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Research Note

First Report on the Prevalence of *Vibrio mimicus* in the Shrimp Aquaculture Farms of Kerala, India: A Surveillance Study

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Among emerging pathogens in aquatic systems, *Vibrio mimicus*—a close genetic relative of *V. cholerae*—has garnered increasing attention in recent years due to its ability to cause gastroenteritis in humans and infections in cultured finfish and shellfish (Baker-Austin et al., 2018). This bacterium is typically a non-halophile associated with freshwater and low-salinity environments, and several reports from aquaculture settings worldwide have identified it as an important pathogen in farmed species (Banerjee, Ooi, Shariff, & Khatoon, 2012; Geng et al., 2014; Raja, Panigrahi, De, & Kumar, 2017; Feng et al., 2024). In recent years, *V. mimicus* carries a dual implication as it compromises both human health and aquaculture productivity. Further, it may also act as a carrier of antimicrobial resistance (AMR), facilitating the spread of resistance determinants in aquatic environments (Baker-Austin et al., 2018; Alam et al., 2023; Food and Agriculture Organization of the United Nations [FAO], 2024).

Keywords: *Vibrio mimicus,* shrimp aquaculture, AMR, multi-drug resistance, coastal aquaculture

Kerala is a coastal state and one of India's major shrimp-producing regions, with an annual production of 2,952 MT (Sahadevan & Sureshkumar, 2020); yet no study has examined the prevalence of *V. mimicus* in shellfish aquaculture farms in Kerala. Hence, the present study was designed to investigate the occurrence, molecular characteristics, and antibiotic resistance profile of *V. mimicus* isolated from 78 commercial shrimp farms of Kerala located in Kannur (n=18), Thrissur (n=20), Alappuzha (n=20), and Ernakulam (n=20) districts. The results provide the first baseline evidence of *V. mimicus* in Kerala's shrimp-farming sector and underscore the organism's emerging relevance in coastal aquaculture.

A total of 156 samples, comprising 78 shrimp (*P. vannamei* = 40; *P. monodon* = 25; *P. indicus* = 13) and 78 pond-water samples, were screened according to the methodology described by Tendencia and de la Peña (2001). All samples were collected aseptically from commercial farms, transported on ice (4–8 °C), and processed within 6–8 h. Approximately 25 g of pooled shrimp tissues (hepatopancreas, gills, and muscle) or 10 mL of pond water were homogenized with alkaline peptone water (APW; 1% peptone, 1–2% NaCl; pH 8.4–8.6) at a 1:10 ratio, following the USFDA BAM (Kaysner, DePaola, & Jones, 2004) protocols. Enrichment broths were incubated at 37 °C for 18 h, after which the surface layer was streaked onto TCBS and HiCrome Vibrio agar. After incubation at 35–37 °C for 18–24 h, colonies showing *Vibrio*-like morphology—particularly green, non-sucrose-fermenting colonies on TCBS and mauve colonies on Hicrome Vibrio agar were purified on tryptic soy agar with 1% NaCl. Purified isolates underwent Gram staining, oxidase testing, motility assessment, salt-tolerance assays (0–8% NaCl), fermentation profiles, and other standard biochemical

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Table 1. Antibiotic Susceptibility Test results of *Vibrio mimicus* isolated from farmed Shrimp (n=7)

Antibiotic	Antibiotic class	Resistant (%)	Intermediate (%)	Susceptible (%)
Cefoxitin	Cephems	100.0	0.0	0.0
Ciprofloxacin	Quinolones	0.0	0.0	100.0
Chloramphenicol	Phenicols	0.0	0.0	100.0
Tetracycline	Tetracyclines	0.0	0.0	100.0
Trimethoprim/ Sulfamethoxazole	Folate pathway inhibitors	0.0	0.0	100.0
Gentamicin	Aminoglycosides	0.0	0.0	100.0
Amoxicillin/ Clavulanic acid	Beta-lactam+Inhibitors	14.3	57.1	28.6
Ampicillin	Penicillin	28.6	57.1	14.3
Cefepime	Cephems	0.0	71.4	28.6
Cefotaxime	Cephems	42.9	14.3	42.9
Ceftazidime	Cephems	0.0	0.0	100.0
Imipenem	Penems	0.0	28.6	71.4

