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Aureimonas altamirensis: First Isolation from a Chicken Slaughterhouse in Italy Followed by Genotype and Phenotype Evaluations

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Abstract: The presence of foodborne pathogens in meat is linked to several contamination sources, and the slaughterhouse environment represents a relevant reservoir of contamination. *Aureimonas altamirensis* is a Gram-negative bacteria associated with different isolation sources, including human clinical cases. This study aims to identify and characterize an *A. altamirensis* isolate from chicken guts collected in an Italian slaughterhouse. The study approach includes whole-genome analysis jointly with phenotypical tests. Whole-genome sequencing (WGS) confirms the initial MALDI-TOF MS identification, finding putative virulence and biofilm-related genes. Moreover, the gene class evaluation reveals that the numerically largest gene category in the *A. altamirensis* genome is related to amino acid metabolism and transport. The analyses performed on a human gut mucus-producing cell line (HT29-MTX-E12) demonstrated the ability of *A. altamirensis* to colonize the host cell layer. Moreover, the antibiotic resistance test showed a high resistance of *A. altamirensis* to gentamicin (MIC 0.5 mg/L). The detection of a potential pathogenic and antibiotic-resistant *A. altamirensis* strain isolated from a slaughterhouse underlines the necessity of active surveillance studies focused on this species and the need for further studies about *A. altamirensis* in foods.

Keywords: antibiotic resistance; food safety; cell lines; biofilm; genomics; bacteria; abattoir; poultry; whole-genome sequencing

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1. Introduction

Foodborne pathogens are of great concern for human health [1], and meat is one of the principal foods subjected to contamination [1]. Among pathogenic bacteria, many Gram-negative species are isolated from meat [1]. *Campylobacter jejuni*, *Campylobacter coli*, and *Salmonella enterica* are often isolated from poultry meat [2]. The slaughtering process represents a possible point of bacterial contamination; for this reason, methods of prevention, disinfection, and control must be followed to reduce the risk of meat contamination by pathogenic species [2,3]. The presence of antibiotic-resistant (AR) strains increases the level of risk related to their presence in meat [4,5]. This is linked to a risk to human health due to antibiotic ineffectiveness and possible transmission of genes related to antibiotic resistance between bacteria [6]. These aspects underline the necessity of active slaughterhouse surveillance [7]. Meat contamination during slaughtering is linked to the presence of bacterial contaminants in the chicken's gastrointestinal tract (GIT), on processing surfaces, and in the environment [8]. Among animal products, poultry meat is considered a vector of human bacterial pathogens [9].

Aureimonas (formerly *Aurantimonas*) *altamirensis* is considered a potential pathogen associated with human illness [10–14], but not yet well considered in the food sector as a foodborne pathogen. This Gram-negative bacterium is included in the *Aurantimonadaceae*

family [15] and was isolated for the first time from the Altamira caves in Spain [16]; later, it was found in plants, like ash tree [17] and rice [18], and from a swollen testicle of a dog [19], while in humans, *A. altamirensis* was isolated in patients with a dendritic corneal ulcer with perforation, keratitis [20], bacteriemia [12], and a biliary infection [14] and from pleural effusion [13], wound culture [21], and skin [22]. The *A. altamirensis* strains are normally antibiotic-susceptible [10,14]; however, resistance was observed against erythromycin, clindamycin [19], gentamicin, and tobramycin [23].

This study reports the detection and characterization of an *A. altamirensis* strain isolated for the first time in a chicken slaughterhouse in Northern Italy. *A. altamirensis* was isolated during a wider sampling procedure focused on *Arcobacter* spp. following the isolation procedure designed by Houf and colleagues [24]. The study was focused on *Arcobacter* spp. (whose name *Aliarcobacter* spp. has recently been questioned) [25], considering its importance as a foodborne pathogen present in poultry meat [26]. The isolation of *A. altamirensis* inside the slaughterhouse caught the interest of the authors, and an in-depth analysis of this microorganism, through genomic and physiological characterizations, was performed. *A. altamirensis* virulence, antibiotic resistance, biofilm production, and genomic features were investigated to evaluate possible hazards related to its presence in food processing plants. Moreover, to study its in vitro pathogenic potential, the ability of *A. altamirensis* to colonize a human mucus-producing cell layer was evaluated. The physiological evaluation was performed jointly with the whole-genome evaluation to detect gene class composition and the presence of genes related to virulence and antibiotic resistance. Although *A. altamirensis* is not currently considered a foodborne pathogen, the characterization of a strain detected in a slaughterhouse environment is important to increase the knowledge related to its presence in a new context other than those contexts already mentioned in the literature.

