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Characterization of *Arcobacter* spp. isolated from retail seafood in Germany

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ABSTRACT

Arcobacter species are considered emerging zoonotic pathogens associated with human gastroenteritis. They were already isolated from a wide range of habitats and hosts worldwide. However, information about the prevalence of *Arcobacter* in retail seafood products is still scarce. This study aimed to evaluate the presence of *Arcobacter* in retail seafood and characterize *Arcobacter* isolates derived from these matrices. In total, seven species of *Arcobacter* were isolated from 56 of 318 (17.6%) seafood samples, including bivalves (mussels, clams and razor clams), shrimps and cephalopods (squids and octopuses). The highest prevalence was detected in cephalopods (27.4%), followed by bivalves (18%) and lowest in shrimps (8.5%). PCRs of 10 putative virulence genes demonstrated higher prevalences of these genes among *A. butzleri,* compared to other species, such as *A. cryaerophilus*, *A. aquimarinus* and *A. venerupis*. Further, high genetic diversity could be determined by ERIC-PCR. Our study indicates the potential transmission of *Arcobacter* to humans by consuming uncooked or undercooked seafood.

1. Introduction

Since several human cases of gastrointestinal disease and bacteremia associated with *Arcobacter* spp. were reported, attention on *Arcobacter* as emerging foodborne pathogen increased in recent years (Arguello et al., 2015; Figueras et al., 2014; Kayman et al., 2012; Lappi et al., 2013; Prouzet-Mauleon et al., 2006; Vandamme et al., 1992; Vandenberg et al., 2004). In 2002, the International Commission on Microbiological Specifications for Foods (ICMSF, 2002) classified *Arcobacter* (*A.*) *butzleri* and *A. cryaerophilus* as a serious hazard to human health.

At present, 29 different species within the genus *Arcobacter* were isolated from a wide variety of environmental, animal, and food sources, such as poultry, beef, pork, water etc. (Collado et al., 2009; Collado et al., 2010; Fera et al., 2004; Houf et al., 2002b; Ramees et al., 2017; Shah et al., 2012; Son et al., 2007; Zacharow et al., 2015; Perez-Cataluna et al., 2018a).

More than half of the species of this genus were recovered from aquatic environments and water-borne animals. Consuming contaminated water or seafood products such as bivalves and cephalopods with inappropriate or inadequate treatment is considered as a transmission route to humans (Girbau et al., 2015). Therefore, the understanding and evaluation of distribution of *Arcobacter* in foods from aquatic origin and their putative pathogenicity is important to develop further food control strategies (Snelling et al., 2006; Talay et al., 2016).

So far, the pathogenicity mechanisms of *Arcobacter* are still poorly understood. However, the whole genome sequence of *A. butzleri* RM4018 demonstrated the presence of 10 putative virulence genes: *ciaB* (encodes the *Campylobacter* invasive antigen B), *mviN* (required for peptidoglycan biosynthesis), *pldA* (encodes an outer membrane phospholipase A associated with lysis of erythrocytes), *tlyA* (encodes a hemolysin), *irgA* (encodes an iron-regulated outer membrane protein), *hecA* (encodes a filamentous hemagglutinin), *hecB* (encodes a hemolysin activation protein), *cj1349* and *cadF* (encoding fibronectin-binding proteins), and *iroE* (encodes a periplasmic enzyme for the iron acquisition) (Miller et al., 2007).

The aim of this study was to investigate the prevalence of *Arcobacter* spp. in retail seafood like bivalves (mussels and clams), shrimps and cephalopods (squids and octopuses), to genotype isolates with ERIC-PCR and to analyze the presence of 10 putative virulence genes.

2. Materials and methods

2.1. Sampling

A total of 318 seafood samples originating from several countries or locations, including 106 bivalve samples (81 mussels, 7 razor clams and 18 clams), 106 shrimp samples and 106 cephalopod samples (81 squids and 25 octopuses), were collected from local retail seafood markets and supermarkets in Berlin, Germany, between November 2015 and

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December 2016. All samples were transported to the laboratory cooled at 4 °C and processed within 72 h.

