

27<sup>th</sup> **ECCMID**

**Vienna, Austria**  
22 – 25 April 2017

The congress of  ESCMID

Session: EV015 Molecular diagnostics and MALDI-TOF

**Category: 9a. Microbial pathogenesis & virulence**

22 April 2017, 08:45 - 15:30  
EV0205

**Novel real-time multiplex PCR for rapid identification of pathogenic *Yersinia* species**

Emmanuel Andre\*<sup>1</sup>, Philippe De Sany<sup>2</sup>, Morgane Darricades<sup>3</sup>, Léonie Goeminne<sup>4</sup>, Thomas Michiels<sup>5</sup>, Michèle Janssens<sup>2</sup>, Georges Wauters<sup>2</sup>, Michel Delmée<sup>6</sup>

<sup>1</sup>*Institut de Recherche Expérimentale et Clinique; Cliniques Universitaires Saint-Luc; Service de Microbiologie*

<sup>2</sup>*Institut de Recherche Expérimentale et Clinique; Université Catholique de Louvain; Pôle de Microbiologie Médicale*

<sup>3</sup>*Pôle de Microbiologie Médicale, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain*

<sup>4</sup>*Institut de Recherche Expérimentale et Clinique; Mblg*

<sup>5</sup>*Institut de Duve, Université Catholique de Louvain*

<sup>6</sup>*Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain; Cliniques Universitaires St Luc; Pôle de Microbiologie*

**Background:** Among the 18 species of the genus *Yersinia*, only three species are responsible for human diseases: *Y. pestis* is the causative agent of plague and has become uncommon in Europe. *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 1<sup>B</sup>, 2, 3, 4, 5 are responsible for gastrointestinal diseases and are found occasionally from stool cultures in bacteriology laboratories. The virulence of these bacteria is related to the presence of virulence factors located in the chromosome and in virulence plasmids. The National Reference Center (NRC) for *Yersinia* currently performs identification of *Yersinia* strains received from clinical specimen, with the aim to determine the pathogenicity based on the species, biotype and serotype determination. Due to the complexity of these gold-standard techniques, time to mean time-to-result is 4 days. In our experience, only 45% of *Yersinia* species isolated from clinical samples are pathogenic.

**Material/methods:** We designed a real-time multiplex PCR allowing rapid identification of pathogenic *Yersinia* species. The technique targets three genes. *hrpA* is a pan-*Yersinia* gene and is used as positive control. *inV* is a gene specific for *Y. pseudotuberculosis* and *Y. pestis*. *yopM* is present in the virulence plasmid of all pathogenic *Yersinia* species. This combination of three targets allows to rapidly distinguish non-pathogenic species from those responsible for human disease (figure 1). The multiplex PCR was performed on purified DNA using the Lightcycler© 96 system (Roche Diagnostics).

**Results:** We performed the multiplex PCR in parallel with Gold Standard techniques on a panel of *Yersinia* species, 13 enteric species and 19 consecutive strains received by the NRC for identification (Table 1). Only two discrepancies were observed, as the PCR did not detect the *hrpA* gene of the non-pathogenic *Y. massiliensis* and *Y. frederiksenii* strains. These discrepancies did not have a clinical impact. All PCR test results were available on the same day.

**Conclusions:** We developed a new multiplex PCR method allowing the rapid identification of pathogenic *Yersinia* species. This should allow to accelerate the response for clinicians awaiting the clinical relevance of a positive *Yersinia* culture.