The aim of this work is therefore the obtainment of genomic and physiological data of *A. altamirensis* as a potential meat contaminant isolated from a meat processing plant to investigate its potential hazard to food safety.

2. Materials and Methods

2.1. Isolation and Phenotypical Characterization

Sample collection for each flock was performed from the caecum of ten Ross 308 chickens (*Gallus gallus domesticus*) of six flocks. The chickens of the sample positive for *A. altamirensis* were slaughtered at the age of 47 days at an average weight of 2.9 kg. Farm and slaughterhouse were in Northern Italy, and the positive flocks were 40 km from the slaughterhouse. The chickens of the other five flocks were slaughtered at the age of 50 (three flocks), 54 (one flock), and 47 (one flock) days at an average weight of 3.44 kg ($\pm$0.22). This sample group is part of a larger sampling campaign (49 flocks) focused on the study of *Arcobacter* spp.

The gut samples collected after evisceration were processed at the laboratory of the Department of Agricultural, Forest and Food Sciences (DISAFA) within 2 h from the sampling following the isolation methods designed for *Arcobacter* spp. by Houf and colleagues [24]. One gram from each gut (ten guts per sample) was pooled to obtain a homogenous sample from different animals and intestinal tracts. The final 10 g was placed in 100 mL of *Arcobacter* broth (Oxoid, United Kingdom, CM0965) supplemented with 5% v/v laked horse blood (Microbiol s.r.l., Macchiareddu, Italy, 17.0156) and 16 mg/L cefoperazone (Merck, St. Louis, MO, USA, C4292), 10 mg/L amphotericin B (Merck, A2411), 100 mg/L 5-fluorouracil (Merck, F6627), 32 mg/L novobiocin sodium salt (Merck, 74675), and 64 mg/L trimethoprim (Merck, T7883) and homogenized for 2 min (VWR; Radnor, PA, USA, 129-0734). The enrichment broth was incubated for 48 h at 28 °C in microaerophilic conditions (Thermo Fisher Scientific, Waltam, MA, USA, 10729393). Ten microliters of the enrichment broth was placed on *Arcobacter* agar supplemented as the enrichment broth with and without 10% v/v of laked horse blood. These plates were incubated for 96 h at 28 °C in microaerophilic conditions. The bacterial cultures necessary for subsequent

experiments were prepared by placing a single colony in 1 mL of *Arcobacter* broth for 24 h at 30 °C and proceeding to inoculate 100 µL of bacterial suspension in 1 mL of *Arcobacter* broth incubated overnight at 30 °C (normal atmosphere).

An oxidase test was performed on the isolates placing a colony on an oxidase strip test (Sigma-Aldrich; St. Louis, MO, USA, 40560-100STRIPS-F). The catalase activity was tested on a single colony placed in hydrogen peroxide (Sigma-Aldrich; H1009). The sugar fermentation of *A. altamirensis* was evaluated in Phenol Red Broth Base with 10% *w*/*v* of maltose and glucose (Liofilchem, Roseto degli Abruzzi, Italy, 610174). The isolate was cultivated on MacConkey agar (MAC) (Merck, USA, M7408) to evaluate lactose fermentation ability.

2.2. DNA Extraction and Genome Sequencing

The genomic DNA (gDNA) extraction was performed following the Epicentre MasterPure™ kit method. A single fresh colony was placed in 3 mL of BHI broth (VWR, 84626.0500) and incubated at 30 °C for 48 h. The bacterial pellet was obtained from 1 mL of the bacterial suspension through centrifugation (16,000 rcf for 10 min at 4 °C; Eppendorf Centrifuge 5415R) and subjected to DNA extraction. The samples were treated with RNAse A (Thermo Scientific, EN0531) for 30 min at 37 °C.

The DNA quality check was performed with an electrophoretic run of 30 min on 0.8% agarose gel (VWR, 0710-500G) at 100 V. The DNA quality was evaluated by Nanodrop spectrophotometry (Thermo Scientific, Nanodrop ND-1000 spectrophotometer, 2353-30-0010). The DNA quantification was performed with Qubit 3.0 fluorometer (Invitrogen, Waltam, MA, USA, 15387293).

The Illumina whole-genome sequencing was performed by the company Novogene (Cambridge, UK). The genomic DNA was randomly fragmented into short fragments, end-repaired, A-tailed, and ligated with Illumina adapter (NEBNext® library prep Kit). The fragments with adapters were PCR-amplified, size-selected, and purified. The library was checked with Qubit and real-time PCR for quantification. A bioanalyzer analysis (Agilent 2100 Bioanalyzer) was performed to check the size distribution. Quantified library was pooled and sequenced on the Illumina platform Novaseq6000 in paired-end 150.

