Comparative genomics analysis and virulence-related factors in novel *Aliarcobacter faecis* and *Aliarcobacter lanthieri* species identified as potential opportunistic pathogens

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**Abstract**

**Background:** Emerging pathogenic bacteria are an increasing threat to public health. Two recently described species of the genus *Aliarcobacter, A. faecis* and *A. lanthieri*, isolated from human or livestock feces, are closely related to *Aliarcobacter* zoonotic pathogens (*A. cryaerophilus, A. skirrowii*, and *A. butzleri*). In this study, comparative genomics analysis was carried out to examine the virulence-related, including virulence, antibiotic, and toxin (VAT) factors in the reference strains of *A. faecis* and *A. lanthieri* that may enable them to become potentially opportunistic zoonotic pathogens.

**Results:** Our results showed that the genomes of the reference strains of both species have flagella genes (*flaA, flaB, flgG, flhA, flhB, fluP, motA* and *cheY1*) as motility and export apparatus, as well as genes encoding the Twin-arginine translocation (Tat) (*tatA, tatB* and *tatC*), type II (*pulE* and *pulF*) and III (*fliF, fliN* and *ylqH*) secretory pathways, allowing them to secrete proteins into the periplasm and host cells. Invasion and immune evasion genes (*ciaB, iamA, mviN, pldA, irgA* and *fur2*) are found in both species, while adherence genes (*cadF* and *cj1349*) are only found in *A. lanthieri*. Acid (*clpB*), heat (*clpA* and *clpB*), osmotic (*mviN*), and low-iron (*irgA* and *fur2*) stress resistance genes were observed in both species, although urease genes were not found in them. In addition, *arcB, gyrA* and *gyrB* were found in both species, mutations of which may mediate the resistance to quaternary ammonium compounds (QACs). Furthermore, 11 VAT genes including six virulence (*cadF, ciaB, irgA, mviN, pldA, and tlyA*), two antibiotic resistance [tet(O) and tet(W)] and three cytolethal distending toxin (*cdtA, cdtB*, and *cdtC*) genes were validated with the PCR assays. *A. lanthieri* tested positive for all 11 VAT genes. By contrast, *A. faecis* showed positive for ten genes except for *cdtB* because no PCR assay for this gene was available for this species.

**Conclusions:** The identification of the virulence, antibiotic-resistance, and toxin genes in the genomes of *A. faecis* and *A. lanthieri* reference strains through comparative genomics analysis and PCR assays highlighted the potential...
Background

The genus Aliarcobacter (formerly Arcobacter) belongs to the family Campylobacteraceae in Epsilonproteobacteria [1–3]. To date, Aliarcobacter consists of nine Gram-negative species reclassified from Arcobacter sensu lato species, including A. butzleri, A. cibarius, A. cryaerophilus, A. faecis, A. lanthieri, A. skirrowii, A. thereius, A. trophiarum, and A. vitoriensis [2–4]. Aliarcobacter species are motile by single polar flagellum and can survive in micro-aerobic and aerobic conditions [2, 5]. Aliarcobacter species have been commonly detected in a variety of foods, including chicken, beef, pork, shellfish, and aquatic niches [6–8], where they can be contaminated by livestock and poultry wastes, agricultural runoff, septic leakages, and wildlife fecal matter [9, 10]. Among the nine Aliarcobacter species, A. butzleri, A. cryaerophilus, and A. skirrowii are associated with human and animal infections, including gastroenteritis, bacteremia, sepsis, mastitis, diarrhea, abortion, and reproductive disorders [5]. In addition, antimicrobial susceptibility and the detection of virulence factors confirmed A. thereius as a zoonotic pathogen [11–13]. Although the physiology and genetics of Aliarcobacter are still poorly understood, comparative genomics analysis can help in deciphering the genetic codes of Aliarcobacter species and elucidate their ecological roles and pathogenic potential. It is worth noting that a recent genome-based study proposed to include Aliarcobacter, Halarcobacter, Malaciobacter, Pseudarcobacter, Poseidonibacter, and Arcobacter sensu stricto in a single genus, Arcobacter [14].

To date, only the genomes of A. butzleri, A. cibarius, A. cryaerophilus, and A. thereius have been characterized in detail [11, 15–17]. These genomes are featured as low GC content (ca. 27%), with the genome sizes ranging from 1.8 to 2.3 Mb [11, 15, 17]. Comparative genomics further identified several sets of genes or proteins that may be associated with the pathophysiology of pathogenic Aliarcobacter species. Strains of A. butzleri often carry a full or partial set of the nine virulence determinants that are homologous to genes with known pathogenic mechanisms, including the putative virulent factor mviN [18] or genes associated with adherence (cadiF, cjl349, hecA and irgA), invasion (ciaB) or destruction (hecB, tlyA, pldA) of host cell walls [5]. Genes or gene clusters involved in the biosynthesis of lipooligosaccharides and flagella, chemotaxis, and antimicrobial resistance have also been identified in A. butzleri and A. thereius [11, 15]. Genome analysis combined with laboratory experiments suggested that A. butzleri, A. cryaerophilus, and A. skirrowii may survive in cold and oligotrophic environments, disinfection regimes, food process procedures, and storage conditions [5]. It was reported that the antimicrobial resistance of pathogenic Aliarcobacter strains might be chromosomally determined and associated with the activity of efflux pumps or the presence of degrading enzymes encoded by genes such as cat (chloramphenicol resistance) [5, 11, 12, 15]. It has also shown that mutations in the quinolone-resistance-determining region of gyrA mediate bacterial susceptibility to fluoroquinolones [19, 20]. In addition, exotoxins and endotoxins and toxin-antitoxin (TA) systems are critical self-defense mechanisms for bacteria that determine a pathogen’s capacity and persistency of pathogenicity [21].

Aliarcobacter lanthieri strain AF1440T, AF1430, and AF1581 were isolated from pig and dairy cattle manure [22], and A. faecis strain AF1078T was isolated from a human septic tank [23]. A. lanthieri and A. faecis are phylogenetically closely related and clustered with A. cryaerophilus, A. skirrowii, and A. butzleri, based on the phylogenetic analysis using 16S rRNA and housekeeping (gyrB, rpoB, cpn60, gyrA, and atpA) genes; and equipped with short flagellum for mobility [22, 23]. Besides, a recent study isolated and identified A. lanthieri strain R-75363 from the stool culture of an immunocompetent patient who developed persistent abdominal bloating and cramps without fever or diarrhea [24]. Therefore, the focus of this study was to assess the virulence-related factors of these two species through comparative genomics analysis. The objectives of this study were to i) perform whole-genome assembly of the reference strains of A. lanthieri and A. faecis; ii) assess the taxonomic position of A. lanthieri and A. faecis based on genome homology; and iii) identify virulence-, antimicrobial resistance- and toxin-related genes in A. lanthieri and A. faecis. This study provided information on the antibiotic resistance, virulence potential, and general fitness of these two new Aliarcobacter species in natural environments.

Materials and methods

Culturing and DNA extraction

A. faecis AF1078T (= LMG 28519T) and A. lanthieri AF1440T (= LMG 28516T) type strains, isolated from
livestock and human fecal sources, were cultured on modified Agarose Medium (m-AAM) (Oxoid) containing selective antibiotic (cefoperazone, amphotericin-B and teicoplanin) supplements. The plates were incubated at 30 °C under microaerophilic conditions (85% N₂, 10% CO₂ and 5% O₂) for 3 to 6 days as described previously [22, 23]. Total genomic DNA was extracted and purified using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). The concentration of DNA was determined using the Qubit™ 2.0 Fluorometer (Life Technologies, Burlington, ON, Canada). Purified DNA was stored at −20 °C for further use.

**Genome sequencing**

Library preparation and paired-end whole-genome sequencing of *A. faecis* AF1078ᵀ and *A. lanthieri* AF1440ᵀ reference strains were performed at the National Research Council Canada (Saskatoon, Saskatchewan, Canada). In brief, high-molecular-weight genomic DNA was used as input for library preparation using the Illumina TrueSeq DNA library preparation kit (Illumina Inc.) to obtain a library with a median insert size of 300 bp. After PCR enrichment, the resultant library was checked on a Bioanalyzer (Agilent Technologies Inc., Mississauga, ON, Canada) and quantified. The libraries were equimolarly pooled and sequenced on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA), generating 2 × 10⁶ bp paired-end reads for each sequenced fragment. Base-calling and primary quality assessments were performed using the Illumina CASAVA pipeline (v1.8.2).

The mate-pair sequencing was performed at the Ottawa Research and Development Centre, Agriculture and Agri-Food Canada (Ottawa, Ontario, Canada). The mate-paired DNA library was prepared using the Nextera® Mate Pair kit (Illumina, San Diego, CA, USA). DNA fragments with three size ranges (1.8–3.5 Kb, 4.0–7.0 Kb, and 8.0–12.0 Kb) were selected using Pippen SageELF (Sage Scientific, Beverly, MA, USA) and pooled with a mean insert size of 6.1 Kb. The libraries were examined on a Bioanalyzer and then quantified using the KAPA qPCR assay (Wilmington, MA, USA). The sequencing libraries were normalized to 2 nM and then diluted to 6 pM prior to loading on a MiSeq Illumina sequencing platform (Illumina, San Diego, CA, USA), which generated 500 bp mate-paired reads for each sequenced fragment.

