Journal of Fisheries
Volume 15 Issue 1 April 2027
Open Access

eISSN 2311-3111
pISSN 2311-729X

DOI: https://doi.org/10.17017/j.fish.1446

Original Article

Phenotypic and molecular characterisation of multidrug-resistant *Enterobacter cloacae* isolated from two cyprinid carp species and evaluation of the antibacterial activity of *Curcuma longa* aqueous extract

Vishal Soni[1] • Sanat Chandravanshi[2] • Rachna Gulati[1] • Bharat A. Kandoriya[3] • Krina D. Patel[4] • Shivam Arunkumar Patel[5] • Alli Asritha[6] • P. Shivani[7] • Julie M.P.[8] • Avadhutha Nikhil[9] • Priyanka Acharya[10] • Sakthibalan S.[11] • P. Geetharani[12]

[1] *College of Fisheries Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, Haryana, India*
[2] *Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata 700094, West Bengal, India*
[3] *College of Fisheries Science, Junagadh Agricultural University, Veraval 362265, Gujarat, India*
[4] *Centre of Excellence in Aquaculture, Kamdhenu University, Ukai, Gujarat 394680, India*
[5] *University of Naples Federico II, Naples 80138, Italy*
[6] *College of Fishery Science, Andhra Pradesh Fisheries University, Muthukur, Nellore 524344, Andhra Pradesh, India*
[7] *ICAR–Central Institute of Fisheries Education, Mumbai 400061, Maharashtra, India*
[8] *Acharya Nagarjuna University, Guntur 522510, Andhra Pradesh, India*
[9] *Dr. M.G.R. Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Ponneri 601204, Tamil Nadu, India*
[10] *Fisheries Polytechnic, Dhamdha, Durg 491331, Chhattisgarh, India*
[11] *Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Thoothukudi 628008, Tamil Nadu, India*
[12] *Kerala University of Fisheries and Ocean Studies, Kochi 682506, Kerala, India*

Correspondence
Vishal Soni; College of Fisheries Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, Haryana, India
vishalsoni68001@gmail.com

Manuscript history
Received 6 August 2026 | Accepted 1 November 2026 | Published online 5 November 2026

Citation
Soni V, Chandravanshi S, Gulati R, Kandoriya BA, Patel KD, Patel SA, Asritha A, Shivani P, Julie MP, Nikhil A, Acharya P, Sakthibalan S, Geetharani P (2027) Phenotypic and molecular characterisation of multidrug-resistant *Enterobacter cloacae* isolated from two cyprinid carp species and evaluation of the antibacterial activity of *Curcuma longa* aqueous extract. Journal of Fisheries 15(1): 151214. DOI: 10.17017/j.fish.1446

Abstract
The emergence of multidrug-resistant (MDR) bacterial pathogens poses a major challenge to freshwater aquaculture by compromising fish health, production sustainability and antimicrobial stewardship. This study investigated the phenotypic and molecular characteristics of multidrug-resistant members of the *Enterobacter cloacae* complex isolated from naturally diseased fish of two cyprinid carps (*Labeo rohita* and *Catla catla*) and evaluated the *in vitro* antibacterial activity of aqueous *Curcuma longa* extract. Eight naturally diseased fish belonging to two cyprinid carp species were collected from three independent disease outbreaks and subjected to bacteriological examination. Morphological and biochemical analyses, together with 16S rRNA gene sequencing, identified all isolates as members of the *E. cloacae* complex with 99.86–100% 16S rRNA gene sequence similarity to reference sequences in the NCBI GenBank database. Antimicrobial susceptibility testing against 12 antibiotics demonstrated that all isolates were multidrug resistant, with multiple antibiotic

resistance (MAR) indices ranging from 0.25 to 0.33. Universal resistance to tetracycline was observed, whereas all isolates remained susceptible to chloramphenicol, levofloxacin and ofloxacin. PCR analysis detected multiple antimicrobial resistance genes, while phenotypic characterisation revealed isolate-dependent variation in biofilm formation, protease and lipase activities. The aqueous *C. longa* extract exhibited significant concentration-dependent antibacterial activity, producing inhibition zones of 18.0–20.0 mm at 100 mg mL^{-1} and minimum inhibitory concentration values of 12.5–25 mg mL^{-1}. These findings identify MDR members of the *E. cloacae* complex as emerging opportunistic pathogens of two cyprinid carps and highlight the potential of aqueous *C. longa* extract as a sustainable plant-derived antibacterial agent for freshwater aquaculture.

Keywords: antibacterial activity; *Catla catla*; *Curcuma longa*; *Enterobacter cloacae* complex; freshwater aquaculture; *Labeo rohita*; multidrug resistance

1 | INTRODUCTION

Aquaculture is one of the fastest-growing food-producing sectors globally and plays a critical role in food security, nutrition and rural livelihoods. According to the Food and Agriculture Organization (FAO), aquaculture now supplies more than half of the aquatic animals consumed worldwide, with freshwater aquaculture contributing substantially to global production (FAO 2024). India is among the world's leading freshwater fish-producing countries, where Indian major carps, including *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*, are the principal cultured species because of their rapid growth, high market demand and economic importance (Jayasankar 2018; FAO 2024). However, production intensification through high stocking densities and intensive farming practices has increased the frequency of infectious disease outbreaks, posing major challenges to fish production, farm profitability and aquaculture sustainability (Bondad-Reantaso *et al.* 2023). Bacterial diseases remain a major constraint, causing mortality, impaired growth, poor feed utilisation and increased production costs. Environmental stressors, including deteriorating water quality, elevated organic loading and husbandry-related stress, further compromise fish immunity and predispose cultured fish to opportunistic bacterial infections (Bondad-Reantaso *et al.* 2023). Among Gram-negative bacteria, members of the family Enterobacteriaceae have emerged as important opportunistic pathogens in aquaculture. *Enterobacter cloacae*, a member of the *Enterobacter cloacae* complex (ECC), has gained increasing attention because of its genomic plasticity, environmental adaptability and broad host range. It has been isolated from diseased freshwater and marine fish, ornamental fish and crustaceans exhibiting haemorrhagic septicaemia, ulcerative lesions, ascites and high mortality, highlighting its growing significance in aquatic animal health (Kumar *et al.* 2013; Dong *et al.* 2020; Preena *et al.* 2021; Anifowose *et al.* 2024; Mabrok *et al.* 2024).

The emergence of *E. cloacae* has coincided with the rapid spread of antimicrobial resistance (AMR), posing a serious threat to aquaculture sustainability and public health. Extensive antibiotic use has accelerated the selection of multidrug-resistant (MDR) bacteria and the dissemination of antimicrobial resistance genes (ARGs) within aquatic ecosystems (Cabello *et al.* 2016; FAO 2024). Owing to its genomic plasticity, *E. cloacae* readily acquires resistance determinants through plasmids, integrons, transposons and other mobile genetic elements, conferring resistance to β-lactams, tetracyclines, aminoglycosides, sulfonamides and fluoroquinolones (Davin-Regli and Pagès 2019; Qiu *et al.* 2024). The dissemination of resistance genes such as tet, sul and bla represents a major One Health concern because these determinants can be horizontally transferred among environmental, animal and human-associated bacteria (WHO 2023; FAO 2024). Continuous surveillance of antimicrobial susceptibility, multidrug resistance, MAR indices and resistance-associated genes is therefore essential for effective disease management and antimicrobial stewardship. In addition to AMR, the pathogenicity of *E. cloacae* is mediated by virulence determinants that promote bacterial persistence and host colonisation. Biofilm formation enhances survival under adverse conditions and antimicrobial tolerance, whereas extracellular enzymes, including proteases and lipases, facilitate tissue invasion and disease progression (Costerton *et al.* 1999; Flemming *et al.* 2016). However, the relationship between antimicrobial resistance and virulence-associated traits in *E. cloacae* infecting Indian major carps remains poorly understood.

