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Antimicrobial susceptibility, virulence potential and sequence types associated with *Arcobacter* strains recovered from human faeces

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Abstract

Purpose. The genus *Arcobacter* includes bacteria that are considered emergent pathogens because they can produce infections in humans and animals. The most common symptoms are bloody and non-bloody persistent diarrhea but cases with abdominal cramps without diarrhea or asymptomatic cases have also been described as well as cases with bacteremia. The objective was to characterize *Arcobacter* clinical strains isolated from the faeces of patients from three Spanish hospitals.

Methodology. We have characterized 28 clinical strains (27 of *A. butzleri* and one of *A. cryaerophilus*) isolated from faeces, analysing their epidemiological relationship using the multilocus sequence typing (MLST) approach and screening them for their antibiotic susceptibility and for the presence of virulence genes.

Results/Key findings. Typing results showed that only one of the 28 identified sequence types (i.e. ST 2) was already present in the MLST database. The other 27 STs constituted new records because they included new alleles for five of the seven genes or new combinations of known alleles of the seven genes. All strains were positive for the *ciaB* virulence gene and sensitive to tetracycline. However, 7.4 % of the *A. butzleri* and *A. cryaerophilus* strains showed resistance to ciprofloxacin.

Conclusion. The fact that epidemiological unrelated strains show the same ST indicates that other techniques with higher resolution should be developed to effectively recognize the infection source. Resistance to ciprofloxacin, one of the antibiotics recommended for the treatment of *Arcobacter* intestinal infections, demonstrated in 10.7 % of the strains, indicates the importance of selecting the most appropriate effective treatment.

INTRODUCTION

The genus *Arcobacter*, considered closely related to the genus *Campylobacter*, belongs to the family *Campylobacteraceae* [1, 2]. The differentiation between *Campylobacter* and *Arcobacter* is based on the capacity of the species of the latter genus to grow at lower temperatures and to tolerate oxygen [1]. Recently, a new classification has been proposed with a description of the new family *Arcobacteraceae* and *Epsilonbacteria* being raised to the phylum level with the new name *Epsilonbacteraeota* [3]. This reclassification was supported by a phylogenetic inference with the 16S and 23S rRNA genes as well as with 120 concatenated conserved proteins extracted from 628 *Epsilonbacteria* genomes and 33 population genomes from metagenome datasets [3]. Since the first description of the genus *Arcobacter* with species previously considered atypical aerotolerant campylobacters in 1991 and 1992 [1, 4], the genus has evolved very fast and currently, in 2017, it includes a total of 26 species [5–7]. *Arcobacter* spp. have been isolated worldwide from various sources including food products mainly of animal origin like meat, milk, as well as from vegetables, shellfish, etc. [2, 8].

In Belgium and France, *Arcobacter* is the fourth most common bacteria belonging to the family *Campylobacteraceae*

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Keywords: *Arcobacter butzleri*; *Arcobacter cryaerophilus*; MLST; virulence genes; antibiotic resistance.

Abbreviations: LMG, Laboratorium voor Microbiologie, Universiteit Gent, Belgium Culture Collection; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MLST, multilocus sequence typing; NJ, neighbour joining; ST, sequence type.

isolated from the faeces of patients with enteric disease, and the third most prevalent in South Africa [9–11]. Bacteria of this genus are considered emergent pathogens according to the International Commission on Microbial Specifications for Foods [12] because they can produce infections in humans and animals ([2, 13] and references therein). Four species, i.e. *A. butzleri*, *A. cryaerophilus*, *A. thereius* and *A. skirrowii,* have so far been related to human infections [11, 13, 14]. Among the cases reported, the most common symptoms are bloody and non-bloody persistent diarrhea but cases with abdominal cramps without diarrhea or asymptomatic cases have also been described as well as cases with bacteremia [11, 13–16]. Cases of person-to-person transmission have been reported but the most common routes have been related to the consumption of contaminated water, raw or poorly cooked vegetables, shellfish, etc. [2, 13]. *Arcobacter* have also been associated with foodborne and waterborne outbreaks [2, 17, 18].