2.2. Isolation of Arcobacter

Arcobacter spp. were isolated according to the protocol described by Lehmann et al. (2015). Briefly, 10 g of the seafood samples were homogenized in 90 ml Arcobacter Broth (Oxoid, Wesel, Germany) with Cefoperazone Amphotericin Teicoplanin selective supplement (Oxoid) and incubated microaerobically (6% O_2, 7.2% CO_2) for 48 h at 28 °C. The enrichment was diluted 1:10 in Brucella Broth (BD Biosciences, Heidelberg, Germany) and 300 µl subsequently applied on a 0.6 µM filter (GE Healthcare Europe, Freiburg, Germany) which was placed on a Mueller Hinton agar plate (Oxoid) with 5% sheep blood (MHB). The filter was discarded after a one-hour aerobic incubation at 28 °C. After that, 100 µl of Brucella Broth was dropped and streaked on the plates for better colony separation. After 48 h of aerobic incubation at 28 °C, up to 5 suspected colonies (white, greyish or translucent colonies with a smooth shape) were picked and enriched on MHB plates incubated for 48 h microaerobically at 28 °C for further identification.

2.3. DNA extraction

DNA of each suspected isolate was extracted using chelex method as previously described (Karadas et al., 2013). Briefly, colonies were suspended in 250 µl 1 × TE buffer (1 mM Tris/HCl, pH 8.0, 100 µM EDTA; Roth) and centrifuged at 16,000 x g for 6 min. The pellets were re-suspended in 250 µl of 5% Chelex (BioRad, Munich, Germany) and incubated for 1 h at 56 °C followed by 15 min at 95 °C. After 6 min centrifugation at 16,000 x g, 100 µl of supernatants were transferred and stored at 4 °C or directly used for multiplex PCR.

2.4. Species-level detection by mPCR

Species-level detection was carried out by mPCR according to Houf et al. (2000). The 25 µl reaction mixtures were composed of autoclaved deionized water, 2 µl DNA, 2.5 µl 10 × buffer (QIAGEN, Venlo, The Netherlands), 2.8 mM $MgCl_2$ (QIAGEN), 0.2 mM of each dNTP (Thermo Fisher Scientific, Waltham, USA), 0.75 U Taq-Polymerase (QIAGEN), 0.5 µM of primer SKIR and 1 µM of each primer ARCO, BUTZ, CRY 1 and CRY 2 (all primers are listed in Table 1). Mixtures were initially heated at 94 °C for 2 min, followed with 32 cycles of denaturation at 94 °C (45 s), annealing at 61 °C (45 s) and chain extension at 72 °C (30 s) with a final elongation at 72 °C (5 min). The PCR products were separated by electrophoresis on 3% agarose gels.

2.5. Species-level detection by rpoB sequencing

All *Arcobacter* isolates identified by mPCR were further analyzed by *rpoB* sequencing according to Korczak et al. (2006).

Briefly, the 50 µl reaction mixtures for PCR were made up of autoclaved deionized water, 4 µl DNA, 5 µl 10 × buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U Taq-Polymerase and 0.4 µM of each primer CamrpoB-L and RpoB-R. The PCR reaction included 35 cycles of denaturation at 94 °C (30 s), annealing at 54 °C (30 s) and chain extension at 72 °C (30 s) with an initial heating at 95 °C (3 min) and a final elongation at 72 °C (7 min).

The PCR products were purified with GeneJET PCR Purification Kit (Thermo Fisher Scientific) and subsequently sequenced (GATC Biotech, Konstanz, Germany). The results were confirmed by BLAST (NCBI).

One strain per species of each positive sample was further characterized by ERIC-PCR and occurrence of virulence genes.

2.6. Species-level identification by 16S rRNA sequencing

The *Arcobacter* isolates which could not be identified to species level by *rpoB* sequencing were characterized by sequencing a fragment of the 16*S* rRNA gene according to Coenye et al. (1999). Briefly, PCR was performed in 50 µl reaction mixture composed of 4 µl DNA, 45 µl ReadyMix PCR Master Mix (Thermo Fisher Scientific) and 1 µM of each primer 16SrRNAF1 and 16SrRNAR1. The amplicons were purified using a GeneJET PCR Purification Kit (Thermo Fisher Scientific) and sequencing was performed (GATC Biotech) using the primers 16F358 and 16R1093 according to Coenye et al. (1999).

2.7. ERIC-PCR

Arcobacter spp. isolates were characterized by ERIC-PCR according to Houf et al. (2002a).

Each 25 µl PCR mixture was composed of autoclaved deionized water, 2.5 µl of 10 × buffer, 4 mM $MgCl_2$, 0.2 mM of each dNTP, 2.5 U Taq-Polymerase and 0.5 µM of each primer ERIC 1R and ERIC 2. The PCR reaction included 40 cycles of denaturation at 94 °C (1 min), annealing at 25 °C (1 min) and chain extension at 72 °C (2 min) with a prior heating at 94 °C (5 min).