The increasing prevalence of MDR bacterial pathogens has stimulated interest in plant-derived therapeutics as sustainable alternatives to antibiotics. *Curcuma longa* L. (turmeric) possesses broad-spectrum antibacterial, antioxidant, anti-inflammatory and immunomodulatory properties, largely attributed to curcumin and other bioactive phenolic compounds (Adamczak *et al.* 2020; Dai *et al.* 2022). These compounds inhibit bacterial growth by disrupting cell membranes, suppressing quorum sensing and biofilm formation, interfering with essential metabolic pathways and attenuating bacterial virulence (Dai *et al.* 2022). Although *C. longa* has demonstrated antibacterial activity against several fish pathogens, including *Aeromonas hydrophila*, *Edwardsiella tarda* and *Enterobacter* spp., its efficacy against MDR *E. cloacae* isolated from Indian major carps remains poorly understood (Harikrishnan and Balasundaram 2008; Al-Niaeem *et al.* 2021; Soni

et al. 2025). Despite these advances, studies integrating molecular identification, antimicrobial susceptibility profiling, AMR gene detection, phenotypic virulence characterisation and evaluation of the antibacterial efficacy of *C. longa* against *E. cloacae* isolated from naturally diseased Indian major carps remain scarce. Therefore, the present study aimed to isolate and molecularly characterise *E. cloacae* from naturally diseased Indian major carps; determine their antimicrobial susceptibility profiles, multidrug resistance status, MAR indices, antimicrobial resistance genes and phenotypic virulence-associated traits; and evaluate the *in vitro* antibacterial efficacy and minimum inhibitory concentration (MIC) of aqueous *C. longa* extract. Collectively, this study provides novel insights into the emergence, antimicrobial resistance and virulence characteristics of *E. cloacae* associated with *Labeo rohita* and *Catla catla* while highlighting the potential of *C. longa* as a sustainable plant-derived antibacterial agent for disease management in freshwater aquaculture.

2 | METHODOLOGY

2.1 Sample collection and bacterial isolation

Disease investigations were conducted during natural outbreaks of bacterial disease in cultured Indian major carps reared in commercial earthen ponds in Ladwa and Sultanpur villages, Hisar district, Haryana, India (Figure 1). A total of eight naturally diseased fish, comprising five rohu (*Labeo rohita*) and three catla (*Catla catla*), were collected from three independent disease outbreaks: two fish from Ladwa in August, three fish from Ladwa in December, and three fish from Sultanpur in December. The sampled fish included *L. rohita* and *C. catla* exhibiting characteristic clinical signs, including haemorrhagic patches and ulcerative skin lesions. Fish weighing approximately 85–210 g were transported alive to the laboratory in aerated containers for immediate bacteriological examination. The external surface surrounding each lesion was disinfected with 70% ethanol and lesion samples were aseptically collected using sterile cotton swabs. Each sample was streaked individually onto Nutrient Agar (NA; HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 32±2°C for 24–48 h. The predominant bacterial colony morphotype from each lesion sample was selected and purified by repeated streaking on fresh NA plates to obtain pure cultures. Purified isolates were maintained on NA slants at 4°C for routine laboratory use and preserved in sterile 20% (v/v) glycerol at −80°C for long-term storage.

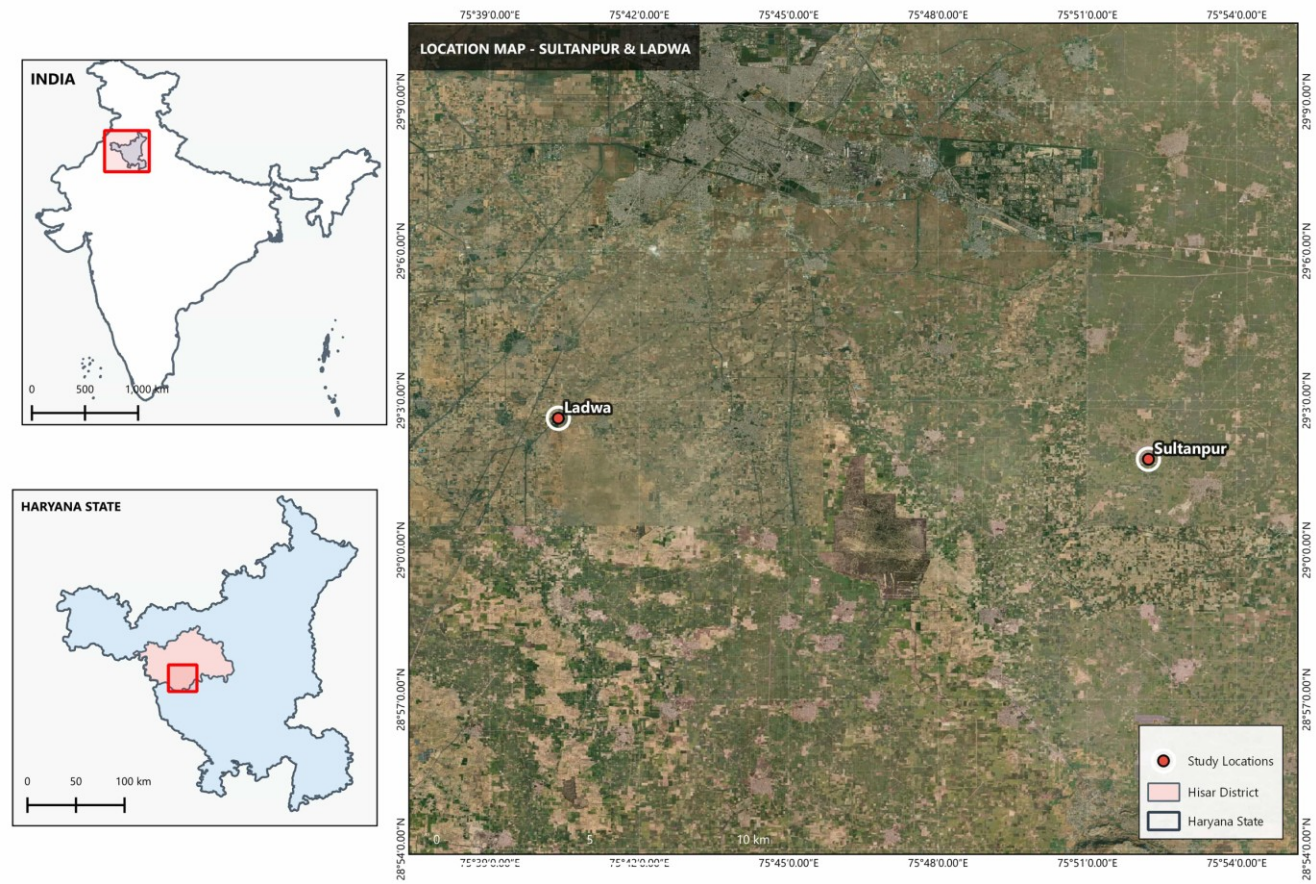


FIGURE 1 Fish sampling locations in Hisar District of Haryana, India.