**Genome assembly and annotation**

Automatic trimming (based on a threshold of Q=25) using Trimmomatic scanning and de novo assembly using SPAdes genome assembler version 3.11.1 [25] with combined Illumina NextSeq500 data set of paired-end and mate-pair reads for each species was performed. The contigs were assembled into scaffolds using Medusa [26], where *A. nitrofigilis* DSM 7299, *A. butzleri* RM 4018, and *A. cryaerophilus* L406 genomes were used as scaffolding references. GapFiller [27] closed scaffold gaps using raw paired-end sequencing data. Finally, both de novo assembled genomes were annotated with Prokka version 1.12 [28]. The genome sequences and annotations were deposited to the JGI IMG/MER under analysis IDs Ga0136198 (*A. faecis*) and Ga0136182 (*A. lanthieri*) [29].

An Unweighted Pair Group Method with Arithmetic mean (UPGMA) phylogenetic tree was built using the MASH tool version 2.3 with a sketch size of s=1000, a k-mer size of k=21, and 100 random seeds for bootstrap [30]. MASH uses the MinHash technique to assess the pairwise distance between sequences [30]. The bootstrapped phylogenetic tree was visualized using the R package ggtree version 3.2.1 [31]. In this analysis, we included the genomes of strains of nine *Aliarcobacter* species fetched from the National Center for Biotechnology Information (NCBI) database: *A. butzleri* (strain RM 4018: NC_009850.1; strain ED-1: NC_017187.1; strain NCTC 12481ᵀ: JGI Ga0136182), *A. cibarius* (strain LMG 21996ᵀ; draft genome NZ_JABW01000000.1; strain H73: NZ_CP043857.1), *A. cryaerophilus* (strain ATCC 43158ᵀ; NZ_CP032823.1; strain D2610: NZ_CP032825.1), *A. lanthieri* (strain AF 1581: NZ_JARV01000000.1), *A. skirrowii* (strain CCUG 10374ᵀ: NZ_CP032099.1; strain A256: NZ_CP034309.1), *A. thereius* (strain LMG 24486ᵀ; draft genome NZ_LKKQ01000000.1; strain DU22: draft genome NZ_LCUJ01000000.1), *A. trophiarum* (strain LMG 25534ᵀ; NZ_CP031367.1; strain CECT 7650: draft genome NZ_PDIS01000000.1), *A. vitreiensis* (strain CECT 9230: draft genome NZ_PKBO01000000.1), as well as more distant members of *Epsilonproteobacteria* in the order *Campylobacterales*: *Helicobacter pylori* (strain 26,695: NC_000915.1), *Campylobacter fetus* (strain 82-40ᵀ: NC_008599.1), *Sulfuropsirillum delelyanum* (strain DSM 6946ᵀ: NC_013512.1). The tree was rooted in *Wolinella succinogenes* (strain DSM 1740ᵀ: NC_005090.1).

Gene synteny and homology of *A. faecis* strain AF1078ᵀ and *A. lanthieri* strain AF1440ᵀ were computed using BLASTp and MCScanX with default parameters (match score ≥ 50, E-value ≤ 10⁻⁵, max gaps ≤ 25) [32, 33].

**Genome annotation for pathogenicity assessment**

VFAnalyzer is an automatic pipeline for a systematic screen of potential virulence factors (VFs) against the Virulence Factor Database (VFDB) [34]. VFAnalyzer was used to identify VFs from the predicted genes of *A. faecis* strain AF1078ᵀ, *A. lanthieri* strain AF1440ᵀ, *A. butzleri* strain NCTC 12481ᵀ, *A. cryaerophilus* strain ATCC
A. skirrowii strain CCUG 10374T, A. cibarius strain LMG 21996T, A. thereius strain LMG 24486T, and A. trophiarum strain LMG 25534T with default parameters. The genome sequence of the Campylobacter jejuni strain NCTC 11168T was used as a reference. Moreover, amino acid sequences related to previously studied virulence factors of Aliarcobacter species [35] were collected in a custom database, including cadF (Abu_0481), cj1349 (Abu_0067), ciaB (Abu_1549), irgA (Abu_0726), pldA (Abu_0861), hecA (Abu_0940), hecB (Abu_0939), tlyA (Abu_1835), waaF (Abu_1800), waaC (Abu_1822), htrA (Abu_2099), iamA (Abu_0107), fur1 (Abu_0717), fur2 (Abu_1770), luxS (Abu_0111), ureB (Abu_0807), ureD (Abu_0805), ureE (Abu_0808), ureG (Abu_0810), flaA (Abu_2254), flaB (Abu_2255), flgH (Abu_0208), motA (Abu_0271) and mvnN (Abu_0878) from A. butzleri strain RM4018T (GenBank assembly accession: GCA_000014025.1) and iroE (AA20_05105) from A. butzleri strain L348 (GenBank assembly accession: GCA_0001010585.1), and virF (AAX29_00642) from A. thereius strain DU22 (GenBank assembly accession: GCA_001695335.1).

TA system is a set of genes encoding a pair of stable toxin and unstable anti-toxin. TAfinder was used to predict type II TA loci in A. faecis strain AF1078T and A. lanthieri strain AF1440T with default parameters [36].

The VF and TA genes in the complete genome of A. faecis AF1078T and A. lanthieri AF1440T strains were visualized using the circlize package in R [37].

PCR-based assays for validation of virulence, antibiotic resistance, and toxin (VAT) genes

The detection of VAT genes was carried out using our previously developed species- and gene-specific primer pairs, mono- and multiplex Polymerase Chain Reaction (PCR) protocols [38]. For A. lanthieri, a total of 11 including six virulence (cadF, ciaB, irgA, mvnN, pldA, and tlyA), two antibiotic resistance [tet(O) and tet(W)] and three cytolethal distending toxin (cdtA, cdtB, and cdtC) genes were tested. However, six virulence (cadF, ciaB irgA, mvnN, pldA and tlyA), two antibiotic resistance [tet(O) and tet(W)] and two cytolethal distending toxin (cdtA and cdtC) genes were tested for A. faecis. No cdtB-based PCR assay was available for A. faecis.

The amplicon sizes of each mono- and multiplex PCR reaction were confirmed by 2.5% agarose gel electrophoresis (Fisher Scientific) using a 100bp DNA size marker (Life Technologies, Grand Island, NY). The agarose gels were stained in ethidium bromide (0.5μg/mL), and Alpha Imager (Fisher Scientific) was used for scanning and documentation.

Table 1 Statistical summary of assembly and annotation of reference genomes of Aliarcobacter lanthieri and A. faecis

| A. lanthieri AF1440T | A. faecis AF1078T |
|---------------------|-------------------|
| No.                 | %                 | No.   | %                 |
| DNA, total number of bases | 2,234,737   | 100   | 2,327,155 | 100 |
| DNA coding number of bases | 2,109,823  | 94.41 | 2,180,685 | 93.71 |
| DNA G+C number of bases | 589,766   | 26.39 | 627,419  | 26.96 |
| Genes total number | 2268     | 100   | 2351     | 100  |
| Protein coding genes | 2230     | 98.32 | 2319     | 98.64 |
| Genes with function prediction | 1749   | 77.12 | 1745   | 74.22 |
| Protein coding genes with COGs | 1491  | 65.74 | 1484  | 63.12 |
| COG clusters | 1097    | 73.57 | 1086   | 73.18 |

Results and discussion

General features of A. faecis and A. lanthieri genomes

The genome of A. faecis AF1078T (= LMG 28519T) reference strain contained 2,327,155bp in one scaffold, and the genome of A. lanthieri AF1440T (= LMG 28516T) reference strain contained 2,234,737bp in one scaffold. The overall GC contents of the two genomes were 27.0 and 26.4%, respectively, which were consistent with other Arcobacter sensu lato species (Table 1). The numbers of protein-coding genes predicted in A. faecis and A. lanthieri genomes were 2319 and 2230, respectively (Table 1). A. lanthieri and A. faecis had a similar percentage (73%) of functionally annotated protein-coding genes with Clusters of Orthologous Groups (COGs) (Table 1). The phylogenetic tree of whole genome comparison shows that the strains of the two new species clustered with A. vitoriensis and A. cibarius, respectively (Fig. 1). This confirms previous maximum-likelihood phylogenetic analysis based on 16S rRNA and housekeeping genes [22, 23].
strain D4963 [39], suggesting significant genetic variation between strains isolated from different geographical locations. Besides, genes (tatA, tatB, and tatC) involved in the Twin-arginine translocation (Tat) secretion pathway were found in A. faecis, A. lanthieri and A. butzleri (Table S1). Furthermore, the mobilome COG category (code X) was underrepresented in the reference genome of A. lanthieri, carrying only one gene associated with COG2932 from that group (Table 2). A. faecis and A. butzleri had 11 and seven genes, respectively, related to COG code X (Table 2). The presence of the mobile genetic elements, such as prophages and transposon, may suggest horizontal gene transfer of potentially antimicrobial resistance and/or adaptation genes.

