Prevalence and characterization of *Yersinia enterocolitica* in retail seafood

Chenyang Li[1], Thomas Alter[1], Stefan Hertwig[2], Andrea Barac[2], Carolin Riedel[1]
[1]Institute of Food Safety and Hygiene, Freie Universität Berlin
[2]Federal Institute for Risk Assessment (BfR)

Introduction

Yersinia (Y.) enterocolitica is a zoonotic enteropathogen widely distributed in Europe, which can cause acute gastroenteritis and mesenteric lymphadenitis mimicking appendicitis. It has extensive distribution and can frequently be isolated from animals and from environmental and food sources. Several studies report a high prevalence of *Y. enterocolitica* in pigs and wild boars. However, no data are available on the prevalence in seafood. The objective of this study was to investigate the prevalence and pathogenic potential of *Y. enterocolitica* in seafood.

Material und Method

Sampling

A total of 220 fresh seafood samples like mussels (n = 90), shrimp (n = 89) and squid (n = 41) were purchased randomly from different retail shops and markets in Berlin (09/2015 - 04/2016). The tissues from seafood were used for further bacterio-logical analyses.

Isolation of *Y. enterocolitica*

Tissue samples (10 g) were diluted 1:10 with PMB (phosphate buffered saline and bile salt) and were crushed and homogenized (Figure 1). Sample bags were enriched at 4 °C for 14 days. About 10 µl of enrichment was applied on CIN agar (Cefsulodin-Irgasan-Novobiocin agar), and incubated at 28 °C for 24 h. Suspicious colonies were transferred onto CIN agar and incubated at 28 ° C for 24 h to obtain pure cultures. DNA was extracted by a Chelex method from isolates tested negative for oxidase activity, and positive for urease activity.

Figure 1: Samples from different retail shops in Berlin. **A:** shrimp samples were homogenized after removing the exoskeleton, **B:** mussel samples were homogenized after removing the shell, **C:** squid samples.

Identification and characterization of *Y. entercolitica* isolates

The confirmation and molecular serotyping of *Yersinia* isolates from seafood were based on multiplex PCR: The 16S rRNA gene fragment was used for *Y. enterocolitica* detection. Strains were also tested for the presence of *ail* and *inv*. If both genes were present, strains were considered to be virulent. Strains tested positive for *yst*B are non-virulent (Garzetti et al. 2014). Molecular serotyping included the genes *per (*O:9), *wzt* (O:5), *wbb*U (O:3) and *wbc*A (O:8) according to Garzetti et al. (2014). The confirmation of biotypes was performed biochemically according to Cornelis et al. (1987) while serotypes were verified by auto-agglutination.

MALDI-TOF

The microbial species identification was further confirmed by MALDI TOF MS as described by Murugaiyan et al. (2014).

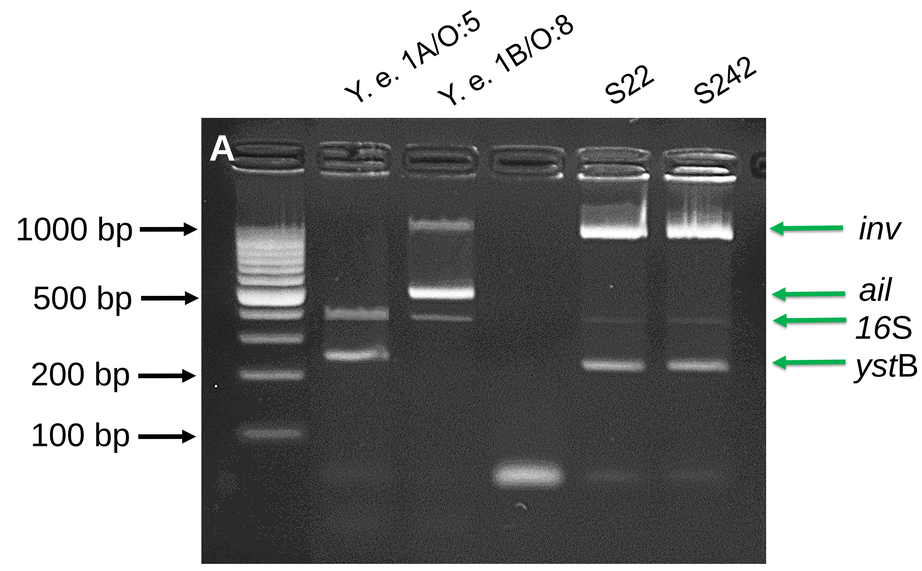
Results

The prevalence of *Y. enterocolitica* in seafood was 2.7% (6/220). Mussel (2/90), shrimp (1/89) and squid (3/41) samples were positive for *Y. enterocolitica* (Table1).

