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

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Introduction

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^B, 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 technics, time-to-result is 4 days. In our experience, only 45% of *Yersinia* species isolated from clinical samples are pathogenic.

Materials and 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.

The combination of these three targets allows to rapidly distinguish nonpathogenic species from those responsible for human disease (figure 1).

The multiplex PCR was performed on purified DNA using the Lightcycler© 96 system (Roche Diagnostics)

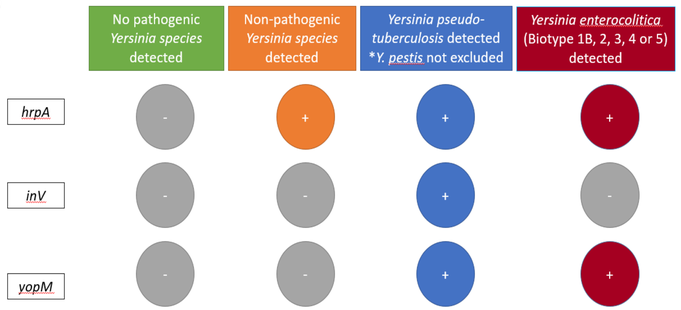

Fig. 1: Design of the multiplex PCR.

Results

We tested 159 clinical *Yersinia* strains sent by different Belgian hospitals (Table 1) and 13 other enteric bacteria.

In summary, 64% (n=102) strains were nonpathogenic *Yersinia* species, and *Y. enterocolitica* Biotype 1^A was the most frequent species recovered (n=88). 36% (n=56) were confirmed as pathogenic *Yersinia* types. *Y. enterocolitica* Biotype 4, Serotype 0:3 was the most frequent pathogenic species recovered (n=41) followed by *Y. enterocolitica* Biotype 2, Serotype 0:9 (n=5), *Y. pseudotuberculosis* (n=4), *Y. enterocolitica* Biotype 2, Serotype 0:5,27 (n=3), *Y. enterocolitica* Biotype 3, Serotype 0:3 (n=2) and *Y. enterocolitica* Biotype 1^B, Serotype 0:8 (n=1). The patient presenting the *Y. enterocolitica* Biotype 1^B, Serotype 0:8 strain had no recent history of travelling to the United States.
One *Y. enterocolitica* Biotype 4,
Serotype 0:3 was classified among the nonpathogenic species as it had lost its virulence plasmid. This was confirmed by a negative PCR targeting l*crV* gene, which is also present on the virulence plasmid.

Table 1: Frequency of Yersinia pathogenic and nonpathogenic species received by the Belgium National Reference Laboratory for *Yersinia*

	Number of clinical isolates recieved	Frequency
Pathogenic Yersinia species	**56**	**36%**
enterocolitica Biotype 4, Serotype 0:3	41	26%
enterocolitica Biotype 2, Serotype 0:9	5	3%
pseudotuberculosis	4	3%
enterocolitica Biotype 2, Serotype 0:5,27	3	2%
enterocolitica Biotype 3, Serotype 0:3	2	1%
enterocolitica Biotype 1^B, Serotype 0:8	1	1%
Nonpathogenic Yersinia species	**102**	**64%**
enterocolitica Biotype 1^A	88	55%
frederiksenii	6	4%
bercovieri	4	3%
intermedia	2	1%
enterocolitica Biotype 4, Serotype 0:3 with no virulence plasmid*	1	1%
massiliensis	1	1%
kristensenii	1	1%
Total	**159**	**100%**

* Rough colony. Loss of virulence plasmid was confirmed by negative *lcrV* PCR

Table 2 shows the results of the multiplex PCR. All 56 pathogenic *Yersinia* species presented a positive signal for *hrpA* and *yopM*. Among these, the four *Y. pseudotuberculosis* strains had in addition a positive signal for the species-specific *inV* target.
Among the nonpathogenic species, the *hrpA* signal was positive among 96/103 (93,2%) strains. The species *Y. frederiksenii* (n=6) and *Y. massiliensis* (n=1) had a constant negative signal for this target.

None of the 13 other enteric bacterial species gave a positive signal for any of the three targeted genes.

	Number of strains tested	*hrpA*	*yopM*	*inV*
Pathogenic Yersinia species	**56**	**56**	**56**	**4**
Y. enterocolitica Biotype 4, Serotype 0:3	41	41	41	0
Y. enterocolitica Biotype 2, Serotype 0:9	5	5	5	0
Y. pseudotuberculosis	4	4	4	4
Y. enterocolitica Biotype 2, Serotype 0:5,27	3	3	3	0
Y. enterocolitica Biotype 3, Serotype 0:3	2	2	2	0
Y. enterocolitica Biotype 1^B, Serotype 0:8	1	1	1	0
Nonpathogenic Yersinia species	**103**	**96**	**0**	**0**
Y. enterocolitica Biotype 1^A	88	88	0	0
Y. frederiksenii	6	1	0	0
Y. bercovieri	4	4	0	0
Y. intermedia	2	2	0	0
Y. enterocolitica Biotype 4, Serotype 0:3 with no virulence plasmid	1	1	0	0
Y. massiliensis	1	0	0	0
Y. kristensenii	1	1	0	0
Other gastrointestinal bacteria	**13**	**0**	**0**	**0**
Campylobacter coli	1	0	0	0
Citrobacter freundii	1	0	0	0
Clostridium difficile	1	0	0	0
Enterobacter cloacae	1	0	0	0
Escherichia coli	1	0	0	0
Hafnia alvei	1	0	0	0
Klebsiella oxytoca	1	0	0	0
Morganella morganii	1	0	0	0
Proteus vulgaris	1	0	0	0
Providencia stuartii	1	0	0	0
Salmonella enterica	1	0	0	0
Serratia marcescens	1	0	0	0
Shigella sonnei	1	0	0	0
Total	**172**			

Table 2: PCR results

Conclusion and discussion

Yersinia infections is a relatively rare disease in Belgium, but conventional microbiology cultures present a lack of specificity because selective agar plates recover other bacteria and nonpathogenic *Yersinia* species.
Current methods for confirmation of the pathogenicity of *Yersinia* species are fastidious and have a turn-around-time incompatible with an adequate clinical management of *Yersinia* infections.

We developed a new multiplex PCR method allowing the rapid identification of pathogenic *Yersinia* species.
This study could demonstrate the accuracy and the feasibility of replacing conventional methods by a rapid multiplex PCR.

This should allow to accelerate the response for clinicians awaiting the clinical relevance of a positive *Yersinia* culture.