2.2 Morphological and biochemical characterisation

All eight bacterial isolates recovered from the diseased fish were initially subjected to preliminary phenotypic characterisation based on colony morphology, including colony size, colour, pigmentation, opacity, surface texture, elevation and margin, after incubation on Nutrient Agar at 32±2°C for 24 h. Gram reaction was determined by Gram staining and the potassium hydroxide (KOH) string test. Preliminary phenotypic identification was performed according to Brenner *et al.* (2005) using conventional biochemical tests, including catalase, oxidase, motility, citrate utilisation, urease, indole production, methyl red (MR), Voges–Proskauer (VP), nitrate reduction and triple sugar iron (TSI) test. Following this initial screening, one isolate from each of the three epidemiologically distinct outbreak events was selected for further investigation. This selection strategy was adopted to ensure representation of all three outbreak events while avoiding redundant detailed characterisation of multiple isolates from the same outbreak. The three selected isolates were designated EC-LA1, EC-LD1 and EC-ST1 and were subjected to further molecular and antimicrobial characterisation.

2.3 Molecular identification by 16S rRNA gene sequencing

Genomic DNA was extracted from purified bacterial isolates using the HiPurA® Bacterial Genomic DNA Purification Kit (MB505, HiMedia Laboratories Pvt. Ltd., Mumbai, India) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were determined using a NanoDrop spectrophotometer prior to PCR amplification. The nearly full-length 16S rRNA gene was amplified using the universal bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) (Weisburg *et al.* 1991) procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. PCR amplification was performed in a 50 µL reaction mixture containing 25 µL of 2× PCR Master Mix (HiMedia Laboratories Pvt. Ltd., Mumbai, India), 0.2 µM of each primer, 2 µL of genomic DNA template and nuclease-free water to the final volume. Amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 45 sec, with a final extension at 72°C for 5 min. PCR products were resolved by electrophoresis on 1.5% agarose gel, visualised using a gel documentation system and the purified amplicons were commercially sequenced by the Sanger method (HiMedia Laboratories Pvt. Ltd., Mumbai, India). The obtained sequences were analysed using the BLASTn algorithm against the NCBI GenBank database for species-level identification. Phylogenetic analysis was performed in MEGA 12. Sequences were aligned using MUSCLE, and the final alignment comprised 1,160 nucleotide positions. Phylogenetic relationships were inferred using the Neighbor-Joining method (Saitou and Nei 1987) with the Kimura two-parameter model and 1,000 bootstrap replicates. Reference sequences were retrieved from NCBI GenBank based on BLASTn sequence identity and query coverage. Gaps and missing data were handled by pairwise deletion.

2.4 Antibiotic susceptibility testing

Antimicrobial susceptibility of the bacterial isolates was evaluated using the Kirby–Bauer disc diffusion method on Mueller–Hinton Agar (MHA) according to the standard procedure described by Bauer *et al.* (1966). Fresh overnight bacterial cultures were adjusted to the 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU mL^{-1}) and uniformly spread onto MHA plates using sterile cotton swabs. Commercial antibiotic discs (HiMedia Laboratories Pvt. Ltd., Mumbai, India) were aseptically placed on the inoculated agar surface and the plates were incubated at 32±2°C for 24 h. A total of 12 antibiotics representing different antimicrobial classes were evaluated: ampicillin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), levofloxacin (5 µg), tetracycline (30 µg), amikacin (30 µg), tobramycin (10 µg), cefotaxime (30 µg), nalidixic acid (30 µg), ofloxacin (5 µg) and cefixime (5 µg). Following incubation, the diameters of the inhibition zones were measured in millimetres and interpreted as susceptible (S), intermediate (I), or resistant (R) according to the Clinical and Laboratory Standards Institute (CLSI) M100, 33rd edition (2023) guidelines. The Multiple Antibiotic Resistance (MAR) index was calculated as per Krumperman (1983) and multidrug-resistance (MDR) classification was determined according to the criteria of Magiorakos *et al.* (2012).

2.5 Detection of antimicrobial resistance (AMR) genes

The presence of selected antimicrobial resistance (AMR) genes, including tetracycline resistance genes (tetA, tetC and tetS), sulfonamide resistance genes (sul1 and sul2) and the β-lactamase gene (blaSHV), was investigated by conventional PCR using gene-specific primers (Table 1). Genomic DNA extracted from the bacterial isolates served as the template. Each 25 µL PCR reaction contained 12.5 µL of 2× PCR Master Mix (HiMedia Laboratories Pvt. Ltd., Mumbai, India), 0.5 µL each of forward and reverse primers (10 µM), 2 µL of template DNA and nuclease-free water. Nuclease-free water was included as a no-template negative control in each PCR run. A positive control was not included. PCR amplification was performed under gene-specific cycling conditions (Table 1). Amplified products were resolved by electrophoresis on 1.5% agarose gels, stained with a nucleic acid stain, visualised under UV illumination using a gel documentation system, and assessed based on the expected amplicon sizes.

TABLE 1 Primers used for PCR amplification of antimicrobial resistance (AMR) genes and PCR conditions.

Gene	Primer sequence (5'–3')	Amplicon size (bp)	PCR conditions	Reference
tetA	F: GCTACATCCTGCTTGCCTT R: CATAGATCGCCGTGAAGAGG	210	95°C, 5 sec; 60°C, 30 sec; 72°C, 30 sec (35 cycles)	Ayoub *et al.* (2024)
tetC	F: TCTAACAAATGCGCTCATCGT R: GGTTGAAGGCTCTCAAGGC	570	94°C, 30 sec; 62°C, 30 sec; 72°C, 45 sec (35 cycles)	Schmidt *et al.* (2001)
tetS	F: CATAGACAAGCCGTTGACC R: ATGTTTTTGGAACGACAGAG	667	95°C, 30 sec; 56°C, 60 sec; 72°C, 45 sec (35 cycles)	Ng *et al.* (2001)
sul1	F: CGCACCGGAAACATCGCTGCAC R: TGAAGTTCCGCCCAAGGCTCG	163	94°C, 30 sec; 62°C, 30 sec; 72°C, 45 sec (35 cycles)	Ayoub *et al.* (2024)
sul2	F: TGCGGATGAAGTCAGCTCC R: GGGGGCAGATGTGATCGAC	623	94°C, 30 sec; 60°C, 30 sec; 72°C, 30 sec (35 cycles)	Mala *et al.* (2017)
blaSHV	F: AGGATTGACTGCCTTTTTG R: ATTTGCTGATTTCGCTCG	392	94°C, 30 s; 54°C, 40 s; 72°C, 40 sec (35 cycles)	Ayoub *et al.* (2024)

2.6 Determination of phenotypic virulence factors

The phenotypic virulence potential of the *E. cloacae* isolates was evaluated by assessing biofilm formation, protease and lipase production following standard protocols. Biofilm formation was determined by the qualitative tube adherence method (Christensen *et al.* 1982), whereas protease and lipase activities were evaluated on skim milk agar and tributyrin agar according to Smibert and Krieg (1994) and Sierra (1957), respectively. The presence of a crystal violet-stained film (biofilm) or a clear hydrolytic zone (protease and lipase) was recorded as positive (+), while the absence of these characteristics was recorded as negative (−).