2.3. Identification by MALDI-TOF MS and 16S rDNA Analysis

The Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) analysis was performed with the instrument Microflex (Bruker, Billerica, MA, USA). The colonies selected for their morphology were placed on a Micro Scout Plate spot MSP 96 to which 1 µL of matrix α-Cyano-4-hydroxycinnamic acid (CHCA; bioMérieux, C8982) was added. After the matrix crystallization on the samples, the plate was read at MALDI-TOF MS comparing the spectra obtained with those present in the instrument database. Identifications with a score higher than 1.9 were considered reliable.

The MALDI-TOF MS bacterial identification was confirmed by species-specific PCR of 16S rDNA amplification [27]. The PCR was performed with the following reagents at the final concentrations of 1.5 mM of $MgCl_2$ (Sigma-Aldrich; DNTP100), 0.2 µM of primer FD1 (5′-AGAGTTTGATCCTGGCTCAG–3′) and RD1 (5′-AAGGAGGTGATCCAGCC-3′), 0.5 units of Taq polymerase (Sigma-Aldrich; D4184), 0.2 mM of deoxyribonucleotides, and buffer 1X (Sigma-Aldrich; P8317-1) filled with water for molecular biology (Sartorius; Goettingen, Germany, 01-869-1B) to a final volume of 25 µL. The PCR products were sequenced by Sanger sequencing by Genewiz company (https://www.genewiz.com/en-GB/, accessed on 4 September 2023). The PCR products were uploaded on BLASTn to identify the isolate.

2.4. Bioinformatic Analysis

The raw reads quality was checked with FastQC (https://github.com/s-andrews/FastQC, accessed on 4 September 2023). The sequence assembly was performed on BV-BRC vr. 3.28.9 (https://www.bv-brc.org/, accessed on 4 September 2023) using SPAdes (v. 3.13.0) and enabling sequence trimming performed by Trim Galore [28,29]. The software

QUAST vr. 5.0.2 was used to obtain information about contigs [30]. The whole-genome identification was performed using the bacterial Similar Genome Finder Service of BV-BRC vr. 3.28.9 suite [28] and LINbase [31]. The functional annotation was performed with Prokka vr. 1.14.5 [32], RAST tool kit (RASTtk) on BV-BRC vr. 3.28.9 [33], Dfast vr. 1.2.18 [34], and Emapper vr. 2.1.9 [35]. The 16S rDNA was compared to sequences present in NCBI using Mega vr. 11.0.13 (unweighted pair group method with arithmetic mean, UPGMA, bootstrap 1000) to obtain a phylogenetic tree [36] visualized on iTol vr. 6.6 [37]. The clustered regularly interspaced short palindromic repeat (CRISPR) sequences were detected using CRISPR-Cas finder vr. 1.1.2 [38], while prophagic sequences and antibiotic resistance genes were detected with Phaster (https://phaster.ca/, accessed on 4 September 2023) [39] and RGI 6.0.1 (Comprehensive Antibiotic Resistance Database, https://card.mcmaster.ca/analyze/rgi, accessed on 4 September 2023), respectively [40].

2.5. Antibiotic Resistance Evaluation

Antibiotic resistance was evaluated against gentamicin (Biowest; P4020-5GR), erythromycin (Sigma-Aldrich; E5389-1G), ampicillin sodium salt (Sigma-Aldrich; A9518-5G), amoxicillin (Sigma-Aldrich; A8523-5G), and amoxicillin trihydrate:potassium clavulanate (4:1) (Sigma-Aldrich; SMB00607-1G). The antibiotics were tested at the minimum inhibitory concentrations (MICs; 1X) and double (2X) and triple (3X) MICs indicated on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Non-Species Breakpoint tables vr. 12.0. Growth in broth with concentrations above the indicated values was considered as resistance to the corresponding antibiotic and concentration.

The test was performed in 96-well microplates (Enrico Bruno; 05327393) inoculating 5 µL of bacterial suspension standardized to 0.5 McFarland (Remel, San Diego, CA, USA; R20410) in 195 µL of Muller–Hinton (Biomaxima, Lublin, Poland; PS15). The microplates were incubated at 30 °C for 48 h. In the case of turbidity, the strain was considered resistant to the corresponding antibiotic and concentration. The antibiotic resistance test was performed in three biological replicates.

