Does infrared spectroscopy allow the identification of *Yersinia enterocolitica* BT4 strains of porcine origin responsible for human's infection?

Martine DENIS[1], Emmanuelle HOUARD[1], Guillaume LARIVIÈRE-GAUTHIER[2], Amandine WILHELM[3], Benoit GASSILLOUD[3], Carole FEURER[4], Javier PIZARRO-CERDA[5], Cyril SAVIN[5], Anne-Sophie LE GUERN[5]

1. Anses, Ploufragan-Plouzané-Niort Laboratory, Hygiene and Quality of Poultry and Pig Products Unit, Ploufragan, France.
2. Cnam, Chaire agroalimentaire, Ploufragan, France
3. Anses, Hydrology Laboratory, Water Microbiology Unit, Nancy, France
4. IFIP, The French Institute for the Pig and Pork Industry, 35650 Le Rheu, France.
5. Institut Pasteur, Université Paris Cité, CNRS UMR6047, *Yersinia* Research Unit, National Reference Laboratory 'Plague & Other Yersiniosis', WHO Collaborating Center for Plague FRA-140, Paris, France

Background

In 2021, yersiniosis was still, the third most frequently reported human zoonosis in Europe, (EFSA and ECDC, 2021) with *Yersinia enterocolitica* being the most common *Yersinia* species reported in human cases. Pigs are frequently described as a source of human contamination by *Y. enterocolitica* through consumption of raw or undercooked meat or by direct contact with contaminated carcasses. Pigs carry *Y. enterocolitica* in their oral cavities, and they excrete this bacterium in their feces. A one-year French survey at slaughterhouses indicated that 13.7% of the pigs were found positive for pathogenic *Y. enterocolitica* and 74.3% of the pig batches contained at least one positive pig (Fondrevez et al., 2014). Biotype 4 (BT4) was the most prevalent biotype among the isolated strains (91.9% of the isolates). This BT4 is also the most common biotype found in human infections in France (79% according to the *Yersinia* French National Reference Laboratory).

Different molecular techniques exist to type bacterial strains and different studies identified FT-IR 5Fourier-Transform infrared) spectroscopy as an alternative technique for bacterial typing. The objective of this project was to test the performance of FT-IR in the identification of BT4 pig strains responsible for human infections. This tool is easy to use and inexpensive compared to other typing techniques such as WGS (Whole Genome Sequencing), which also requires bioinformatic skills.

Materials and methods

The 100 strains of *Yersinia enterocolitica* from biotype BT4 used in this study were part of 10-year-old collections. Among them, 50 were of porcine origin (Anses and IFIP's collections), and 50 of human cases *(Yersinia* National Reference Laboratory collection).

Genomic DNA was extracted from fresh colonies using the PureLink™ Genomic DNA Mini Kit from Thermo Fisher Scientific and genomes were sequenced at the P2M sequencing platform (Institut Pasteur, Paris, France) using Illumina NextSeq technology. Genomes were assembled and isolates were characterized using the *Yersinia* spp. 500-gene cgMLST analysis as described by Savin et al. (2019). Another cgMLST, based on 1,727 genes and restricted to the *Y. enterocolitica* species, was used as a molecular typing tool. Strains with a maximum of five allelic differences were considered as belonging to the same cluster and therefore genetically closely related.

For IR analysis, the samples were prepared according to Bruker recommendations. All strains were typed with five replicates using IR Biotyper® system from Bruker Daltonics. The spectra data were recovered from the IR Biotyper software, in the wavelength range corresponding to lipids (3000-2800 cm^{-1}), proteins (1800-1500 cm^{-1}) and polysaccharides (1300-800 cm^{-1}) regions. Thus, variables corresponding to numbers of waves were considered for statistical analyses, first by considering all the data and secondly, only the data from the polysaccharide region (Region of interest according to Bruker for typing). The average spectra values of the five replicates of each strain were used for comparison using Partial least square discriminant analysis (PLS-DA) models with the ropls package under R.

Results

With cgMLST, 14 clusters were identified, 9 of which included only porcine strains, 2 included only human strains, and 3 included both human and porcine strains. Human (4) and porcine (4) strains that were in the same cluster were identified as P+H+. Other strains of porcine or human origin were identified as P+H- and P-H+, respectively.

On the basis of this classification obtained by cgMLST, a discriminant analysis by partial least squares PLS-DA was carried out on the spectral data in order to determine whether it was possible to differentiate the 3 groups P+H+, P+H- and P-H+.

On the PLS-DA graphs, whether for the entire spectrum or just the polysaccharide region, we detected a separation between the P-H+ and P+H- groups, but the P+H+ phenotype was mixed across the two other groups. The R2Y and Q2 values obtained for this model were low (less than 0.5) indicating that this model was not able to discriminate the strains. However, when we did a pairwise comparison, there was a good discrimination between the P+H- and P-H+ groups, with a model that allowed a good prediction.

Considered regions	Value	3 groups together	P+H-/P-H+	P-H+/P+H+	P+H-/P+H+
Entire spectrum	R2Y	0.452	0.769	0.487	0.498
	Q2	0.349	0.669	0.186	0.154
Polysaccharide region	R2Y	0.413	0.712	0.265	0.258
	Q2	0.363	0.672	-0.0954	0.049

Finally, looking at the origin of the strains, analyzes on the IR data showed that the porcine and human strains were clearly separated. Looking, specifically at the P+H+ strains, we observed that the porcine strains of these clusters were mostly with the other porcine strains and that human strains of these clusters were mostly with the other human strains

Conclusion

The cgMLST revealed within our collection only four porcine strains involved in human yersiniosis over the same period. FT-IR did not allowed us to identify porcine and human strains grouped in the same cgMLST clusters. On the other hand, this approach showed that porcine strains differed from human strains. It would be interesting to identify the numbers of wave that differentiate these two populations (in progress). Finally, this tool, in the context of our study, did not fully satisfy our objective, which was to identify porcine strains involved in human toxi-infections.
In conclusion, the cgMLST and FT-IR results minimize the importance of the involvement of pigs in BT4 yersiniosis, in France.

References

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