The PCR products were separated by electrophoresis on 3% agarose gels. Band patterns were analyzed using BioNumerics version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). After normalization, the similarities between profiles, based on peak position, were calculated using Jaccard similarity coefficient. For cluster analysis, the UPGMA algorithm was used.

2.8. Detection of virulence gene

Detection of virulence genes *pldA*, *irgA*, *hecA*, *hecB*, *cj1349*, *ciaB*, *mviN* and *tlyA* in all *Arcobacter* isolates was performed by PCR according to Whiteduck-Leveillee et al. (2016) and *iroE* and *cadF* according to Karadas et al. (2013).

Briefly, 25 µl PCR mixture was composed of autoclaved deionized water, 2.5 µl of 10 × buffer, 1.5 mM MgCl, 0.2 mM each dNTP, 0.5 U Taq-Polymerase and 0.1 µM Primer (1 µM for *iroE* and *cadF* gene). The PCR reaction included 30 cycles of denaturation at 95 °C (30 s), annealing at 56 °C (45 s) and chain extension at 72 °C (45 s) with a prior heating at 95 °C (4 min). For *iroE* gene, the annealing temperature was 50 °C for 30 s, while denaturation and chain extension duration were both 30 s.

2.9. Statistical analysis

The differences in the prevalence levels across the matrices were analyzed using Fisher's exact test (GraphPad Prism v6.07, La Jolla, California, USA) and two-tailed p-value < 0.05 were considered as statistically significant.

3. Results and discussion

3.1. Prevalence of Arcobacter spp.

Arcobacter spp. were isolated from 56 of 318 (17.6%) seafood samples, including bivalves (mussels, clams and razor clams), shrimps and cephalopods (squids and octopuses). In six samples (1 bivalve and 5 cephalopods), two different *Arcobacter* spp. were detected simultaneously (Table 2). Among all 62 isolates, 53% belonged to *A. butzleri*, followed by 15% of *A. venerupis*, 13% of *A. cryaerophilus* and 11% of *A. aquimarinus*. *A. skirrowii* and *A. thereius* were only detected once (Table 2), and three isolates could not be specified to species level by both *rpoB* and 16S rRNA gene sequencing. The *rpoB* and 16s rRNA sequencing of these *Arcobacter* sp. showed more than 99.9% similarity with each other, 93.2% and 98.9% homology with both *A. aquimarinus* and *A. ellisii*, respectively. The most prevalent species in all three matrices was *A. butzleri*, followed by *A. aquimarinus* for bivalves, *A. venerupis* for cephalopods and *A. cryaerophilus* for shrimps (Table 2).

Table 1
List of primers used in PCR assays for identification, ERIC-PCR and detection of virulence genes of *Arcobacter* strains isolated form retail seafood.

Primer	Target gene	Nucleotide sequence (5′-3′)	Amplicon (bp)	Reference
ARCO R	16S rRNA	CGTATTCACCGTAGCATAGC		Houf et al. (2000)
BUTZ F	16S rRNA	CCTGGACTTGACATAGTAAGAATGA	401	
SKIR F	16S rRNA	GGCGATTTACTGGAACACA	641	
CRY 1	23S rRNA	TGCTGGAGCGGATAGAAGTA	257	
CRY 2		AACAACCTACGTCCTTCGAC		
CamrpoB-L	*rpoB*	CCAATTTATGGATCAAAC		Korczak et al. (2006)
RpoB-R		GTTGCATGTTNGNACCCAT		
16SrRNAF1	16S rRNA	AGAGTTTGATCCTGGCTGAG	1500	Coenye et al. (1999)
16SrRNAR1		AAGGAGGTGATCCAGCCGCA		
16F358		CTCCTACGGGAGGCAGCAGT		
16R1093		GTTGCGCTCGTTGCGGGACT		
ERIC 1R		ATGTAAGCTCCTGGGGATTCAC		Houf et al. (2002a)
ERIC 2		AAGTAAGTGACTGGGGTGAGCG		
ciaB F	*ciaB*	TGGGCAGATGTGGATAGAGCTTGGA	284	Whiteduck-Leveillee et al. (2016)
ciaB R		TAGTGCTGGTCGTCCCACATAAAG		
cj1349 F	*cj1349*	CCAGAAATCACTGGCTTTTGAG	659	
cj1349 R		GGGCATAAGTTAGATGAGGTTCC		
pldA F	*pldA*	TTGACGAGACAATAAGTGCAGC	293	
pldA R		CGTCTTTATCTTTGCTTTCAGGGA		
irgA F	*irgA*	TGCAGAGGATACTTGGAGCGTAACT	437	
irgA R		GTATAACCCCATTGATGAGGAGCA		
hecA F	*hecA*	GTGGAAGTACAACGATAGCAGGCTC	537	
hecA R		GTCTGTTTTAGTTGCTCTGCACTC		
hecB F	*hecB*	CTAAACTCTACAAATCGTGC	528	
hecB R		CTTTTGAGTGTTGACCTC		
mviN F	*mviN*	TGCACTTGTTGCAAAACGGTG	294	
mviN R		TGCTGATGGAGCTTTTACGCAAGC		
tlyA F	*tlyA*	CAAAGTCGAAACAAAGCGACTG	230	
tlyA R		TCCACCAGTGCTACTTCCTATA		
cadF F	*cadF*	TTACTCCTACACCGTAGT	283	Karadas et al. (2013)
cadF R		AAACTATGCTAACGCTGGTT		
iroE F	*iroE*	AATGGCTATGATGTTGTTTAC	415	
iroE R		TTGCTGCTATGAAGTTTTG		