**Figure 1:**

	No pathogenic <i>Yersinia</i> species detected	Non-pathogenic <i>Yersinia</i> species detected	<i>Yersinia pseudo-tuberculosis</i> detected * <i>Y. pestis</i> not excluded	<i>Yersinia enterocolitica</i> (Biotype 1B, 2, 3, 4 or 5) detected
<i>hrpA</i>	-	+	+	+
<i>inV</i>	-	-	+	-
<i>yopM</i>	-	-	+	+

**Table 1:**

	Gold-Standard method (Time to response = 4 days)					Novel real-time PCR method (Time to response = same day)			Concordant results?	
	Strain #	Species	Biotype	Serotype	Pathogenic strain?	Pan-Yersinia target ( <i>hrpA</i> )	Yersinia pseudotuberculosis target ( <i>inv</i> )	Virulence plasmid target ( <i>yopM</i> )		Multiplex PCR interpretation
Technical validation	1	<i>Y. enterocolitica</i>	1A	O: 41,43	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	2	<i>Y. enterocolitica</i>	1A	O: 10	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	3	<i>Y. enterocolitica</i>	1A	O: 36	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	4	<i>Y. enterocolitica</i>	2	O: 5,27	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
	5	<i>Y. enterocolitica</i>	2	O: 9	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
	6	<i>Y. enterocolitica</i>	2	O: 5,27	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
	7	<i>Y. enterocolitica</i>	4	O: 3	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
	8	<i>Y. enterocolitica</i>	4	O: 3	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
	9	<i>Y. enterocolitica</i>	4	O: 3	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
	10	<i>Y. pseudotuberculosis</i>	/	Type: I	Yes	+	+	+	Pathogenic <i>Yersinia pseudotuberculosis</i> detected	Yes
	11	<i>Y. pseudotuberculosis</i>	/	Type: I	Yes	+	+	+	Pathogenic <i>Yersinia pseudotuberculosis</i> detected	Yes
	12	<i>Y. pseudotuberculosis</i>	/	Type: III	Yes	+	+	+	Pathogenic <i>Yersinia pseudotuberculosis</i> detected	Yes
Specificity evaluation	1	<i>E. coli</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	2	<i>Shigella sonnei</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	3	<i>K. oxytoca</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	4	<i>Salmonella enterica</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	5	<i>proteus vulgaris</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	6	<i>E. cloacae</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	7	<i>Serratia marcescens</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	8	<i>Citrobacter freundii</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	9	<i>Hafnia alvei</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	10	<i>Morganella morganii</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	11	<i>Providencia stuartii</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	12	<i>C. difficile</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
	13	<i>campylobacter coli</i>	NA	NA	NA	-	-	-	No pathogenic <i>Yersinia</i> species detected	Yes
Clinical validation	1	<i>Y. kristensenii</i>	/	O: 12	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	2	<i>Y. intermedia</i>	/	O: 76	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	3	<i>Y. enterocolitica</i>	1A	O: 5	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	4	<i>Y. enterocolitica</i>	4	O: 3	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
	5	<i>Y. enterocolitica</i>	1A	O: 6	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	6	<i>Y. enterocolitica</i>	1A	O: 6	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	7	<i>Y. enterocolitica</i>	1A	O: 5	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	8	<i>Y. frederiksenii</i>	/	O: 52	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	9	<i>Y. enterocolitica</i>	1A	O: 13,7	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	10	<i>Y. massiliensis</i>	/	/	No	-	-	-	No pathogenic <i>Yersinia</i> species detected	No. But no clinical impact.
	11	<i>Y. enterocolitica</i>	1A	O: 6	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	12	<i>Y. enterocolitica</i>	1A	O: 6	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	13	<i>Y. enterocolitica</i>	1A	O: 13,7	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	14	<i>Y. enterocolitica</i>	1A	O: 5	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	15	<i>Y. enterocolitica</i>	1A	O: 36	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes
	16	<i>Y. enterocolitica</i>	4	O: 3	Yes	+	-	+	Pathogenic <i>Yersinia enterocolitica</i> detected	Yes
17	<i>Y. enterocolitica</i>	1A	O: 57	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes	
18	<i>Y. enterocolitica</i>	1A	O: 6	No	+	-	-	Non-pathogenic <i>Yersinia</i> species	Yes	
19	<i>Y. frederiksenii</i>	/	O: 76	No	-	-	-	No pathogenic <i>Yersinia</i> species detected	No. But no clinical impact.	