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Original article

Identification and specificity validation of unique and antimicrobial resistance genes to trace suspected pathogenic AMR bacteria and to monitor the development of AMR in non-AMR strains in the environment and clinical settings

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ABSTRACT

The detection of developing antimicrobial resistance (AMR) has become a global issue. The detection of developing antimicrobial resistance has become a global issue. The growing number of AMR bacteria poses a new threat to public health. Therefore, a less laborious and quick confirmatory test becomes important for further investigations into developing AMR in the environment and in clinical settings. This study aims to present a comprehensive analysis and validation of unique and antimicrobial-resistant strains from the WHO priority list of antimicrobial-resistant bacteria and previously reported AMR strains such as *Acinetobacter baumannii, Aeromonas* spp., *Anaeromonas frigoriresistens, Anaeromonas gelatinfytica, Bacillus* spp., *Campylobacter jejuni* subsp. *jejuni, Enterococcus faecalis, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumonia subsp. pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* subsp. *enterica* serovar Typhimurium, *Thermanaeromonas toyohensis,* and *Vibrio proteolyticus*. Using in-house designed gene-specific primers, 18 different antibiotic resistance genes (*algJ, alpB, AQU-1, CEPH-A3, ciaB, CMY-1-MOX-7, CMY-1-MOX-9, CMY-1/MOX, cphA2, cphA5, cphA7, ebpA, ECP_4655, fliC, OXA-51, RfbU, ThiU2,* and *tolB*) from 46 strains were selected and validated. Hence, this study provides insight into the identification of strain-specific, unique antimicrobial resistance genes. Targeted amplification and verification using selected unique marker genes have been reported. Thus, the present detection and validation use a robust method for the entire experiment. Results also highlight the presence of another set of 18 antibiotic-resistant and unique genes (*Aqu1, cphA2, cphA3, cphA5, cphA7, cmy1/mox7, cmy1/mox9, asaI, ascV, asoB, oxa-12, acr-2, pepA, uo65, pliI, dr0274, tapY2,* and *cpeT*). Of these sets of genes, 15 were found to be suitable for the detection of pathogenic strains belonging to the genera *Aeromonas, Pseudomonas, Helicobacter, Campylobacter, Enterococcus, Klebsiella, Acinetobacter, Salmonella, Haemophilus,* and *Bacillus*. Thus, we have detected and verified sets of unique and antimicrobial resistance genes in bacteria on the WHO Priority List and from published reports on AMR bacteria. This study offers advantages for confirming antimicrobial resistance in all suspected AMR bacteria and monitoring the development of AMR in non-AMR bacteria, in the environment, and in clinical settings.

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1. Introduction

The life forms that inhabit this world range from inconspicuous organisms to large, multicellular, advanced beings. Realizing that the most significant proportion of earth dwellers are microscopic organisms is fascinating. Bacteria are highly cosmopolitan in existence. Even a square area of the soil is colonized by a cocktail of millions of bacteria. However, the diversity and relative abundance of bacterial phyla vary from soil to soil (Gupta et al. 2017). A minor proportion of these bacteria are pathogenic and can result in catastrophic events in humans (Khan et al. 2022). Antimicrobial resistance studies are crucial because they address the growing threat of bacteria and other pathogens becoming resistant to antibiotics (Ventola, 2015). This phenomenon endangers global health, making previously treatable infections deadly (Serwecińska, 2020). Understanding and combating antimicrobial resistance is vital to preserving the effectiveness of our current medical arsenal and ensuring a healthier future (Annunziato, 2019). Antimicrobial resistance genes in bacteria on the WHO priority list are of paramount importance because they pose severe threats to human health. These genes can render antibiotics ineffective, making infections harder to treat, leading to prolonged illness, increased mortality rates, and higher healthcare costs. Urgent research and action are essential to combat this global health crisis (WHO, https://www.who.int/). Therefore, the health and well-being of humans rely on the rapid and early detection of pathogenic organisms. The conventional detection methods of identification of bacteria by isolation and culturing on agar media and confirmation by biochemical and serological testing are cumbersome and time-consuming (Rajapaksha et al. 2019). In addition, the finding that less than 10 % of soil bacteria can be cultured fueled the need for rapid detection techniques. Cutting down the culturing step facilitates the detection of bacteria using PCR amplification (Petti, 2007; Gupta et al. 2017). Diverse forms of PCR, namely, real-time PCR, multiplex PCR, RT-PCR, and droplet digital PCR (ddPCR), are currently used for bacterial detection and quantification. Although techniques such as gene sequencing (Petti, 2007), flow cytometry, optical biosensors, and bioluminescent sensors are available for bacterial detection, PCR remains the most commonly used detection method that utilizes DNA- and RNA-based assays for bacterial identification. Molecular detection methods are rapid and sensitive for bacterial detection, including the identification of emerging pathogens. The sensitivity of the detection assay can be further improved by designing new primers (Rajapaksha et al. 2019). Several gene targets act as important tools in molecular detection assays. The functionally constant, conserved regions of the genes provide universality of the targets annealed by PCR primers. Generally, bacterial identification is performed using the 16S rRNA gene (Petti, 2007). However, the use of strain-specific genes for the identification of bacteria has been reported in recent studies. The detection of bacteria using strain-specific genes proves to be fast, efficient, inexpensive, and reliable. This technique allows the differentiation of bacterial strains that share a significant level of similarity in their morphology and physiology. Here, PCR primers are designed to target the single-copy genes present exclusively in a particular strain. It is worth noting that combining the primer pairs and running multiplexing PCR helps to characterize mixtures of strains simultaneously. This detection assay is highly flexible, as identifying a strain-specific gene helps to determine and distinguish specific bacterial strains (Ferrandis-Vila et al. 2022).

The present study employs the use of strain-specific genes identified in silico to detect and characterize intended bacterial strains from coastal soil samples employing multiplexing PCR. The strain-specific genes of *Aeromonas* spp., *Helicobacter pylori*, *Campylobacter jejuni*, *Salmonella enterica*, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Klebsiella pneumonia*, and *Enterococcus faecalis* identified through BV-BRC and BLAST analysis were used to design PCR primers. Multiplexing PCR was used to validate the presence of these bacterial strains.

2. Materials and methods

2.1. Computational analysis for identification of strain-specific genes

Forty-six pathogenic bacterial strains were selected for the study. The unique genes of the respective bacterial strains were obtained through annotation using the Bacterial and Viral Bioinformatics Resource Centre (BV-BRC) webserver (Olson et al. 2022). The NCBI database was screened for previously reported papers and data available on the selected genes. NCBI-BLAST analysis was performed to confirm the identity and specific genes. The uniqueness of the selected genes to a specific bacterial strain was determined from the percentage sequence similarity obtained from BLAST analysis. Fifteen genes were identified to be uniquely expressed individually in 20 bacterial strains and were used in designing primers for laboratory validation (Fig. S1).

2.2. Criteria for selection of the reference genomes and bioprojects

The reference genomes and bioprojects of 46 selected pathogenic bacteria that are highly prevalent in environmental samples were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). This included *Acinetobacter baumannii* K09 (NZ_CP043953.1), *Aeromonas allosaccharophila* FDAARGOS_933 (GCF_016026615.1), *Aeromonas allosaccharophila* FDAARGOS_933 (NZ_CP065745.1), *Aeromonas aquatic* AE235 contig7 (NZ_JRGL01000007.1), *Aeromonas australiensis* CECT 8023 (NZ_CDDH01000062.1), *Aeromonas bestiarum* GA97-22 Contig0001 (NZ_PPUX01000001.1), *Aeromonas bivalvium* ZJ19-2 NODE_1 (NZ_NXBQ01000001.1), *Aeromonas cavernicola* DSM 24474 (NZ_PGGC01000005.1), *Aeromonas caviae* WP8-S18-ESBL-04 (NZ_AP022254.1), *Aeromonas dhakensis* 71,431 (NZ_CP084351.1), *Aeromonas diversa* CDC 2478–85 (NZ_CDCE01000029.1), *Aeromonas encheleia* NCTC12917 (NZ_LR134376.1), *Aeromonas enteropelogenes* FDAARGOS_1537 (NZ_CP084358.1), *Aeromonas eucrenophila* CECT 4224 (NZ_CDDF01000005.1), *Aeromonas finlandensis* 4287D contig286 (NZ_JRGK01000286.1), *Aeromonas fluvialis* LMG 24681 (NZ_CDBO01000011.1), *Aeromonas hydrophila* FDAARGOS_916 (NZ_CP065651.1), *Aeromonas jandaei* FDAARGOS_986 (NZ_CP066092.1), *Aeromonas lacus* AE122 Contig147 (NZ_JRGM01000147.1), *Aeromonas lusitana* MDC 2473 A (NZ_PGCP01000003.1), *Aeromonas media* TR3_1 (NZ_CP075564.1), *Aeromonas molluskorum* 848 Cont1 (NZ_AQGQ01000001.1), *Aeromonas piscicola* LMG 24783 (NZ_CDBL01000052.1), *Aeromonas popoffii* CIP 105493 (NZ_CDBI01000014.1), *Aeromonas rivipollensis* G78 G78_contig_29 (NZ_JAAILA010000003.1), *Aeromonas rivuli* 20-VB00005 (NZ_CP079742.1), *Aeromonas salmonicida* SRW-OG1(NZ_CP051883.1), *Aeromonas sanarellii* LMG 24682 (NZ_CDBN01000021.1), *Aeromonas schubertii* ATCC 43700 Scaffold1 (NZ_LPUO01000001.1), *Aeromonas simiae* A6 (NZ_CP040449.1), *Aeromonas sobria* CECT 4245 (NZ_CDBW01000006.1), *Aeromonas taiwanensis* LMG 24683 (NZ_CDDD01000101.1), *Aeromonas tecta* CECT 7082 NZ_CDCA01000036.1), *Aeromonas veronii* FDAARGOS_632 NZ_CP044060.1), *Anaeromonas frigoriresistens* D2Q NZ_WSFT01000053.1 & NZ_WSFU01000119.1), *Campylobacter jejuni* subsp. *jejuni* NCTC 11168 (NC_002163.1), *Enterococcus faecalis* EnGen0336 (NZ_KB944666.1), *Escherichia coli O157 H7* Sakai (NC_002695.2), *Haemophilus influenzae* 477 (NZ_CP007470.1), *Helicobacter pylori* MT5135 (NZ_CP071982.1), *Klebsiella pneumonia* subsp. *pneumoniae* HS11286 (NC_016845.1), *Pseudomonas aeruginosa* PAO1 (NC_002516.2), *Salmonella enterica* subsp. *enterica* serovar *typhimurium* LT2 (NC_003197.2), *Thermanaeromonas toyohensis* ToBE chromosome I (NZ_LT838272.1), and *Vibrio proteolyticus* NBRC 13287 (NZ_BATJ01000001.1). A total of 46 complete genomes were retrieved in the FASTA file format (Table S1). *Bacillus subtilis* and *Bacillus cereus*-group strains were accessed for presence of AMR genes from the BV-BRC web server (https://www.bv-brc.org/) (Olson et al. 2022) for confirmation.