**Detection and comparison of virulence-associated genes of Aliarcobacter species**

Twenty-six virulence-associated genes were previously reported in A. butzleri and other Aliarcobacter species [15, 35]. This study compared the 26 genes and identified 15 in A. faecis and 20 in A. lanthieri (Table 3, Fig. 2). Besides, additional putative VF and TA genes of A. faecis and A. lanthieri were identified using VFanalyser and TAfinder (Table 4; Fig. 2) against the VFDB and TADB databases, respectively [34, 36]. Other known and putative zoonotic pathogens in the genus Aliarcobacter were also annotated using VFanalyser (Table 4). The E-values of putative VFs were $10^{-10}$, and in general, the coverages were > 90% (Table 4). Here, we present these genes into functional categories, including motility and export apparatus, invasion and stress resistance, adherence, antimicrobial resistance, TA systems, and general resistance.

**Motility and export apparatus**

Bacterial flagellum can affect its virulence by determining the physical motility and act as a secretion system for other virulence factors [40]. Flagella genes flaA, flaB, flgG, flhA, flhB, flhC, flIP, cheY1, and motA were found in both A. faecis and A. lanthieri, reference strain genomes (Tables 3 and 4). The flagellum apparatus of pathogenic bacteria is considered a secretion system composed of flagellar proteins, which forms a needle to inject bacterial toxins into the host cell. For example, it was reported that the flagellum of H. pylori is required to colonize the mucosal membrane of the stomach as opposed to penetrating the gastric mucosa [41]. Comparative genomics analysis also claimed that some non-flagellum type III secretion systems were evolved from flagellar secretion systems through a series of genetic deletions, innovations, and recruitments of components from other cellular structures [42].
Orthologs of virulence factors ciaB, iamA, and mviN were detected in the genomes of both A. faecis and A. lanthieri reference strains. These genes provide pathogens a competitive advantage to survive in the bacterial community (Table 3). Gene ciaB encodes one of the invasion antigens (Cia proteins), deletion of which resulted in significantly attenuated virulence in C. jejuni [43]. In addition, it has been suggested that flagellum serves as an export apparatus or secretion channel for Cia proteins [43]. Studies showed that mutants of Yersinia and C. jejuni without functional flagellar apparatus lack the ability to secrete Cia proteins in comparison to wild type [43]. Another secretion-associated gene, mviN, encodes peptidoglycan (a.k.a murein) flippase. Murein protects the gram-negative bacterial cell membrane from osmotic stress and serves as an anchor for virulence factors [44, 45]. The murein layer is vitally important for bacterial cells’ survival and is shown in Table 3. It shows that A. faecis and A. lanthieri carry a single copy of mviN ortholog.

Similarly, orthologs of iamA and pldA were found in both A. faecis and A. lanthieri genomes (Table 3). Of these, iamA, an invasion-associated marker gene, was also found in C. jejuni and reported to be associated with diarrhea [46, 47]. Previous studies showed that the PLA activity in Legionella spp., E. coli and Mycoplasma hyorhinis was associated with the impairment of host intestine cell membranes through hydrolyzation [48]. The lysis property of PLA also helps bacteria to acquire iron from erythrocytes by penetrating the host cell membranes [49]. Orthologs of waaC and waaF were discovered in A. lanthieri but not in A. faecis (Table 3). These two genes were also virulence determinants involved in the biosynthesis of liposaccharide in A. thereius and other species of the family Campylobacteraceae [50].

**Table 2** Number of predicted orthologous genes associated with COG functional categories in *Aliarcobacter* species

| COG Category | A. faecis AF1078T | A. lanthieri AF1440T | A. butzleri NCTC 12481T | A. cryearophilus ATCC 43158T | A. skirrowii CCUG 10374T | A. trophiarum LMG 25534T | Description |
|--------------|-------------------|----------------------|-------------------------|---------------------------|------------------------|------------------------|--------------|
| A            | 0                 | 0                    | 0                       | 0                         | 0                      | 0                      | RNA processing and modification |
| B            | 0                 | 0                    | 0                       | 0                         | 0                      | 0                      | Chromatin structure and dynamics |
| C            | 100               | 90                   | 110                     | 117                       | 119                    | 125                    | Energy production and conversion |
| D            | 18                | 15                   | 16                      | 30                        | 26                     | 24                     | Cell cycle control, cell division, chromosome partitioning |
| E            | 116               | 126                  | 139                     | 149                       | 143                    | 145                    | Amino acid transport and metabolism |
| F            | 56                | 53                   | 57                      | 63                        | 62                     | 62                     | Nucleotide transport and metabolism |
| G            | 41                | 46                   | 52                      | 63                        | 62                     | 63                     | Carbohydrate transport and metabolism |
| H            | 117               | 115                  | 108                     | 127                       | 126                    | 122                    | Coenzyme transport and metabolism |
| I            | 49                | 49                   | 47                      | 63                        | 50                     | 59                     | Lipid transport and metabolism |
| J            | 155               | 160                  | 161                     | 183                       | 177                    | 177                    | Translation, ribosomal structure and biogenesis |
| K            | 81                | 78                   | 92                      | 76                        | 77                     | 65                     | Transcription |
| L            | 80                | 78                   | 71                      | 107                       | 106                    | 87                     | Replication, recombination and repair |
| M            | 123               | 133                  | 141                     | 167                       | 129                    | 149                    | Cell wall/membrane/envelope biogenesis |
| N            | 33                | 34                   | 35                      | 56                        | 53                     | 49                     | Cell motility |
| O            | 65                | 72                   | 76                      | 98                        | 95                     | 100                    | Posttranslational modification, protein turnover, chaperones |
| P            | 118               | 142                  | 118                     | 107                       | 119                    | 113                    | Inorganic ion transport and metabolism |
| Q            | 9                 | 6                    | 7                       | 13                        | 10                     | 9                      | Secondary metabolites biosynthesis, transport and catabolism |
| R            | 99                | 100                  | 104                     | 130                       | 128                    | 118                    | General function prediction only |
| S            | 36                | 29                   | 39                      | 41                        | 51                     | 36                     | Function unknown |
| T            | 133               | 117                  | 143                     | 185                       | 156                    | 155                    | Signal transduction mechanisms |
| U            | 38                | 25                   | 22                      | 45                        | 57                     | 44                     | Intracellular trafficking, secretion, and vesicular transport |
| V            | 43                | 34                   | 41                      | 46                        | 43                     | 36                     | Defense mechanisms |
| W            | 2                 | 2                    | 2                       | 6                         | 4                      | 3                      | Extracellular structures |
| X            | 11                | 1                    | 7                       | 11                        | 13                     | 31                     | Mobilome: prophages, transposons |
| Y            | 0                 | 0                    | 0                       | 0                         | 0                      | 0                      | Nuclear structure |
| Z            | 2                 | 2                    | 2                       | 3                         | 4                      | 3                      | Cytoskeleton |

**Invasion and stress resistance**

Orthologs of virulence factors ciaB, iamA, and mviN were detected in the genomes of both *A. faecis* and *A. lanthieri* reference strains. These genes provide pathogens a competitive advantage to survive in the bacterial community (Table 3). Gene ciaB encodes one of the invasion antigens (Cia proteins), deletion of which resulted in significantly attenuated virulence in *C. jejuni* [43]. In addition, it has been suggested that flagellum serves as an export apparatus or secretion channel for Cia proteins [43]. Studies showed that mutants of *Yersinia* and *C. jejuni* without functional flagellar apparatus lack the ability to secrete Cia proteins in comparison to wild type [43]. Another secretion-associated gene, mviN, encodes peptidoglycan (a.k.a murein) flippase. Murein protects the gram-negative bacterial cell membrane from osmotic stress and serves as an anchor for virulence factors [44, 45]. The murein layer is vitally important for bacterial cells’ survival and is shown in Table 3. It shows that *A. faecis* and *A. lanthieri* carry a single copy of mviN ortholog.

Similarly, orthologs of iamA and pldA were found in both *A. faecis* and *A. lanthieri* genomes (Table 3). Of these, iamA, an invasion-associated marker gene, was also found in *C. jejuni* and reported to be associated with diarrhea [46, 47]. Previous studies showed that the PLA activity in *Legionella* spp., *E. coli* and *Mycoplasma hyorhinis* was associated with the impairment of host intestine cell membranes through hydrolyzation [48]. The lysis property of PLA also helps bacteria to acquire iron from erythrocytes by penetrating the host cell membranes [49]. Orthologs of waaC and waaF were discovered in *A. lanthieri* but not in *A. faecis* (Table 3). These two genes were also virulence determinants involved in the biosynthesis of liposaccharide in *A. thereius* and other species of the family *Campylobacteraceae* [50].
The orthologs of *irgA* and *fur2* were identified in both *A. faecis* and *A. lanthieri* genomes (Table 3). It was previously suggested that *irgA*, the enterobactin receptor gene, is induced by low iron, and the regulation depends on the iron-responsive master regulator Fur [51]. In addition, *irgA* ortholog was described for *A. butzleri* [15] and to a lesser degree to some *Campylobacter* species [52].