In 2009, Miller and co-workers developed an *Arcobacter* multilocus sequence typing (MLST) scheme and PubMLST database (http://pubmlst.org/arcobacter/) based on seven housekeeping genes to determine the epidemiological relationship of the studied isolates [19]. Since then this epidemiological typing method has been used in several studies [20–24]. However, at the time of writing, only 14.8 % (127/859) of the isolates deposited in the PubMLST database were from human origin. Considering the limited number of sequences from clinical sources, it is important to continue studying these kinds of isolates to create a more robust database that can be used in epidemiological studies of the genus. Therefore, the aim of this work was to characterize clinical strains isolated from the faeces of patients from three Spanish hospitals, analysing their epidemiological relationship using the MLST approach and screening them for their antibiotic susceptibility and for the presence of virulence genes.

METHODS

Strain isolation

A total of 28 strains recovered from faeces were obtained from three different Spanish University hospitals between 2013 and 2017. One strain came from the University Hospital Miguel Servet (prefix strain HUMS) from the city of Zaragoza, five from the University Hospital Sant Joan de Reus (prefix strain HSJR) from Reus and 22 strains were received from the University Hospital Joan XXIII (prefix strain HJXXIII) from Tarragona. All strains came from patients with gastrointestinal symptoms except one that was isolated from a healthy patient during a medical check-up after returning from Africa (HSJR-6). All strains were isolated from Yersinia Selective Agar (CIN agar, Cefsulodin-Irgasan-Novobiocin, BD, Madrid, Spain) or Campylosel agar (bioMèrieux, Barcelona, Spain). Strains were identified at the hospital laboratories using phenotypical tests or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) with the Ultraflex instrument, that uses MALDI BIOTYPER 2.0 software (Bruker Daltonics, Bremen, Germany). Strains identified as *Campylobacter* or *Arcobacter* were sent to our laboratory at the University Rovira i Virgili (Reus, Spain) for further analysis. All strains were subcultured in Blood Agar (Merk, Madrid, Spain) and incubated at 30 °C for 24–48 h from where the DNA from pure cultures was extracted using InstaGene DNA Purification Matrix (Bio-Rad Laboratories, Hercules, CA, USA). Each strain was genotyped with the enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), using primers and conditions previously described [25] to find potential clones. In order to verify the species identity, the *rpoB* gene was sequenced using primers (rpoB-Arc-F/rpoB-Arc-R) and conditions described by Levican [26]. The amplification product was confirmed by electrophoresis in a 1.5 % agarose gel with 3.5 µl of RedSafe (INtRON Biotechnology, Lynnwood, WA, USA) nucleic acid staining solution at 100 V for 45 min. The PCR products were diluted to a final concentration of 75 ng μl^{-1} and sequenced by Macrogen Europe. The obtained *rpoB* sequences were aligned using CLUSTALW [27] with those of all type strains of all of the known *Arcobacter* species included in our bona fide in-house database using MEGA v6.0 [28]. A phylogenetic analysis was used to identify the strains at species level using the neighbour joining (NJ) method [29, 30].

Multilocus sequence typing

The seven housekeeping genes (*aspA*, *atpA*, *glnA*, *gltA*, *glyA*, *pgm* and *tkt*) included in the *Arcobacter* scheme of the PubMLST database (http://pubmlst.org/arcobacter/) were amplified and sequenced using primers and protocols described there and published by Miller *et al.* [19]. The allele and sequence type (ST) assignments of the studied isolates were obtained using the database where they were all deposited (http://pubmlst.org/arcobacter/). Recombination and selection tests were performed using s.t.a.r.t.2 software (https://pubmlst.org/software/analysis/start2/). To analyse the clustering of our strains with the clinical strains isolated from humans and deposited in the PubMLST database, sequences of the seven genes were aligned with CLUSTALW [27] and a phylogenetic tree was constructed using the NJ algorithm [29, 30] with MEGA 6.0 [28]. Type strains of *A. thereius* and *A. cibarius* and the strain of *A. skirrowii* LMG 10238 were used as the outgroup (Fig. 1).