However, as we used an enrichment step and first detection by mPCR, some *Arcobacter* species might be underestimated or unidentified.

The highest prevalence of *Arcobacter* spp. with 27.4% was detected in cephalopods (Table 2). To the best of our knowledge, this is the first study reporting cephalopods as a source of *Arcobacter* spp. In addition to the highest prevalence, the highest diversity of *Arcobacter* species was also determined for cephalopod samples in our study (Table 2). Based on the Fisher's exact test ($p < 0.005$), the percentage of positive samples detected in cephalopods (27.4%) is significantly higher than in shrimps (8.5%).

The overall prevalence of *Arcobacter* in bivalves in our study (with 17.0%) is comparable to previous studies, who isolated *Arcobacter* from 14.7% to 20.6% of shellfish samples collected in India (Laishram et al., 2016; Rathlavath et al., 2017b). Mottola et al. (2016) isolated a slightly higher percentage (22.8%) of *Arcobacter* from shellfish samples in the Apulian region in Italy. However, other studies reported rather high prevalence compared to our findings. Morejon et al. (2017) determined an *Arcobacter* spp. prevalence of 37% in shellfish samples, while Salas-Masso et al. (2016) determined a prevalence of 51% in water and shellfish samples collected in Spain, respectively.

The differences in detection rates could be explained by differences in detection methods or the kind of samples included in the studies. Another probable reason is that in the summer season, when the highest prevalence of *Arcobacter* was usually reported (Levican et al., 2014), fresh bivalves are almost unavailable in Germany markets. During the winter season, Levican et al. (2014) also determined a prevalence of 20% in shellfish, which is comparable to our data (17.0%). Moreover, different geographic location could also be responsible for the different prevalences of *Arcobacter*.

3.2. Genotyping of Arcobacter spp. by ERIC-PCR

To determine the genetic diversity of the *Arcobacter* species isolated from seafood, the 62 strains derived from our study and the reference strain *A. butzleri* CCUG 30485 (human source) were genotyped by ERIC-PCR. The three replicates of CCUG 30485 presented identical pattern,

Table 2
Occurrence of *Arcobacter* species isolated from seafood samples.

Matrix	Prevalence	Detected *Arcobacter* species (n)						
		A. butzleri	*A. cryaerophilus*	*A. skirrowii*	*A. venerupis*	*A. aquimarinus*	*A. thereius*	*Arcobacter* sp.
Bivalves	17.0% (18/106)[b]	42% (8)	11% (2)	0% (0)	5% (1)	32% (6)	0% (0)	11% (2)
Cephalopods	27.4% (29/106)[c, d]	62% (21)	9% (3)	3% (1)	21% (7)	0% (0)	3% (1)	3% (1)
Shrimp	8.5% (9/106)	44% (4)	33% (3)	0% (0)	11% (1)	11% (1)	0% (0)	0% (0)
Total	17.6% (56/318)[a]	53% (33)	13% (8)	2% (1)	15% (9)	11% (7)	2% (1)	5% (3)

[a] Incl. samples with more than one *Arcobacter* species.
[b] *A. aquimarinus* + *A. cryaerophilus*.
[c] *A. venerupis* + *A. cryaerophilus*.
[d] *A. venerupis* + *A. butzleri*.