Furthermore, the urease enzyme secreted by bacteria promotes its own persistence in the stomach, allowing them to quickly migrate into the gastric mucosal epithelial line by chemotaxis, where pH is comparatively higher [53]. Although the urease enzyme gene cluster was found along with some accessory genes (*ureB, ureD, ureE, and ureG*) in *A. butzleri* (Table 3) [39], it is not identified in *A. faecis* and *A. lanthieri* genomes (Table 3).

### Table 3  Presence and copy numbers of known virulence factors in *A. faecis* AF1078T and *A. lanthieri* AF1440T

| Gene | COG Category | COG ID | *A. faecis* AF1078T | *A. lanthieri* AF1440T | Product |
|------|--------------|--------|---------------------|------------------------|---------|
| tlyA | J            | COG1189| –                   | 1                      | 23S rRNA (cytidine1920–2′-O)/16S rRNA (cytidine1409–2′-O)-methyltransferase |
| virF | K            | COG2207| 1                   | 1                      | AraC-type DNA-binding protein |
| cadF | M            | COG2885| 1                   | 1                      | OmpA-OmpF porin, OOP family |
| iamA | M            | COG1127| 1                   | 1                      | phospholipid/cholesterol/gamma-HCH transport system ATP-binding protein |
| mviN | M            | COG0728| 1                   | 1                      | putative peptidoglycan lipid II flippase |
| pldA | M            | COG2829| 1                   | 1                      | phospholipase A1 |
| waaC | M            | COG0859| –                   | 1                      | heptosyltransferase-1 |
| waaF | M            | COG0859| –                   | 1                      | heptosyltransferase-2 |
| flaA | N            | COG1344| 1                   | 2                      | flagellin |
| flaB | N            | COG1344| 1                   | 2                      | flagellin |
| flgH | N            | COG2063| 1                   | 1                      | flagellar L-ring protein precursor FlgH |
| htrA | O            | COG0265| 1                   | 1                      | serine protease Do |
| fur1 | P            | COG0735| –                   | 1                      | Fur family transcriptional regulator, ferric uptake regulator |
| fur2 | P            | COG0735| 1                   | 1                      | Fur family transcriptional regulator, ferric uptake regulator |
| irgA | P            | COG4771| 2                   | 2                      | outer membrane receptor for ferrienterochelin and colicins |
| rdeE | E            | COG2819| –                   | 1                      | Predicted hydrolase of the alpha/beta superfamily |
| luxS | T            | COG1854| 1                   | 1                      | S-ribosylhomocysteine lyase /quorum-sensing autoinducer 2 (AI-2) synthesis protein LuxS |
| ciaB | –            | –      | –                   | 1                      | *Campylobacter* invasion antigens; involved in secretion of virulence factors thorough flagellum |
| c1349| –            | –      | 1                   | 1                      | Fibronectin-binding protein A N-terminus (FbpA) |
| motA | –            | –      | –                   | 1                      | MotA/TolQ/ExbB proton channel family protein |
| hecA | –            | –      | –                   | –                      | Adhesive |
| hecB | –            | –      | –                   | –                      | Adhesive |
| ureB | –            | –      | –                   | –                      | Urease gene |
| ureD | –            | –      | –                   | –                      | Urease gene |
| ureE | –            | –      | –                   | –                      | Urease gene |
| ureG | –            | –      | –                   | –                      | Urease gene |

Adhesin encoded by *cadF* was found to mediate binding to Fibronectin, a protein present on the surface of epithelial cells [56]. Studies have also shown that *Campylobacter* cells lacking *cadF* exhibited a 50–90% reduction in adherence to epithelial cells [56]. Both proteins, *cadF* and *cj1349c*, were important for *C. jejuni* to adhere to the outer membrane of chicken cells and increase their virulence [56]. Orthologs of *hecA/hecB*, previously detected exclusively in a few strains [56], were not found in *A. faecis* and *A. lanthieri* genomes (Table 3). Although not all known adherence genes were found in the reference genomes of *A. faecis* and *A. lanthieri* (Table 3 and Table 4), these two species may still exhibit adherence ability linking to pathogenicity.
Antimicrobial resistance

Antimicrobial resistance genes consist of the most abundant group of virulence-related factors. Genomes of reference strains of *A. faecis, A. lanthieri, A. butzleri,* and other *Aliarcobacter* species contain efflux pumps associated with antibiotic resistance (Table 4). The identified pumps belong to the Resistance Nodulation cell Division (RND) protein superfamily, one of the most studied antiporters found in bacteria [57]. The identified genes were highly similar to *acrB,* encoding a multidrug efflux pump [58]. *AcrB* is a well-described antiporter involved in resistance to lipophilic β-lactam antibiotics, such as carbapenems and cephalosporins, fluoroquinolones, tetracyclines (including tigecycline), chloramphenicol, macrolides, trimethoprim, ethidium, rifampicin, and novobiocin [58]. It is of particular interest as previous studies showed that *A. butzleri* strains exhibited resistance to a variety of antibiotics, where the majority of them belong to β-lactams and some to quinolones and coumarins [15].

As indicated above, RND transporters like *AcrB* may determine resistance to quinolones and coumarins [58, 59]. This class of antibiotics targets bacterial DNA gyrase, type II topoisomerase, which plays an essential role in DNA replication [60]. However, significant data accumulated suggests that the resistance to such antibiotics may be acquired through specific mutations in the DNA gyrase gene [19, 20]. According to Vickers [20], resistance to novobiocin (coumarin antibiotic) is acquired through two amino acid residue mutations G(80)K and L(140)R in the B-subunit of DNA gyrase (* gyrB*) gene in *Staphylococcus saprophyticus.* Alignment of * gyrB* gene from novobiocin susceptible strain of *Staphylococcus saprophyticus* with homologs from *A. faecis, A. lanthieri,* and *A. butzleri* showed that these *Aliarcobacter* species carry A(80) and...
| Class                  | Virulence Factors | Gene   | A. faecis AF1078<sup>T</sup> | A. lanthieri AF1440<sup>T</sup> | A. butzleri NCTC 12481<sup>T</sup> | A. cyaerophilus ATCC 43158<sup>B</sup> | A. skirrowii CCUG 10374<sup>T</sup> | A. thetaius LMG 24486<sup>T</sup> | A. cibarius LMG 21996<sup>T</sup> | A. trophiarum LMG 25534<sup>T</sup> |
|-----------------------|-------------------|--------|-----------------------------|-------------------------------|---------------------------------|---------------------------------|-------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Acid resistance       | Urease            | ureA   | –                           | –                             | –                               | –                               | 1 (1.14e-805|60|98)                          | –                               | –                               | –                               |
|                       |                   | ureB   | –                           | –                             | 1 (0.65|100)                        | –                               | –                               | –                               | –                               | –                               |
|                       |                   | ureG   | –                           | –                             | 1 (8.13e-806|97)                        | –                               | –                               | –                               | –                               | –                               |
| Adherence             | LPS O-antigen     | hisF2  | –                           | –                             | –                               | –                               | –                               | –                               | –                               | –                               |
|                       |                   | hisH2  | –                           | –                             | 1 (1.03e-77|51|99)                        | –                               | –                               | –                               | –                               | –                               |
|                       |                   | wbpB   | –                           | –                             | 1 (8.68e-116|53|97)                        | 1 (2.2e-111|56|93)                        | –                               | –                               | –                               | –                               |
|                       |                   | wbpD   | –                           | –                             | 1 (8.26e-70|53|93)                        | 1 (3.37e-70|53|93)                        | –                               | –                               | –                               | –                               |
|                       |                   | wbpE   | –                           | –                             | –                               | –                               | –                               | –                               | 1 (4.52e-131|50|99)                        | –                               |
|                       |                   | wbpG   | –                           | –                             | 1 (3.52e-175|61|100)                      | –                               | –                               | –                               | –                               | –                               |
|                       |                   | wbpH   | –                           | –                             | 1 (7.68e-159|96|99)                        | –                               | –                               | –                               | –                               | –                               |
|                       |                   | wbpI   | –                           | –                             | 1 (1.17e-156|60|99)                        | –                               | –                               | –                               | –                               | –                               |
|                       |                   | Undetermined | –                           | –                             | –                               | –                               | –                               | 1 (3.69e-147|59|99)                        | –                               | –                               |
| Antiphagocytosis       | Capsule           | ugd    | –                           | 1 (0.73|100)                    | –                               | –                               | –                               | –                               | –                               | –                               |
|                       |                   | uge    | 1 (2.88e-150|57|98)            | –                             | 1 (2.6e-158|99)                        | –                               | –                               | –                               | –                               | –                               |
|                       |                   | Undetermined | 1 (0.7|99)                    | 1 (0.12e-65|53|92)                  | 1 (0.72|100)                    | 3 (0.7|100)                      | 3 (0.72|100)                    | 1 (7.17e-69|55|92)                       | 2 (0.73|97)                      | –                               |
| Chemotaxis and motility | Flagella          | cheY   | 1 (2.3e-61|67|98)            | 1 (2.92e-50|66|98)                  | 1 (2.62e-50|66|98)                | –                               | 1 (2.99e-51|66|98)                  | 1 (3.34e-50|64|98)                  | –                               | –                               |
|                       |                   | Undetermined | 1 (1.7e-105|50|98)               | 1 (1.3e-136|53|98)               | –                               | 1 (3.35e-103|50|98)                 | 1 (2.54e-102|51|98)               | 1 (3.39e-101|51|97)               | 2 (2.08e-137|52|99)               | 1 (1.91e-102|50|98)               |
| Colonization and Immune evasion | Capsule biosynthesis and transport | Undetermined | 1 (0.75|98)                    | 1 (0.53|98)                    | 2 (0.55|100)                   | 1 (0.52|98)                     | 1 (0.53|100)                   | 1 (0.52|98)                     | 1 (0.53|98)                   | 1 (0.52|98)                   |
| Efflux pump            | AcrAB             | acrB   | 1 (0.53|98)                    | –                             | –                               | –                               | –                               | –                               | –                               | –                               |
| Endotoxin              | LOS               | lpxA   | –                           | 1 (5.51e-84|50|95)                  | –                               | –                               | –                               | –                               | –                               | –                               |
| Enzyme                 | Streptococcal enolase | eno  | 1 (2.9e-156|54|99)           | 1 (3.68e-160|55|99)                | 1 (4.67e-157|53|97)                | 1 (8.53e-157|50|96)                | 1 (1.23e-157|54|99)                | 1 (1.98e-156|54|99)                | 1 (7.89e-156|54|99)                | 1 (9.52e-157|55|99)                |
Table 4 (continued)