2.6. Biofilm Production Evaluation

The biofilm formation index (BFI) was evaluated following the methods performed by Teh and colleagues with some modifications [41]. An inoculum of 5 µL (optical density 0.2; 630 nm) was placed in 195 µL of *Arcobacter* broth in a polystyrene microplate well (Enrico Bruno; 05327393) and incubated at 30 °C for 48 h. The optical density was measured at 630 nm (Bioteck; Synergy HT, Winooski, VT, USA), and the well was washed with deionized water two times and let dry at 42 °C for 30 min. This washing step was followed by the addition of 200 µL of crystal violet 1% v/v in water (Sigma-Aldrich; C6158) and the incubation at room temperature for 15 min. The crystal violet was eliminated, and the microplate was washed three times with deionized water eliminated by drying at 42 °C for 30 min. To evaluate the crystal violet persistent in the biofilm, 200 µL of ethanol 98% (Supelco, Bellefonte, PA, USA; 1009831011) was added to the well, transferred in a new microplate, and measured at 595 nm. The BFI was calculated as follows: BFI = (optical density of the stained-attached microorganisms/-optical density of the stained wells without bacteria)/optical density of the bacteria in *Arcobacter* broth. The BFI test was performed in three biological replicates. *Listeria monocytogenes* EGD-e was used as a control.

2.7. Evaluation of A. altamirensis Colonization on Human Cells

The cell model preparation and colonization tests were performed in three biological replicates following a protocol used to study *Arcobacter butzleri* with some modifications [42,43]. A human colon carcinoma cell line HT29-MTX-E12 (12040401; ECACC) was cultured in Dulbecco's Modified Eagle Medium (DMEM, 6429; Sigma-Aldrich, St. Louis, MO, USA) with 10% v/v FBS (F7524; Sigma-Aldrich) and EmbryoMax penicillin–streptomycin (TMS-AB2-C; Sigma-Aldrich). The cell line was grown in flasks (Corning, New York, NY, USA) at 37 °C in a humidified atmosphere (5% CO_2), subpassaged every

3 days (Galaxy 170 S; Eppendorf, Hamburg, Germany). The cells were seeded at a density of 35,000 cells/cm^2 on 1.93 cm^2. The cells were grown in a complete culture medium under the same conditions as described above for 14 to 15 days. Three days before the bacterial inoculum, the monolayer was washed twice with phosphate-buffered saline (PBS). After the washing step, the culture medium was replaced with the same medium without antibiotics.

The colonization test was performed by replacing 0.25 mL of DMEM in the model well with 0.25 mL of bacterial suspension normalized to an optical density of 0.1 (630 nm) in DMEM (average initial inoculum Log_{10}, 7.64 CFU/mL, standard. Dev. 0.22). After 90 minutes, the cell models incubated at 37 °C were washed twice with PBS. The bacterial colonization ability (bacterial present in the cell models after washing removal) was evaluated by treating the cell layer with 0.5 mL of Triton X-100 0.25% (*v*/*v*, in PBS). The suspension was analyzed after 30 min of incubation at 37 °C to determine the colonizing bacterial load diluting the samples in Ringer's solution and plating them on *Arcobacter* agar medium. The plates were incubated at 30 °C for 48 h under microaerobic conditions before the colony count. Bacterial colonization was expressed as bacterial load after washing the cell layer minus the load in the initial inoculum. *L. monocytogenes* EGD-e was used as a control.

2.8. Statistical Analysis

RStudio 2022.07.2+576 (R version 4.2.1) was used to perform statistical analysis. Homogeneity tests were performed using Shapiro–Wilk's W and Modified Levene's tests (Brown–Forsythe test). Wilcoxon rank sum test (WRS) and two-sample *t*-test were performed to evaluate differences between two groups, for nonparametric (WRS) and parametric data (*t*-test). The dendrograms were graphically curated using iTol software (https://itol.embl.de/; accessed on 13 May 2023) [37].

