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Conjugal Transfer of the $bla_{CTX\text{-}M\text{-}1}$ Gene Between *Escherichia coli* and *Salmonella enterica* serovar Enteritidis in Seafood

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Abstract

The aim of the present study was to investigate the conjugal transfer of the $bla_{CTX\text{-}M\text{-}1}$ gene from *Escherichia coli* to *E. coli* and from *E. coli* to *Salmonella enteritidis* serovar Enteritidis in *Villorita cyprinoides* and *Penaeus monodon* at different temperatures. An *E. coli* strain (N13) harbouring the $bla_{CTX\text{-}M\text{-}1}$ gene was used as the donor strain. The recipients were *E. coli* (EF2) and *S.* Enteritidis (S3), both nalidixic acid-resistant strains of shellfish origin. Antibiotic susceptibility testing, minimum inhibitory concentration (MIC) determination and PCR detection of the $bla_{CTX\text{-}M\text{-}1}$ gene were performed to verify transconjugants. The clonal relationships between donors, recipients and transconjugants were assessed using ERIC-PCR. The results indicated that transfer frequency was highest at 30°C and lowest at 42°C for both mating pairs (N13-EF2 and N13-S3) in *V. cyprinoides* and *P. monodon*. Both pairs (N13-EF2 and N13-S3) exhibited higher transfer frequencies in *V. cyprinoides* than in *P. monodon*. This study demonstrates that conjugation is a significant factor in the rapid dissemination of beta-lactam resistance among foodborne pathogens and highlights the potential risk of contaminated seafood as a transmission route of the $bla_{CTX\text{-}M\text{-}1}$ gene to consumers.

Keywords: Antibiotic resistance, beta-lactamase, transfer frequency, ERIC-PCR, seafood

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Introduction

The effectiveness of antibiotics is being threatened by their indiscriminate use in aquaculture, human and veterinary medicine, contributing to the rise of multi-drug resistant (MDR) pathogens (Sivan, Hridya, Sukumaran, & Abdulla, 2024; Xie, Hamid, Zhang, Zhang, & Peng, 2024). The global public health crisis caused by MDR underscores the urgent need for the 'One Health' approach (Mudenda et al., 2023). Extended-spectrum beta-lactamases (ESBLs) are bacterial enzymes, that hydrolyze the essential beta-lactam bond, rendering beta-lactam antibiotics like cephalosporins, penicillins and aztreonam ineffective (Zabiszak et al., 2023). The rapid dissemination of extended spectrum beta-lactamase (ESBL) in bacteria is a growing concern, with ESBL-producing Enterobacteriaceae identified as priority pathogens (Tacconelli et al., 2018; WHO, 2024).

The $bla_{CTX\text{-}M\text{-}1}$ gene is among the most prevalent ESBL genes found in Enterobacteriaceae, including *Escherichia coli* and *Klebsiella pneumoniae* (Sivan, Sukumaran, Ezhuthanikkunnel, & Abdulla, 2023; Lubwama et al., 2024). *E. coli*, in particular, shows remarkable genetic flexibility and adaptability, significantly enhancing its ability to disseminate these resistance determinants (Maddamsetti et al., 2024). Antibiotic-resistant bacteria (ARB) from various sources enter aquatic ecosystems, creating a favourable environment for horizontal gene transfer (HGT) (Abe, Nomura, & Suzuki, 2020; Hatha, Divya, Reshma, & Nifty, 2020). Among the HGT mechanisms, bacterial conjugation is the most prevalent, outpacing other methods like transformation and transduction (Amirfard, Moriyama, Suzuki, & Sano, 2024). Through conjugation, plasmids harbouring

antibiotic-resistant genes (ARGs) play a central role in transferring these genes between bacteria. ESBL genes, often carried on plasmids, disseminate through inter- and intraspecies conjugation within the Enterobacteriaceae family (Macesic et al., 2023). The dissemination of antibiotic resistance may be influenced and potentially accelerated by regional temperature variations and future climatic changes (Rzymski, Gwenzi, Poniedzialek, Mangul, & Fal, 2024).

E. coli harbouring $bla_{CTX\text{-}M\text{-}1}$ has been detected in clinical settings, aquatic ecosystems and the seafood industry (Divya & Hatha, 2019; Sivan et al., 2023; Wranne et al., 2024). ESBL producing bacteria can enter fish and shellfish through various routes: during the pre-harvest growth phase, when antibiotics are used for feed and treatment of bacterial infections; through polluted aquatic environments; and during post-harvest processing and handling (Cai et al., 2024).

