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Draft Genome Sequence of *Aeromonas caviae* Isolated from a Newborn with Acute Haemorrhagic Gastroenteritis

Savita Jadhav[1†], Ujjayni Saha[2†], Kunal Dixit[2], Anjali Kher[3], Sourav Sen[4], Nitin Lingayat[3], Vivekanand Jadhav[5], and Sunil Saroj[2*]

[1]*Department of Microbiology, Symbiosis Medical College for Women (SMCW) & Symbiosis University Hospital and Research Centre (SUHRC), Symbiosis International (Deemed University), Lavale, Pune 412115, Maharashtra, India*
[2]*Symbiosis School of Biological Sciences (SSBS), Symbiosis International (Deemed University), Lavale, Pune 412115, Maharashtra, India*
[3]*Department of Paediatrics, Symbiosis Medical College for Women (SMCW) & Symbiosis University Hospital and Research Centre (SUHRC), Symbiosis International (Deemed University), Lavale, Pune 412115, Maharashtra, India*
[4]*University Research Department, Maharashtra University of Health Sciences, Mhasrul, Vani Dindori Road, Nashik 422004, Maharashtra, India*
[5]*Dr Naidu Infectious Diseases Hospital, Pune Municipal Corporation, Pune 411001, Maharashtra, India*

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Aeromonas spp., are Gram-negative rods that can cause infections in healthy and immunocompromised hosts. The clinical presentation of gastroenteritis varies from mild diarrhoea to shigella-like dysentery to severe cholera-like watery diarrhoea. Here, we report a case of acute hemorrhagic gastroenteritis in a newborn infant by *Aeromonas caviae* and its draft genome sequence. It is important to reduce the chance of incorrect isolate identification, which could lead to the exclusion of pathogenic *Aeromonas* spp., from routine laboratory identification in cases of diarrheal diseases. The genome sequence of *A. caviae* SVJ23 represents a significant step forward in understanding the diversity and pathogenesis, virulence, and antimicrobial resistance profile.

Keywords: *Aeromonas caviae*, antimicrobial resistance, haemorrhagic gastroenteritis, genome sequencing

Introduction

Aeromonads are the etiological agents of several infections that can occur in both immunocompetent and immunocompromised people. They have been frequently isolated from foods, soil, and aquatic environments [1]. Aeromonads are causative agents of gastroenteritis, pyogenic infections, septicemia, catheter-related infections, necrotizing fasciitis, and pneumonia [2, 3]. *Aeromonas caviae* is a Gram-negative, motile, rod-shaped facultative anaerobe and is responsible for causing a range of gastroenteritis and extra-intestinal infections in humans [4]. They are becoming widely recognized as a cause of pediatric diarrhoea [5]. It has been shown that the majority of *A. caviae* isolates are resistant to antibiotics commonly used in hospitals, veterinary practices, and agriculture [6]. With the emergence of *Aeromonas* spp. as a critical human pathogen in combination with evolving resistance to antimicrobials, it is critical to investigate the genome of this bacteria thoroughly.

***Corresponding author**
E-mail: sunil.saroj@ssbs.edu.in
†These authors contributed equally to this work.

Case description

The second of the twin's male baby was delivered on 11/03/2022 at 2.39 pm by lower (uterine) segment Caesarean section (LSCS). The baby had 38 weeks of gestational age and weighed 2.84 kg. The baby had two episodes of blood in the stools, due to which the baby was admitted to NICU. The patient was in early hypovolemic shock and treatment was given as per protocol. An ultrasound (USG) abdomen and pelvis, coagulation profile, complete blood count (CBC), C-reactive protein (CRP), urine routine, and urine culture reports were normal. Treatment was started with injectable IV Piptaz and IV Amikacin (10 days therapy), vitamin K, IV fluid maintenance, and EBM with a spoon, and gradually baby started breastfeeding. The stool culture report showed growth of *A. caviae* resistant to third-generation cephalosporins antibiotics, but the baby showed clinical improvement, and therefore antibiotics were kept the same.

Materials and Methods

Laboratory investigations

Stool samples were received in the Department of Microbiology for culture and antimicrobial sensitivity. Isolation and identification of clinical isolates were made by standard and conventional methods [7]. The isolate was inoculated in the identification and susceptibility panels of "The BD Phoenix™ automated identification and susceptibility testing system" (Table 1).