2.3. Annotation of sequence data

The 46 genomes and bioprojects of the selected bacterial strains were annotated on the BV-BRC web server (https://www.bv-brc.org/) (Olson et al. 2022). An individual annotation of each acquired genome was carried out on the BV-BRC Workspace website (Table S1), and some information was retrieved from published literature. Genome-based selective annotation was carried out to identify strain-specific unique genes, specialty genes, domains and motifs, critical pathways, and subsystems.

2.4. Identification of strain-specific unique genes

The termed "specialty" genes contained within the annotated entire reference genomes of each of the different bacterial strains were subjected to a one-by-one examination for the purpose of gene isolation. Through a process known as NCBI-BLAST analysis, the one-of-a-kindness of the gene that was found was determined. The genes that had the lowest percentage of similarity to other gene sequences were chosen. The BV-BRC was consulted to acquire FASTA files containing single-copy gene sequences. The effective publications that were received from the website of the List of Prokaryotic names with Standing in Nomenclature (LPSN) (Parte et al. 2020) were used to initially identify the strain-specific genes of the bacteria that were chosen for the investigation.

2.5. Selective screening for the presence of inter- and intragenus unique genes and detection of point mutations

All 46 inter- and intragenus strains were thoroughly analyzed for the presence of strain-specific unique genes to avoid errors during validation and misinterpretation of results. The presence of similar genes in other strains in the same genus and other strains in another genus were tested and cross-verified. The percentage of similarity among genes was also validated to check for any possible errors. The RIPper - Genome-Wide Repeat-Induced Point (RIP) Mutation Analysis (https://theripper.hawk.rocks/#/home) tool was used to check for point mutations (van Wyk et al., 2019).

2.6. Extraction of DNA from pure cultures

Genomic DNA was extracted from in-house-isolated environmental bacteria from coastal and estuarine soil samples using the phenol:chloroform:isoamyl (25:24:1) (PCA) method (P3803, Merck). This method has been employed on strains of *Kocuria, Acinetobacter, Aeromonas, Pseudomonas, Helicobacter, Campylobacter, Enterococcus, Klebsiella,* and *Salmonella.* Quality control strains such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis* were used to obtain DNA samples for the group that served as a control. The extracted DNA was measured with a NanoDrop spectrophotometer (260/280 ratio). Mix 2 mg of a pure culture of bacteria with 100 µl of lysozyme (20 mg/ml) and incubate overnight at 37 °C. After incubation, 15 µl of proteinase K and 40 µl of 10 % SDS were added and brought up to 400 µl with TE buffer. Five microliters of RNase enzyme (100 µg/ml concentration) were added to the tube, which was incubated at 55 °C to 70 °C for four hours with intermittent vortexing. To this mixture, equal volumes (approximately 400 µl) of freshly prepared phenol, chloroform, and isoamyl alcohol (25:24:1) were added and mixed by gentle inversion. The tubes were centrifuged at 10,000 rpm for 30 min. Upon centrifugation, distinct aqueous and organic phases were formed. The aqueous phase (i.e., the upper layer or aqueous layer containing DNA) was carefully transferred to a fresh tube. The volume of 3 M sodium acetate added was 10 % of the total volume of the aqueous phase. Then, 2.5 times ice-cold absolute ethanol was added to the solution and incubated overnight at −20 °C. After incubation, the tubes were centrifuged at 10,000 rpm for 30 min, and the supernatant was discarded. The DNA pellet was washed twice with freshly prepared 70 % ethanol, and the pellet was allowed to air dry before being suspended in 20 µl of TE buffer. The isolated DNA was visualized on a 0.8 % agarose gel (Otal et al. 1991; de Almeida et al. 2013). The DNA concentration in each sample was adjusted to approximately 100 ng/µl for further analysis.

2.7. Design and validation of primers for PCR amplification and confirmation of unique genes using in silico tools

Selected unique genes were used as target genes for the design of primers for polymerase chain reaction (PCR) and subsequent PCR amplification and confirmation of unique genes in a laboratory. A portion of a specific gene was selected, and primers were designed using the Integrated DNA Technologies, Inc., PrimerQuest™ Tool (IDT, 2023a). The expected properties of your oligos before wet laboratory validation for guanine and cytosine (GC) content, melting temperature (Tm), molecular weight, extinction coefficient, µg/OD, nmol/OD, to identify secondary structure potential, to minimize dimerization, and NCBI BLAST™ analysis have been closely examined using the OligoAnalyzer™ Tool (IDT, 2023b) to avoid further errors in experiments during validation. Designed primers were tested for working ability and confirmation of product generation by in silico PCR amplification (Franklin et al. 1996; Rekadwad et al. 2021). The objective of utilizing in silico PCR is to facilitate the acquisition of anticipated PCR outcomes from DNA through the utilization of contemporary bacterial genome sequences (Bikandi et al. 2004; Brown et al. 2005; Rocco et al. 2016).

2.8. Targeted amplification and wet-lab verification of unique genes by polymerase chain reaction (PCR)

PCR amplification and wet-lab verification of the concerned gene were performed by using in-house designed specific forward and reverse primers for genes - A*QU-1/cphA2 (aqcp_222-F, aqcp_744-R), CMY-1/MOX (cmy-mox_241-F, cmy-mox_769-R), cephA Family (cephA_195-F, cephA_422-R, cephA_327-R), aqu1 (aqu1_128-F, aqu1_1088-R), alpB (alpB_450_F, alpB_1146_R and alpB_1155_F, alpB_1589_R), ciaB (ciaB_154-F, ciaB_1082-R), rfbU (rfbU_34, rfbU_561), Oxa 51 (oxa51_281-F, oxa51_692-R), ThiU2 (thiU2_157-F, thiU2_356-R), and ebpA (ebpA_242-F, ebpA_892-R), and ECP_4655 (ecp427-F, ecp652-R)* (Table 1).

PCR amplification and wet-lab verification were conducted for the selected genes using specific forward and reverse primers designed in-house. The primer pairs used for each gene are listed in Table 2. The PCR master mix was prepared by combining the eDNA template, forward and reverse primers, dNTPs, Taq DNA polymerase, and PCR buffer. The reaction conditions were set according to the annealing temperature specific to each pair of primers. Positive and negative controls were included in the PCRs using known samples. The reaction components were thoroughly mixed and distributed into PCR tubes. The tubes or plates were then placed into a thermal cycler, and PCR amplification was carried out for 35 cycles. The amplification protocol included an initial denaturation step at 95 °C for 5 min, followed by denaturation at 95 °C for 1 min, annealing at the respective temperature for each set of primers, extension at 72 °C for 45 s, and a final extension at 72 °C for 7 min (Rocco et al. 2016). The annealing temperatures varied for each set of primers used for the amplification of the target genes. The annealing time was set to 45 s at temperatures of 63 °C, 63 °C, 64 °C, 53 °C, 61 °C, 55 °C, 57 °C, 57 °C, 53 °C, 56 °C, 55 °C, 43 °C, and 55.5 °C for the genes AQU-1/cphA2, cmy-1/mox, cephA Family (one forward and two reverse primers, separate PCRs were performed for each reverse primer), *aqu1, alpB, ciaB, rfbU, Oxa 51, ThiU2, ebpA,* and *ECP_4655*, respectively. After PCR amplification, the resulting products were analyzed using agarose gel electrophoresis. The DNA bands were visualized under UV light and compared with a DNA ladder (100 bp) to determine the expected sizes for the respective target genes from known quality control strains such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and

Table 1
Primers for unique genes found in a selected set of 20 pathogenic and antibiotic-resistant bacteria.