tests according to Bergey's Manual of Systematic Bacteriology (Farmer, Janda, Brenner, Cameron, & Birkhead, 2005). Molecular confirmation was carried out using PCR assays targeting the *vmh* gene and species-specific markers (Shinoda et al., 2004). The presence of the *ctx* gene was determined by polymerase chain reaction (PCR) using specific primers targeting the cholera toxin gene (Alam et al., 2023). Amplicons were resolved on 1.8% agarose gels to confirm species identity, and representative isolates were preserved in glycerol stocks at -80 °C.

In the present study, the overall prevalence of *V. mimicus* in farmed shrimp was 9% (7/78). Seven positive samples were detected in Ernakulam and Thrissur districts, whereas all samples were found to be negative for *V. mimicus* in Kannur and Alappuzha districts. *V. mimicus* was not detected in any of the water samples. Among the positive samples, the species-wise prevalence was 7.5% (3/40) in *P. vannamei* and 16% (4/25) in *P. monodon*. *V. mimicus* can enter aquaculture system primarily through natural water sources such as estuarine, coastal, and freshwater bodies, where Vibrios are indigenous and persist in the water column and sediments. It can also be introduced via plankton, aquatic animals, and contaminated feed, as *Vibrio* species readily attach to chitinous surfaces and colonize the gastrointestinal tract of fish and shellfish. In addition, farm management practices and water exchange with surrounding environments may facilitate the introduction and dissemination of *V. mimicus* within aquaculture ponds (Thompson, Iida, & Swings, 2004; Baker-Austin et al., 2018). All the isolates carried the *vmh* gene, which aligns with previous studies (Shinoda et al., 2004; Hernández-Robles et al., 2021; Guardiola-Avila et al., 2021), indicating that this gene can be used as a reliable marker for the molecular confirmation of *V. mimicus* (Fig. 1). The *ctx* gene was not detected in any of the

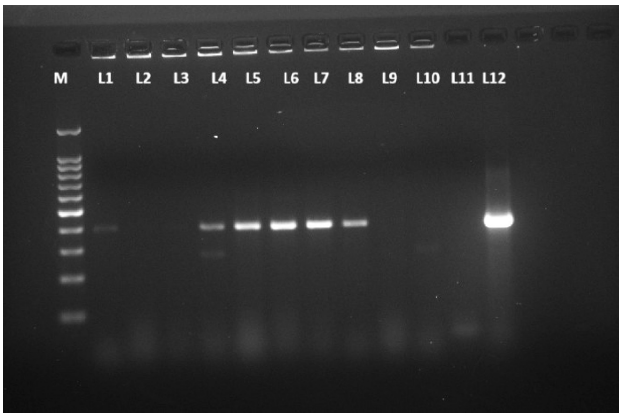

Fig. 1. PCR confirmation of the *vmh* gene of *Vibrio mimicus*

Agarose gel electrophoresis of PCR products targeting the *vmh* gene (expected amplicon = 390 bp). Lane M: 100 bp DNA ladder; Lanes 2,3, 9, 10, shrimp isolates (negative for *vmh*); Lanes 4–8: *vmh*-positive isolates showing the 390 bp band; Lane 11: negative control (no template); Lane 12: positive control (*V. mimicus* MTCC 4434)

tested isolates (Nilavan et al., 2021; Hernández-Robles et al., 2021).

The antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method in accordance with the Clinical and Laboratory Standards Institute [CLSI] (2024) guidelines, and the results were interpreted using WHONET software. All the tested isolates showed resistance to cefoxitin (100%) (Fig. 2a) and moderate levels of resistance to ampicillin (28%) (Fig. 2b) and cefotaxime (42%). On the other hand, the isolates exhibited complete susceptibility (100%) (Fig. 2c) to chloramphenicol, ciprofloxacin, imipenem, and tetracycline (Table 1). Similar antimicrobial trends have been reported in *Vibrio* spp. inhabiting antibiotic-influenced aquaculture environments (Nilavan & Mothadaka, 2023). The AST pattern observed in the present study agrees with previous studies reporting the susceptibility of non-cholera Vibrios to carbapenems and tetracyclines (Preena, Swaminathan, Kumar, & Singh, 2020). The detection of β-lactam and cephalosporin resistance warrants immediate attention, as the widespread and inappropriate use of these antibiotics in aquaculture settings accelerates the development of AMR strains (Preena et al., 2020). Among the antibiotic-resistant isolates, 17% were classified as multi-drug resistant (MDR) strains. The MAR index ranged from 0.08 to 0.33. One isolate, VMW2 exhibited MDR pattern, i.e., AMC-AMP-CTX-CX. Our findings highlight the detection of a few antibiotic-resistant *V. mimicus* strains in commercial shrimp farms across selected districts of Kerala. Regular monitoring of *Vibrio* populations, prudent use of antibiotics, and farm-level biosecurity practices are crucial to curb the establishment and spread of such pathogenic and resistant strains within aquaculture systems.

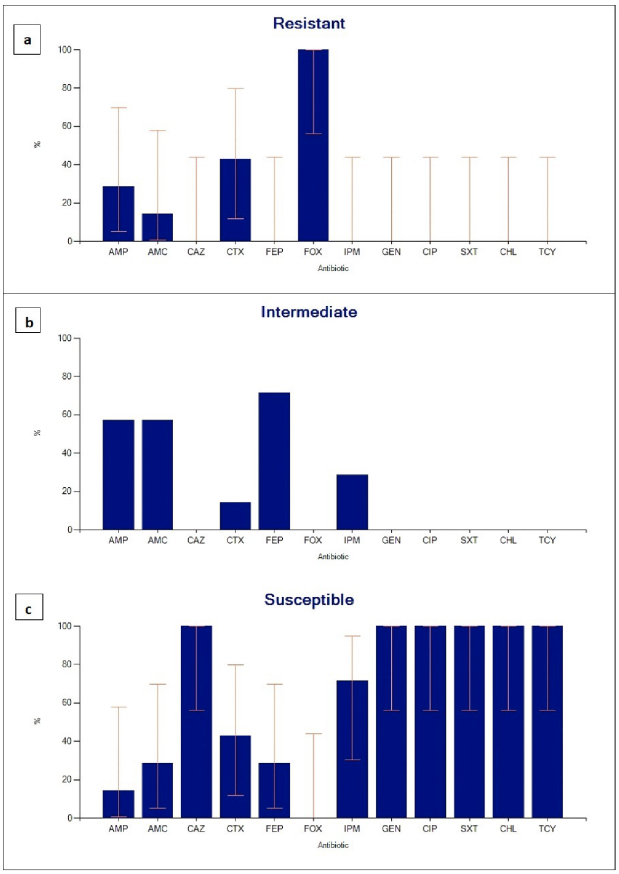

Fig. 2. Antimicrobial susceptibility profile of *Vibrio mimicus* isolates (n = 7). Bar plots illustrating the proportion (%) of *Vibrio mimicus* isolates (n = 7) classified as resistant (R), intermediate (I), or susceptible (S), to each antibiotic tested. Percent values are plotted on the Y-axis, and antibiotics are shown along the X-axis. Interpretations were based on available CLSI breakpoints

The present study confirms the prevalence of antibiotic-resistant *V. mimicus* in shrimp-farming environments in Kerala. Our findings underscore the urgent need for strengthened surveillance and responsible antimicrobial stewardship to mitigate potential risks from farm to fork.

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