3. Results and Discussion

3.1. A. altamirensis Isolation, Identification, and Characterization

The *Arcobacter* media supplemented with antimicrobials typically added for the isolation of *Arcobacter* spp. [24] allowed the isolation of rounded yellowish colonies resulting in a catalase and oxidase-positive bacterium, not able to ferment lactose, glucose, and maltose. After Gram staining, the isolate was observed under the optical microscope showing Gram-negative bacilli (Figure 1). The MALDI-TOF MS analysis identified the isolate as *A. altamirensis* (score 1.9), and this identification was confirmed by 16S rDNA (BLASTn) and whole-genome sequencing comparisons with an average nucleotide identity (ANI) similarity score of 97.6% with *A. altamirensis* ON-56566. The MALDI-TOF MS analysis allowed a fast identification confirmed by PCR and whole-genome analysis, underlying the importance of this method for the identification of *A. altamirensis*. This is made even more evident by the identification of this species via MALDI-TOF MS reported in the literature [14,17,21]. At the same time, *A. butzleri*, identified by MALDI-TOF and 16S rDNA, was isolated from the same sample from which *A. altamirensis* was isolated. The isolation of *A. butzleri* and *A. altamirensis* demonstrates their coexistence in chicken guts after slaughtering. However, the obtainment of a single *A. altamirensis* strain can be associated with the absence of a specific isolation procedure, as the isolation of this bacterium is performed using several nonselective media [10,13,16,19]. The whole-genome analysis (Supplementary Table S1) of the strain isolated in this work (strain AAI) showed a genome of 4.1 Mb with a GC percentage of 64.99%, 4041 coding DNA sequences (CDS), and 3 16S rDNA, 1 23S rRNA, and 47 tRNA demonstrating characteristics comparable to other isolates of the same species [17]. The dendrogram tree (Figure 2) showed a high similarity between AAI and two *A. altamirensis* strains isolated from the oxygen minimum zone (OMZ) water column and pleural fluid showing the absence of similarity of AAI with isolates from a specific matrix. However, the low oxygen concentration used during the isolation procedure of AAI and present in the chicken gut can be related to the lack of oxygen in the OMZ indicating a similarity between strains from low O_2 availability conditions. The absence of 16S rRNA

and genomes related to *A. altamirensis* strains isolated from poultry does not allow the evaluation of its contamination route.

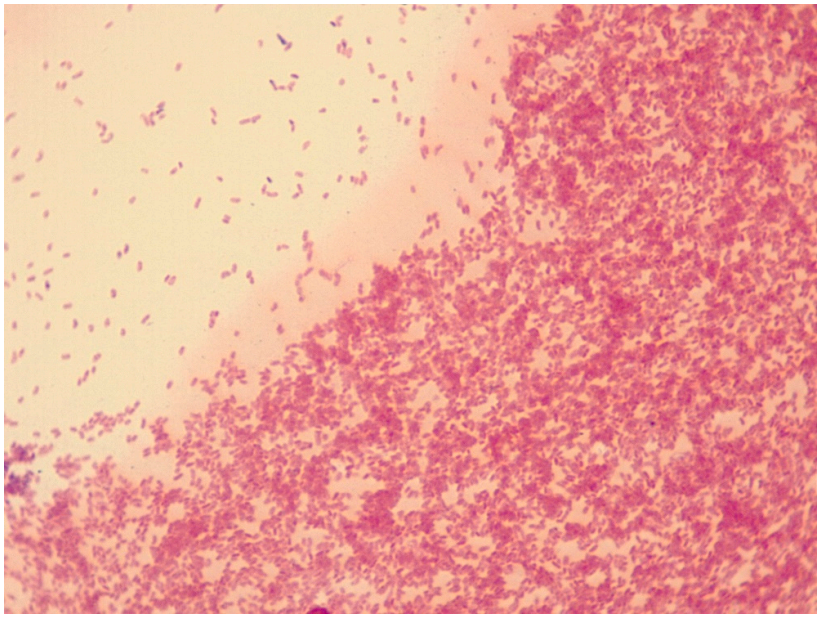

Figure 1. Bacterial cells of *A. altamirensis* AAI. The figure shows Gram-negative bacterial cells under optical microscope (100X).