Gene	Primer F/R	Sequence (5′ to 3′)	Start bp	No of bp	Tm Actual	Total gene bp	ssDNA bp	Product bp	Annealing Temp
AQU-1/ cphA2	aqcp_222-F	GGTCAGCGAGCAGACCCTGTTC	222	22	65.8	1149	927	–	
	aqcp_744-R	GCTGGTCTTGATGCCGTAGGCCTC	744	24	67.8	1149	744	523	63
CMY-1/MOX	cmy-mox_241-F	GTCAGCGAGCAGACCCTGTTCG	241	22	65.8	1167	1026	–	
	cmy-mox_769-R	CCGCCGAGCTGGTCTTGATGCC	769	22	67.7	1167	769	529	63
cephA Family	cephA_195-F	GGCGACCTGGACGCCGGATAC	195	21	67.6	765	570	–	
	cephA_422-R	TCCGGCAGCCCCTTGCGGGT	422	20	67.5	765	422	228	64
	cephA_327-R	GGACTTCCAGTAGGCGTTA	327	19	56.7	765	327	133	53
aqu1	aqu1_128-F	AGCACAGGATCCCGGGCATG	128	20	63.4	1149	1021	–	
	aqu1_1088-R	CGGTTGGCCAGCATGACGATGC	1088	22	65.8	1149	1088	960	61
alpB	alpB_450_F	CCAAGGCAACCTGAGTCTTTAT	450	22	58.4	1596	1146	–	
	alpB_1146_R	GAATGTGGGCTTACGCTACTAC	1146	22	60.3	1596	1146	696	55
	alpB_1155_F	CTTACGCTACTACGGCTTCTTC	1155	22	60.3	1596	434	–	
	alpB_1589_R	CGTAGCCATAGACCCAATACAC	1589	22	60.3	1596	434	434	57
ciaB	ciaB_154-F	GCCATACTTAGGCGTTTGATTG	154	22	58.4	1801	1647	–	
	ciaB_1082-R	GGAACGACTTGAGCTGAGAATA	1082	22	58.4	1801	1082	929	57
rfbU	rfbU_34	GGTACGGGAATGTGGCAATA	34	20	57.3	1001	977	–	
	rfbU_561	CAACTTGCACCAACAGCTAAA	561	21	55.9	1001	561	527	53
Oxa 51	oxa51_281-F	ATAAGGCAACCACCACAGAAG	281	21	57.9	801	520	–	
	oxa51_692-R	GCTGAACAACCCATCCAGTTA	692	21	57.9	801	692	411	56
ThiU2	thiU2_157-F	GCACTTTCCACTTTAGCACTTAC	157	23	58.9	1301	1144	–	
	thiU2_356-R	ACCGATACCTTGCCCAATAC	356	20	57.3	1301	356	200	55
ebpA	ebpA_242-F	CAGCTCAGCCACCTAAGTTATT	242	22	45.5	3301	2945	–	
	ebpA_892-R	ACCGCTATCTGCCAATGTATC	892	21	47.6	3301	892	650	43
ECP_4655	ecp427-F	ATCACCGCAGGATCGTTAATC	427	21	57.9	901	474	–	
	ecp652-R	TGGTGCCGGAGAGGTAATA	652	19	58.4	901	652	225	55.5

Bacillus subtilis. In-house isolated environmental bacteria from coastal soil samples, such *Acinetobacter* spp. and *Kocuria* spp., were used for confirmation of in silico results and experimental validation of unique genes (Mullis et al. 1986; Parkhill et al. 2000; Bikandi et al. 2004; Nallapareddy et al. 2006; San Millán et al. 2013; In silico PCR amplification, 2023). Based on PCR specific to the above bacterial taxa and visualization through gel electrophoresis, the *cmy-1/mox* and *aqu1* genes were successfully validated in *Pseudomonas aeruginosa,* followed by other taxa.

3. Results

3.1. An overview of specialized genes, antibiotic production ability, pathogenicity and reports on pathogenic strains

In the context of bacteria, specialized genes can contribute to their ability to produce antibiotics, enhance pathogenicity, or perform other specialized functions. Almost all analyzed bacterial strains do not have any published reports stating proven antimicrobial resistance or reported unique genes especially in *Aeromonas* in this study. Those reported have functions in antibiotic inactivation enzyme, beta-lactam resistance gene, *Campylobacter* invasion antigen B, virulence factor, adherence, biofilm formation, sortase-assembled pili, adhesion, predicted thiazole transporter, outer membrane protein/porin, protein (regulates length and adhesion of type 1 fimbriae, and mediates mannose binding), antiphagocytosis, serum resistance, LPS O-antigen biosynthesis protein, and Tol-Pal system beta propeller repeat protein as per RAST and BV-BRC analysis. Specialized genes refer to specific genes that are unique to certain organisms or have specific functions within 46 selected strains (Table S1), as mentioned above. Strains belonging to the *Bacillus subtilis* and *Bacillus cereus groups* were not included due to technical reasons in the main analysis. *Bacillus* spp. were accessed on the BV-BRC web server for selected sets of genes for the presence of AMR.

Eighteen genes unique to these microorganisms were selected based on criteria adopted for single copy number (Rekadwad et al., 2021): *OXA-51, algJ, alpB, AQU-1, cphA2, CEPH-A3, ciaB, CMY-1/MOX, cphA5, cphA7, ebpA, ECP_4655, fliC, MOX-7, MOX-9, CMY-1/MOX, RfbU, ThiU2,* and *tolB* (Table 2). These genes can play a crucial role in the adaptation, survival, and specialized functions of an organism.

3.2. Elucidation of unique genes in a taxon based on single copy number

Based on screening criteria single copy number, selected 20 bacterial strains (out of 46 + strains belonging *Bacillus* groups) were screened out for the presence of unique taxa-specific genes and selected present single copy number genes highly specific to taxa or groups of similar strains in taxa, such as K09-14, FDAARGOS_933, WP8-S18-ESBL-04, 71431, FDAARGOS_1537, FDAARGOS_916, FDAARGOS_986, TR3_1, LMG 24783, SRW-OG1, FDAARGOS_632, NCTC 11168, EnGen0336 strain T5 acAro-supercont1.1, 477, MT5135, HS11286, PAO1, ZJ19-2, NCTC 12917, and LT2.

The gene similarity report suggests that the unique genes *tolB, alpB, ecp_4655, fliC, oxa-51, rfbU, thiU2, cmy-1-mox-7, aqu-1, cpha2, cepha3, cpha5, cmy-1/mox,* and *cmy-1-mox-9* were found in selected bacterial strains showing identity with other taxa (Table 3). No point mutations were detected in the selected genes during analysis. This indicates that during pathogenesis, such genes may be acquired by these bacteria either in the environment or through horizontal gene transfer. Further analysis within the genus *Aeromonas* indicates that *cpha5, cmy-1/mox7, and cmy-1/mox7 are unique to the taxon compared to aqu-1, cpha2, cpha3, cpha7,* and *cmy-1/mox* (Table 4).

3.3. Unique genes specific to selected strains in the genus Aeromonas

A total of 37 strains among the genus *Aeromonas* were analyzed for the presence of unique genes and antimicrobial resistance. Of the 37 screened *Aeromonas* strains, 15 strains possessed various unique and antimicrobial resistance genes belonging to *A. allosaccharophila* 71431, *A. allosaccharophila* FDAARGOS_933, *A. bivalvium* ZJ19-2 NODE_1, *A. caviae* WP8-S18-ESBL-04, *A. dhakensis* 71431, *A. encheleia* NCTC12917, *A. enteropelogenes* FDAARGOS_1537, *A. eucrenophila* CECT 4224, *A. hydrophila* FDAARGOS_916, A. jandaei FDAARGOS_986, *A. media* TR3_1, *A. rivuli* 20-VB00005, *A. salmonicida* SRW-OG1, *A. simiae* A6, and *A. veronii* FDAARGOS_632 (Table 5a). Furthermore,

Table 2
List of scrutinized taxa containing inter- and intragenus strain-specific unique genes.

Sl. No.	Taxa	NCBI accession number	Previous reports
1	*Pseudomonas aeruginosa* PAO1	NC_002516.2	Franklin & Ohman, 1996
2	*Helicobacter pylori* MT5135	NZ_CP071982.1	Bai et al., 2002
3	*Campylobacter jejuni* subsp. *jejuni* NCTC 11168	NC_002163.1	Parkhill et al., 2000
4	*Enterococcus faecalis* EnGen0336 strain T5 acAro-supercont1.1	NZ_KB944666.1	Nallapareddy et al., 2006
5	*Klebsiella pneumonia* subsp. *pneumoniae* HS11286	NC_016845.1	No report available
6	*Acinetobacter baumannii* K09-14	NZ_CP043953.1	Brown et al., 2005
7	*Salmonella enterica* subsp. *enterica* serovar *typhimurium* str. LT2	NC_003197.2	Xiang et al., 1994
8	*Haemophilus influenza* 477	NZ_CP007470.1	No report available
9	*Aeromonas dhakensis* 71431	NZ_CP084351.1	Wu et al., 2013
10	*Aeromonas allosaccharophila* FDAARGOS_933	NZ_CP065745.1	Wang et al., 2021
11	*Aeromonas enteropelogenes* FDAARGOS_1537	NZ_CP084358.1	No report available
12	*Aeromonas jandaei* FDAARGOS_986	NZ_CP066092.1	No report available
13	*Aeromonas veronii* FDAARGOS_632	NZ_CP044060.1	Ragupathi et al., 2020
14	*Aeromonas bivalvium* ZJ19-2	NZ_NXBQ01000001.1	No report available
15	*Aeromonas hydrophila* FDAARGOS_916	NZ_CP065651.1	Bottoni et al., 2015
16	*Aeromonas salmonicida* SRW-OG1	NZ_CP051883.1	No report available
17	*Aeromonas encheleia* NCTC12917	NZ_LR134376.1	No report available
18	*Aeromonas piscicola* LMG 24783	NZ_CDBL01000052.1	No report available
19	*Aeromonas caviae* WP8-S18-ESBL-04	NZ_AP022254.1	No report available
20	*Aeromonas media* TR3_1	NZ_CP075564.1	Ebmeyer et al., 2019

18 genes belong to the genus *Aeromonas* were disclosed that governs antimicrobial resistance through various mechanisms viz., Acyl-homoserine-lactone synthase (*asaI*), Type 3 secretion system (*ascV*), Arsenite oxidase subunit (*asoB*), Ambler Class beta-lactamase, carbapenem (*Ceph-A3*), Histidine kinase family (*ChpA*), CMY beta-lactamase (*cmy-1/mox*), Cyanophycin synthetase (*CphA*), OXA β-Lactamases (*Oxa-12*), 4-amino-6-deoxy-N-Acetyl-D-hexosaminyl-(Lipid carrier) acetyltrasferase (*pglD_3*), Acetylcholine receptor subunit beta-type acr-2 protein (*Acr-2*), Aminopeptidase PepA-related protein (*PepA*), Aminopeptidase Y (Arg, Lys, Leu preference) (*UO65*), AQU family (*Aqu*), Inhibitor of invertebrate i-type lysozyme, periplasmic (*PliI*), Bacteriocin lactacin-F subunit (*LafX*), Nudix dNTPase - *MutT/nudix* family protein (*DR0274*), Transporter 2, ATP binding cassette subfamily B member (*TapY2*) and T-type phycobiliprotein lyase (*CpeT*).