| Class                      | Virulence Factors             | Gene   | A. faecis AF1078<sup>T</sup> | A. lanthieri AF1440<sup>T</sup> | A. butzleri NCTC 12481<sup>T</sup> | A. cryaerophilus ATCC 43158<sup>B</sup> | A. skinowii CCUG 10374<sup>T</sup> | A. thereus LMG 24486<sup>6</sup> | A. cibarius LMG 21996<sup>T</sup> | A. trophiarum LMG 25534<sup>T</sup> |
|----------------------------|--------------------------------|--------|-----------------------------|--------------------------------|--------------------------------------|------------------------------------|--------------------------------|-----------------------------|--------------------------------|--------------------------------|
| Glycosylation system       | N-linked protein glycosylation| pgC    | 1.68e-90|61|99 | – | – | – | – | – | – |
|                           |                                | pgD    | 1.12e-15|31|96 | – | – | – | – | – | – |
|                           |                                | pgE    | 8.61e-16|62|99 | – | – | – | – | – | – |
| O-linked flagellar glycosylation | neuB2                          | – | 2.77e-15|65|100 | – | 2.09e-14|61|100 | – | 1.32e-15|65|100 |
|                           |                                | neuC2  | 5.03e-13|95|99 | 6.19e-14|95|99 | – | – | 1.04e-18|74|99 |
|                           |                                | pseB   | 0.73|99 | 2.4e-18|72|99 | 0.75|99 | 1.075|100 | 1.04e-18|74|99 |
|                           |                                | pseI   | 1.92e-13|56|99 | 9.3e-13|56|100 | 1.84e-13|56|99 | 1.7e-13|56|99 |
|                           |                                | ptaB   | – | – | – | – | 1.81e-98|60|97 | – | 8.29e-99|62|99 |
| Immune evasion             | Capsule                        | Undetermined | 2.06|3|100 | 4.07|99 | 3.0|77|99 | 1.82e-80|54|96 | 1.71e-83|54|96 | 3.07|99 |
|                           | Exopolysaccharide              | galE   | – | – | – | 3.7e-15|60|99 | 1.98e-15|62|99 | 1.58e-15|62|99 | 1.31e-15|62|99 |
|                           |                                | galU   | – | – | 7.19e-92|51|93 | – | – | 1.65e-87|50|93 | – | 2.89e-92|50|93 |
|                           | Hyaluronic acid (HA) capsule   | LOS    | Undetermined | 2.18e-15|70|98 | 9.3|6e-17|78|98 | 10.978e-17|79|98 | 13.209e-17|80|98 | 7.17e-85|66|96 | 8.56e-15|78|99 |
|                           |                                | LPS    | 6.99e-22|60|89 | 1.73e-23|62|89 | 1.234e-22|60|89 | 1.79e-23|62|89 | 1.304e-22|60|89 | 1.69e-22|60|89 | 1.276e-23|62|89 |
|                           |                                | LPS    | fabZ   | – | – | 1.64e-44|50|97 | – | – | – | – | – | – | – | – | – |
| Immune modulator           | Neutrophil-activating protein  | napA   | 1.37e-53|50|99 | 1.23e-56|54|99 | 1.44e-56|55|97 | 1.986e-51|51|98 | 1.11e-48|50|98 | 1.78e-50|51|99 | – |
|                           | (HR-NAP)                       |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| Invasion                   | Campylobacter invasion antigen | cwb    | 8.94e-14|80|97 | 6.4e-14|74|97 | 2.14e-14|74|97 | 1.8e-14|74|97 | 1.38e-14|74|97 | 1.29e-14|74|97 | 1.29e-14|74|97 |
|                           | Flagella                       | cheY1  | 8.1e-30|51|93 | 1.23e-28|50|91 | 3.02e-31|52|91 | 7.03e-29|50|91 | – | 2.02e-29|50|91 |
| Iron uptake                | Heme biosynthesis              | hemL   | 0.32e-16|51|99 | 1.94e-16|54|97 | 1.48e-15|59|99 | 2.76e-15|59|99 | 1.67e-15|59|99 | 3.72e-15|59|99 | 2.06e-15|59|99 |
| Lipid and fatty acid metabolism | Pantothenate synthesis | panD   | 4.36e-34|51|91 | 1.04e-36|55|91 | 1.31e-36|55|91 | 9.878e-35|53|88 | 1.19e-34|50|88 | 1.12e-34|53|88 | 4.91e-35|53|88 |

<sup>T</sup> Transcribed; <sup>B</sup> Bacteriophage-temperate; <sup>6</sup> Six transcribed
### Table 4 (continued)

| Class                     | Virulence Factors       | Gene | A. faecis AF1078<sup>T</sup> | A. lanthieri AF1440<sup>T</sup> | A. butzleri NCTC 12481<sup>T</sup> | A. cryaerophilus ATCC 43158<sup>T</sup> | A. skirrowii CCUG 10374<sup>T</sup> | A. thereus LMG 24486<sup>6</sup> | A. cibarius LMG 21996<sup>T</sup> | A. trophiarum LMG 25534<sup>T</sup> |
|---------------------------|-------------------------|------|-----------------------------|---------------------------------|----------------------------------|----------------------------------|---------------------------------|----------------------------------|---------------------------------|---------------------------------|
| Motility and export apparatus | Flagella                | flgG | 1.0 (5.01e-100|58|100) | 1 (2.15e-98|58|100) | 1 (4.53e-101|57|100) | 1 (1.09e-98|59|100) | 1 (4.07e-100|59|100) | 1 (7.31e-102|58|100) | 1 (4.85e-102|58|100) | 1 (2.52e-102|58|100) |
|                           | flhA                    | 1 (2.15e-98|58|100) | 1 (0|50|98) | 1 (0|50|98) | 1 (2.15e-98|58|100) | 1 (0|50|98) | 1 (2.15e-98|58|100) | 1 (0|50|98) | 1 (0|50|98) |
|                           | flhB                    | 1 (4.53e-101|57|100) | 1 (0|50|98) | 1 (0|50|98) | 1 (4.53e-101|57|100) | 1 (0|50|98) | 1 (4.53e-101|57|100) | 1 (0|50|98) | 1 (0|50|98) |
|                           | flhI                    | 1 (1.37e-102|49|99) | 1 (0|50|98) | 1 (0|50|98) | 1 (1.37e-102|49|99) | 1 (0|50|98) | 1 (1.37e-102|49|99) | 1 (0|50|98) | 1 (0|50|98) |
|                           | flhP                    | 1 (1.71e-103|62|100) | 1 (1.71e-103|62|100) | 1 (1.71e-103|62|100) | 1 (1.71e-103|62|100) | 1 (1.71e-103|62|100) | 1 (1.71e-103|62|100) | 1 (1.71e-103|62|100) | 1 (1.71e-103|62|100) |
|                           | motA                    | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
| Nutritional virulence      | Pyrimidine biosynthesis | Undetermined | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
| Secretion system           | T6SS                    | undetermined | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
|                           | T6SS-1                  | Undetermined | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
|                           | T7SS                    | undetermined | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
| Serum resistance and immune evasion | LPS                     | wpbI | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
| Stress adaptation          | Catalase                | undetermined | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
|                           | Catalase-peroxidase     | undetermined | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) | 1 (0|50|98) |
R(140) residues in gyrB gene (Fig. 3). This may indicate partial resistance to novobiocin due to gyrB mutations.