2.7 Evaluation of the antibacterial activity of *Curcuma longa* aqueous extract

2.7.1 Preparation of extract and agar well diffusion assay: Aqueous extract of *C. longa* rhizomes was prepared as described previously (Soni *et al.* 2025). Briefly, fresh rhizomes were washed, shade-dried and ground into powder, mixed with distilled water (1:10, w/v), agitated on an orbital shaker (120 rpm, 48 h), filtered (Whatman No. 1), centrifuged (2,500 × g, 10 min) and the supernatant concentrated under reduced pressure and stored at -20°C. The resulting extract was evaluated for antibacterial activity by the agar well diffusion method. Overnight bacterial cultures adjusted to 0.5 McFarland standard were uniformly spread onto Mueller-Hinton agar (MHA) using sterile cotton swabs and wells of 6 mm diameter were prepared using a sterile cork borer and each well was loaded with 100 µL of aqueous extract at one of four concentrations (100, 50, 25, 12.5 mg mL^{-1}). Sterile distilled water served as the negative control and a chloramphenicol disc (30 µg) served as the positive control. Plates were held at room temperature for 1 h to permit extract diffusion, then incubated at 32±2°C for 24 h and inhibition zone diameters were measured in millimetres in triplicate.

2.7.2 Determination of minimum inhibitory concentration (MIC): MIC of the aqueous extract against *E. cloacae* isolates was determined by broth microdilution in sterile 96-well microtiter plates following Sarker *et al.* (2007), with slight modifications. Two-fold serial dilutions of the extract (100 mg mL^{-1} stock) were prepared in Mueller–Hinton Broth (MHB) to yield final concentrations of 50–0.024 mg mL^{-1}, with each well containing extract and bacterial suspension (~5 × 10^5 CFU mL^{-1}) in a final volume of 100 µL. Plates were incubated at 32±2°C for 24 h, followed by addition of 30 µL of 0.015% (w/v) resazurin as a growth indicator; MIC was recorded as the lowest concentration preventing the blue-to-pink colour change. Sterility, growth and medium controls were included and assays were performed in triplicate.

2.8 Data analysis

Data are expressed as mean ± SD of three technical replicates (n = 3). Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Differences among treatment means were analysed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Statistical significance was accepted at $p < 0.05$.

3 | RESULTS

3.1 Clinical signs and gross pathology

Naturally diseased *L. rohita* and *C. catla* exhibited multifocal ulcerative lesions on the dorsal and lateral body surfaces, accompanied by haemorrhagic patches around the fins and operculum. Affected fish also displayed lethargy, reduced feeding activity and erratic swimming behaviour. Internal examination revealed ascitic fluid accumulation within the peritoneal cavity.

3.2 Colony morphology and biochemical characterisation

Morphological and biochemical characterisation of all purified isolates recovered from the eight diseased fish

revealed similar phenotypic characteristics among isolates obtained from the same disease outbreak. Therefore, one representative isolate from each outbreak (EC-LA1, EC-LD1 and EC-ST1) was selected for detailed characterisation. The representative isolates produced cream-coloured, circular, smooth, convex and opaque colonies on Nutrient Agar after incubation at 32±2°C for 24 h. All isolates were Gram-negative, KOH-positive, catalase-positive, oxidase-negative and motile. They were positive for citrate utilisation, Voges–Proskauer (VP), nitrate reduction and produced an acid slant/acid butt (A/A) reaction with gas but no hydrogen sulfide (H_2S) on Triple Sugar Iron (TSI) agar. All isolates were negative for indole production and methyl red (MR) reaction. Urease activity differed among the isolates, with EC-LA1 and EC-ST1 testing positive and EC-LD1 testing negative. Overall, the morphological and biochemical characteristics were consistent with those of *E. cloacae* (Table 2).

3.3 Molecular identification and phylogenetic analysis

PCR amplification of the 16S rRNA gene yielded a single amplicon of approximately 1,500 bp in all three bacterial isolates (EC-LA1, EC-LD1 and EC-ST1). NCBI BLASTn analysis of the obtained 16S rRNA gene sequences identified all three isolates as *E. cloacae*, with 99.86–100% sequence identity and 100% query coverage against reference sequences available in the NCBI GenBank database. The representative sequences were deposited in GenBank under accession numbers PP987319 (EC-LA1), PP980717 (EC-LD1) and PP981421 (EC-ST1). Phylogenetic analysis revealed that all three isolates clustered with reference *E. cloacae* sequences and were clearly separated from the outgroup *Escherichia coli* (X80724) (Figure 2). Isolate EC-LD1 clustered most closely with the reference *E. cloacae* strain PV882369, with a bootstrap value of 100%. This cluster was further associated with isolate EC-ST1 and reference strain PX272174, supported by bootstrap values of 84% and 80%, respectively. In contrast, isolate EC-LA1 formed a separate subclade with reference strains KY930712 and KJ607605, supported by bootstrap values of 78% and 92%, respectively. Overall, the phylogenetic relationships were consistent with the molecular identification of all three isolates as *E. cloacae*.

TABLE 2 Morphological and biochemical characteristics of *Enterobacter cloacae* isolates.

Characteristic	*E. cloacae* EC-LA1	*E. cloacae* EC-LD1	*E. cloacae* EC-ST1
Gram stain	–	–	–
KOH string test	+	+	+
Catalase	+	+	+
Oxidase	–	–	–
Motility	+	+	+
Citrate utilisation	+	+	+
Indole production	–	–	–
Urease	+	–	+
Methyl red (MR)	–	–	–
Voges–Proskauer (VP)	+	+	+
Nitrate reduction	+	+	+
Triple Sugar Iron (TSI)	A/A, Gas, H_2S–	A/A, Gas, H_2S–	A/A, Gas, H_2S–

Note: +, positive reaction; –, negative reaction; A/A, acid slant/acid butt; Gas, gas production; H_2S–, no hydrogen sulfide production.

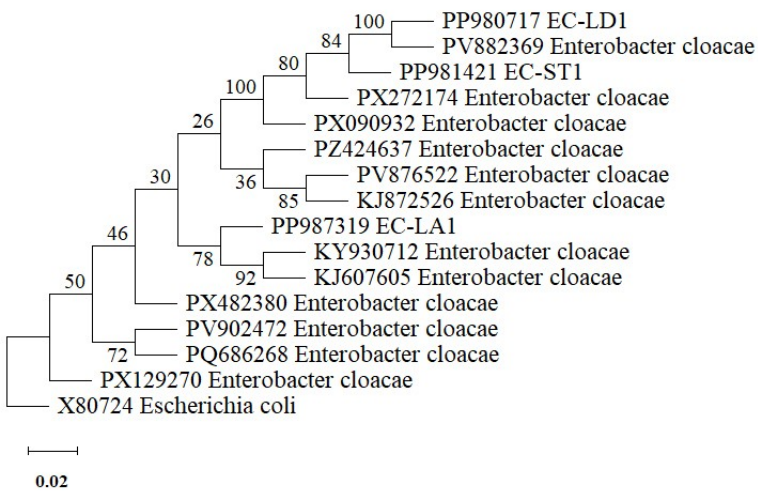


FIGURE 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing relationships of *Enterobacter cloacae* isolates EC-LA1 (PP987319), EC-LD1 (PP980717) and EC-ST1 (PP981421) with GenBank reference sequences, using *Escherichia coli* (X80724) as the outgroup. Bootstrap values (%) from 1,000 replications are shown at branch nodes. Scale bar = 0.02 substitutions per nucleotide position.