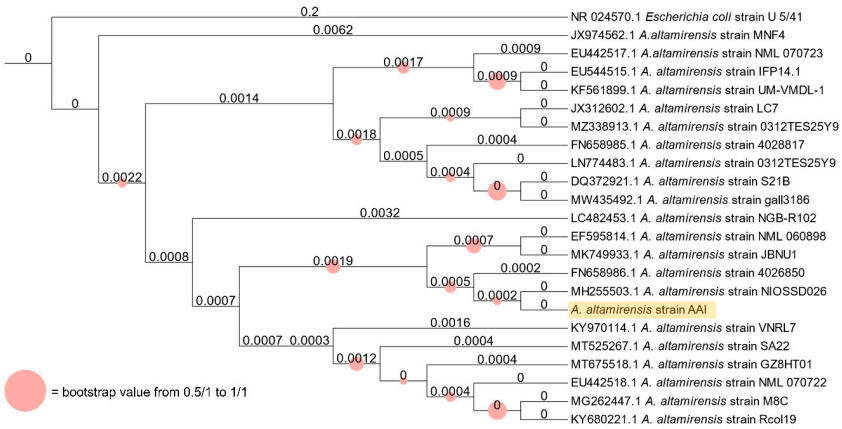

Figure 2. 16S rDNA UPGMA tree. The UPGMA dendrogram shows the similarities between twenty *A. altamirensis* 16S rDNA sequences available on NCBI (https://www.ncbi.nlm.nih.gov/; accessed on 4 April 2023) and the strain object of study (AAI). The bootstrap values are indicated by pink circles on the tree branches, and values below 0.5/1 (bootstrap 1000) are not included. The distance values are indicated on the branches. The sequences included are related to *A. altamirensis* strains isolated from the environment (Altamira caves), air samples, and human (blood, bile, corneal ulcer, sputum from cystic fibrosis patient, contact lens solution from a patient with keratitis, and pleural fluid), sunflower (endorhizosphere), dog, soil, and surface samples from MARS 500 facility, *Rhodnius colombiensis* (midgut), *Leptocybe invasa*, *Triticum aestivum* (rhizosphere), fecal pellet in the gut of scallop, and OMZ water column. *Escherichia coli* DSM30083 was included as an outgroup.

3.2. Human Cell Colonization, BFI, and Antibiotic Resistance of A. altamirensis AAI

The *A. altamirensis* strain demonstrated the ability to colonize the human gut cell layer in vitro (Figure 3A). This aspect suggests the ability of *A. altamirensis* AAI to colonize the gut epithelium in the presence of mucus colonizing this barrier. The in vitro colonization of the cell layer in the presence of mucus is an aspect already observed for other Gram-negative bacteria, like *C. jejuni*, Helicobacter pylori [44], and *A. butzleri* [42,44]. The strain

AAI showed a decrease from the initial inoculum of Log_{10} 1.55 CFU/mL (st. err., 0.17), demonstrating a lower colonization compared to *L. monocytogenes* EGD-e that showed a bacterial load increase of Log_{10} 0.20 CFU/mL (st. err., 0.56, t = −2.98, *p*-value < 0.05) (Figure 3A). Although *A. altamirensis* showed a lower colonization compared to the well-considered foodborne pathogen *L. monocytogenes*, its in vitro colonization against human gut cells suggests a possible risk in the case of the assumption of contaminated food or contact with contaminated material (e.g., work surfaces). Even considering that at the moment *A. altamirensis* is not considered a foodborne pathogen, the risk to human health is more evident considering the isolation of this bacterium from blood cultures [15], bacteriemia [12], and pleural empyema cases [11].

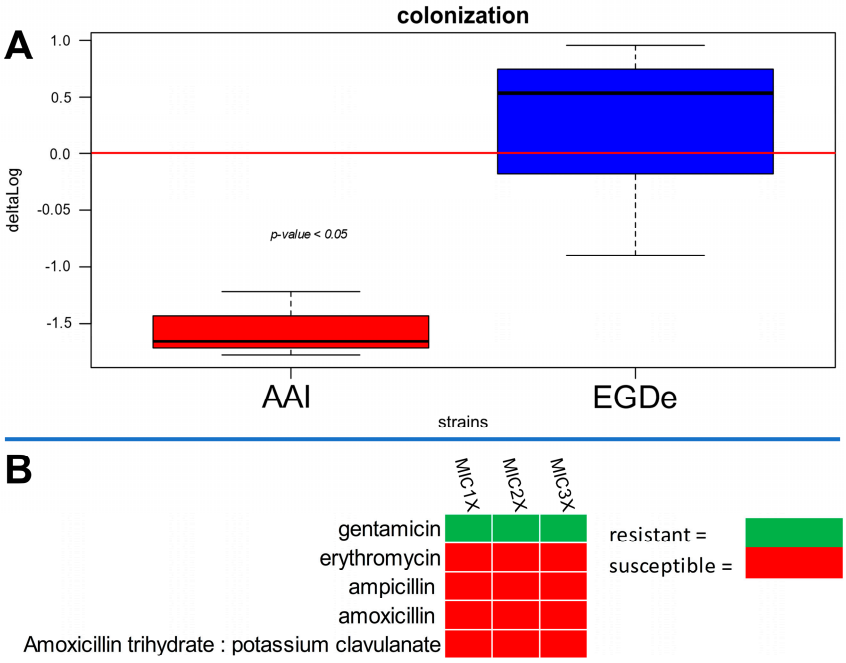

Figure 3. Cell colonization and antibiotic resistance test. The box plot (**A**) shows data about *A. altamirensis* AAI (red box) and *L. monocytogenes* EGDe (blue box) colonization on a HT29−MTX−E12 gut cell layer after 90 min from the inoculum. The red line shows a delta log of 0, corresponding to a stable colonized bacterial load if compared to the initial inoculum (colonization = bacterial load after washing steps (90 min of in vitro infection)—bacterial load of inoculum). The lower colonization ability of *A. altamirensis* (*p*−value < 0.05) when compared to *L. monocytogenes* is evident from the shift of the blue box toward positive values. Positive delta log values indicate an increase in bacterial load compared to the initial inoculum (growth). The antibiotic resistance (green) and susceptibility (red) results for five antibiotics and three concentrations are shown in panel (**B**).