Subunit-A of DNA gyrase (gyrA) may also define resistance to quinolones. According to a previous study, the mutations of two amino acid residues in gyrA, T(83) and D(87) are enough to gain resistance to a variety of quinolones [61]. The alignment of gyrA genes of five Arcobacter strains with its orthologs in Pseudomonas aeruginosa strain ATCC 27853, susceptible to quinolones, showed that residues T(83) and D(87) marked on P. aeruginosa sequence remain intact for most strains including A. butzleri (Fig. 3). On the other hand, A. lanthieri showed Serine at position 83 instead of Thrreonine, which still indicates susceptibility to quinolones [61]. As shown in previous studies, A. butzleri is susceptible to a high concentration of quinolones, much higher than A. lanthieri.

### Table 5

Toxin-antitoxin systems annotated with TAfinder in A. faecis AF1078\(^T\) and A. lanthieri AF1440\(^T\)

| Genome          | JGI Gene ID   | Product                                      | Toxin/Anti-toxin | Domain Annotation       |
|-----------------|---------------|----------------------------------------------|------------------|-------------------------|
| A. faecis AF1078\(^T\) | 2,690,352,548 | MFS transporter, DHA1 family, bicyclomycin/chloramphenicol resistance protein | Toxin            | pfam12568               |
|                 | 2,690,352,549 | transcriptional regulator, TetR family       | Anti-toxin       | pfam00440               |
|                 | 2,690,352,895 | HTH-type transcriptional regulator / antitoxin HigA | Antitoxin       | COG5499; Xre like domain |
|                 | 2,690,352,896 | mRNA interferase HigB                       | Toxin            | COG4680; relE like domain |
|                 | 2,690,353,381 | hypothetical protein                         | Anti-toxin       | cd00093                 |
|                 | 2,690,353,382 | serine/threonine-protein kinase HipA         | Toxin            | COG3550                 |
|                 | 2,690,353,395 | DNA-binding response regulator, the OmpR family, contains REC and winged-helix (wHTH) domain | Anti-toxin       | smart00862             |
|                 | 2,690,353,396 | hypothetical protein                         | Toxin            | TIGR03694               |
|                 | 2,690,353,532 | hypothetical protein                         | Toxin            | COG4891                 |
|                 | 2,690,353,533 | Transglutaminase-like superfAMILY protein    | Antitoxin        | pfam01047               |
|                 | 2,690,353,533 | Transglutaminase-like superfAMILY protein    | Toxin            | COG1246                 |
|                 | 2,690,354,744 | Putative activation module component         | Anti-toxin       | cd06171                 |
|                 | 2,690,354,745 | ParE toxin of type II toxin-antitoxin system, parDE | Toxin            | relE like domain        |
| A. lanthieri AF1440\(^T\) | 2,690,288,241 | transcriptional regulator, TetR family       | Antitoxin        | pfam00440               |
|                 | 2,690,288,242 | MFS transporter, DHA1 family, bicyclomycin/chloramphenicol resistance protein | Toxin            | pfam12568               |
|                 | 2,690,288,868 | Helix-turn-helix domain-containing protein    | Anti-toxin       | COG5606; Xre like domain |
|                 | 2,690,288,869 | serine/threonine-protein kinase HipA         | Toxin            | COG3550; HipA like domain |
|                 | 2,690,289,495 | AraC-type DNA-binding protein                | Anti-toxin       | Xre like domain         |
|                 | 2,690,289,496 | putative acetyltransferase                   | Toxin            | GNAT like domain        |
those determined by mutations in gyrA [15, 62]. Thus, it is suggested that RND transporters are the main contributors to quinolones resistance in \textit{Aliarcobacter} species, making Gyrase mutations less significant.

**Toxin-antitoxin (TA) systems**

TA system is a pair of genes encoding a toxin and its cognate anti-toxin, and it helps bacteria withstand lethal antibiotic exposure or environmental stresses [63]. We identified seven TA systems in \textit{A. faecis} and three in \textit{A. lanthieri} (Table 5). The TetR-type transcriptional regulator is located near a gene encoding a major facilitator superfamily (MFS) efflux transporter (Table 5), showing the resistance to disinfectants of quaternary ammonium compounds (QACs), including benzalkonium chloride (BAC) [64]. Also, hipBA TA systems are present in both species (Table 5). The hipB anti-toxin neutralizes the HipA toxin, a serine/threonine kinase inhibiting cell growth where hipBA modules are found in divergent bacterial genomes, and many are related to the persistence of antibiotic resistance [63].

In \textit{A. lanthieri}, the AraC-type DNA-binding protein, which regulates the expression of the proteins requiring the sugar L-arabinose, is adjacent to a putative acetyltransferase (Table 5) conserved in most environmental mycobacterial species, such as \textit{Mycobacterium smegmatis} [65].

On the other hand, in \textit{A. faecis}, the HigB/HigA TA system was found (Table 5), which regulates VFs pyochelin, pyocyanin, swarming, and biofilm formation in \textit{Pseudomonas aeruginosa} [66]. Besides, the ParDE TA system was also identified. This TA system helps bacteria resist heat and antibiotics [67]. We also found a TA system in \textit{A. faecis} related to the OmpR family DNA binding response regulator and a putative gene of acetyltransferase (Table 5). The OmpR protein was found to regulate the expression of a type III secretion system at the transcriptional level in Enterohemorrhagic \textit{E. coli} [68].

**General resistance**

Conservatively, general resistance factors are not VFs. They determine overall cell stability as part of the housekeeping processes. We identified five chaperone genes, \textit{clpA}, \textit{clpB}, \textit{groEL}, \textit{dnaK} and EF-Tu, as general resistance factors in \textit{A. faecis}, \textit{A. lanthieri}, and \textit{A. butzleri}, which previously showed a connection to bacterial virulence.

The main function of chaperones is protein folding, and it might determine cell resistance against abiotic stress [69]. In particular, genes \textit{clpA} and \textit{clpB}, encoding members of the Hsp100/Clp ATPases family in chaperones, were found necessarily required for intracellular multiplication and heat tolerance [70]. These chaperones, identified as a part of the Clp proteolytic complex, were first reported in \textit{E. coli} and later identified in other bacteria, such as \textit{Staphylococcus aureus} [70]. A study of \textit{C. jejuni} confirmed that \textit{clpB} acts in acid resistance and stomach transit [71].

In addition, \textit{dnaK} and \textit{groEL} are the significant heat shock genes, helping bacteria to overcome stressful environmental conditions, such as heat and acid environments [72]. Of these genes, \textit{dnaK}, encoding \textit{hsp70}, assists in the protein folding process through their substrate binding and ATPase domains [72, 73], while \textit{groEL}, encoding \textit{hsp60}, provides a protected cavity in a double heptameric ring structure for the folding of newly synthesized proteins [72].

Another general resistance VF is the elongation factor TU (EF-tu), the most abundant protein in bacterial cells [74]. EF-tu is a GTP-transferase that catalyzes the binding of aminoacyl-tRNA to the ribosome during the elongation stage of cell growth [75]. Current data shows that EF-tu can be inhibited by aminoglycoside antibiotics, which induces mistranslation and bacterial death [76].

**Validation of in silico identified virulence-related genes using PCR assays**

Furthermore, we validated the existence of 11 VAT genes, including six virulence (\textit{cadE}, \textit{ciaB}, \textit{irmA}, \textit{mvIN}, \textit{pldA}, and \textit{tlyA}), two antibiotic resistance (\textit{tet(O) and tet(W)}), and three cytolethal distending toxin (\textit{cdtA}, \textit{cdtB}, and \textit{cdtC}) genes, in \textit{A. faecis} and \textit{A. lanthieri} using species-specific PCR-based assays [38]. \textit{A. lanthieri} tested positive for all 11 VAT genes. By contrast, \textit{A. faecis} showed positive for ten genes except for \textit{cdtB} because no PCR assay for this gene was available for this species [38]. However, our comparative genomics analysis identified all three \textit{cdt} (\textit{cdtA}, \textit{cdtB}, and \textit{cdtC}) genes in the reference genomes of \textit{A. faecis} and \textit{A. lanthieri} strains. To validate our detection of the \textit{cdtB} in \textit{A. faecis} AF1078\textsuperscript{T} genome, we aligned the \textit{cdtB} gene of \textit{A. lanthieri} (UniProt ID: A0A2K9YS5C) against the protein sequences of \textit{A. faecis} strain AF1078\textsuperscript{T} using BLASTp and identified gene 2,690,353,140 as the \textit{cdtB} gene of \textit{A. faecis} (identity 78%; E-value = 0) (Supplementary Fig. S1). Similarly, \textit{Campylobacter} spp. also showed variable frequency of the \textit{cdt} genes [78].

Of the Cytolethal Distending Toxin encoded by the \textit{cdt} operon [77], \textit{cdtB} is the active subunit, while \textit{cdtA} and \textit{cdtC} work as two regulatory subunits that bind to \textit{cdtB} [77]. The presence of all three genes of the \textit{cdt} operon may indicate that the \textit{A. faecis} strain AF1078\textsuperscript{T} and \textit{A. lanthieri} strain AF1440\textsuperscript{T} could potentially be pathogenic; therefore, further in vitro research is warranted to investigate risk assessment analysis associated with human and animal health. In contrary to these results, studies
have shown the absence of the cdt genes in A. butzleri [15, 79].