3.4 Antibiotic susceptibility test

The antimicrobial susceptibility profiles of the three *E. cloacae* isolates against 12 antimicrobial agents are presented in Figure 3, while the MAR index and MDR profiles are summarised in Table 3. All isolates were uniformly susceptible to chloramphenicol, levofloxacin and ofloxacin, whereas complete resistance to tetracycline was observed in all isolates. Isolate-specific resistance patterns varied: EC-LA1 was resistant to ampicillin, nalidixic acid, cefixime and tetracycline; EC-LD1 was resistant to ciprofloxacin, cefixime and tetracycline, with intermediate susceptibility to gentamicin and amikacin; and EC-ST1 was resistant to ampicillin, ciprofloxacin, tobramycin and tetracycline, with intermediate susceptibility to cefotaxime. Overall, all three isolates exhibited resistance to multiple antimicrobial classes and were therefore classified as multidrug-resistant (MDR). The MAR index ranged from 0.25 to 0.33, with EC-LA1 and EC-ST1 each exhibiting resistance to four antibiotics representing four antimicrobial classes (MAR = 0.33), whereas EC-LD1 exhibited resistance to three antibiotics belonging to three antimicrobial classes (MAR = 0.25).

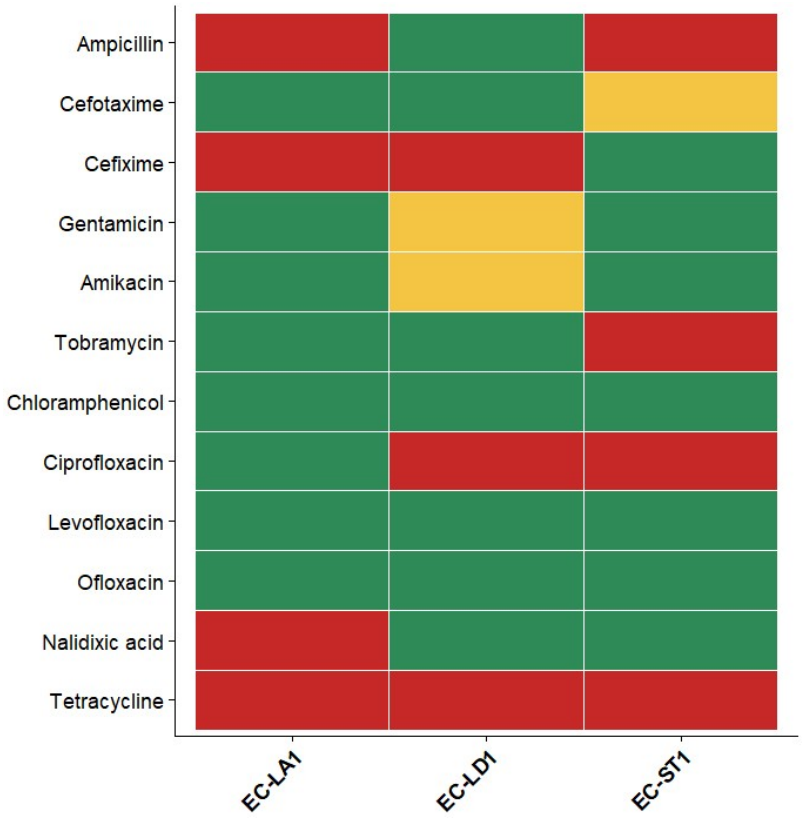


FIGURE 3 Heatmap showing the antimicrobial susceptibility profiles of *Enterobacter cloacae* isolates (EC-LA1, EC-LD1 and EC-ST1) against 12 antimicrobial agents, interpreted according to CLSI (2023) guidelines. Antibiotics are grouped according to their antimicrobial classes. Green, yellow and red indicate susceptible (S), intermediate (I) and resistant (R), respectively.

TABLE 3 Multiple antibiotic resistance (MAR) index and multidrug resistance (MDR) profile of *Enterobacter cloacae* isolates.

Isolate	MAR index	No. of resistant antimicrobial classes	MDR status
E. cloacae EC-LA1	0.33	4 (Penicillin, Quinolone, Tetracycline, Cephalosporin)	MDR
E. cloacae EC-LD1	0.25	3 (Fluoroquinolone, Tetracycline, Cephalosporin)	MDR
E. cloacae EC-ST1	0.33	4 (Penicillin, Fluoroquinolone, Tetracycline, Aminoglycoside)	MDR

3.5 Antibacterial activity of *Curcuma longa* aqueous extract

The antibacterial activity of the aqueous extract of Curcuma longa against *E. cloacae* isolates was evaluated by the agar well diffusion assay, and the minimum inhibitory concentration (MIC) was determined by the broth microdilution method (Table 4; Figure 4). The aqueous extract exhibited a clear concentration-dependent antibacterial effect against all three isolates. The largest inhibition zones were observed at the highest extract concentration (100 mg mL^{-1}), ranging from 18.0±0.8 mm to 20.0±1.0 mm, whereas the smallest inhibition zones were recorded at 12.5 mg mL^{-1}, ranging from 7.0±0.6 mm to 9.0±1.0 mm. No inhibition was observed in the negative control, while

the positive control, chloramphenicol (30 µg), produced inhibition zones ranging from 18.9±0.8 mm to 22.1±0.4 mm. The positive and negative controls were included to validate assay performance and were not subjected to statistical comparison with the extract treatments. Among the tested isolates, *E. cloacae* EC-LA1 exhibited the greatest susceptibility to the aqueous extract, producing the largest inhibition zones at all tested concentrations and the lowest MIC value (12.5 mg mL^{-1}). In contrast, EC-LD1 and EC-ST1 exhibited comparatively lower susceptibility, with identical MIC values of 25 mg mL^{-1}. One-way analysis of variance (ANOVA) revealed that extract concentration had a highly significant effect on the inhibition zone diameter of EC-LA1 ($F_{3,8}$ = 112.27, p < 0.001), EC-LD1 ($F_{3,8}$ = 97.87, p < 0.001) and EC-ST1 ($F_{3,8}$ = 147.26, p < 0.001). Tukey's HSD test further confirmed significant differences in inhibition zone diameters among all tested extract concentrations for each isolate (p < 0.05). Collectively, these findings demonstrate that the aqueous extract of *C. longa* possesses significant concentration-dependent antibacterial activity against MDR *E. cloacae* isolates.