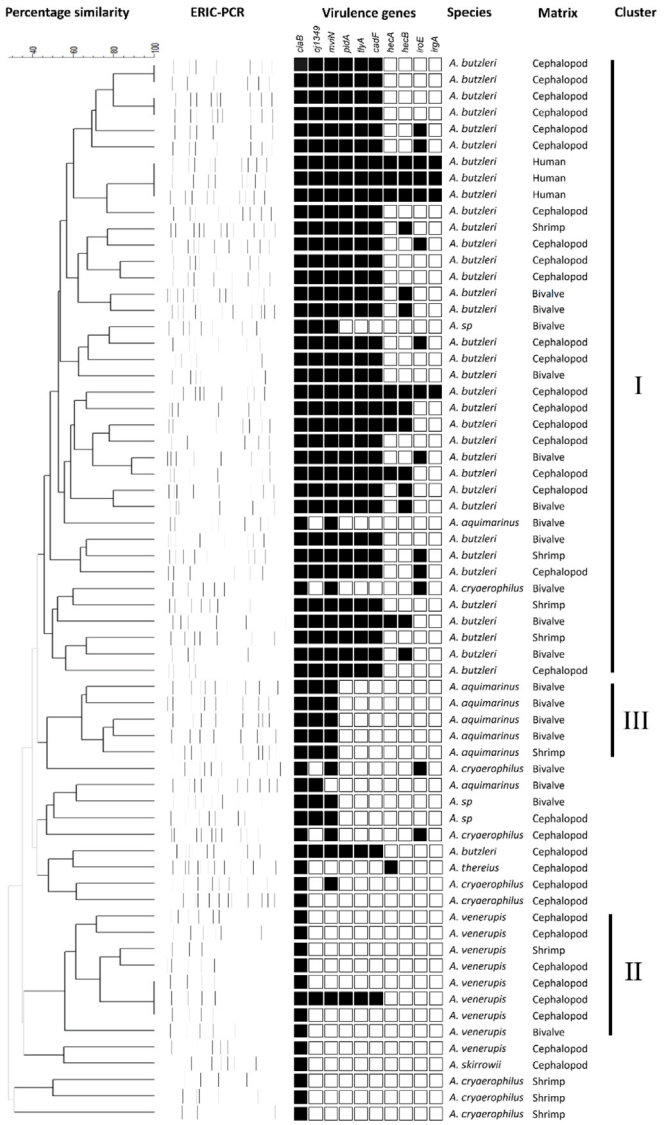

Fig. 1. Characterization of 62 *Arcobacter* spp. strains isolated from retail seafood. Based on the ERIC-PCR pattern a cluster analysis was performed by the Jaccard similarity coefficient and the UPGMA algorithm. The presence of virulence genes, species and source for each isolate are shown. (Virulence gene pattern: black = gene present, white = gene absent).

showing the stability of this method (Fig. 1). Most strains belonging to the species *A. butzleri* were well grouped in one large cluster (Cluster I) while only one *A. butzleri* strain was excluded from this cluster. However, the similarity within that cluster ranged from 45.9% up to 100%. Levican et al. (2014) analyzed 118 isolates of different *Arcobacter* species and found that only *A. butzleri* and *A. molluscorum* were well clustered among 11 *Arcobacter* species derived from shellfish, but still high genotypic diversity can be observed both at intraspecies and interspecies level. Similar intraspecies heterogeneity could also be demonstrated for *A. butzleri* strains isolated from chicken and fish (Lehmann et al., 2015). Further, the *A. aquimarinus* strains (Cluster III), and the *A. venerupis* strains (Cluster II) were grouped in clusters with only two or one strain excluded, respectively. The similarity of *A. aquimarinus* strains ranged from 64.4% to 80%, while the similarity of *A. venerupis* strains ranged from 55.8% to 100%. This could be explained by a higher genetic homogeneity or the lower strain numbers investigated for the two species. In contrast, most strains of the species *A. cryaerophilus* seemed to group more randomly, indicating an even higher genetic heterogeneity compared to the other investigated *Arcobacter* species. This observation was already described in some previous studies (Houf et al., 2003; Levican et al., 2014). A recent study of Perez-Cataluna et al. (2018b) evaluated 52 temporally and geographically dispersed *A. cryaerophilus* strains from nine countries and found that these strains formed four clusters by a Multilocus Phylogenetic Analysis and phenotypic characterization, suggesting this species represents four separated genomovares, which might explain the high genetic heterogeneity described also in former studies. As we characterized only one isolate per species of each *Arcobacter* positive sample, the genetic diversity of all species might even be higher as described in our study.

