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Characterization of Campylobacter coli isolated from pig, sheep, poultry, wild bird, river and shellfish using MALDI-TOF and comparison of their protein spectra to identify relationships between sources

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Introduction

The sanitary quality of shellfish-harvesting areas is a key issue in France, the leading shellfish producer in Europe. *Campylobacter* spp was detected in coastal catchments and shellfish-harvesting areas in Brittany and Normandy, France (Rincé et al., 2018). This pathogen is excreted by many animals whether wild birds or livestock (Mughini-Gras et al., 2016). These participate in the contamination of the environment directly or via manure spreading.

Comparison of PFGE profiles or MLST types have proved their effectiveness in determining the origin of human cases of campylobacteriosis or surface water contamination (Denis et al., 2009; Denis et al., 2011a; Clark et al., 2011; Jonas et al., 2015; Mughini-Gras et al., 2016). Identify the sources of shellfish contamination with these techniques is possible but these latter are expensive and long to implement. An alternative may be the typing of strains by MALDI-TOF MS. Links of MALDI-types of *Campylobacter* to their MLST types were described (Zautner et al., 2013). Moreover, the MALDI-TOF MS technique is easy to perform and inexpensive.

In this study, we characterize *Campylobacter coli* isolated from pig, sheep, poultry and shorebird fecal samples, river water samples and shellfish batches using MALDI-TOF and compare their protein spectra to identify relationships between sources and the source of shellfish contamination. We focused on *C. coli*, the only species detected in pig in France (Denis et al., 2011b).

Material and Methods

We considered 144 *C. coli* isolated from feces of pigs (60), sheep (15), poultry (30), and shorebirds (10) and from river water samples (24) and shellfish batches (5). They were isolated in Brittany and Normandy, which are the two main areas of shellfish production in France with an important livestock production. After culture on blood agar (24h, 37°C) in microaerophilic conditions, proteins of each strain were extracted as recommended by Bruker. Then, 1 µl of each extract was deposited 8 times on spots of MSP 48 target polished steel plate and included in 1 µl of IVD matrix HCCA. The steel plates were sent to MALDI-TOF Platform of Anses where four reads per spot were realized (32 protein spectra per strain). Under BioNumerics, an average spectrum of protein was obtained for each strain, and all the average spectra were clustered in a dendrogram using UPGMA method and Pearson's coefficient.

Results

The strains were distributed in 14 clusters (Tab1). *Campylobacter coli* of pigs were mostly distinguishable from *C. coli* of other animal reservoirs. They never clustered with *C. coli* from poultry. Seventy percent of pig strains clustered together; the others (28.3%) clustered with sheep (3) strains and, one pig strain (1.6%) with sheep (12), shorebird (4) and river water (3) strains. Sheep strains (80%) clustered with wild bird strains (4) and river water strains (3). Poultry strains (86.6%) clustered with shorebird (3), river (11) and shellfish (2) strains. Shorebird strains (90%) clustered with river water strains (19) and shellfish strains (2). Finally, two strains of shellfish were grouped with strains of river water, shorebird and poultry; the three other strains were only grouped with *C. coli* of river water.

Table 1: Clustering with 93% of similarity of average protein spectrum under BioNumerics

Cluster	Pig	Sheep	Poultry	Wild bird	River	Shellfish	Total
C1	2						2
C2	17	3					20
C3			1				1
C4	39						39
C5	1						1
C6	1	12		4	3		20
C7			26	3	11	2	42
C8				1	3		4
C9					3	2	5
C10					2	1	3
C11			2				2
C12			1				1
C13				1	2		3
C14				1			1
Total	60	15	30	10	24	5	144

Discussion and Conclusion

Although in France, *C. coli* is rarely isolated from shellfish, it is important to identify the animal reservoir that causes this *C. coli* contamination. Especially since the water, from rivers of the upstream catchments, arriving in these shellfish-harvesting areas mainly contains *C. coli* (Rincé et al., 2018).

With the use of MALDI-TOF, we observed that *C. coli* of pigs were mostly distinguishable from *C. coli* of other animal reservoirs, and particularly from *C. coli* isolated from poultry. PFGE already highlighted that *C. coli* of pigs differed genetically from *C. coli* of poultry in France (Denis et al., 2009) while MLST showed common STs in these two reservoirs in other countries (Mughini-Gras et al., 2016).

Our study suggests also that pig is very weakly involved in river contamination by *C. coli* as already described by PFGE or MLST in other studies (Denis et al., 2011a, Mughini-Gras et al., 2016). This may explain why *C. coli* of pigs was not linked to shellfish.

C. coli of sheep clustered with pig strains. Another study showed that very few STs of *C. coli* of ruminants shared common STs with *C. coli* of pigs (Mughini-Gras et al., 2016).

Our study suggests that poultry, shorebirds and sheep could contribute to the contamination of rivers by *C. coli*. This is consistent with MLST results (Mughini-Gras et al., 2016) showing that *Campylobacter* in surface water were mostly attributed to wild birds and poultry followed by ruminants. The contamination of the rivers by *C. coli* can thus contribute to that of shellfish.

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