A. altamirensis AAI did not show biofilm production (BFI = 0.0; st. err., 0.01), indicating the absence of surface adhesion and biofilm production on polystyrene. However, *A. altamirensis* biofilm production can be related to specific environmental conditions and surface materials as genes associated with biofilm production were detected (Section 3.3). The antibiotic resistance tests performed using five antibiotics (gentamicin, erythromycin, ampicillin sodium salt, amoxicillin, and amoxicillin trihydrate:potassium clavulanate) demonstrated that *A. altamirensis* AAI was resistant to gentamicin at the maximum concentration tested (MIX 3X; 1.5 mg/L), while it was susceptible to the other antibiotics at MIC 1X (Figure 3B). The resistance of *A. altamirensis* to gentamicin was already observed in an isolate from ascites fluid [23]. The gentamicin resistance can be related to efflux pumps and to ribosomal mutations that can confer resistance to aminoglycosides [45]. The resistance to gentamicin together with the ability to colonize the intestinal epithelium in vitro increases the relevance of the study regarding this bacterium. The antibiotic resistance of

AAI suggests a risk in the case of ingestion and potential transfer of antibiotic resistance genes to host gut microbiota [46].

3.3. Gene Enrichment and Virulence—Antibiotic Resistance Gene Traits

The gene enrichment analysis demonstrated that the most numerous gene classes detected in the *A. altamirensis* genome are related to amino acid transport and metabolism (409 genes). However, most of the genes have not been classified, and these genes have been placed in the category "unknown function" (Figure 4). The high number of genes related to amino acid transport and metabolism is an aspect observed in other Gram-negative bacteria, like *Arcobacter* spp. [42,47]. The similar gene class composition between *A. altamirensis* and *Arcobacter* spp. is not surprising considering the growth ability of AAI on *Arcobacter* media normally used to isolate *Arcobacter* spp.

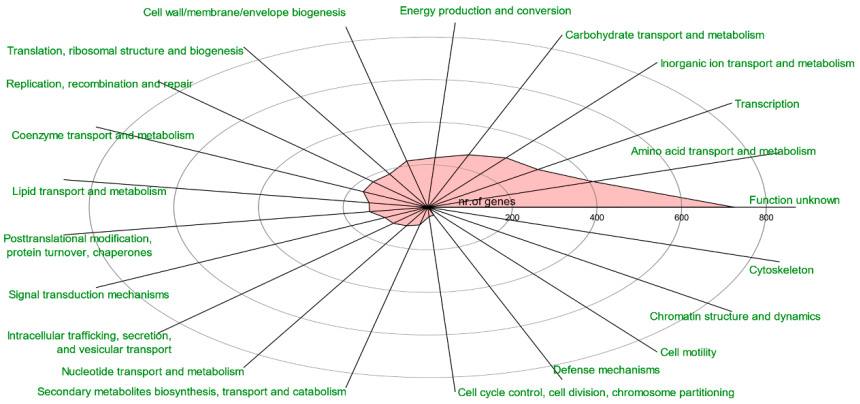

Figure 4. Genes class composition in AAI. The polar plot shows the number of genes related to different gene classes. The results show a high number of sequences with unknown functions followed by amino acid transport- and metabolism-related genes.

The analysis of antibiotic resistance genes (ARGs) revealed the presence of *adeF* (resistance-nodulation-cell division (RND) antibiotic efflux pump) and *tetR* (Tet Repressor protein). As stated, the efflux pump can be related to gentamicin resistance [45]. However, the CARD tool did not allow the detection of other ARGs. Although AAI was resistant only to gentamicin, the detection of a few ARGs suggests the presence of other genes related to antibiotic resistance not present in the database.

Phagic sequence detection showed two phage sequences in the AAI genome and did not contain CRISPR-CAS sequences, suggesting the absence of this immune defense mechanism [48] or the presence of sequence variants not included in the database and not detectable through comparison. Moreover, the absence of CRISPR-CAS sequences in animal-related bacteria was already observed in other Gram-negative bacteria, like *C. jejuni* [49] and *Arcobacter* spp. [25].