In accordance to Lehmann et al. (2015), no obvious correlation between the ERIC-PCR pattern and the source of isolation was observed for the *A. butzleri* strains in our study. Since both the *A. aquimarinus* and the *A. venerupis* strains were almost all isolated from a single source (bivalves or cephalopods, respectively), no conclusion on a correlation between the ERIC-PCR pattern and the source can be deduced (Fig. 1).

3.3. Occurrence of putative virulence genes

To further characterize the *Arcobacter* strains isolated from seafood, the presence of virulence associated genes in all 62 *Arcobacter* strains was investigated by PCR.

All *A. butzleri* strains encoded for *ciaB*, *cj1349*, *mviN*, *pldA*, *tlyA* and *cadF,* while only 33% of the *A. butzleri* strains encoded for *hecB*, 24% for *iroE*, 15% for *hecA* and 3% for *irgA* (Fig. 1). Such tendency was also reported in other studies, where a predominance of the anterior six genes was detected in *A. butzleri* isolated from various food matrices and environments (Laishram et al., 2016; Lehmann et al., 2015; Rathlavath et al., 2017a; Tabatabaei et al., 2014).

Among all eight *A. cryaerophilus* isolates, *ciaB* (100%) was detected most frequently, followed by *mviN* (50%) and *iroE* (38%), while none of these strains possessed any of the other seven genes. However, other studies described the detection of at least nine of the ten putative virulence genes in *A. cryaerophilus* with the primers used in our study.

All *A. venerupis* isolates (n = 9) encoded for *ciaB*, and only one encoded for other five virulence genes, a pattern only detected in *A. butzleri* strains in our study. The single *A. venerupis* strain investigated by Levican et al. (2013) also only encoded for *ciaB*, as most of our strains. Likewise, all *A. thereius* strains (n = 5) were negative for all five tested genes in their study, while the one *A. thereius* we isolated encoded for *ciaB* and *hecA*. The only *A. skirrowii* strain included in our study was negative in all virulence genes except *ciaB*.

To the best of our knowledge, so far no other study investigated the occurrence of the putative virulence genes in *A. aquimarinus*. In our study, the three genes *ciaB* (100%), *mviN* (86%) and *cj1349* (86%) were detected in a total of seven *A. aquimarinus* isolates. These three genes were also present in the three *Arcobacter* strains only identified up to genus level.

Altogether, these data indicate that the presence of *ciaB* is highly conserved within *Arcobacter* spp. investigated in our study, while the occurrence of the other virulence associated genes were less conserved. Regarding the fact that all virulence gene primers were designed on the basis of the genome of *A. butzleri*, as well as the high heterogeneity in genomes of different *Arcobacter* spp., the true presence of putative virulence genes might be underestimated in other *Arcobacter* spp. So far, no correlation between the presence of the putative virulence genes and their pathogenicity has been detected.

Nevertheless, the virulence gene pattern do not seem to be related to either the source of isolation or the ERIC-PCR pattern (Fig. 1).

4. Conclusion

In this study, the prevalence of *Arcobacter* in retail seafood was 17.6% with *A. butzleri* as predominant species. This data support that retail seafood, such as bivalves, shrimps and cephalopods, can act as a

potential source of *Arcobacter* infection in humans. Based on the ERIC-PCR pattern and the occurrence of virulence genes, a high genetic diversity of *Arcobacter* strains isolated from retail seafood samples in Germany has been demonstrated.

Declarations of interest

All authors declare they have no competing interests.

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References

Arguello, E., Otto, C.C., Mead, P., Babady, N.E., 2015. Bacteremia caused by *Arcobacter butzleri* in an immunocompromised host. J. Clin. Microbiol. 53, 1448–1451.

Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J.R., Kersters, K., Vandamme, P., 1999. Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. Int J Syst Bacteriol 49 (Pt 2), 405–413.

Collado, L., Guarro, J., Figueras, M.J., 2009. Prevalence of *Arcobacter* in meat and shellfish. J. Food Protect. 72, 1102–1106.

Collado, L., Kasimir, G., Perez, U., Bosch, A., Pinto, R., Saucedo, G., Huguet, J.M., Figueras, M.J., 2010. Occurrence and diversity of *Arcobacter* spp. along the Llobregat River catchment, at sewage effluents and in a drinking water treatment plant. Water Res. 44, 3696–3702.

Fera, M.T., Maugeri, T.L., Gugliandolo, C., Beninati, C., Giannone, M., La Camera, E., Carbone, M., 2004. Detection of *Arcobacter* spp. in the coastal environment of the mediterranean sea. Appl. Environ. Microbiol. 70, 1271–1276.