We have found that some *Aeromonas* taxa possesses completely unique and novel genes not showing identity with any other genes in the existing database such as *asaI* (*A. allosaccharophila* 71431, and *A. piscicola* LMG 24783), *ascV* (*A. diversa* CECT 4254), *asoB* (*A. allosaccharophila* 71431, *A. bivalvium* ZJ19-2 NODE_1, and *A. eucrenophila* CECT 4224), *ChpA* (*A. allosaccharophila* 71431, and *A. piscicola* LMG 24783), *CMY-1/MOX* (*A. bivalvium* ZJ19-2 NODE_1), *cphA* (*A. allosaccharophila* 71431, and A. piscicola LMG 24783), OXA-12 (*A. allosaccharophila* 71431), *Acr-2* (*A. diversa* CECT 4254), *PepA* (*A. allosaccharophila* 71431), UO65 (*A. allosaccharophila* 71431, and *A. tecta* CECT 7082) Aqu (*A. allosaccharophila* 71431), *PliI* (*A. bivalvium* ZJ19-2 NODE_1, and *A. eucrenophila* CECT 4224), DR0274 (*A. allosaccharophila* 71431, and A. tecta CECT 7082), *TapY2* (*A. piscicola* LMG 24783), and CpeT (*A. allosaccharophila* 71431) (Table 5b). This suggests that some important strains, such as *A. allosaccharophila* 71431, *A. bivalvium* ZJ19-2 NODE_1, *A. diversa* CECT 4254, *A. eucrenophila* CECT 4224, A. piscicola LMG 24783, and A. tecta CECT 7082, and other strains, except those that show 95–100 % identity of genes (Table 5a), are potential bacteria to explore for deep analysis to find significant differences among strains belonging to the genus *Aeromonas*.

A total of 17 strains belonging to the genera *Aeromonas* (*A. allosaccharophila* 71,431 (NZ_CP084351.1), *A. allosaccharophila* 71,431 (NZ_CP084351.1), *A. dhakensis* 71,431 (NZ_CP084351.1), *A. dhakensis* 71,431 (NZ_CP084351.1), *A. hydrophila* FDAARGOS_916 (NZ_CP065651.1), *A. hydrophila* FDAARGOS_916 (NZ_CP065651.1), *A. allosaccharophila* FDAARGOS_933 (NZ_CP065745.1), *A. enteropelogenes* FDAARGOS_1537 (NZ_CP084358.1), *A. jandaei* FDAARGOS_986 (NZ_CP066092.1), *A. veronii* FDAARGOS_632 (NZ_CP044060.1), *A. bivalvium* ZJ19-2 NODE_1 (NZ_NXBQ01000001.1), *A. salmonicida* SRW-OG1 (NZ_CP051883.1), *A. encheleia* NCTC12917 (NZ_LR134376.1), *A. encheleia* NCTC12917 (NZ_LR134376.1), *A. piscicola* LMG 24783 (NZ_CDBL01000052.1), *A. caviae* WP8-S18-ESBL-04 (NZ_AP022254.1), and *A. media* TR3_1 (NZ_CP075564.1)) were further investigated for disclosed unique genes such as *aqu-1, cpha2, aqu-1_d, cpha2, aqu-1_h, cpha2, cepha3_a, cepha3_e, cepha3_j, cepha3_v, cmy-1/mox, cpha5, cpha7_e, cmy-1/mox, cpha7_p, cmy-1-mox-7,* and *cmy-1-mox-9* to infer either significant differences or similarities among genes (Fig. 1). The heatmap suggests that almost all unique genes in the genus *Aeromonas* have significant differences rather than similarities among unique genes on a scale of 0 to 1. It has been recorded that *aqu1, cepha2,* and those showing values less than 0.85 have significant differences among genes.

3.4. Inference from specific validation of novel and unique genes

Antibiotic resistance genes belonging to the genera *Aeromonas, Pseudomonas, Helicobacter, Campylobacter, Enterococcus, Klebsiella, Acinetobacter, Salmonella, Bacillus, and Haemophilus* have been identified. They found that some unique genes in these strains showed similarity with genes from other taxa with antibiotic production ability or resistance to antibiotics (Table 5b). *Aeromonas* strains are known pathogens that infect fish, animals and humans. Hence, pathogenic strains belonging to the *Aeromonas* genera were analyzed in this study for the presence of the abovementioned genes and identified based on single copy 15 unique gene number criteria. These genes play a crucial role in the adaptation, survival, and specialized functions of organisms. Some of these genes were found to be involved in antibiotic resistance, pathogenicity, adherence, biofilm formation, and other functions. This investigation of the genus *Aeromonas* shows that 15 out of 37 strains were found to possess unique and antimicrobial resistance genes. Additionally, 18 genes related to antimicrobial resistance were identified within the genus. Some strains within the genus *Aeromonas* showed completely unique and novel genes not found in other strains. Further analysis using a heatmap showed significant differences among the unique genes in the genus *Aeromonas*.

4. Discussion

The 46 strains chosen were from *Aeromonas, Pseudomonas, Helicobacter, Campylobacter, Enterococcus, Klebsiella, Acinetobacter, Salmonella, Haemophilus,* and *Bacillus* genera. They all had 18 single-copy unique genes such as *algJ, alpB, AQU-1, CEPH-A3, ciaB, CMY-1-MOX-7, CMY-1-MOX-9, CMY-1/MOX, cphA2, cphA5, cphA7, ebpA, ECP_4655, fliC, OXA-51, RfbU, ThiU2,* and *tolB* (Tables 2 and 3) that were involved in antibiotic resistance, pathogenicity, adherence, and biofilm formation. These suggest that identified genes can play a crucial role in the adaptation, survival, and specialized functions of an organism (Evans

Table 3
Presence of 18 unique taxa-specific genes in another genera or domain.