Detection of virulence genes

The DNA from each strain was also screened for the presence of five virulence genes (*ciaB*, *cadF*, *cj1349*, *hecA* and *irgA*) using the PCR primers and conditions described by Douidah *et al.* [31], using strain *A. butzleri* LMG 10828^T as a positive control and water as a negative control. Amplification products were analysed on 2 % agarose gels at 80 V for 90 min using a 100 bp ladder as a weight marker (Thermo Fisher Scientific, Madrid, Spain). Gels were prepared in buffer 1x Tris-Borate-EDTA (TBE) and stained with RedSafe (INtRON Biotechnology, Lynnwood, WA, USA). Image capture was performed using Gel Doc XR System (Bio-Rad, Madrid, Spain). To ensure that the

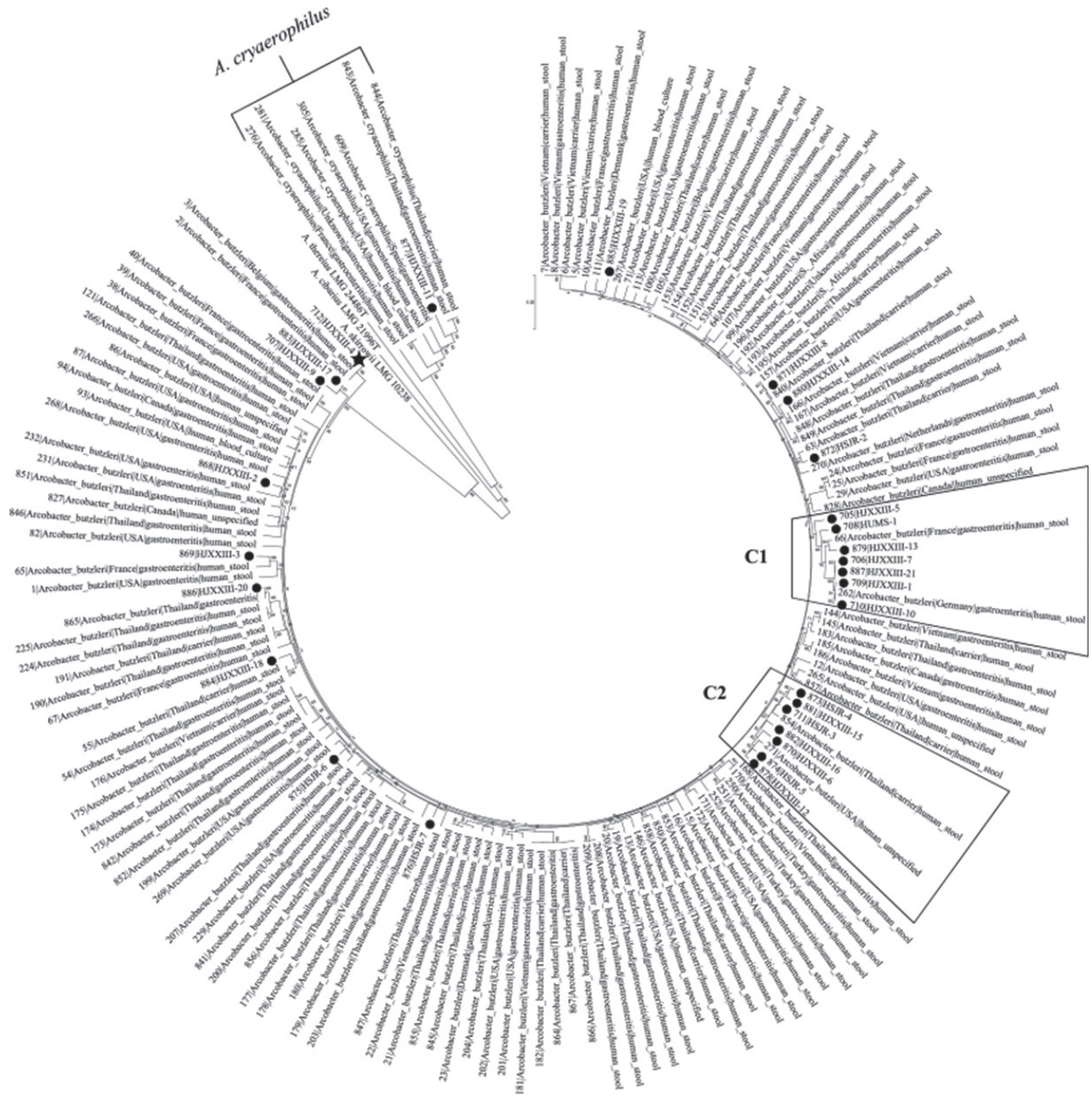

Fig. 1. NJ tree showing the distribution of the Spanish STs (black dot and black star) among the human clinical strains of *A. butzleri* and *A. cryaerophilus* available in the PubMLST database. Notice that the Spanish strain (HJXXX-4) assigned to ST2 (black star) cluster with the only two ST2 strains of the database that corresponded to gastrointestinal cases from France and Belgium. Two groups of seven Spanish strains each cluster together with other STs of the database (C1 and C2).

amplification products belonged to the expected virulence genes the PCR products were sequenced with the same primers used in the amplification.

Antibiotic resistance

The antibiotic susceptibility of each strain was assayed using the disk diffusion method following recommendations of the Clinical and Laboratory Standards Institute for *Campylobacter* with some modifications [32]. Tested antibiotics and concentrations were amoxicillin/clavulanate (20/10 µg), erythromycin (15 µg), gentamycin (10 µg) tetracycline (30 µg) and ciprofloxacin (5 µg) using BBL Sensi-Disc Susceptibility Test Discs (BD, Madrid, Spain). For each strain, 100 µl of a standardized inoculum containing 10^6 c.f.u. were

plated in Blood Agar and each disc was placed on top of the agar. Plates were incubated at 30 °C in air and the inhibition zones were measured at 24, 48 and 72 h. Each experiment was repeated twice in parallel.