The absence of virulence-related genes was observed by Becker and colleagues in the genomes of three *A. altamirensis* strains (C2P003, DSM 21988, and ON-56566), while biofilm-related genes were detected [17]. In the AAI genome, the genes *tonB* and *exbB*, *exbD*, and *tolB* (TonB-related functions) are present. The protein TonB has been associated with virulence functions and host colonization in Gram-negative pathogens, like *Escherichia coli* and *A. butzleri* [43,50]. Even if the detection of other virulence genes was not possible, similarly to other *A. altamirensis* [17], the presence of *tonB* and related genes agrees with its in vitro colonization ability. The presence of biofilm-related genes in the *A. altamirensis* genome [17] was confirmed in AAI that presents twelve *exo* (P, O, M, A, L, K, W, U, Y, F, U, Q, and a repressor) genes. AAI did not demonstrate the ability to produce biofilms; however, the presence of *exo* genes suggests the potential production of biofilm under conditions that remain to be investigated. The *exo* genes can be expressed only under specific environmental stimuli [51] leading to biofilm production. Biofilm can increase

surface adhesion influenced by different types of materials [52]. The biofilm production of *A. altamirensis* on the materials present in the slaughterhouse cannot be excluded.

4. Conclusions

MALDI-TOF MS is a useful instrument for rapid bacterial identification [53], whose efficacy was also confirmed in the case of *A. altamirensis* AAI isolation. The presence of *A. altamirensis* along the slaughterhouse production chain suggests the possible risk of meat cross-contamination linked to its presence in meat processing plants [2,3]. The in vitro colonization ability of *A. altamirensis* leads to focus attention on this bacterium, which can potentially cause human infections. The reported infections in humans and animals support the virulence potential of this bacterial species [10–14], with the risk being increased by AAI antibiotic resistance and the presence of putative virulence genes. The small number of genomes in the public databases and strains in open collections do not allow a pangenome study of the *A. altamirensis* species. Moreover, a high number of genes were not identified. These aspects suggest the necessity of future studies about pangenome and functional genome annotation related to this bacterium to link genomic information to physiological characteristics, including antibiotic resistance, in vitro pathogenicity, and biofilm formation index evaluation. The isolation and characterization of *A. altamirensis* are important in a "one health" context for detecting a potentially pathogenic species in a meat-handling environment [54], including the determination of antibiotic resistance [5]. Future studies are needed to investigate the presence of *A. altamirensis* in other sources by isolating strains with different characteristics and isolation sources to perform a pangenome analysis and evaluating its possible role as a foodborne pathogen. The isolation of AAI from a slaughterhouse and the analysis of its virulence potential in vitro and in silico led to emphasize the necessity of new studies about *A. altamirensis* in poultry meat production and the distribution chain. The isolation of *A. altamirensis* from a meat-handling place, like the slaughterhouse object of study, suggests the possible role of this bacterium as a food contaminant. However, the isolation procedure followed was not specifically designed for *A. altamirensis*, suggesting the necessity of new specific culture-dependent methods for its isolation. The study of the characteristics of *A. altamirensis* is pivotal to develop new dedicated media. Culture-independent analyses (e.g., metagenomics and 16S rDNA sequencing) can overcome the detection limit of *A. altamirensis*, obtaining additional information about its presence in the food supply and production chains. Finally, culture-independent methods will allow the evaluation of the presence of *A. altamirensis* on retail meat even in the absence of a specific isolation protocol exploring its possible relevance as a food contaminant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres14030089/s1, Supplementary Table S1. QUAST statistics about AAI genome.

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Abbreviations

Average nucleotide identity (ANI), antibiotic-resistant (AR), antibiotic resistance gene (ARG), biofilm formation index (BFI), Comprehensive Antibiotic Resistance Database (CARD), CRISPR-associated protein (CAS), coding DNA sequences (CDSs), colony-forming unit (CFU), clusters of orthologous groups (COGs), clustered regularly interspaced short palindromic repeats (CRISPRs), Department of Agricultural, Forest and Food Sciences (DISAFA), Dulbecco's modified Eagle medium (DMEM), genomic DNA (gDNA), gastrointestinal tract (GIT), Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), minimum inhibitory concentrations (MICs), oxygen minimum zone (OMZ), resistance-nodulation-cell division (RND), phosphate-buffered saline (PBS), unweighted pair group method with arithmetic mean (UPGMA).

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