Gene	Taxon	Gene ID in other taxa, if available	Name and NCBI Accession number of reported taxa
AQU-1, cphA2	*Aeromonas allosaccharophila* 71431 (NZ_CP084351.1)	114286262 (AQU-1)	*Camellia sinensis* cultivar Shuchazao unplaced genomic scaffold AHAU_CSS_1 Scaffold3615 (NW_021027072.1)
AQU-1, cphA2	*Aeromonas dhakensis* 71431 (NZ_CP084351.1)	NG_050396.1 (cphA2)	*Aeromonas hydrophila* AER 19 cphA gene for subclass B2 metallo-beta-lactamase CphA2 (NG_050396.1)
AQU-1, cphA2	*Aeromonas hydrophila* FDAARGOS_916 (NZ_CP065651.1)	———	———
CEPH-A3	*Aeromonas allosaccharophila* FDAARGOS_933 (NZ_CP065745.1)	———	———
CEPH-A3	*Aeromonas enteropelogenes* FDAARGOS_1537 (NZ_CP084358.1)	———	———
CEPH-A3	*Aeromonas jandaei* FDAARGOS_986 (NZ_CP066092.1)	———	———
CEPH-A3	*Aeromonas veronii* FDAARGOS_632 (NZ_CP044060.1)	———	———
CMY-1/MOX	*Aeromonas bivalvium* ZJ19-2 NODE_1 (NZ_NXBQ01000001.1)	———	———
cphA5	*Aeromonas salmonicida* SRW-OG1 (NZ_CP051883.1)	———	———
cphA7, CMY-1/MOX	*Aeromonas encheleia* NCTC12917 (NZ_LR134376.1)	NG_050400.1 (cphA7)	*Aeromonas jandaei* ATCC 49568 cphA gene for subclass B2 metallo-beta-lactamase CphA7 (NG_050400.1)
cphA7	*Aeromonas piscicola* LMG 24783 (NZ_CDBL01000052.1)	NG_050400.1 (cphA7)	*Aeromonas jandaei* ATCC 49568 cphA gene for subclass B2 metallo-beta-lactamase CphA7 (NG_050400.1)
CMY-1-MOX-7	*Aeromonas caviae* WP8-S18-ESBL-04 (NZ_AP022254.1)	18813570 (MOX-7)	*Serpula lacrymans* var. *lacrymans* S7.9 unplaced genomic scaffold SERLAscaffold_11 (NW_006763300.1)
CMY-1-MOX-9	*Aeromonas media* TR3_1 (NZ_CP075564.1)	18815923 (MOX-9)	*Serpula lacrymans* var. *lacrymans* S7.9 unplaced genomic scaffold SERLAscaffold_18 (NW_006763307.1)
algJ	*Pseudomonas aeruginosa* PAO1 (NC_002516.2)	77219964	*Pseudomonas paraeruginosa* strain Cr1 (NZ_CP020560.1)
		66491330	*Legionella pneumophila* strain C9_S (NZ_CP015941.1)
		45624672	*Pseudomonas simiae* strain PCL1751 (NZ_CP010896.1)
		78258735	*Butyrivibrio crossotus* isolate MGYG-HGUT-01319 (NZ_CABKNR010000014.1)
		72998054	*Pseudomonas citronellolis* strain P3B5 (NZ_CP014158.1)
		72394459	*Pseudomonas coronafaciens* pv. *oryzae* str. 1_6 (NZ_CP046035.1)
		61792614	*Pseudomonas avellanae* strain CC1416 contig318.1 (NZ_AVEP02000318.1)
		61708583	*Pseudomonas lactis* strain SS101 (NZ_CM001513.1)
		57261476	*Pseudomonas brassicacearum* strain 3Re2-7 (NZ_CP034725.1)
		45621619	*Pseudomonas simiae* strain PCL1751 (NZ_CP010896.1)
		45522320	*Pseudomonas putida* NBRC 14164 (NC_021505.1)
		1182870	*Pseudomonas syringae* pv. *tomato* str. DC3000 (NC_004578.1)
		73734174	Pseudomonas tremae strain PA-1-10F (NZ_CP066270.1)
		78554965	*Pseudomonas extremaustralis* strain DSM 17835 (NZ_LT629689.1)
		72192903	Pseudomonas umsongensis strain CY-1 (NZ_CP051487.1)
		69747833	*Pseudomonas alloputida* strain NMI2441_06 (NZ_JAJSPR010000005.1)
		66647291	*Pseudomonas mandelii* strain KGI_MA19 (NZ_CP081178.1)
		65075219	*Pseudomonas congelans* strain DSM 14939 (NZ_FNJH01000002.1)
		61868467	*Pseudomonas amygdali* pv. *tabaci* str. ATCC 11528 (NZ_CP042804.1)
		61648697	*Pseudomonas chlororaphis* strain qlu-1 (NZ_CP061079.1)
		58532891	*Pseudomonas asiatica* strain RYU5 RYU5_unitig_0 (NZ_BLJF01000001.1)
		57607844	*Pseudomonas mendocina* S5.2 (NZ_CP013124.1)
		57474020	*Pseudomonas protegens* CHA0 (NZ_LS999205.1)
		55845753	*Pseudomonas tolaasii* NCPPB 2192 Ga0070648_11 (NZ_PHHD01000001.1)
		49870618	*Pseudomonas monteilii* strain B5 (NZ_CP022562.1)
		49614069	*Pseudomonas plecoglossicida* strain XSDHY-P (NZ_CP031146.1)
		77179175	*Pseudomonas guariconensis* strain MR119 MR119_8 (NZ_PJCP01000008.1)
		64093427	Pseudomonas fulva strain YAB-1 contig13 (NZ_LAWW01000013.1)
		57399847	*Pseudomonas otitidis* strain MrB4 (NZ_AP022642.1)
		56069949	*Pseudomonas yamanorum* strain LBUM636 (NZ_CP012400.2)
		47554532	*Pseudomonas veronii* strain R02 (NZ_CP018420.1)
		57518971	*Pseudomonas proteolytica* strain WS 5126 4_283282_20.4743 (NZ_JAAQXL010000004.1)
		78504381	*Pseudomonas parafulva* NBRC 16636 (NZ_BBIU01000020.1)
		77277010	*Pseudomonas syringae* strain Susan2139 (NZ_CP074578.1)
		77257756	*Marinobacter salarius* strain SMR5 (NZ_CP020931.1)
		77247859	*Pseudomonas carnis* strain NWU Be30 (NZ_JAMKPY010000008.1)
		77187064	*Pseudomonas capsici* strain NCPPB2479 (NZ_JAOXME010000028.1)
		76211923	*Pseudomonas mediterranea* strain DSM 16733 (NZ_LT629790.1)
		75527915	*Pseudomonas atacamensis* strain SM1 (NZ_CP070503.1)
		75198874	*Pseudomonas kurunegalensis* strain T2909-1 1 (NZ_JALKHE010000002.1)
		75192006	*Pseudomonas siliginis* strain OTU6BANIB1 (NZ_CP099598.1)
		72498365	*Pseudomonas marginalis* strain PgKB35 contig2 (NZ_VTFG01000002.1)
		72478030	*Pseudomonas moraviensis* strain LMG 24280 (NZ_LT629788.1)
		72422112	*Pseudomonas juntendi* strain PP_2463 (NZ_CP091088.1)
		70103785	*Pseudomonas gessardii* strain LMG 21604 (NZ_FNKR01000003.1)
		69858097	*Pseudomonas savastanoi* strain MHT1 (NZ_CP076652.1)
		66761385	*Pseudomonas poae* strain LMG 21465 (NZ_LT629706.1)

(*continued on next page*)

Table 3 (*continued*)

Gene	Taxon	Gene ID in other taxa, if available	Name and NCBI Accession number of reported taxa
		64467080	*Pseudomonas cannabina* pv. *alisalensis* strain MAFF 301419 (NZ_CP067022.1)
		61932304	*Azotobacter chroococcum* strain B3 (NZ_CP011835.1)
		61881971	*Pseudomonas lundensis* strain 2T.2.5.2 (NZ_CP062158.2)
		61828961	*Pseudomonas synxantha* strain R6-28-08 (NZ_CP027756.1)
		61637163	*Pseudomonas fluorescens* strain ATCC 13525 (NZ_LT907842.1)
		58768986	*Pseudomonas mosselii* strain PtA1 (NZ_CP024159.1)
		58766660	*Pseudomonas mosselii* strain PtA1 (NZ_CP024159.1)
		58694899	*Pseudomonas rhodesiae* strain NL2019 (NZ_CP054205.1)
		57661674	*Pseudomonas gingeri* strain A6001 (NZ_JACAOR010000008.1)
		57633271	*Pseudomonas koreensis* strain LMG 21318 (NZ_LT629687.1)
		57377827	*Pseudomonas azotoformans* strain LMG 21611 (NZ_LT629702.1)
		55644702	*Pseudomonas corrugata* strain RM1-1-4 (NZ_CP014262.1)
		47765936	*Pseudomonas viridiflava* strain CFBP 1590 isolate E12-5 (NZ_LT855380.1)
		45541106	*Pseudomonas cichorii* JBC1 (NZ_CP007039.1)
		42930483	*Pseudomonas alcaligenes* strain NEB 585 (NZ_CP014784.1)
		31709204	*Pseudomonas lurida* strain L228 (NZ_CP015639.1)
		878551	*Pseudomonas aeruginosa* PAO1 algX (NC_002516.2)
alpB	*Helicobacter pylori* MT5135 (NZ_CP071982.1)	124639028	*Helicoverpa zea* isolate HzStark_Cry1AcR (NC_061469.1)
		100125692	*Triticum aestivum* cultivar Chinese Spring chromosome 4A (NC_057803.1)
		542895	*Triticum aestivum* cultivar Chinese Spring chromosome 7D (NC_057814.1)
ciaB	*Campylobacter jejuni subsp. jejuni* NCTC 11168 (NC_002163.1)	7411001	*Campylobacter lari* RM2100 (NC_012039.1)
		78326632	*Arcobacter nitrofigilis* DSM 7299 (NC_014166.1)
		77176464	*Campylobacter ureolyticus* strain LMG 6451 (NZ_CP053832.1)
		66544092	*Campylobacter coli* strain FDAARGOS_735 (NZ_CP046317.1)
		61065105	*Campylobacter fetus* strain CFF00A031 (NZ_CP059443.1)
		61001623	*Campylobacter curvus* strain ATCC 35224 (NZ_CP053826.1)
		60991106	*Campylobacter showae* strain ATCC 51146 (NZ_CP012544.1)
		77266361	*Campylobacter vulpis* strain 251/13 (NZ_CP041617.1)
		56587051	*Campylobacter armoricus* strain CCUG 73571 (NZ_CP053825.1)
		56509597	*Campylobacter hyointestinalis* subsp. *lawsonii* strain CHY5 (NZ_CP053828.1)
		44004343	*Campylobacter hepaticus* strain HV10 (NZ_CP031611.1)
		39299964	*Aliarcobacter thereius* LMG 24486 AA347_contig000001 (NZ_LLKQ01000001.1)
		905214	*Campylobacter jejuni* subsp. *jejuni* NCTC 11168 (NC_002163.1)
		66539337	*Helicobacter cinaedi* PAGU611 (NC_017761.1)
		56463744	*Aliarcobacter butzleri* ED-1 (NC_017187.1)
		61153890	*Campylobacter pinnipediorum* subsp. *pinnipediorum* strain RM17261 (NZ_CP012547.1)
		68759512	*Campylobacter sputorum* bv. *paraureolyticus* LMG 11764 strain LMG 17589 (NZ_CP019684.1)
		46921585	*Campylobacter lanienae* NCTC 13004 (NZ_CP015578.1)
		74432015	*Campylobacter insulaenigrae* NCTC 12927 (NZ_CP007770.1)
		77230936	*Campylobacter upsaliensis* 17-M197059 (NZ_OU701459.1)
		66287961	*Campylobacter volucris* strain LMG 24380 (NZ_CP043428.1)
		61750346	*Aliarcobacter skirrowii* CCUG 10374 (NZ_CP032099.1)
		57004565	Helicobacter pullorum *strain NCTC13156 (NZ*_UGJF01000001.1)
		56461800	*Aliarcobacter cryaerophilus* ATCC 43158 (NZ_CP032823.1)
		52037112	*Campylobacter helveticus* strain ATCC 51209 (NZ_CP020478.1)
		28663259	*Campylobacter concisus* strain ATCC 33237 (NZ_CP012541.1)
		61924358	*Clostridium innocuum* strain ATCC 14501 (NZ_CP048838.1)
		68118740	*Naegleria fowleri* strain ATCC 30894 (NW_025407941.1)
		54452205	*Macroventuria anomochaeta* strain CBS 525.71 (NW_022985375.1)
ebpA	*Enterococcus faecalis* EnGen0336 (NZ_KB944666.1)	1050	*Homo sapiens* chromosome 19, GRCh38.p14 (NC_000019.10)
		12606	*Mus musculus* strain C57BL/6J chromosome 7, GRCm39 (NC_000073.7)
		110596866	*Homo sapiens* chromosome 8, GRCh38.p14 (NC_000008.11)
		12608	*Mus musculus* strain C57BL/6J chromosome 2, GRCm39 (NC_000068.8)
		19016	*Mus musculus* strain C57BL/6J chromosome 6, GRCm39 (NC_000072.7)
		111832672	*Mus musculus* strain C57BL/6J chromosome 5, GRCm39 (NC_000071.7)
		73389	*Mus musculus* strain C57BL/6J chromosome 12, GRCm39 (NC_000078.7)
		5468	Homo sapiens chromosome 3, GRCh38.p14 (NC_000003.12)
		861	*Homo sapiens* chromosome 21, GRCh38.p14 (NC_000021.9)
		5241	*Homo sapiens* chromosome 11, GRCh38.p14 (NC_000011.10)
		56729	*Homo sapiens* chromosome 19, GRCh38.p14 (NC_000019.10)
		4297	*Homo sapiens* chromosome 11, GRCh38.p14 (NC_000011.10)
		1051	Homo sapiens chromosome 20, GRCh38.p14 (NC_000020.11)
		13653	*Mus musculus* strain C57BL/6J chromosome 18, GRCm39 (NC_000084.7)
		387173	*Mus musculus* strain C57BL/6J chromosome 16, GRCm39 (NC_000082.7)
		6548	*Homo sapiens* chromosome 1, GRCh38.p14 (NC_000001.11)
		20787	*Mus musculus* strain C57BL/6J chromosome 11, GRCm39 (NC_000077.7)
		6659	*Homo sapiens* chromosome 6, GRCh38.p14 (NC_000006.12)
		5598	*Homo sapiens* chromosome 17, GRCh38.p14 (NC_000017.11)
		723848	*Mus musculus* strain C57BL/6J chromosome 4, GRCm39 (NC_000070.7)
		11091	*Homo sapiens* chromosome 9, GRCh38.p14 (NC_000009.12)