RESULTS AND DISCUSSION

Strain identification and multilocus sequence typing

The 28 strains received at the Universitat Rovira i Virgili were identified at the hospitals as *A. butzleri* (*n*=22), *Arcobacter* sp. (*n*=5) and *Campylobacter* (*n*=1). The *rpoB* gene analysis showed that 27 strains belonged to *A. butzleri* and one to *A. cryaerophilus* (HSJXII-11) (data not shown). These results are in agreement with the *Arcobacter* spp. of human origin present in the PubMLST database dominated by the species *A. butzleri* (94.7 %) and *A. cryaerophilus* (5.3 %) (http://pubmlst.org/arcobacter/). The 22 strains identified with MALDI-TOF as *A. butzleri* at the hospitals were correctly assigned at species level.

The MLST alleles and ST found for the 28 strains are listed in Table 1 and their relationship with the ST of other strains of human origin included in the database is shown in Fig. 1. Only one strain (HJXXIII-4) belonged to the already known ST 2 (*aspA*-2; *atpA*-2; *glnA*-10; *gltA*-10; *glyA*-10; *pgm*-11; *tkt*-10) available in the database from only two clinical strains from France and Belgium related to gastrointestinal cases (Fig. 1). The fact that apparently epidemiologically unrelated clinical strains show the same ST, as has been previously reported for the *Legionella pneumophila* MLST scheme, indicates that this approach does not show enough resolution to discriminate the infection source, especially for worldwide distributed STs [33]. The other 27 STs were all new and therefore our study increased the number of clinical STs in the database, from 77 (at the time of writing) to 104. However, of these new STs the majority (20/27,

Table 1. MLST results of the 28 clinical strains with allelic profiles according to the *Arcobacter* MLST database

The new alleles and the resulting new ST are in bold. Strain HJXXIII-11, reference 877 at the PubMLST database is the one corresponding to *A. cryaerophilus*.