In summary, the results of our PCR assays are in congruence with previous studies where a high frequency of cadd, ciaB, mviN, pldA, and thyA virulence genes was reported in A. butzleri and A. skirrowii strains [80, 81]. Similarly, tet(O) and tet(W) antibiotic resistance genes were also detected in both species, which has also been reported in A. cryaerophilus [15]. Our findings indicate that tetracycline resistance is prevalent in the genus Aliarcobacter.

Conclusion
This study provided insights into the virulence-related factors identified in the reference genomes of two new Aliarcobacter species, A. faecis and A. lanthieri, using whole genome sequencing, comparative genomics analysis, and qPCR validation. Our results generally showed genes encoding motility and export apparatus, secretory pathways, biostress resistance, and antimicrobial resistance were found in both A. faecis and A. lanthieri. However, unique genes were also identified for individual species. We acknowledge that further research in vitro and in vivo assays are required to evaluate the roles of virulence-related factors in the pathogenicity of A. faecis and A. lanthieri in human and animal infections.

Abbreviations
COG: Clusters of Orthologous Group; EF-tu: Elongation Factor TU; Tat: Twin-arginine translocation; NCBI: National Center for Biotechnology Information; QAC: Quaternary Ammonium Compound; PCR: Polymerase Chain Reaction; RND: Resistance Nodulation cell Division; TA: Toxin-antitoxin; UPGMA: Unweighted Pair Group Method with Arithmetic mean; VF: Virulence Factor; VFDB: Virulence Factor Database; VAT: Virulence, Antibiotic resistance and Toxin.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08663-w.

Additional file 1: Table S1. COG gene occurrence in Aliarcobacter spp.

Additional file 2: Figure S1. The amino acid alignment of the cdtB gene of A. faecis AF1078 (top) and A. lanthieri AF1440 (bottom).

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Authors’ contributions
JC and AB performed the comparative genomics analysis under WC’s supervision. JK and MC carried out qPCR tests. All co-authors drafted, reviewed, and edited the manuscript. All authors read and approved the final version of this manuscript.

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Availability of data and materials
The genome annotations generated during the current study are available in the JGI IMG/MER repository, https://img.jgi.doe.gov, under analysis ID Ga0136198 (Aliarcobacter faecis strain LMG 28519T), Ga0136182 (A. lanthieri strain LMG 28516T), and Ga0225945 (A. butzleri strain NCTC 12481T).

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Collado L, Figueras MJ. Taxonomy, epidemiology, and clinical relevance of the genus Arcobacter. Clin Microbiol Rev. 2011;24(1):174–92.
2. Perez-Cataluna A, Salas-Masso N, Dieguez AL, Balboa S, Lema A, Romalde JL, et al. Revisiting the taxonomy of the genus Arcobacter: getting order from the Chaos. Front Microbiol. 2018;9(2077):2077.
3. On SUW, Miller WG, Biggi PJ, Cornelius AJ, Vandamme P. A critical rebuttal of the proposed division of the genus Arcobacter into six genera using comparative genomic, phylogenetic, and phenotypic criteria. Syst Appl Microbiol. 2020;43(5):126108.
4. Alonso R, Girbau C, Martinez-Malaxetxebarria I, Perez-Cataluna A, Salas-Masso N, Romalde JL, et al. Aliarcobacter vittoriiens sp. nov., isolated from carrot and urban wastewater. Syst Appl Microbiol. 2020;43(4):126091.
5. Ferreira S, Queiroz JA,OLEASTRO M, Domingues FC. Insights in the pathogenesis and resistance of Arcobacter: a review. Crit Rev Microbiol. 2016;42(3):364–83.
6. Rice EW, Rodgers MR, Wesley IV, Johnson CH, Tanner SA. Isolation of Arcobacter butzleri from ground water. Lett Appl Microbiol. 1999;28(1):31–5.
7. Fera MT, Maugeri TL, Gugliandolo C, Beninati C, Giannone M, La Camera E, et al. Detection of Arcobacter spp. in the coastal environment of the Mediterranean Sea. Appl Environ Microbiol. 2004;70(5):1271–6.
8. Collado L, Guarro J, Figueras MJ. Prevalence of Arcobacter in meat and shellfish. J Food Prot. 2009;72(5):1102–6.
9. Houf K, Stephan R. Isolation and characterization of the emerging foodborn pathogen Arcobacter from human stool. J Microbiol Methods. 2007;68(2):408–13.
10. Miltenburg MG, Cloutier M, Craiovan E, Lapen DR, Wilkes G, Topp E, et al. Real-time quantitative PCR assay development and application for assessment of agricultural surfactant water and various fecal matter for prevalence of Aliarcobacter faecis and Aliarcobacter lanthieri. BMC Microbiol. 2020;20(1):1–13.
11. Roverto F, Carlier A, Van den Abeele AM, Illeghems K, Van Nieuwerburgh F, Cocobin L, et al. Characterization of the emerging zoonotic pathogen Arcobacter thermus by whole genome sequencing and comparative genomics. PLoS One. 2017;12(7):e0180493.
12. Hanel I, Muller E, Santamarina BG, Tomaso H, Kerkhof PJ, Kohn S, et al. Antimicrobial susceptibility and genomic analysis of Arcobacter cibarius and Arcobacter butzleri from food samples in Germany. Berl Munch Tierarztl Wochenschr. 2008;111(3–4):163–7.
13. Levican A, Alkeskas A, Günter C, Forothy SJ, Figueras MJ. Adherence to and invasion of human intestinal cells by Arcobacter species and their virulence genotypes. Appl Environ Microbiol. 2013;79(6):4951–7.
14. On SLW, Miller WG, Bansa P, Cornelius AJ, Vandamme P. Arcobacter, Haliarococcus, Malacicobacter, Pseudarcobacter and Poseidonibacter are later synonyms of Arcobacter: transfer of Poseidonibacter parus, Poseidonibacter antarcticus, Haliarococcus arenosus, and Arcobacter vitioriensis to Arcobacter as Arcobacter parus comb. nov., Arcobacter antarcticus comb. nov., Arcobacter arenosus comb. nov. and Arcobacter vitioriensis comb. nov. International Journal of systematic and evolutionary microbiology 2021;71(1):005133.
15. Miller WG, Parker CT, Rubenfield M, Mendz GL, Wosten MW, Ussery DW, et al. The complete genome sequence and analysis of the epsilonproteobacterium Arcobacter butzleri. PLoS One. 2007;2(12):e1358.
16. Toh H, Sharma VK, Oshima K, Kondo M, Ward FB, et al. Complete genome sequences of Arcobacter butzleri ED-1 and Arcobacter sp. strain L, both isolated from a microbial fuel cell. J Bacteriol. 2011;193(22):6411–2.
17. Menga Jr, Winstanley C, Williams NJ, Ye E, Miller WG. Complete genome sequence of the Arcobacter butzleri cattle isolate 7H1. Genome Announc. 2013;1(4).
18. Douda L, de Zutter L, Bare J, De Vos P, Vandamme P, Vandenberg O, et al. Occurrence of putative virulence genes in arcobacter species isolated from humans and animals. J Clin Microbiol. 2012;50(3):735–41.
19. Fujimoto-Nakamura M, Ito H, Oyamada Y, Nishino T, Yamagishi J-i. Accumulation of mutations in both gyrB and parE genes is associated with high-level resistance to novobiocin in Staphylococcus aureus. Antimicrob Agents Chemother. 2005;49(9):3810–5.
20. Vickers AA, Chopra I, O’Neill AJ. Intrinsic novobiocin resistance in staphylococcus saprophyticus. Antimicrob Agents Chemother. 2007;51(12):4484–5.
21. Fernandez-Garci A, Blasco L, Lopez M, Bou G, Garcia-Contrenas R, Wood T, et al. Toxin-antitoxin Systems in Clinical Pathogens (Basel). 2016;8;7.
22. Whiteduck-Leveillee K, Whiteduck-Leveillee J, Cloutier M, Tambong JT, Xu T, et al. Intrinsic novobiocin resistance in staphylococcus saprophyticus. Antimicrob Agents Chemother. 2007;51(12):4484–5.
23. Bankievich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455–77.
24. Medusa Github page [https://github.com/combogenomics/medusa/]
25. Bohter M, Pirovano W, Toward almost closed genomes with GapFiller. Genome Biol. 2012;13(6):R65.
26. Seemann T, Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9.
27. JGI IMG Integrated Microbial Genomes & Microbiomes [https://img.jgi.doe.gov/]
28. Ondov BD, Teangen TJ, Meldset P, Mallonee AB, Bergman NH, Koren S, et al. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol. 2016;17(1):132.
29. Yu G. Using ggtree to visualize data on tree-like structures. Curr Protoc Bioinformatics. 2020;69(1):e96.
30. Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res. 2012;40(7):e49–9.
31. Campache C, Coulot-Ferrat C, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+ architecture and applications. BMC Bioinform. 2009;10:421.
32. Liu B, Zheng D, Jiu Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res. 2019;47(D1):D687–92.
33. Cheifetti D, Fanelli F, Fusco V. Arcobacter butzleri: up-to-date taxonomy, ecology, and pathogenicity of an emerging pathogen. Compr Rev Food Sci Food Saf. 2020;19(4):2071–109.
34. Xie Y, Wei Y, Shen Y, Li X, Zhou H, Tai C, et al. TADB 2.0: an updated database of bacterial type II toxin-antitoxin loci. Nucleic Acids Res. 2018;46(11):D749–53.
35. Gu Z, Liu G, Eilts R, Schlesner M, Brox B. Circles implements and enhances circular visualization in R. Bioinformatics. 2014;30(19):2811–2.
36. Zambri M, Cloutier M, Adam Z, Lapen DR, Wilkes G, Sunohara M, et al. Novel virulence, antibiotic resistance and toxin gene specific PCR-based assays for rapid pathogenicity assessment of Arcobacter faecii and Arcobacter butzleri. BMC Microbiol. 2019;19(1):11.
37. Isidro J, Ferreira S, Pinto M, Domingues F, Oleario M, Gomes JP, et al. Virulence and antibiotic resistance placticity of Arcobacter butzleri: insights on the genomic diversity of an emerging human pathogen. Infect Genet Evol. 2020;80:104213.
38. Hako I, Westerlund-Wikstrom B. The role of the bacterial flagellum in adherence and virulence. Biology (Basel). 2013;2(4):1242–67.
39. Ottemann KM, Loventhal AC. Helicobacter pylori uses motility for initial colonization and to attain robust infection. Infect Immun. 2002;70(4):1984–00.
40. Abby SS, Rocha EPC. The non-flagellar type III secretion system evolved from the bacterial flagellum and diversified into host-cell adapted systems, 2012.
41. Konkel ME, Koen JD, Riviere-Amili V, Monteville MR, Biswas D, Raphael B, et al. Secretion of virulence proteins from Campylobacter jejuni is dependent on a functional flagellar export apparatus. J Bacteriol. 2004;186(11):3296–303.
42. Damski S, Magnet S, Davison S, Arthur M. Covalent attachment of proteins to peptidoglycan. FEMS Microbiol Rev. 2008;32(2):307–20.
43. Ruiz N. Bioinformatic analysis of virulence genes in helicobacter pylori using Motility. J Microbiol. 2004;13:1553–3.
44. Talukder KA, Aslam M, Islam Z, Azmi IU, Dutta DK, Hossain S, et al. Prevalence of virulence genes and cytolethal distending toxin production in Campylobacter jejuni isolates from diarrheal patients in Bangladesh. J Clin Microbiol. 2008;46(4):1485–8.
45. Han X, Guan X, Zeng H, Liu J, Huang X, Wen Y, et al. Prevalence, antimicrobial resistance profiles and virulence-associated genes of thermophilic Campylobacter spp. isolated from ducks in a Chinese slaughterhouse. Food Control. 2019;104:157–66.
46. Dorrell N, Martino MC, Stabler RA, Ward SJ, Zhang ZW, McColm AA, et al. Characterization of helicobacter pylori PhaA, a phospholipase with a role in colonization of the gastric mucosa. Gastroenterology. 1999;115:1598–104.
47. van der Meer-Janssen YP, van Gaalen J, Batenburg JJ, Helms JB. Lipids in host-pathogen interactions: pathogens exploit the complexity of the host lipidome. Prog Lipid Res. 2010;49(1):1–26.
48. Fanelli D, Di Pinto A, Mottola A, Mule G, Cheifetti D, Baruzzi F, et al. Genomic characterization of Arcobacter butzleri isolated from shellfish: novel insight into antibiotic resistance and virulence determinants. Front Microbiol. 2019;10:670:670.
49. Mey AR, Wyckoff EE, Kanukurthy V, Fisher CR, Payne SM. Iron and fur regulation of virulence and antibiotic resistance plasticity of Arcobacter butzleri. Infect Genet Evol. 2020;80:104213.
56. Girbau C, Guerra C, Martinez-Malaxetbarria I, Alonso R, Fernandez-Astorga A. Prevalence of ten putative virulence genes in the emerging foodborne pathogen Acrobacter isolated from food products. Food Microbiol. 2015;52:146–9.