Table 1: Prevalence of *Y. enterocolitica* in seafood samples

Seafood	n	*Y. enterocolitica* positive	95% CI
Mussels	90	2 (2.22%)	0.00 - 5.33
Shrimp	89	1 (1.12%)	0.00 - 3.36
Squid	41	3 (7.32%)	0.00 -15.64
Total	220	6 (2.73%)	0.56 - 4.89

The identification and the characterization of *Y. enterocolitica* (Figure 2, Table 2) indicated that all isolates are non-virulent (biotype 1A). While two samples showed an unknown serotype, three isolates could be determined as serotype O:8 and one sample as serotype O:5.



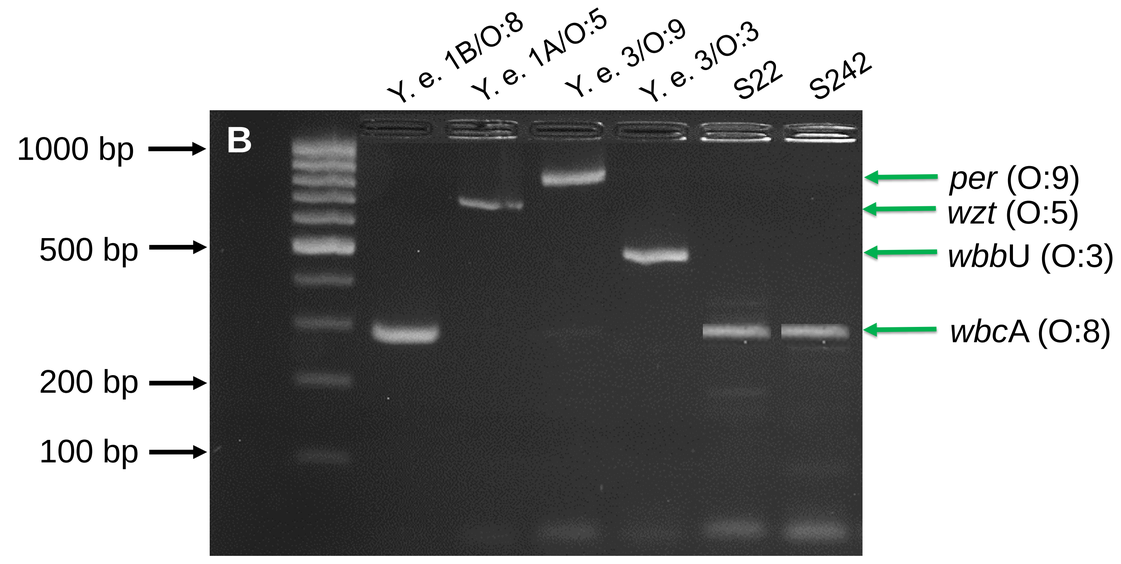


Figure 2: Identification and serotyping by PCR method exemplarily shown for two positive samples (S22 and S242). **A**: *inv*, *ail*, 16S and *yst*B were amplified for detection and molecular biotyping of isolates. Y. e. 1B/O:8 (virulent) and Y. e. 1A/O:5 (non-virulent) were used as reference strains. **B:** *per*, *wzt*, *wbb*U and *wbc*A were amplified for determination of serotype. Y. e. 1B/O:8, Y. e. 1A/O:5, Y. e. 3/O:9 and Y. e. 3/O:3 represent positive controls for different serotypes.

Table 2: Identification and characterization of *Y. enterocolitica* isolates

Seafood	n	Genotype	Serotype	Biotype	Virulence
Mussel	2	16S+*inv*+*yst*B (2)	O:8 (2)	1A (2)	non
Shrimp	1	16S+*yst*B (1)	unknown (1)	1A (1)	non
Squid	3	16S+*yst*B (1) 16S+*inv*+*yst*B (2)	O:5 (1) O:8 (1) unknown (1)	1A (3)	non

Conclusion

This study provides the first systematic prevalence study of *Y. enterocolitica* in retail seafood in Germany. Although the prevalence was quite low (2.73%) and all isolates were characterized as non-virulent strains, this study shows that seafood might be a potential source of infection with *Y. enterocolitica*. However, seafood seems not to play a prominent role in the epidemiology of Yersiniosis.

Reference

- Garzetti et al. 2014. A molecular scheme for *Yersinia enterocolitica* patho-serotyping derived from genome-wide analysis. *International Journal of Medical Microbiology*.
- Murugaiyan et al. 2014. Species differentiation within the *Staphylococcus intermedius* group using a refined MALDI-TOF MS database. *Clinical Microbiology and Infection*.
- Cornelis et al. 1987. *Yersinia-Enterocolitica*, a Primary Model for Bacterial Invasiveness. *Reviews of Infectious Diseases*.

Institute of Food Safety and Hygiene • FU Berlin • Königsweg 69 • 14163 Berlin • Tel. +49 30 - 838 - 62545 • Fax +49 30 - 838 - 462029 • chenyang.li@fu-berlin.de • www.vetmed.fu-berlin.de