3.6 Phenotypic virulence potential of the isolates

The phenotypic virulence-associated traits of the three *E. cloacae* isolates were evaluated based on biofilm formation, protease production and lipase activity (Table 5). Biofilm formation was observed in isolates EC-LA1 and EC-LD1, whereas EC-ST1 was biofilm-negative. Protease activity was detected in EC-LA1 and EC-ST1 but was absent in EC-LD1. In contrast, lipase activity was detected in EC-LD1 and EC-ST1 but not in EC-LA1. None of the isolates exhibited all three virulence-associated traits simultaneously, indicating variability in the phenotypic expression of virulence factors among the *E. cloacae* isolates.

TABLE 4 Antibacterial activity of *Curcuma longa* aqueous extract against *Enterobacter cloacae* isolates determined by the agar well diffusion assay and minimum inhibitory concentration (MIC).

Isolate	Zone of inhibition (mm)						MIC (mg mL^{-1})
	100 mg mL^{-1}	50 mg mL^{-1}	25 mg mL^{-1}	12.5 mg mL^{-1}	Negative control	Chloramphenicol (30 µg)	
E. cloacae EC-LA1	20.0±1.0^{a}	17.3±0.6^{b}	13.0±0.5^{c}	9.0±1.0^{d}	No inhibition	22.1±0.4	12.5
E. cloacae EC-LD1	18.0±0.8^{a}	15.0±1.0^{b}	11.0±0.9^{c}	7.0±0.6^{d}	No inhibition	18.9±0.8	25.0
E. cloacae EC-ST1	18.5±0.7^{a}	13.9±0.4^{b}	10.1±0.7^{c}	7.2±0.9^{d}	No inhibition	21.5±0.9	25.0

Values are presented as mean ± SD of three technical replicates (n = 3). Different lowercase superscript letters within the same row indicate significant differences among extract concentrations (one-way ANOVA followed by Tukey's HSD test, p < 0.05). Positive (chloramphenicol) and negative controls were included for assay validation only and were excluded from statistical comparisons.

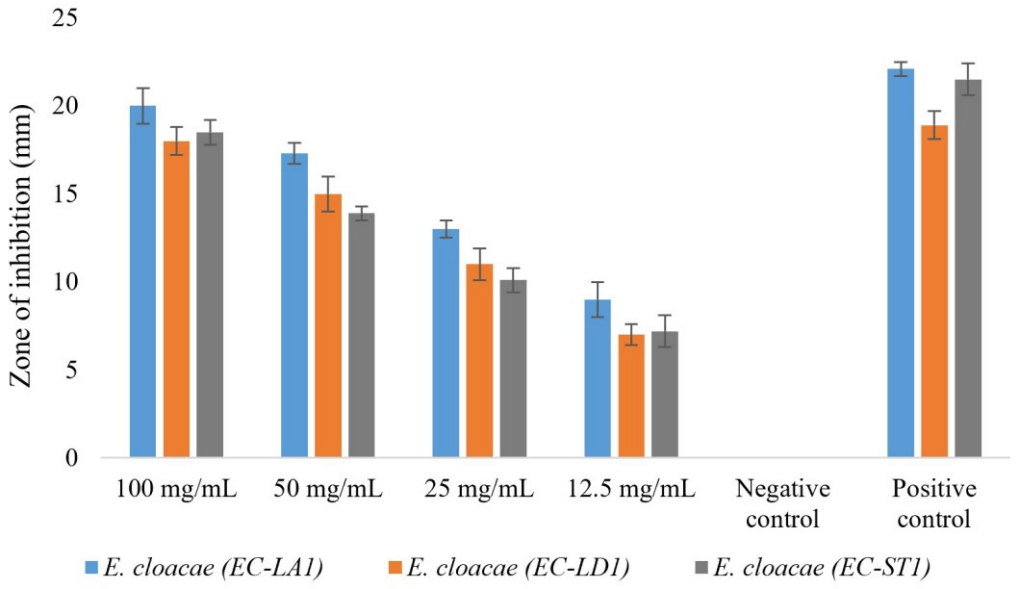


FIGURE 4 Antibacterial activity of the aqueous extract of *Curcuma longa* against *Enterobacter cloacae* isolates determined by the agar well diffusion assay at different extract concentrations. Bars data are presented as mean ± SD of three technical replicates (n = 3).

TABLE 5 Phenotypic virulence-associated traits of *Enterobacter cloacae* isolates.

Isolate	Biofilm	Protease	Lipase
E. cloacae EC-LA1	+	+	–
E. cloacae EC-LD1	+	–	+
E. cloacae EC-ST1	–	+	+

3.7 Distribution of antimicrobial resistance (AMR) genes

The distribution of tetracycline resistance genes (tetA, tetC, tetS), sulfonamide resistance genes (sul1, sul2) and the β-lactamase gene (blaSHV) among the three *E. cloacae* isolates is presented in Table 6. The tetA gene was detected in EC-LA1 and EC-LD1 but was absent in EC-ST1, whereas tetC was detected exclusively in EC-LD1. The tetS gene was present in both EC-LD1 and EC-ST1 but absent in EC-LA1. Among the sulfonamide resistance genes, sul1 was detected exclusively in EC-LA1, while sul2 was detected in EC-LA1 and EC-ST1 but absent in EC-LD1. The β-lactamase gene blaSHV was detected in EC-LA1 and EC-LD1 but not in EC-ST1. Overall, each isolate carried a distinct combination of resistance genes. EC-LA1 and EC-LD1 each harboured the highest number of AMR genes (four genes each), comprising tetA, sul1, sul2 and blaSHV in EC-LA1 and tetA, tetC, tetS and blaSHV in EC-LD1. In contrast, EC-ST1 carried the fewest resistance genes, harbouring only tetS and sul2. Notably, the co-occurrence of tetracycline and sulfonamide or β-lactamase resistance genes across isolates suggests the possible presence of resistance gene clusters or co-transfer via mobile genetic elements, warranting further investigation.

TABLE 6 Distribution of AMR genes among *Enterobacter cloacae* isolates.

Isolate	*tetA*	*tetC*	*tetS*	*sul1*	*sul2*	*blaSHV*
E. cloacae EC-LA1	+	–	–	+	+	+
E. cloacae EC-LD1	+	+	+	–	–	+
E. cloacae EC-ST1	–	–	+	–	+	–

Note: + = gene detected; − = gene not detected

4 | DISCUSSION

4.1 Phenotypic and molecular characterisation

The present study characterised three representative *E. cloacae* isolates recovered from naturally diseased *L. rohita* and *C. catla* during bacterial disease outbreaks in Haryana, India, through integrated phenotypic, molecular, antimicrobial resistance and virulence profiling. The clinical signs observed in naturally infected *L. rohita* and *C. catla*, including multifocal ulcerative lesions, haemorrhagic patches, lethargy, erratic swimming behaviour and ascitic fluid accumulation, are consistent with systemic bacterial septicaemia associated with opportunistic Gram-negative pathogens. Comparable pathological manifestations have been reported in *E. cloacae*-infected *Pangasianodon hypophthalmus* (Kumar *et al.* 2013), mullets (*Mugil cephalus* and *M. capito*) (Mabrok *et al.* 2024), African catfish (*Clarias gariepinus*) (Anifowose *et al.* 2024) and experimentally infected red swamp crayfish (*Procambarus clarkii*) (Dong *et al.* 2020). Although similar clinical manifestations have been reported in different aquatic hosts, these signs are not specific to *E. cloacae* and may occur in infections caused by other pathogens. Environmental stress, deteriorating water quality and compromised host immunity may also contribute to disease development by facilitating bacterial colonisation and dissemination. Therefore, the recovery of *E. cloacae* from the diseased carp examined in this study indicates an association with the investigated outbreaks but does not, by itself, establish causation.