Genome sequencing

The strain was grown overnight on a Luria-Bertani (LB) agar plate after being kept in glycerol stock at -80℃, and colonies were picked and transferred into LB broth at 37℃. DNA was extracted using the alkaline lysis method [8]. Whole genome sequencing was performed at D and B Genomics Pvt. Ltd., Kolhapur, Maharashtra, India. DNA was quantified using Nanodrop 1000, and the quality was confirmed using 1% Agarose gel electrophoresis. In brief, 2 µl of DNA was mixed with 2 µl of 6X Loading dye (Invitrogen, India), and electrophoresis at 120 V for 30 min was performed. The TruSeq® DNA Nano LP kit (Illumina #15041877, Illumina #20665713, India) was used to prepare the library. Following the manufacturer's protocol, final libraries were quantified using a Qubit 4.0 fluorometer (Thermofisher #Q33238, India) and a DNA HS assay kit (Thermofisher #Q32851). To determine the insert size of the library, Tapestation 4150 (Agilent) and highly sensitive D1000 screentapes (Agilent # 5067-5582, India) in accordance with the manufacturer's protocol were used. The Illumina Novaseq 6000 was used for genome sequencing. A manual bash script was used to count the total number of raw reads and average read length (ARL) as part of a standard quality control process. The quality of the raw reads was determined using the FastQC tool v0.11.5 (https://github.com/s-andrews/FastQC), and the report was summarised using MulitQC v1.8 (https://multiqc.info/). As part of a standard quality control procedure, the raw reads were filtered, and the average read length (ARL) was calculated. Trimmomatic v0.361 was used to remove adapter sequence, leading- and trailing-low-quality bases (below quality 3), and reads less than 36 bp from suboptimal quality reads with a sliding window of 4:15 and a minimum Phred score of 33. The quality of the Trimmomatic filtered reads was determined using the FastQC tool v0.11.5, and the report was summarised using MulitQC v1.8. The assembly was performed using Unicycler v0.4.8.1[2] with SPAdes for obtaining the pseudo-genome at default parameters like moderate contig size and misassembly rate. Linear sequences were filtered out, and contigs with a length shorter than 500 basepairs were excluded from the assembly. Contigs were further used to form a scaffold using *Aeromonas caviae* GSH8M-1 as the reference genome. Scaffolds were further patched to form a single contig using Ragtag v2.1.0 with the help of the same reference genome. Genome completeness and the quality check was performed using QUAST [9] and CheckM [10]. Further, the genome was annotated using Prokka 1.14.5 and a web-based PATRIC annotation server [11]. 16S rRNA sequence was extracted from the genome with the help of the PATRIC annotation server, and the closest reference match was identified using the NCBI BLASTn with 100% query coverage and 100% identity score. The full genome sequence of closest match *Aeromonas caviae* GSH8M-1 DNA was downloaded in '.fasta' format and was used to calculate average nucleotide identity (ANI) and average amino acid identity (AAI) using a web-based tool Kostas lab (http://enveomics.ce.gatech.edu/ani/). Additionally, the presence of

virulence and antimicrobial resistance genes were reported using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [12].

Data availability

The genome sequence of *Aeromonas caviae* strain SVJ23 has been deposited at GenBank under the accession number CP110176.2 and NCBI BioProject ID PRJNA893029.

Results

Antimicrobial susceptibility testing to *A. caviae* demonstrated extended-spectrum beta-lactamases (ESBL) producer. It was resistant to a broad spectrum of antibiotics (Table 1).

After sequencing, 27.89 M reads were obtained. 83.88% of reads were retained after filtering, which resulted in the genome sequence of the *A. caviae* SVJ23 strain that was 4556225 bp in length. The overall G + C content of the assembled genome was 61.54%. The percentage of single-copy orthologous genes was 98.65%. Additionally, 100% completeness and 0% contamination of the genome were confirmed using a web-based PATRIC annotation server. Our query genome showed an

Table 1. Results of Antibiotic susceptibility testing of *A. caviae*.

Antimicrobial	MIC	Resistance pattern
Amikacin	32	Resistant
Gentamicin	4	Sensitive
Ertapenem	>1	Resistant
Imipenem	2	Intermediate
Meropenem	0.5	Sensitive
Cefazolin	16	Resistant
Cefuroxime	16	Resistant
Cefoxitin	16	Resistant
Ceftazidime	16	Resistant
Ceftazidime-Avibactam	<=0.25/4	Sensitive
Ceftriaxone	>4	Resistant
Cefepime	16	Resistant
Aztreonam	<=1	Sensitive
Ampicillin	16	Resistant
Ampicillin-Sulbactam	16/8	Sensitive
Piperacillin- Tazobactam	64/4	Resistant
Trimethoprim-Sulfamethoxazole	2/38	Sensitive
Ciprofloxacin	0.5	Sensitive
Levofloxacin	<=1	Sensitive

Table 2. Genome summary table.

