaves and water)
 - Comparison to culture-based identification by plating enrichments using SCAI medium and MALDI-TOF mass spectrometry

RESULTS

• The assay targets an intergenic region (*zkir*) that we found to be uniquely present in genomes of the Kp complex.



Figure 3 Standard curve for zkir assay

Sensitivity on soil microcosms spiked with Kp1 ATCC13883T

 \rightarrow Detection down to 7.0 × 10⁻¹ cfu per g after a 24h-enrichment step

Higher detection of Kp from environmental samples

→ 28/84 (33.3 %) environmental samples positive with the zkir assay (Ct: 18 to 36, melt curve peak: 78,5 to $80,1^{\circ}$ C)

 \rightarrow 24/84 (28.5 %) positive with culture-based method and MALDI-TOF MS identification.

CONCLUSION AND PERSPECTIVES



Figure 1. zkir (zinc-khe intergenic region) real time PCR assay

- We developed a specific and sensitive real time PCR assay for the rapid detection of all phylogroups of the Kp complex.
- This system can detect as low as 1 bacteria in a 5 g sample after a 24h-enrichment step, with higher sensitivity than culture-based methods.
- Inter-laboratory validation on food samples and on stools of animals and intensive care unit patients is ongoing. It is also used for broad screening of potential environmental sources of Kp.

This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.