The combined application of conventional bacteriological methods and 16S rRNA gene sequencing enabled identification of the representative isolates as *E. cloacae*. Their morphological and biochemical characteristics were consistent with previous descriptions from diseased fish (Kumar *et al.* 2013; Preena *et al.* 2021; Anifowose *et al.* 2024; Mabrok *et al.* 2024), while sequencing revealed 99.86–100% similarity to reference sequences in GenBank. Phylogenetic analysis revealed that the isolates clustered within the *E. cloacae* lineage but formed distinct subclades, indicating sequence variation among the investigated isolates. The integration of phenotypic and molecular approaches strengthened the identification process, which is particularly important because members of the *E. cloacae* complex exhibit substantial phenotypic similarity despite considerable genomic diversity. Accordingly, although 16S rRNA gene sequencing remains useful for routine bacterial identification, multilocus sequence analysis or whole-genome sequencing would provide improved resolution among closely related strains (Davin-Regli and Pagès 2019; Rahi *et al.* 2024; Qiu *et al.* 2024). Consequently, the identification should be interpreted in view of the limited discriminatory power of 16S rRNA gene sequencing for distinguishing closely related members of the *E. cloacae* complex.

4.2 Antimicrobial resistance and resistance gene profiles

All isolates exhibited multidrug resistance, with MAR index values ranging from 0.25 to 0.33, indicating exposure to environments under considerable antimicrobial selection pressure. According to Krumperman (1983), MAR values exceeding 0.20 are indicative of high-risk environments with frequent antibiotic exposure. Similar multidrug-resistant *E. cloacae* isolates with elevated MAR indices have been reported from diseased mullets (Mabrok *et al.* 2024) and African catfish (Anifowose *et al.* 2024), whereas higher resistance levels have been described in isolates recovered from diseased crayfish and ornamental fish (Dong *et al.* 2020; Preena *et al.* 2021). Although the

observed MAR indices suggest possible exposure to antimicrobial selection pressure, antimicrobial usage and the source of resistance were not directly investigated in this study. Universal resistance to tetracycline further indicates a consistent tetracycline-resistant phenotype among the tested isolates, whereas chloramphenicol, levofloxacin and ofloxacin remained effective against all isolates under the conditions of the susceptibility tests performed. These findings highlight the importance of routine antimicrobial susceptibility testing for evidence-based therapeutic decisions.

The detection of tetA, tetC, tetS, sul1, sul2 and blaSHV based on the expected PCR amplicon sizes was consistent with the observed resistance phenotypes and agrees with previous reports of multidrug-resistant *E. cloacae* from aquatic environments (Anifowose *et al.* 2024; Mabrok *et al.* 2024). However, the identities of these PCR products were not confirmed by sequencing; therefore, their identification should be interpreted as presumptive. Variation in the distribution of tet genes despite universal tetracycline resistance, together with the broad resistance profile of EC-ST1 despite harbouring fewer PCR-detected resistance genes, suggests that additional mechanisms, including chromosomal mutations, multidrug efflux systems or other uncharacterised resistance determinants, may also contribute to the resistant phenotype. Such genotype–phenotype discrepancies are consistent with the genomic plasticity of the *E. cloacae* complex, which facilitates the acquisition, loss and regulation of diverse antimicrobial resistance determinants (Davin-Regli and Pagès 2019). Consequently, phenotypic resistance cannot always be fully explained by the detection of a limited number of resistance genes. Furthermore, as the PCR screening targeted only selected resistance genes, other resistance determinants may have remained undetected. The distinct combinations of PCR-detected resistance determinants among the isolates further demonstrate variation in the resistance gene profiles of the three investigated isolates.

4.3 Phenotypic virulence-associated traits

The three representative *E. cloacae* isolates exhibited strain-dependent variation in biofilm formation, protease production and lipase activity, consistent with previous observations in *E. cloacae* and other opportunistic Enterobacteriaceae (Davin-Regli and Pagès 2019; Mabrok *et al.* 2024). Biofilm formation was observed in EC-LA1 and EC-LD1, whereas extracellular enzyme production differed among isolates, indicating that the virulence-associated traits assessed in this study varied among the isolates. Biofilms enhance bacterial persistence by protecting cells from environmental stress and host immune responses, whereas extracellular proteases and lipases may facilitate tissue interactions and nutrient acquisition (Costerton *et al.* 1999; Flemming *et al.* 2016). However, these *in vitro* observations do not directly establish the contribution of individual traits to disease severity in carp. No consistent relationship was observed between individual virulence traits and susceptibility to *C. longa*, suggesting that antibacterial susceptibility is influenced by multiple physiological and genetic factors rather than by any single phenotypic characteristic.

4.4 Antibacterial activity of aqueous *Curcuma longa* extract

The aqueous extract of *C. longa* exhibited concentration-dependent in vitro antibacterial activity against all three multidrug-resistant *E. cloacae* isolates. EC-LA1 showed the greatest susceptibility, with the largest inhibition zones and the lowest MIC (12.5 mg mL^{-1}), whereas EC-LD1 and EC-ST1 exhibited higher MIC values (25 mg mL^{-1}). Comparable antibacterial activity of *C. longa* against fish-associated Gram-negative bacteria, including *E. cloacae*, has been reported previously, although differences in antibacterial efficacy among studies are expected because of variations in extraction methods, phytochemical composition and bacterial strains (Harikrishnan and Balasundaram 2008; Adamczak *et al.* 2020; Al-Niaeem *et al.* 2021). The observed antibacterial activity may be associated with curcuminoids and other phenolic constituents reported to affect bacterial membrane integrity, cellular metabolism and biofilm formation (Dai *et al.* 2022). However, the active constituents and their mechanisms of action were not directly established in this study. The variation in susceptibility among the investigated isolates most likely reflects strain-specific physiological or genetic characteristics, although this requires further investigation.

A limitation of this study is the small sample size (eight diseased fish) and restricted geographical coverage (three outbreaks), with only three representative isolates selected for detailed characterisation. Selecting one isolate per outbreak may have limited the assessment of within-outbreak bacterial diversity. Therefore, these findings should be considered preliminary and not generalised to carp populations across India. Future studies should include larger sample sizes, broader geographical coverage, multiple isolates per outbreak where appropriate, and higher-resolution genomic approaches. Further investigations should also assess the safety and in vivo efficacy of aqueous *C. longa* extract before its potential application in aquaculture.