Figueras, M.J., Levican, A., Pujol, I., Ballester, F., Rabada Quilez, M.J., Gomez-Bertomeu, F., 2014. A severe case of persistent diarrhoea associated with *Arcobacter cryaerophilus* but attributed to *Campylobacter* sp. and a review of the clinical incidence of *Arcobacter* spp. New Microbes New Infect 2, 31–37.

Girbau, C., Guerra, C., Martinez-Malaxetxebarria, I., Alonso, R., Fernandez-Astorga, A., 2015. Prevalence of ten putative virulence genes in the emerging foodborne pathogen *Arcobacter* isolated from food products. Food Microbiol. 52, 146–149.

Houf, K., Tutenel, A., De Zutter, L., Van Hoof, J., Vandamme, P., 2000. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiol. Lett. 193, 89–94.

Houf, K., De Zutter, L., Van Hoof, J., Vandamme, P., 2002a. Assessment of the genetic diversity among Arcobacters isolated from poultry products by using two PCR-based typing methods. Appl. Environ. Microbiol. 68, 2172–2178.

Houf, K., De Zutter, L., Van Hoof, J., Vandamme, P., 2002b. Occurrence and distribution of *Arcobacter* species in poultry processing. J. Food Protect. 65, 1233–1239.

Houf, K., De Zutter, L., Verbeke, B., Van Hoof, J., Vandamme, P., 2003. Molecular characterization of *Arcobacter* isolates collected in a poultry slaughterhouse. J. Food Protect. 66, 364–369.

ICMSF Microorganisms in Foods 7, 2002. Microbiological testing in food safety management. In: International Commission on Microbiological Specifications for Foods. Kluwer Academic/Plenum Publishers, New York.

Karadas, G., Sharbati, S., Hanel, I., Messelhausser, U., Glocker, E., Alter, T., Gölz, G., 2013. Presence of virulence genes, adhesion and invasion of *Arcobacter butzleri*. J. Appl. Microbiol. 115, 583–590.

Kayman, T., Abay, S., Hizlisoy, H., Atabay, H.I., Diker, K.S., Aydin, F., 2012. Emerging pathogen *Arcobacter* spp. in acute gastroenteritis: molecular identification, antibiotic susceptibilities and genotyping of the isolated arcobacters. J. Med. Microbiol. 61, 1439–1444.

Korczak, B.M., Stieber, R., Emler, S., Burnens, A.P., Frey, J., Kuhnert, P., 2006. Genetic relatedness within the genus *Campylobacter* inferred from *rpoB* sequences. Int. J. Syst. Evol. Microbiol. 56, 937–945.

Laishram, M., Rathlavath, S., Lekshmi, M., Kumar, S., Nayak, B.B., 2016. Isolation and characterization of *Arcobacter* spp. from fresh seafood and the aquatic environment. Int. J. Food Microbiol. 232, 87–89.

Lappi, V., Archer, J.R., Cebelinski, E., Leano, F., Besser, J.M., Klos, R.F., Medus, C., Smith, K.E., Fitzgerald, C., Davis, J.P., 2013. An outbreak of foodborne illness among attendees of a wedding reception in Wisconsin likely caused by *Arcobacter butzleri*. Foodb. Pathog. Dis. 10, 250–255.

Lehmann, D., Alter, T., Lehmann, L., Uherkova, S., Seidler, T., Gölz, G., 2015. Prevalence, virulence gene distribution and genetic diversity of *Arcobacter* in food samples in Germany. Berl. Münchener Tierärztliche Wochenschr. 128, 163–168.

Levican, A., Alkeskas, A., Gunter, C., Forsythe, S.J., Figueras, M.J., 2013. Adherence to and invasion of human intestinal cells by *Arcobacter* species and their virulence genotypes. Appl. Environ. Microbiol. 79, 4951–4957.

Levican, A., Collado, L., Yustes, C., Aguilar, C., Figueras, M.J., 2014. Higher water temperature and incubation under aerobic and microaerobic conditions increase the recovery and diversity of *Arcobacter* spp. from shellfish. Appl. Environ. Microbiol. 80, 385–391.

Miller, W.G., Parker, C.T., Rubenfield, M., Mendz, G.L., Wosten, M.M., Ussery, D.W., Stolz, J.F., Binnewies, T.T., Hallin, P.F., Wang, G., Malek, J.A., Rogosin, A., Stanker, L.H., Mandrell, R.E., 2007. The complete genome sequence and analysis of the epsilonproteobacterium *Arcobacter butzleri*. PLoS One 2, e1358.