(*continued on next page*)

Table 3 (*continued*)

Gene	Taxon	Gene ID in other taxa, if available	Name and NCBI Accession number of reported taxa
		723814	*Mus musculus* strain C57BL/6J chromosome X, GRCm39 (NC_000086.8)
		57264	*Mus musculus* strain C57BL6J chromosome 8, GRCm39 (NC_000074.7)
		20471	*Mus musculus* strain C57BL/6J chromosome 12, GRCm39 (NC_000078.7)
		28951	*Homo sapiens* chromosome 2, GRCh38.p14 (NC_000002.12)
		13592	*Mus musculus* strain C57BL/6J chromosome 14, GRCm39 (NC_000080.7)
		140815	Danio rerio strain Tuebingen chromosome 7, GRCz11 (NC_007118.7)
		18105	*Mus musculus* strain C57BL/6J chromosome 13, GRCm39 (NC_000079.7)
		396728	Sus scrofa isolate TJ Tabasco breed Duroc chromosome 13 Sscrofa11.1 (NC_010455.5)
ECP_4655	*Klebsiella pneumoniae subsp. pneumoniae* HS11286 (NC_016845.1)	ABG72591.1	FimH protein precursor [*Escherichia coli* 536] (ABG72591.1)
fliC	*Escherichia coli* O157 H7 str. Sakai (NC_002695.2)	Many	————
OXA-51	*Acinetobacter baumannii* K09-14 (NZ_CP043953.1)	————	————
RfbU	*Salmonella enterica subsp. enterica serovar Typhimurium str.* LT2 (NC_003197.2)	1238129	*Shigella flexneri* 2a str. 301 plasmid pCP301 (NC_004851.1)
		1789671	*Escherichia coli* O157H7 str. Sakai plasmid pO157 (NC_002128.1)
ThiU2	*Haemophilus influenzae* 477 (NZ_CP007470.1)	————	————
tolB	*Vibrio proteolyticus* NBRC 13287 (NZ_BATJ01000001.1)	Many	————

Table 4
Inter- and intrataxa presence of discovered unique genes.

Strain	Unique gene	*aqu-1*	*cpha2*	*cepha3*	*cmy-1/mox*	*cpha5*	*cpha7*	*cmy-1-mox-7*	*cmy-1-mox-9*
Aeromonas allosaccharophila 71431 (NZ_CP084351.1)	*AQU-1, cphA2*	■	■						
Aeromonas dhakensis 71431 (NZ_CP084351.1)	*AQU-1, cphA2*	■	■						
Aeromonas hydrophila FDAARGOS_916 (NZ_CP065651.1)	*AQU-1, cphA2*	■	■						
Aeromonas allosaccharophila FDAARGOS_933 (NZ_CP065745.1)	*CEPH-A3*			■					
Aeromonas enteropelogenes FDAARGOS_1537 (NZ_CP084358.1)	*CEPH-A3*		■	■			■		
Aeromonas jandaei FDAARGOS_986 (NZ_CP066092.1)	*CEPH-A3*		■	■			■		
Aeromonas veronii FDAARGOS_632 (NZ_CP044060.1)	*CEPH-A3*			■					
Aeromonas bivalvium ZJ19-2 NODE_1 (NZ_NXBQ01000001.1)	*CMY-1/MOX*				■				
Aeromonas salmonicida SRW-OG1 (NZ_CP051883.1)	*cphA5*		■			■			
Aeromonas encheleia NCTC12917 (NZ_LR134376.1)	*CMY-1/MOX, cphA7*		■		■		■		
Aeromonas piscicola LMG 24783 (NZ_CDBL01000052.1)	*cphA7*		■				■		
Aeromonas caviae WP8-S18-ESBL-04 (NZ_AP022254.1)	*CMY-1-MOX-7*				■			■	
Aeromonas media TR3_1 (NZ_CP075564.1)	*CMY-1-MOX-9*				■				■

Note: Cells highlighted in green color represent inter- and intra-taxa presence of genes

and Amyes, 2014; McMillan et al. 2019). According to a few investigations, bacterial strains such as *Staphylococcus aureus* (Oogai et al. 2011), *Helicobacter pylori* (Yamaoka, 2010; Wang et al. 2015), *Escherichia coli* (Bidet et al. 2012), *Salmonella* spp. (Jennings et al. 2017), *Pseudomonas aeruginosa* (Olejnickova et al. 2014), and *Streptococcus suis* (Wu et al., 2013) produce a variety of virulence factors, including toxins, immune-modulating agents, and exoenzymes. Those strains were investigated in this paper, and one of the most interesting findings of the

Table 5a
Presence of 18 unique or antimicrobial resistance genes in the *Aeromonas*.

Unique/AMR gene	Code	Strains	Taxa	Accession No.	Product size (bp)	Identity (%) in BLAST
Acyl-homoserine-lactone synthase	*asaI*	12	*A. allosaccharophila* 71,431	NZ_CP084351.1	624	0
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	651	1
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	630	51
			A. dhakensis 71,431	NZ_CP084351.1	624	24
			A. encheleia NCTC12917	NZ_LR134376.1	627	3
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	651	5
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	624	79
			A. media TR3_1	NZ_CP075564.1	627	14
			A. piscicola LMG 24783	NZ_CDBL01000052.1	624	0
			A. rivuli 20-VB00005	NZ_CP079742.1	657	1.9
			A. salmonicida SRW-OG1	NZ_CP051883.1	624	37
			A. veronii FDAARGOS_632	NZ_CP044060.1	651	37
Type 3 secretion system	*ascV*	5	*A. allosaccharophila* FDAARGOS_933	NZ_CP065745.1	2118	3
			A. diversa CECT 4254	NZ_CDCE01000029.1	2115	0
			A. encheleia NCTC12917	NZ_LR134376.1	2118	2
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	2115	24
			A. jandaei FDAARGOS_986	NZ_CP066092.1	2118	11
Arsenite oxidase subunit	*asoB*	15	*A. allosaccharophila* 71,431	NZ_CP084351.1	1281	0
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	1320	2
			A. bivalvium ZJ19-2 NODE_1	NZ_NXBQ01000001.1	1278	0
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	1281	52
			A. dhakensis 71,431	NZ_CP084351.1	1281	27
			A. encheleia NCTC12917	NZ_LR134376.1	1278	3
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	1281	6
			A. eucrenophila CECT 4224	NZ_CDDF01000005.1	1287	0
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	1281	71
			A. jandaei FDAARGOS_986	NZ_CP066092.1	1278	13
			A. media TR3_1	NZ_CP075564.1	1278	14
			A. rivuli 20-VB00005	NZ_CP079742.1	1290	1
			A. salmonicida SRW-OG1	NZ_CP051883.1	1281	34
			A. simiae A6	NZ_CP040449.1	1272	1
			A. veronii FDAARGOS_632	NZ_CP044060.1	1281	43
Ambler Class beta-lactamase, carbapenem	*CEPH-A3*	4	A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	765	6
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	603	1
			A. jandaei FDAARGOS_986	NZ_CP066092.1	765	15
			A. veronii FDAARGOS_632	NZ_CP044060.1	762	47
Histidine kinase family	*ChpA*	11	*A. allosaccharophila* 71,431	NZ_CP084351.1	762	0
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	765	6
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	210	68.75
			A. dhakensis 71,431	NZ_CP084351.1	762	35
			A. encheleia NCTC12917	NZ_LR134376.1	663	3
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	603	1
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	765	64
			A. jandaei FDAARGOS_986	NZ_CP066092.1	765	15
			A. piscicola LMG 24783	NZ_CDBL01000052.1	765	0
			A. salmonicida SRW-OG1	NZ_CP051883.1	762	27
			A. veronii FDAARGOS_632	NZ_CP044060.1	762	47
CMY beta-lactamase	*CMY-1/MOX*	4	*A. bivalvium* ZJ19-2 NODE_1	NZ_NXBQ01000001.1	1170	0
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	1152	81
			A. encheleia NCTC12917	NZ_LR134376.1	1167	3
			A. media TR3_1	NZ_CP075564.1	1152	16
Cyanophycin synthetase	*cphA*	10	*A. allosaccharophila* 71,431	NZ_CP084351.1	762	0
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	765	6
			A. dhakensis 71,431	NZ_CP084351.1	762	35
			A. encheleia NCTC12917	NZ_LR134376.1	663	3
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	603	1
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	765	64
			A. jandaei FDAARGOS_986	NZ_CP066092.1	765	15
			A. piscicola LMG 24783	NZ_CDBL01000052.1	765	0
			A. salmonicida SRW-OG1	NZ_CP051883.1	762	27
			A. veronii FDAARGOS_632	NZ_CP044060.1	762	47
OXA β-Lactamases	*OXA-12*	7	*A. allosaccharophila* 71,431	NZ_CP084351.1	795	0