5 | CONCLUSIONS

This study characterised three multidrug-resistant *Enterobacter cloacae* isolates recovered from naturally diseased *Labeo rohita* and *Catla catla* during three disease outbreaks in Haryana, India. Phenotypic characterisation, 16S rRNA gene sequencing, antimicrobial susceptibility

testing, resistance gene screening and virulence profiling revealed variation in antimicrobial resistance patterns and virulence-associated traits among the selected isolates. The aqueous extract of *Curcuma longa* exhibited in vitro antibacterial activity against the tested isolates, with MIC values ranging from 12.5 to 25 mg mL^{-1}. These findings provide preliminary evidence of antibacterial activity against the isolates examined but do not establish the prevalence or wider epidemiological significance of multidrug-resistant *E. cloacae* in carp populations across India. Further studies are needed to characterise a larger and geographically broader collection of isolates and to determine the safety and *in vivo* efficacy of aqueous *C. longa* extract before its potential use in aquaculture can be established.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Professor and Head, Department of Aquatic Animal Health, College of Fisheries, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India, for providing the necessary laboratory facilities and institutional support to carry out this research.

ETHICAL APPROVAL

All procedures involving fish sampling and bacterial isolation were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC), Directorate of Research, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) at its meeting held on 11 October 2023..

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Vishal Soni: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft; Sanat Chandravanshi: Writing – review & editing; Rachna Gulati: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing; Bharat A. Kandoriya: Writing – review & editing; Krina D. Patel: Writing – review & editing; Shivam Arunkumar Patel: Writing – review & editing; Alli Asritha: Writing – review & editing; P. Shivani: Writing – review & editing; Julie M.P.: Writing – review & editing; Avadhutha Nikhil: Writing – review & editing; Priyanka Acharya: Writing – review & editing; Sakthibalan S: Writing – review & editing; P. Geetharani: Writing – review & editing.

AI-USE STATEMENT

Grammarly was used for grammar and language correction. The authors reviewed all suggested corrections and take full responsibility for the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

REFERENCES

Adamczak A, Ożarowski M, Karpiński TM (2020) Curcumin, a natural antimicrobial agent with strain-specific activity. Pharmaceuticals 13(7): 153.

Al-Niaeem KS, Resen AK, Al-Haider SM (2021) Potential of herbal extracts to avoid the bacterial infection of *Enterobacter cloacae* in common carp, *Cyprinus carpio* L. IOP Conference Series: Earth and Environmental Science 779: 012118.

Anifowose OR, Ajiboye AO, Olakojo TA (2024) Isolation, phenotypic and genotypic characterization of multidrug-resistant *Enterobacter cloacae* from diseased African catfish in Lagos State. SVU-International Journal of Veterinary Sciences 7: 40–50.

Ayoub HF, Khafagy AR, Esawy AM, El-Moaty NA, Alwutayd KM,... El-Tarabili RM (2024) Phenotypic, molecular detection, and antibiotic resistance profile (MDR and XDR) of *Aeromonas hydrophila* isolated from farmed *Tilapia zillii* and *Mugil cephalus*. BMC Veterinary Research 20(1): 84.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45: 493–496.

Bondad-Reantaso MG, MacKinnon B, Karunasagar I, Fridman S, Alday-Sanz V, ... Caputo A (2023) Review of alternatives to antibiotic use in aquaculture. Reviews in Aquaculture 15(4): 1421–1451.

Brenner DJ, Krieg NR, Staley JT, Garrity GM (2005) Bergey's manual of systematic bacteriology, volume 2, part B. Springer, Berlin.

Cabello FC, Godfrey HP, Buschmann AH, Dölz HJ (2016) Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. The Lancet Infectious Diseases 16: e127–e133.

Christensen GD, Simpson WA, Bisno AL, Beachey EH (1982) Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infection and Immunity 37: 318–326.

CLSI (2023) Performance standards for antimicrobial susceptibility testing, 33rd edition. CLSI Supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284: 1318–1322.

Dai C, Lin J, Li H, Shen Z, Wang Y, ... Shen J (2022) The natural product curcumin as an antibacterial agent: current achievements and problems. Antioxidants 11: 459.

Davin-Regli A, Pagès JM (2019) *Enterobacter* spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clinical Microbiology Reviews 32: e00002–19.

Dong J, Zhang L, Zhou S, Xu N, Yang Q, ... Ai X (2020) Identification of a multi-resistant *Enterobacter cloacae* strain from diseased crayfish (*Procambarus clarkii*). Aquaculture Reports 17: 100405.

FAO (2024) The state of world fisheries and aquaculture 2024: blue transformation in action. Food and Agriculture Organization of the United Nations, Rome.

Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology 14: 563–575.

Harikrishnan R, Balasundaram C (2008) *In vitro* and *in vivo* studies of the use of some medicinal herbals against the pathogen *Aeromonas hydrophila* in goldfish. Journal of Aquatic Animal Health 20: 165–176.

Jayasankar P (2018) Present status of freshwater aquaculture in India: a review. Indian Journal of Fisheries 65: 157–165.

Krumperman PH (1983) Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Applied and Environmental Microbiology 46: 165–170.

Kumar K, Prasad KP, Raman RP, Kumar S, Purushothaman CS (2013) Association of *Enterobacter cloacae* in the mortality of *Pangasianodon hypophthalmus* reared in culture pond in Bhimavaram, Andhra Pradesh, India. Indian Journal of Fisheries 60: 147–149.

Mabrok M, Algammal AM, El-Tarabili RM, Dessouki AA, ElBanna NI, ... Rodkhum C (2024) *Enterobacter cloacae* as a re-emerging pathogen affecting mullets (*Mugil* spp.): pathogenicity testing, LD_{50}, antibiogram, and encoded antimicrobial resistance genes. Aquaculture 583: 740619.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, ... Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 18(3): 268–281.

Mala W, Faksri K, Samerpitak K, Yordpratum U, Kaewkes W, ... Chomvarin C (2017) Antimicrobial resistance and genetic diversity of the SXT element in *Vibrio cholerae* from clinical and environmental water samples in northeastern Thailand. Infection, Genetics and Evolution 52: 89–95.

Ng LK, Martin I, Alfa M, Mulvey M (2001) Multiplex PCR for the detection of tetracycline resistant genes. Molecular and Cellular Probes 15: 209–215.

Preena PG, Dharmaratnam A, Raj NS, Raja SA, Nair RR, Swaminathan TR (2021) Antibiotic-resistant Enterobacteriaceae from diseased freshwater goldfish. Archives of Microbiology 203: 219–231.

Qiu X, Ye K, Ma Y, Zhao Q, Wang L, Yang J (2024) Genome sequence-based species classification of *Enterobacter cloacae* complex: a study among clinical isolates. Microbiology Spectrum 12: e04312–23.

Rahi P, Mühle E, Scandola C, Touak G, Clermont D (2024) Genome sequence-based identification of Enterobacter strains and description of *Enterobacter pasteurii* sp. nov. Microbiology Spectrum 12: e03150–23.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Sarker SD, Nahar L, Kumarasamy Y (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 42: 321–324.

Schmidt AS, Bruun MS, Dalsgaard I, Larsen JL (2001) Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment. Applied and Environmental Microbiology 67: 5675–5682.

Sierra G (1957) A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek 23: 15–22.

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (Eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC.

Soni V, Gulati R, Tiwari VK, Tripathi V, Singh S, ... Chaudhary V (2025) Evaluation of *Curcuma longa* aqueous extract for bactericidal activity toward *Enterobacter ludwigii* isolated from diseased *Catla catla*. Journal of Advances in Biology & Biotechnology 28(10): 1048–1058.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173: 697–703.

WHO (2023) Global antimicrobial resistance and use surveillance system (GLASS) report 2023. World Health Organization, Geneva.