Strain	PubMLST ID	*aspA*	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST
HUMS-1	**708**	30^{a-d}	5^{a-d}	1^{a-e}	65^{a}	**517**	$16^{a,b,d}$	$9^{a,b,d,e}$	**483**
HSJR-2	**872**	6^{a-d}	34^{a-d}	1^{a-e}	12^{a-d}	**522**	$50^{a,b,d}$	55^{a-d}	**493**
HSJR-3	**711**	80^{a}	67^{a}	49^{a}	12^{a-d}	47^{a}	44^{a}	33^{a-d}	**485**
HSJR-4	**873**	80^{a}	67^{a}	49^{a}	12^{a-d}	**524**	$2^{a,b,d}$	33^{a-d}	**494**
HSJR-5	**874**	80^{a}	67^{a}	49^{a}	$25^{a,d}$	171^{a}	**265**	$26^{a,d}$	**495**
HSJR-6	**875**	$38^{a,b}$	30^{a-c}	11^{a-d}	20^{b-d}	**518**	**264**	**208**	**496**
HSJR-7	**876**	39^{a-c}	33^{a-d}	2^{a-d}	128^{b}	**595**	**317**	4^{a-d}	**642**
HJXXIII-1	**709**	73^{a-e}	12^{a-e}	1^{a-e}	$9^{a,d,e}$	**517**	$10^{a,d,e}$	$9^{a,b,d,e}$	**484**
HJXXIII-2	**868**	**237**	45^{b}	26^{a-e}	$48^{a,b,d}$	$113^{b,e}$	85^{b}	**205**	**490**
HJXXIII-3	**869**	$8^{a,e}$	8^{a-e}	137^{c}	$166^{c,e}$	**519**	19^{d}	204^{c}	**491**
HJXXIII-4	**712**	$2^{a,b,d}$	2^{a-d}	10^{a}	10^{a}	10^{a}	11^{a-d}	$10^{a,b,d}$	2^{a}
HJXXIII-5	**705**	30^{a-d}	5^{a-d}	7^{a-d}	$26^{a,d}$	125^{d}	102^{a-e}	$58^{b,d,e}$	**470***
HJXXIII-6	**870**	80^{a}	67^{a}	49^{a}	$25^{a,d}$	**523**	**266**	$9^{a,b,d,e}$	**492**
HJXXIII-7	**706**	73^{a-e}	12^{a-e}	$30^{a,d}$	$9^{a,d,e}$	220^{e}	$10^{a,d,e}$	179^{d}	**478***
HJXXIII-8	**871**	30^{a-d}	5^{a-d}	5^{a-e}	30^{a-d}	**525**	35^{a-d}	4^{a-d}	**497**
HJXXIII-9	**707**	$24^{a,d}$	23^{a-d}	22^{d}	$25^{a,d}$	10^{a}	$86^{b,d}$	$26^{a,d}$	**482***
HJXXIII-10	**710**	73^{a-e}	12^{a-e}	1^{a-e}	65^{a}	**517**	$10^{a,d,e}$	$9^{a,b,d,e}$	**486**
HJXXIII-11	**877**	**260**	$81^{b,d}$	$61^{a,b,d}$	**177**	**560**	319^{f}	**227**	**638**
HJXXIII-12	**878**	80^{a}	67^{a}	49^{a}	$25^{a,d}$	171^{a}	**290**	$26^{a,d}$	**639**
HJXXIII-13	**879**	30^{a-d}	5^{a-d}	5^{a-e}	65^{a}	44^{a}	$10^{a,d,e}$	$9^{a,b,d,e}$	**640***
HJXXIII-14	**880**	30^{a-d}	5^{a-d}	9^{a-d}	30^{a-d}	**559**	35^{a-d}	4^{a-d}	**641**
HJXXIII-15	**881**	80^{a}	67^{a}	49^{a}	12^{a-d}	**524**	**321**	33^{a-d}	**643**
HJXXIII-16	**882**	80^{a}	67^{a}	49^{a}	23^{a-d}	**524**	30^{d}	$23^{b,d}$	**679**
HJXXIII-17	**883**	$24^{a,d}$	23^{a-d}	22^{d}	$25^{a,d}$	$56^{a,b}$	$86^{b,d}$	$26^{a,d}$	**644***
HJXXIII-18	**884**	73^{a-e}	12^{a-e}	1^{a-e}	$9^{a,d,e}$	385^{e}	263^{e}	14^{a-e}	**645***
HJXXIII-19	**885**	$15^{b,d,e}$	$10^{a,b,d,e}$	$34^{a,d}$	23^{a-d}	441^{d}	**368**	$51^{a,d}$	**677**
HJXXIII-20	**886**	$50^{a,d}$	$40^{a,d}$	$19^{a,d}$	12^{a-d}	165^{d}	$68^{a,d}$	$48^{a,d}$	**678***
HJXXIII-21	**887**	73^{a-e}	12^{a-e}	1^{a-e}	$9^{a,d,e}$	220^{e}	$10^{a,d,e}$	179^{d}	**676***