57. Colelough AL, Alav J, Whittle EE, Pugh HL, Darby EM, Legood SW, et al. RND efflux pumps in gram-negative bacteria: regulation, structure and role in antibiotic resistance. Future Microbiol. 2020;15(2):143–57.

58. Ornkh-Cha A, Wilhelm J, Kobylika J, Sjuts H, Vargiu AV, Mallici G, et al. Structural and functional analysis of the promiscuous AcrB and AdeB efflux pumps suggests different drug binding mechanisms. Nat Commun. 2021;12(1):6919.

59. Verma P, Tiwari M, Tiwari V. Strategies to combat bacterial antimicrobial resistance: a focus on mechanism of the efflux pumps inhibitors. SN Comprehensive Clinical Medicine. 2021:1–18.

60. Dighe SN, Collot TA. Recent advances in DNA gyrase-targeted antimicrobial agents. Eur J Med Chem. 2020;199:112326.

61. Nakano M, Deguchi T, Kawamura T, Yasuda M, Kimura M, Okano Y, et al. Mutations in the gyrA and parC genes in fluoroquinolone-resistant clinical isolates of Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1997;41(10):2289–91.

62. Abdelbaqi K, Menard A, Prouzet-Mauleon V, Bringaud F, Lehours P, Megraud F. Nucleotide sequence of the gyrA gene of Acrobacter species and characterization of human ciprofloxacin-resistant clinical isolates. FEMS Immunol Med Microbiol. 2007;49(3):337–45.

63. Huang CY, Gonzalez-Lopez C, Henry C, Mijakovic I, Ryan KR. hspBA toxin-antitoxin systems mediate persistence in Caulobacter crescentus. Sci Rep. 2020;10(1):2865.

64. Chitrakaranwong J, Charoenlap N, Vanitshavit V, Sowtad A, Mongkolsum S, Vattanaviboon P. The role of MfsR, a TetR-type transcriptional regulator, in adaptive protection of Stenotrophomonas maltophilia against benzonikolnium chloride via the regulation of mfsQ. FEMS Microbiol Lett. 2021;368(15):fnab098.

65. Evangelopoulos D, Gupta A, Lack NA, Maitra A, ten Bokum AM, Kendall S, et al. Characterisation of a putative AraC transcriptional regulator from mycobacterium smegmatis. Tuberculosis (Edinb). 2014;94(6):664–71.

66. Wood TL, Wood TK. The HigB/HigA toxin/antitoxin system of Pseudomonas aeruginosa influences the virulence factors pyochelin, pyocyanin, and biofilm formation. Microbiologyopen. 2016;5(3):499–511.

67. Kamruzzaman M, Iredell J. A ParDE-family toxin antitoxin system in major resistance plasmids of Enterobacteriaceae confers antibiotic and heat tolerance. Sci Rep. 2019;9(1):9872.

68. Wang S-T, Kuo C-J, Huang C-W, Lee T-M, Chen J-W, Chen C-S. OmpR coordinates the expression of virulence factors of Enterohemorrhagic Escherichia coli in the alimentary tract of Caenorhabditis elegans. Mol Microbiol. 2021;116(1):168–83.

69. Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci. 2005;62(6):670–84.

70. Frees D, Chastanet A, Qazi S, Sorensen K, Hill P, Msadek T, et al. Clp ATPases and Aminoacyl-tRNA. Science. 2009;326(5953):688–94.

71. Reid AN, Pandey R, Palyada K, Naikare H, Stintzi A. Identification of Campylobacter jejuni genes involved in the response to acidic pH and stomach transit. Appl Environ Microbiol. 2008;74(5):1583–97.

72. Neckers L, Tatu U. Molecular chaperones in pathogen virulence: emerging new targets for therapy. Cell Host Microbe. 2008;4(6):519–27.

73. Harrison CJ, Hayer-Hartl M, Di Liberto M, Hartl F, Kuriyan J. Crystal structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK. Science. 1997;276(5311):431–5.

74. Smiline Girja AS, Ganesh PS. Virulence of Acinetobacter baumannii in proteins moonlighting. Arch Microbiol. 2021;204(1):96.

75. Schmeing TM, Voorhees Rebecca M, Kelley Ann C, Gao Y-G, Murphy Frank V, Weir John R, et al. The crystal structure of the ribosome bound to EF-Tu and Aminoacyl-tRNA. Science. 2009;326(5953):688–94.

76. Wohlgemuth I, Garofalo R, Samatova E, Günenç AN, Lenz C, Urlaub H, et al. Translation error clusters induced by aminoglycoside antibiotics. Nat Commun. 2021;12(1):1830.

77. Purdy D, Buswell CM, Hodgson AE, McAlpine K, Henderson I, Leach SA. Characterisation of cytolethal distending toxin (CDT) mutants of Campylobacter jejuni. J Med Microbiol. 2000;49(5):473–9.

78. Karadas G, Sharbati S, Hänel I, Messelhäuser U, Glocker E, Alter T, et al. Presence of virulence genes, adhesion and invasion of a rocbacter but- zleri. J Appl Microbiol. 2013;115(2):583–90.

79. Tabataeabi M, Askì HS, Shayegh H, Khoshbakht R. Occurrence of six virulence-associated genes in Acrobacter species isolated from various sources in shiraz, Southern Iran. Microbial Pathogenesis. 2014;66:1–4.

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