(*continued on next page*)

Table 5a (*continued*)

Unique/AMR gene	Code	Strains	Taxa	Accession No.	Product size (bp)	Identity (%) in BLAST
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	795	4
			A. dhakensis 71,431	NZ_CP084351.1	795	33
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	795	78
			A. jandaei FDAARGOS_986	NZ_CP066092.1	795	13
			A. salmonicida SRW-OG1	NZ_CP051883.1	795	34
			A. veronii FDAARGOS_632	NZ_CP044060.1	795	49
4-amino-6-deoxy-N-Acetyl-D-hexosaminyl-(Lipid carrier) acetyltrasferase	*pglD_3*	3	*A. caviae* WP8-S18-ESBL-04	NZ_AP022254.1	597	40
			A. salmonicida SRW-OG1	NZ_CP051883.1	609	100
			A. simiae A6	NZ_CP040449.1	645	100
Acetylcholine receptor subunit beta-type acr-2 protein	*Acr-2*	2	*A. diversa* CECT 4254	NZ_CDCE01000029.1	372	0
			A. encheleia NCTC12917	NZ_LR134376.1	372	3.1
Aminopeptidase PepA-related protein	*PepA*	14	*A. allosaccharophila* 71,431	NZ_CP084351.1	1497	0
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	1500	2
			A. bestiarum GA97-22 Contig0001	NZ_PPUX01000001.1	1497	1
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	1482	52
			A. dhakensis 71,431	NZ_CP084351.1	1497	25
			A. encheleia NCTC12917	NZ_LR134376.1	1491	3
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	1515	5
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	1497	70
			A. jandaei FDAARGOS_986	NZ_CP066092.1	1506	12
			A. media TR3_1	NZ_CP075564.1	1494	14
			A. rivuli 20-VB00005	NZ_CP079742.1	1509	1
			A. salmonicida SRW-OG1	NZ_CP051883.1	1497	33
			A. simiae A6	NZ_CP040449.1	771	1
			A. veronii FDAARGOS_632	NZ_CP044060.1	1500	42
Aminopeptidase Y (Arg, Lys, Leu preference)	*UO65*	10	*A. allosaccharophila* 71,431	NZ_CP084351.1	1068	0
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	1068	52
			A. dhakensis 71,431	NZ_CP084351.1	1068	29
			A. encheleia NCTC12917	NZ_LR134376.1	1068	3
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	1068	72
			A. media TR3_1	NZ_CP075564.1	1068	14
			A. rivuli 20-VB00005	NZ_CP079742.1	1077	1
			A. salmonicida SRW-OG1	NZ_CP051883.1	1068	33
			A. simiae A6	NZ_CP040449.1	1080	1
			A. tecta CECT 7082	NZ_CDCA01000036.1	1068	0
AQU family	*Aqu*	3	*A. allosaccharophila* 71,431	NZ_CP084351.1	1143	0
			A. dhakensis 71,431	NZ_CP084351.1	1143	45
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	1149	74
Inhibitor of invertebrate i-type lysozyme, periplasmic	*PliI*	13	*A. allosaccharophila* 71,431	NZ_CP084351.1	438	25
			A. bivalvium ZJ19-2 NODE_1	NZ_NXBQ01000001.1	438	0
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	438	52
			A. dhakensis 71,431	NZ_CP084351.1	438	25
			A. encheleia NCTC12917	NZ_LR134376.1	438	3
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	453	5
			A. eucrenophila CECT 4224	NZ_CDDF01000005.1	438	0
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	438	72
			A. jandaei FDAARGOS_986	NZ_CP066092.1	438	13
			A. media TR3_1	NZ_CP075564.1	438	13
			A. rivuli 20-VB00005	NZ_CP079742.1	438	3.5
			A. salmonicida SRW-OG1	NZ_CP051883.1	438	33
			A. veronii FDAARGOS_632	NZ_CP044060.1	438	75.9
Bacteriocin lactacin-F subunit	*LafX*	5	*A. finlandensis* 4287D contig286	NZ_JRGK01000286.1	339	0
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	339	57.8
			A. jandaei FDAARGOS_986	NZ_CP066092.1	339	91.6
			A. lacus AE122 Contig147	NZ_JRGM01000147.1	339	0
			A. rivuli 20-VB00005	NZ_CP079742.1	339	100
Nudix dNTPase - MutT/nudix family protein	*DR0274*	13	*A. allosaccharophila* 71,431	NZ_CP084351.1	558	0
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	564	2
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	558	52
			A. dhakensis 71,431	NZ_CP084351.1	558	29
			A. encheleia NCTC12917	NZ_LR134376.1	552	3.7
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	561	6
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	558	73
			A. jandaei FDAARGOS_986	NZ_CP066092.1	561	12
			A. media TR3_1	NZ_CP075564.1	558	14
			A. rivuli 20-VB00005	NZ_CP079742.1	579	16.6
			A. salmonicida SRW-OG1	NZ_CP051883.1	558	33

(*continued on next page*)

Table 5a (*continued*)

Unique/AMR gene	Code	Strains	Taxa	Accession No.	Product size (bp)	Identity (%) in BLAST
			A. tecta CECT 7082	NZ_CDCA01000036.1	558	0
			A. veronii FDAARGOS_632	NZ_CP044060.1	564	43
Transporter 2, ATP binding cassette subfamily B member	*TapY2*	4	*A. allosaccharophila* FDAARGOS_933	NZ_CP065745.1	282	3.3
			A. jandaei FDAARGOS_986	NZ_CP066092.1	276	90.9
			A. piscicola LMG 24783	NZ_CDBL01000052.1	279	0
			A. veronii FDAARGOS_632	NZ_CP044060.1	282	66.6
T-type phycobiliprotein lyase	*CpeT*	12	*A. allosaccharophila* 71,431	NZ_CP084351.1	675	0
			A. allosaccharophila FDAARGOS_933	NZ_CP065745.1	651	2.3
			A. caviae WP8-S18-ESBL-04	NZ_AP022254.1	666	52
			A. dhakensis 71,431	NZ_CP084351.1	675	25
			A. enteropelogenes FDAARGOS_1537	NZ_CP084358.1	651	31.5
			A. hydrophila FDAARGOS_916	NZ_CP065651.1	675	72
			A. jandaei FDAARGOS_986	NZ_CP066092.1	645	11
			A. media TR3_1	NZ_CP075564.1	660	14
			A. rivuli 20-VB00005	NZ_CP079742.1	684	1
			A. salmonicida SRW-OG1	NZ_CP051883.1	684	24
			A. simiae A6	NZ_CP040449.1	636	100
			A. veronii FDAARGOS_632	NZ_CP044060.1	651	53.1

Table 5b
Presence of 15 unique or antimicrobial resistance genes in the *Aeromonas*.