*New ST resulting from new combinations of known alleles. Source of isolation for the already existing alleles in the database are: *a*, human; *b*, animals; *c*, environmental water; *d*, food; *e*, shellfish; *f*, unknown.

74 %) were generated by the incorporation of new alleles for five of the seven genes (*glyA* 12 new alleles, *pgm* 7, *tkt* 3, *aspA* 2 and *gltA* 1) as shown in Table 1.

Genes *atpA* and *glnA* did not show any new allele. In the remaining strains (7/27, 25 %), the new STs were due to the presence, in our strains, of new combinations of known alleles. All of the known alleles were previously isolated from humans, among other sources as indicated in Table 1. In relation to the other strains and as expected, the *A. cryaerophilus* strain (HJXXIII-11, 877) was the most different, with four of the seven genes presenting new alleles, including one for the *gltA* gene, for which none of the *A. butzleri* strain showed any new allele (Table 1). The number of different alleles found for the 28 strains and for each of the seven genes was gene-specific, the genes *pgm* (22 alleles) and *glyA* (21 alleles) being the most diverse. The high diversity of *glyA* and *pmg* agrees with these being the genes with more alleles in the database (http://pubmlst.org/arcobacter/) with 425 and 286, respectively.

When the composition of the allelic profiles was analysed in our strains, an apparent association between the alleles *aspA*-80, *atpA*-67 and *glnA*-49 known only from human origin was observed (Table 1). This was suggested by the fact that the combination of these three alleles occurred in seven of our strains (25 %, Table 1) and also in isolate 271 of the database that was also isolated from humans. None of the other strains in the database showed these allele combinations nor the alleles *atpA*-67 and/or *gln*A-49, and the allele *aspA*-80 was only found in another strain (strain 270, LMG 15577) recovered from the stool of a patient with gastroenteritis. These findings support the hypothesis that these alleles could be a human signature. Further studies would be required to confirm this observation.

The number of alleles, the synonym substitutions (d_S) and the non-synonymous substitutions (d_N) and the polymorphic sites for each locus are listed in Table 2. In fact, genes *pgm* and *glyA* that showed a higher number of alleles, i.e. 22 and 21, respectively, were also the genes that showed more polymorphic sites (Table 2). Several studies have evaluated the ratio of non-synonymous to synonymous evolutionary substitutions (d_N/d_S) of the seven genes included in the MLST approaches of several genera including *Arcobacter* [24, 34–37]. In our study, the ratios ranged from 0.0000 for the *gltA* gene to 0.1379 for the *glyA* gene. These results (d_N/d_S<1) evidenced that a negative selection occurs within these genes, in agreement with the *A. butzleri* data obtained by De Cesare *et al.* [24] with d_N/d_S ratios ranging between 0.0000 for *gltA* to 0.1246 for *atpA* among the seven loci.