Genome Size	4556225 bp
G + C content	61.54%
rRNA's (5S, 6S, 23S)	1,1,1
tRNA's	85
Pseudogenes	74
Contigs (>= 0 bp)	182
Contigs (>= 0 bp)	150
Contigs (>= 5000 bp)	98
Contigs (>= 10000 bp)	79
Contigs (>= 25000 bp)	51
Contigs (>= 50000 bp)	23
Total length (>= 0 bp)	4556225
Total length (>= 1000 bp)	4533205
Total length (>= 5000 bp)	4421560
Total length (>= 10000 bp)	4289212
Total length (>= 25000 bp)	3813380
Total length (>= 50000 bp)	2724871
Largest contig	283253
Total length	4556225
N50	76772
N75	36580
L50	16
L75	39
N's per 100 kb	0.00
Complete BUSCO (%)	98.65%
Partial BUSCO (%)	0.00
Completeness (%)	100%
Contamination (%)	0%
Predicted genes (unique)	4055
Predicted genes (>= 0 bp)	4008 + 47 part
Predicted genes (>= 300 bp)	3650 + 42 part
Predicted genes (>= 1500 bp)	651 + 6 part
Predicted genes (>= 3000 bp)	70 + 2 part
Protein-encoding genes with Functional Assignment	2944
Protein-encoding genes without Functional Assignment	1448
% Protein-encoding feature coverage	96.4
% Features that are hypothetical	32.97
% Features that are in local protein families	95.17
Accession number of GenBank	CP110176.2

Table 3. Presence of virulence and antimicrobial resistance genes in the draft genome sequence of *Aeromonas caviae* SVJ23.

Strain	Virulence genes	Antimicrobial resistance genes
Aeromonas caviae SVJ23	*che*A, *che*B, *che*B1, *che*R2, *che*V_1, *che*V_2, *che*V_3, *che*W, *che*Y, *che*Z, *fli*A, *fli*D, *fli*E, *fli*F, *fli*G, *fli*L, *fli*M, *fli*N, *fli*P, *fli*Q, *fli*R, *flh*B, *flb*A, *hly*C, *pom*A_1, *pom*A_2, *cp*A, *ecp*B, *ecp*C, *ecp*D, *ecp*E, *hly*C, *flg*B, *flg*C, *flg*E, *flg*F, *flg*G, *flg*H, *flg*I, *flg*J, *exe*D	*amp*C, *emr*, bla_{oxa}, bla_{mox}, *tet*R, *fsr*, *aba*F, *fos*

ANI value of 98.5% (SD-2.29%) and AAI value of 98.3% (SD-6.75%), confirming the species similarity of the query genome with *Aeromonas caviae* GSH8M-1 DNA complete genome. Additional genome statistics for the query genome are provided in Table 2.

Numerous putative virulence genes are found in *A. caviae* SVJ23, including those for type I, II, III, and IV secretion systems, type IV pilus, hemolysin, adhesin, flagella, collagenase, etc., and antibiotic resistance genes conferring resistance to fluoroquinolones and fosfomycin along with multidrug resistance efflux pumps, and quorum sensing genes like *lux*R, *lux*S, *lux*O. In any case, insights into its genome content suggest that the strain is well adapted to unfavourable conditions via various stress response mechanisms and contains multiple antibiotic and heavy metal resistance genes and several efflux transporters that extrude a variety of compounds from the cell (Table 3).

Discussion

To ensure accurate identification of the pathogenic *Aeromonas* spp., standardized bacterial identification procedures must be used with a high suspicion index regarding the potential pathogen(s). The genome sequencing of *A. caviae* SVJ23 represents a substantial advancement in our knowledge of the pathogenesis of this genus, enabling additional investigation into microbial virulence and host-pathogen interaction and the development of efficient diagnostics.

Authors' Contributions

Dr Savita Jadhav: Investigation of the case and the original first draft; Ms Ujjayni Saha: DNA isolation, genome sequencing and draft preparation; Mr Kunal Dixit: genome analysis and revision; Dr Anjali Kher and Dr Nitin Lingayat: Clinical treatment of the present case. Dr Sourav Sen: Editing and finalising the draft version; Dr Vivekanand Jadhav: Investigation of the case, Dr Sunil Saroj: Corresponding author, editing and finalising the draft version; all authors reviewed the manuscript.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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