Morejon, I.F., Gonzalez, A., Ferrus, M.A., 2017. Detection, identification, and antimicrobial susceptibility of *arcobacter* spp. isolated from shellfish in Spain. Foodb. Pathog. Dis. 14, 238–243.

Mottola, A., Bonerba, E., Figueras, M.J., Perez-Cataluna, A., Marchetti, P., Serraino, A., Bozzo, G., Terio, V., Tantillo, G., Di Pinto, A., 2016. Occurrence of potentially pathogenic arcobacters in shellfish. Food Microbiol. 57, 23–27.

Perez-Cataluna, A., Collado, L., Salgado, O., Lefinanco, V., Figueras, M.J., 2018a. A polyphasic and taxogenomic evaluation uncovers *Arcobacter cryaerophilus* as a species complex that embraces four genomovars. Front. Microbiol. 9, 805.

Perez-Cataluna, A., Salas-Masso, N., Dieguez, A.L., Balboa, S., Lema, A., Romalde, J.L., Figueras, M.J., 2018b. Revisiting the taxonomy of the genus *Arcobacter*: getting order from the chaos. Front. Microbiol. 9, 2077.

Prouzet-Mauleon, V., Labadi, L., Bouges, N., Menard, A., Megraud, F., 2006. *Arcobacter butzleri*: underestimated enteropathogen. Emerg. Infect. Dis. 12, 307–309.

Ramees, T.P., Dhama, K., Karthik, K., Rathore, R.S., Kumar, A., Saminathan, M., Tiwari, R., Malik, Y.S., Singh, R.K., 2017. *Arcobacter*: an emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - a comprehensive review. Vet. Q. 37, 136–161.

Rathlavath, S., Kohli, V., Singh, A.S., Lekshmi, M., Tripathi, G., Kumar, S., Nayak, B.B., 2017a. Virulence genotypes and antimicrobial susceptibility patterns of *Arcobacter butzleri* isolated from seafood and its environment. Int. J. Food Microbiol. 263, 32–37.

Rathlavath, S., Kumar, S., Nayak, B.B., 2017b. Comparative isolation and genetic diversity of *Arcobacter* sp. from fish and the coastal environment. Lett. Appl. Microbiol. 65, 42–49.

Salas-Masso, N., Andree, K.B., Furones, M.D., Figueras, M.J., 2016. Enhanced recovery of *Arcobacter* spp. using NaCl in culture media and re-assessment of the traits of *Arcobacter marinus and Arcobacter halophilus* isolated from marine water and shellfish. Sci. Total Environ. 566–567, 1355–1361.

Shah, A.H., Saleha, A.A., Murugaiyah, M., Zunita, Z., Memon, A.A., 2012. Prevalence and distribution of *Arcobacter* spp. in raw milk and retail raw beef. J. Food Protect. 75, 1474–1478.

Snelling, W.J., Matsuda, M., Moore, J.E., Dooley, J.S., 2006. Under the microscope: *Arcobacter*. Lett. Appl. Microbiol. 42, 7–14.

Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J., Harrison, M.A., 2007. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. Int. J. Food Microbiol. 113, 16–22.

Tabatabaei, M., Shirzad Aski, H., Shayegh, H., Khoshbakht, R., 2014. Occurrence of six virulence-associated genes in *Arcobacter* species isolated from various sources in Shiraz, Southern Iran. Microb. Pathog. 66, 1–4.

Talay, F., Molva, C., Atabay, H.I., 2016. Isolation and identification of *Arcobacter* species from environmental and drinking water samples. Folia Microbiol. 61, 479–484.

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D., Vlaes, L., van den Borre, C., Higgins, R., Hommez, J., et al., 1992. Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. Int. J. Syst. Bacteriol. 42, 344–356.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G., Butzler, J.P., Vandamme, P., 2004. *Arcobacter* species in humans. Emerg. Infect. Dis. 10, 1863–1867.

Whiteduck-Leveillee, J., Cloutier, M., Topp, E., Lapen, D.R., Talbot, G., Villemur, R., Khan, I.U., 2016. Development and evaluation of multiplex PCR assays for rapid detection of virulence-associated genes in *Arcobacter* species. J. Microbiol. Methods 121, 59–65.

Zacharow, I., Bystron, J., Walecka-Zacharska, E., Podkowik, M., Bania, J., 2015. Genetic diversity and incidence of virulence-associated genes of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolates from pork, beef, and chicken meat in Poland. BioMed Res. Int. 2015, 956507.