While previous studies have demonstrated the transferability of CTX-M genes in liquid media (Siddaramappa et al., 2018), there is a lack of research on the transferability of $bla_{CTX\text{-}M\text{-}1}$ gene between *E. coli* to *E. coli*, as well as *E. coli* to *S. enterica* ser. Enteritidis in shellfish such as *V. cyprinoides* and *P. monodon.* Carbapenem-resistant and third-generation cephalosporin resistant *E. coli* are listed on the WHO global priority pathogens list, due to their role in hospital and community-acquired infections (WHO, 2024). This study aims to investigate the HGT (conjugation) of the $bla_{CTX\text{-}M\text{-}1}$ gene from *E. coli* to *E. coli* and from *E. coli* to *S.* Enteritidis in *V. cyprinoides* and *P. monodon* at various temperatures.

Materials and Methods

E. coli strain N13 (ceftazidime-resistant), harbouring the ESBL-encoding gene $bla_{CTX\text{-}M\text{-}1}$, was used as the donor strain. The recipient strains included *E. coli* (EF2) and *S.* Enteritidis (S3), both of which are resistant to nalidixic acid and were isolated from shellfish sources.

Antibiotic susceptibility testing of the donor and recipient strains was performed using the disc diffusion method on Mueller-Hinton agar (Hi-Media, India) (Bauer, Kirby, Sherris, & Turck, 1966). The antibiotics tested, along with their concentrations, were as follows: ampicillin (AMP, 10 mcg), cefotaxime (CTX, 30 mcg), cefoxitin (CFX, 30mcg), cefpodoxime (CPD, 10mcg), ceftazidime (CAZ, 30 mcg), ceftriaxone (CTR, 30 mcg), cefuroxime (CXM, 30 mcg), chloramphenicol (CHL, 30 mcg), ciprofloxacin (CIP, 5 mcg), co-trimoxazole (COT, 25 mcg), gentamicin (GEN, 10 mcg), nalidixic acid (NAL, 30 mcg), streptomycin (STR, 10 mcg), tetracycline (TET, 30 mcg), and trimethoprim (TMP, 5 mcg). The results were interpreted according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2019). The minimum inhibitory concentrations (MICs) of the donor and recipient strains were also determined based on the CLSI guidelines (CLSI, 2019). The MIC was defined as the lowest concentration of an antibiotic that inhibited visible growth of the organism after overnight incubation at 37°C for 18 hours (Andrews, 2001).

Conjugation experiments in liquid medium were performed in triplicate (Jung & Matthews, 2016). Donor (N13) and recipient (EF2 and S3) strains were inoculated in Luria-Bertani broth (LB) and incubated at 37°C. Overnight cultures of the donor and recipient cells were harvested, and mixed at a 1:1 ratio. A 100 μL aliquot of the mixed preparation was dispensed into 5 ml of fresh LB broth. LB broth tubes were incubated overnight statically at 24°C, 30°C, 37°C, and 42°C. An aliquot of the overnight mixed cultures was serially diluted and spread plated on LB agar plates containing 256 μg/mL nalidixic acid and 16 μg/mL ceftazidime. Three colonies of transconjugants from each plate were picked and stored in LB agar slants for further characterization. Transfer frequency was calculated as the number of transconjugant cells (expressed as cfu/mL) divided by the number of donor cells (Sparo et al., 2012).

Food matrices used for in vitro mating included *V. cyprinoides* and *P. monodon.* Seafood samples were sterilised to remove indigenous bacteria. Seafood samples were weighed into different sets of 25 g, and transferred into sterile petri plates. Donor and recipient strains were grown overnight in Luria-Bertani broth to a final concentration of 10^8 cfu/mL, and 0.3 mL of the donor and recipient suspensions were spread onto the seafood samples (25 g each). Inoculated seafood samples in sterile petri plates were sealed in sterile plastic bags to avoid any contamination and incubated at 24°C, 30°C, 37°C, and 42°C without shaking for 48 hours. After incubation, each shellfish sample was placed into a sterile plastic bag containing 225 mL sterile distilled water and homogenized for 1 min using a masticator (IUL instruments, Spain). Serially diluted homogenate was spread on LB agar plates containing 256

μg/mL nalidixic acid and 16 μg/mL ceftazidime. Three colonies of transconjugants from each plate were picked and stored in LB agar slants for further characterization. Transfer frequency was calculated as the number of transconjugant cells (expressed as CFU/mL) divided by the number of donor cells (Sparo et al., 2012).