Table 2. Results of recombination and selection tests for each locus

Locus	Alleles	Polymorphic sites	d_N	d_S	d_N/d_S
aspA	11	20	0.0009	0.0585	0.0161
atpA	13	23	0.0039	0.0424	0.0923
glnA	16	20	0.0015	0.0365	0.0402
gltA	14	13	0.0000	0.0473	0.0000
glyA	21	45	0.0086	0.0624	0.1379
pgm	22	38	0.0016	0.0836	0.0187
tkt	15	21	0.0014	0.0610	0.0226

Results of negative selection have also been observed in other MLST schemes using different loci for *Leptospira* species [34], *Lactococcus lactis* [36] or *Mycoplasma hominis* [37] with results of d_N/d_S in most of the cases below 0.1. The only exception is the MLST study for *Staphylococcus lugdunensis* where four of the seven genes showed d_N/d_S ratios higher than 0.2, but there was no evidence of positive selection when other selection tests (i.e. Tajima's test) were performed [35].

Epidemiological analysis

A phylogenetic analysis (Fig. 1) was performed comparing the 28 STs from Spain with all those from human origin (n=132) present in the MLST database, at the time of writing. This comparison showed that half of the Spanish STs (n=14) grouped randomly with the STs already included in the database, while the other half (14, 50 %) formed two clusters (Fig. 1). Cluster 1 (C1) included seven Spanish STs [i.e. six from the same hospital (HJXXIII) not related in time and the ST isolated from the city of Zaragoza] and two STs from other European countries (one from Germany and one from France). However, cluster 2 (C2) grouped seven Spanish STs, recovered from two hospitals [i.e. HSJR (n=3) and HJXXIII (n=4)], with two STs from strains from the USA and Thailand (Fig. 1). Apart from these associations, no other ones could be observed.

Virulence genes and antibiotic susceptibility

The pathogenic mechanisms of *Arcobacter* spp. are relatively poorly known [2]. However, the prevalence of nine putative virulence genes (*cadF*, *cj1349*, *ciaB*, *mviN*, *pldA*, *tlyA*, *hecA*, *hecB* and *irgA*) have been screened for using the PCR described by Douidah *et al.* [31]. The primers in the latter study were developed on the basis of only the sequences of these genes being retrieved from the genome of strain *A. butzleri* (ATCC49616^T). However, only five genes (*cadF*, *cj1349*, *ciaB*, *hecA* and *irgA*) have shown to be more abundant [31] and are those that have been previously studied [38, 39] and analysed in this study. Genes *cadF* and *cj1349* encode two fibronectin-binding proteins (CadF and Cj1349); *ciaB* encodes the invasion protein CiaB; the *hecA* gene encodes for an adhesin of the filamentous haemagglutinin family and the gene *irgA* encodes the iron-regulated outer membrane protein IrgA. The PCR results for the five virulence genes found in our study are shown in Table 3. In the *A. cryaerophilus* strain only the gene *ciaB* was detected and the absence of the other genes in this species can be due to the absence of them or to the heterogeneity of the gene sequences which may hamper the amplification [31, 38]. Genes *ciaB* and *cj1349* were present in all *A. butzleri* strains tested while gene *cadF* was detected in 96.4 % of the strains. Genes *hecA* and *irgA* were only detected in 3.6 % (n=1) and 7.1 % (n=2) of the strains, respectively. Interestingly, the

Table 3. Presence of virulence genes and antibiotic susceptibility of the 28 clinical strains