Unique/AMR gene	Code	Taxa	Accession No.	Identity (%) in BLAST
Acyl-homoserine-lactone synthase	*asaI*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
		A. piscicola LMG 24783	NZ_CDBL01000052.1	0
Type 3 secretion system	*ascV*	*A. diversa* CECT 4254	NZ_CDCE01000029.1	0
Arsenite oxidase subunit	*asoB*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
		A. bivalvium ZJ19-2 NODE_1	NZ_NXBQ01000001.1	0
		A. eucrenophila CECT 4224	NZ_CDDF01000005.1	0
Histidine kinase family	*ChpA*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
		A. piscicola LMG 24783	NZ_CDBL01000052.1	0
CMY beta-lactamase	*CMY-1/MOX*	*A. bivalvium* ZJ19-2 NODE_1	NZ_NXBQ01000001.1	0
Cyanophycin synthetase	*cphA*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
		A. piscicola LMG 24783	NZ_CDBL01000052.1	0
OXA β-Lactamases	*OXA-12*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
Acetylcholine receptor subunit beta-type acr-2 protein	*Acr-2*	*A. diversa* CECT 4254	NZ_CDCE01000029.1	0
Aminopeptidase PepA-related protein	*PepA*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
Aminopeptidase Y (Arg, Lys, Leu preference)	*UO65*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
		A. tecta CECT 7082	NZ_CDCA01000036.1	0
AQU family	*Aqu*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
Inhibitor of invertebrate i-type lysozyme, periplasmic	*PliI*	*A. bivalvium* ZJ19-2 NODE_1	NZ_NXBQ01000001.1	0
		A. eucrenophila CECT 4224	NZ_CDDF01000005.1	0
Nudix dNTPase - MutT/nudix family protein	*DR0274*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0
		A. tecta CECT 7082	NZ_CDCA01000036.1	0
Transporter 2, ATP binding cassette subfamily B member	*TapY2*	*A. piscicola* LMG 24783		
T-type phycobiliprotein lyase	*CpeT*	*A. allosaccharophila* 71,431	NZ_CP084351.1	0

study is the discovery of completely unique and novel genes with significant differences (Xiang et al. 1994; Bai et al. 2002; Wu et al. 2011; Wang et al. 2021) among them and common in all strains were found in one genus, Aeromonas. That means Aeromonas is the hub for all those antimicrobial genes found (Piotrowska and Popowska, 2014; Luo et al. 2022; Dubey et al. 2022) in other genera investigated under this study. This information can be used to better understand the adaptation, survival, and virulence of antimicrobial resistance strains. It can also be used to develop new strategies for preventing and treating antmicrobial resistance and virulence (Beceiro et al. 2013) in Pseudomonas, Helicobacter, Campylobacter, Enterococcus, Klebsiella, Acinetobacter, Salmonella, Haemophilus, Bacillus, and Aeromonas infections. Research from several groups in the last ten years (Martino et al. 2011; Liang et al. 2022; Zhang et al. 2023) backs up the idea that some genes found in other genera were unique to Aeromonas or even to certain strains of Aeromonas. This was the prime reason to explore the genus Aeromonas in the later part of the investigations. The heatmap analysis showed that almost all unique genes in the genus Aeromonas have significant differences rather than similarities. This suggests that the unique genes are highly diverse and may play a role in the diversity of Aeromonas strains. Hence, the findings of this study have a number of potential implications for the prevention, diagnosis, and treatment of antimicrobial-resistant bacteria (Bottoni et al. 2015; Ebmeyer et al. 2019; Ragupathi et al. 2020), not limited to *Aeromonas* infections. Hence, the identification of unique genes in antimicrobial resistance strains may lead to the development of new diagnostic tools, such as PCR tests, to detect specific unique genes detected in the above genera (Galhano et al. 2021). Furthermore, the identification of unique genes in certain strains could also lead to the development of new therapeutic targets for effective treatments for infections caused by antimicrobial-resistant bacteria in the case of some important diseases such as diabetes, malaria, tuberculosis, AIDS, cancer, etc., (Dadgostar, 2019; Demain and Sanchez, 2009; Qadri et al. 2023). Moreover, the findings of this study may help to understand the process of evolution and acquiring unique genes from taxa. This may help trace the emergence of new strains that may be more virulent or resistant to existing antibiotics.

Strain	Gene list	aqu-1	cpha2	aqu-1	cpha2	aqu-1	cpha2	cepha3	cepha3	cepha3	cepha3	cmy-1/ Mox	cpha5	cpha7	cmy-1/ Mox
A. allosaccharophila 71431 (NZ_CP084351.1)	AQU-1	0	1	0	0.87	0	0	0	0	0	0.78	0	0	0.8	0
A. allosaccharophila 71431 (NZ_CP084351.1)	cphA2	1	0	1	0	0.95	0.94	0.95	0.93	0.93	0	0.93	0.9	0	0.92
A. dhakensis 71431 (NZ_CP084351.1)	AQU-1	0	1	0	0.87	0	0	0	0	0	0.78	0	0	0.8	0
A. dhakensis 71431 (NZ_CP084351.1)	cphA2	1	0	1	0	0.95	0.94	0.95	0.93	0.93	0	0.93	0.9	0	0.92
A. hydrophila FDAARGOS_916 (NZ_CP065651.1)	AQU-1	0	0.87	0	1	0	0	0	0	0	0	0	0	0.8	0
A. hydrophila FDAARGOS_916 (NZ_CP065651.1)	cphA2	0.95	0	0.95	0	1	0.92	0.92	0.91	0.92	0	0.91	0.87	0	0.9
A. allosaccharophila FDAARGOS_933 (NZ_CP065745.1	CEPH-A3	0.94	0	0.94	0	0.92	1	0.96	0.96	0.95	0	0.91	0.89	0	0.92
A. enteropelogenes FDAARGOS_1537 (NZ_CP084358.1)	CEPH-A3	0.95	0	0.95	0	0.92	0.96	1	0.97	0.96	0	0.92	0.88	0	0.93
A. jandaei FDAARGOS_986 (NZ_CP066092.1)	CEPH-A3	0.93	0	0.93	0	0.91	0.96	0.97	1	0.95	0	0.91	0.89	0	0.92
A. veronii FDAARGOS_632 (NZ_CP044060.1)	CEPH-A3	0.93	0	0.93	0	0.91	0.95	0.96	0.96	1	0	0.91	0.88	0	0.91
A. bivalvium ZJ19-2 NODE_1 (NZ_NXBQ01000001.1)	CMY-1/MOX	0	0.78	0	0	0	0	0	0	0	1	0	0	0.79	0
A. salmonicida SRW-OG1 (NZ_CP051883.1)	cphA5	0.93	0	0.93	0	0.91	0.91	0.92	0.91	0.91	0	1	0.88	0	0.93
A. encheleia NCTC12917 (NZ_LR134376.1)	cphA7	0.9	0	0.9	0	0.87	0.89	0.88	0.89	0.88	0	0.89	1	0	0.89
A. encheleia NCTC12917 (NZ_LR134376.1)	CMY-1/MOX	0	0.8	0	0.8	0	0	0	0	0	0.79	0	0	1	0
A. piscicola LMG 24783 (NZ_CDBL01000052.1)	cphA7	0.92	0	0.92	0	0.9	0.92	0.93	0.92	0.91	0	0.93	0.88	0	1
A. caviae WP8-S18-ESBL-04 (NZ_AP022254.1)	CMY-1-MOX-7	0	0.82	0	0.82	0	0	0	0	0	0.78	0	0	0.8	0
A. media TR3_1 (NZ_CP075564.1)	CMY-1-MOX-9	0	0.82	0	0.8	0	0	0	0	0	0.82	0	0	0.81	0

Fig. 1. Heatmap of *aqu-1, cpha2, aqu-1_d, cpha2, aqu-1_h, cpha2, cepha3_a, cepha3_e, cepha3_j, cepha3_v, cmy-1/mox, cpha5, cpha7_e, cmy-1/mox, cpha7_p, cmy-1-mox-7,* and *cmy-1-mox-9* in the genus *Aeromonas*. (Note: 1. Similar genes found in a taxon have been suffixed by the letter of the strain name. For example, the aqu1 gene, if found in multiple taxa, is suffixed with_d in the case of Aeromonas dhakensis. 2. 0 = indicates 100 % difference, while 1 = indicates 100 % similarity).

5. Conclusions

Infections caused by antimicrobial-resistant bacteria such as WHO priority list of antimicrobial-resistant bacteria and previously reported AMR strains such as *Acinetobacter baumannii, Aeromonas* spp., *Anaeromonas frigoriresistens, Anaeromonas gelatinfytica, Bacillus* spp., *Campylobacter jejuni* subsp. *jejuni, Enterococcus faecalis, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumonia subsp. pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* subsp. *enterica* serovar Typhimurium, *Thermanaeromonas toyohensis,* and *Vibrio proteolyticus* are difficult to detect and treat and have become a global public health threat. This investigation discloses and presents a comprehensive analysis of 46 antimicrobial-resistant strains of 20 pathogenic bacterial taxa. Additionally, two different sets of 18 antibiotic-resistant and unique genes in WHO priority list bacterial strains and in *Aeromonas* spp., were identified. It was observed that 15 single-copy genes may be suitable for the detection of these pathogenic strains, which belong to 10 different genera, such as *Aeromonas, Pseudomonas, Helicobacter, Campylobacter, Enterococcus, Klebsiella, Acinetobacter, Salmonella, Haemophilus,* and *Bacillus*. Identified sets of strain-specific, unique genes that can be used to develop new diagnostic tools to confirm AMR genes in suspected AMR bacteria and track the spread of AMR in non-AMR strains in the environment and clinical settings such as a hospital, laboratories, department, outpatient facility, or primary clinic (medicine, rehabilitation, or wellness), mobile hospitals, and tertiary care hospitals. Thus, this research can be used to develop more effective strategies for surveillance, preventing and combating AMR.

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CRediT authorship contribution statement

Bhagwan Narayan Rekadwad: Conceptualization, Formal analysis, Project administration, Resources, Supervision, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Nanditha Pramod:** Methodology, Data curation, Formal analysis, Visualization, Writing – original draft. **Manik Prabhu Narsing Rao:** Resources, Data curation, Formal analysis, Writing – review & editing. **Abeer Hashem:** Resources, Data curation, Formal analysis, Writing – review & editing. **Graciela Dolores Avila-Quezada:** Resources, Data curation, Formal analysis, Writing – review & editing. **Elsayed Fathi Abd_Allah:** Project administration, Funding acquisition, Resources, Data curation, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103869.

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