	Virulence genes					Antibiotic susceptibility				
	ciaB	*cadF*	*cj1349*	*hecA*	*irgA*	GM	AMC	E	TE	CIP
HUMS-1	+	+	+	−	−	S	S	S	S	S
HSJR-2	+	+	+	−	−	S	S	S	S	S
HSJR-3	+	+	+	−	−	S	S	S	S	S
HSJR-4	+	+	+	−	−	S	S	S	S	S
HSJR-5	+	+	+	−	−	S	S	S	S	S
HSJR-6	+	+	+	−	−	S	R	S	S	S
HSJR-7	+	+	+	+	−	S	S	S	S	S
HJXXIII-1	+	+	+	−	−	S	S	S	S	S
HJXXIII-2	+	+	+	−	+	R	R	S	S	S
HJXXIII-3	+	+	+	−	−	S	R	S	S	S
HJXXIII-4	+	+	+	−	−	S	R	S	S	S
HJXXIII-5	+	+	+	−	−	S	S	S	S	S
HJXXIII-6	+	+	+	−	−	S	S	S	S	S
HJXXIII-7	+	+	+	−	−	S	S	R	S	S
HJXXIII-8	+	+	+	−	−	S	S	S	S	S
HJXXIII-9	+	+	+	−	−	S	S	S	S	S
HJXXIII-10	+	−	+	−	−	S	S	S	S	S
HJXXIII-11	+	−	−	−	−	S	S	S	S	R
HJXXIII-12	+	+	+	−	−	S	S	S	S	S
HJXXIII-13	+	+	+	−	−	S	S	S	S	S
HJXXIII-14	+	+	+	−	−	S	S	S	S	S
HJXXIII-15	+	+	+	−	−	S	S	S	S	R
HJXXIII-16	+	+	+	−	−	S	S	S	S	S
HJXXIII-17	+	+	+	−	−	S	R	S	S	S
HJXXIII-18	+	+	+	−	−	S	S	S	S	S
HJXXIII-19	+	−	+	−	−	S	S	S	S	S
HJXXIII-20	+	+	+	−	+	S	S	S	S	R
HJXXIII-21	+	+	+	−	−	S	S	S	S	S

GM, Gentamycin; AMC, Amoxicillin/Clavulate; E, Erythromycin; TE, Tetracycline; CIP, Ciprofloxacin; R, Resistant; S, Sensitive.

only strain that was positive for the *hecA* gene was the one isolated from the healthy patient coming from Africa. The detected genes in our study are in concordance with those found from human strains by Karadas *et al.* [40] or for strains from different sources (animals, foods…) studied by Tabatabaei *et al.* [41]. However, in those studies the *cadF* gene was detected in all of the tested *A. butzleri* strains, in contrast with the studies of Levican *et al.* [38] and Collado *et al.* [42] in which they reported a lower presence of this gene in strains isolated from shellfish and sewage.

The susceptibility results (Table 3) showed that nine of the 28 tested strains (32.1 %) were resistant to at least one of the analysed antibiotics. The strain *A. cryaerophilus* HSJXXIII-11 and 7.4 % of the *A. butzleri* strains showed resistance to ciprofloxacin. Previous studies also showed resistance to this fluoroquinolone by strains of *Arcobacter* spp. of human clinical origin [43, 44]. However, ciprofloxacin has previously been recommended for the treatment of *Arcobacter* intestinal infections. The highest resistance detected among our strains (17.9 %) was for amoxicillin combined with the β-lactamases inhibitor clavulanic acid. Resistance to β-lactam antibiotics has been reported previously [45–47].

In our study only one strain of *A. butzleri* showed resistance to erythromycin (3.6 %) and the same occurred for gentamycin. However, Vandenberg *et al.* [48] found that 21.3 % of the strains were resistant to erythromycin and none of them to gentamycin. Interestingly, none of our strains showed resistance to tetracycline which is another drug recommended for intestinal infections [2, 39]. The latter seems to be the most effective treatment due to the high susceptibility observed among clinical strains [44, 48, 49].

Conclusions

In the present study 28 clinical *Arcobacter* strains were analysed using a MLST approach and only one ST (ST 2) was already present in the database and the other 27 constituted new records. The latter resulted from the presence of new alleles or new combinations of known allele, evidencing a

high diversity among the clinical strains. More studies are needed to clarify the epidemiology and the molecular relationships among the different *Arcobacter* strains around the world. Most importantly, clinicians should be alerted to the acquisition of resistances to ciprofloxacin, one of the recommended treatments for intestinal *Arcobacter* infections. Furthermore, antibiotic susceptibility tests of the recovered strains should be performed before selecting any empirical treatment to ensure the use of the most effective antimicrobials and to prevent the development of more resistant clinical strains.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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