Confirmatory phenotypic and genotypic methods were performed to verify the presence of a transconjugant. In this study, a transconjugant was defined as a recipient that acquired the $bla_{CTX-M-1}$ gene from the donor. The stability of the transferred $bla_{CTX-M-1}$ gene was evaluated by transferring a transconjugant colony onto LB agar (without antibiotics), incubating overnight, and then streaking for isolation on LB agar. An isolated colony from the LB agar plate was picked, and used in PCR to amplify the $bla_{CTX-M-1}$ gene. After the characterization of transconjugants, the antibiotic resistance patterns were determined by the disc diffusion method (Bauer et al., 1966).

PCR was performed on presumptive transconjugants to detect the $bla_{CTX-M-1}$ gene (Dallenne, da Costa, Decré, Favier, & Arlet, 2010). DNA from the bacterial genome was extracted using the standard Proteinase-K digestion method (Sambrook & Russell, 2001). DNA was then amplified under the following conditions: 5 min at 94°C, 30 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 1.5 min and a final extension at 72°C for 5 min. PCR products were electrophoresed on a 1.5% agarose gel (Sigma-Aldrich, United States) stained with ethidium bromide (GeNei™, India).

Transconjugants were analysed using the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) with primer ERIC-2 (Meacham, Zhang, Foxman, Bauer, & Marrs, 2003). The PCR amplifications were performed in 25 μL volumes containing 5 mM $MgCl_2$, 2 U of *Taq* polymerase, 0.4 mM deoxy-nucleoside triphosphates, 10 ng of crude template DNA, and 25 pmol of the ERIC2 primer (5′-AAGTAAGTGACTGGGGTGAGCG-3′). PCR amplification included an initial denaturation at 94°C for 2 min, denaturation at 94°C for 30 sec, annealing at 60°C for 1 min, and extension at 72°C for 4.5 min and, after 35 cycles, a final extension for 1 min at 72°C. PCR products were then electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized by Gel Documentation (BioRad Gel Doc™ EZ Imager, USA). Fingerprint data analysis GelCompare II version 6.0 software (Applied Maths, Texas) was used to analyze the ERIC-PCR fingerprints of the *E. coli* isolates. Dendrograms were constructed using the unweighted pair group method with arithmetic means (UPGMA).

The mean transfer frequency was statistically compared (one-way analysis of variance, Duncan's post hoc analysis) using IBM SPSS version 22 (IBM Corporation, United States). Statistical significance was defined as a p-value of <0.05.

Results and Discussion

The donor strain N13 (*E. coli*) exhibited resistance to nine antibiotics (AMP, CTX, CFX, CPD, CAZ, CTR, CXM, STR, TET, and TMP). The recipient strain EF2 (*E. coli*) showed resistance to AMP, COT, GEN, NAL, STR, and TMP, while the recipient strain S3 (*S.* Enteritidis) exhibited resistance to AMP, COT, NAL, STR, TET, and TMP. The donor *E. coli* strain N13 had a high MIC for CAZ (>128 μg/mL), whereas the recipient strains *E. coli* EF2 and *S.* Enteritidis S3 had a high MIC for NAL (>128 μg/mL) (Table 1).

Transconjugants growing on LB agar containing NAL and CAZ were selected from three colonies on each plate. The donor strain N13 (*E. coli*) success-

Table 1. Antibiotic resistance profiles and Minimum Inhibitory Concentrations (MICs) of ceftazidime (CAZ) and nalidixic acid (NAL) in donor and recipient strains.

Strains	Antibiotic resistance profile	MIC of CAZ	MIC of NAL
Donor strain (*Escherichia coli*, N13)	AMP CTX CFX CPD CAZ CTR CXM STR TET	>128 μg/mL	0
Recipient strain 1 (*Escherichia coli*, EF2)	AMP COT GEN NAL STR TMP	0	>128 μg/mL
Recipient strain 2 (*Salmonella*enteritidis, S3)	AMP COT NAL STR TET TMP	0	>128 μg/mL

fully transferred its $bla_{CTX\text{-}M\text{-}1}$ gene to the recipient strains *E. coli* (EF2) and *S.* Enteritidis (S3). The conjugative transfer frequency from *E. coli* to *E. coli* (N13-EF2) was highest at 37°C (1.31×10^{-1}) and lowest at 42°C (0.90×10^{-2}). In contrast, the transfer frequency from *E. coli* to *S.* Enteritidis (N13-S3) was highest at 37°C (1.25×10^{-4}) and lowest at 24°C (1.58×10^{-5}). The transfer frequency was higher from *E. coli* to *E. coli* compared to *E. coli* to *S.* Enteritidis at all tested temperature (Table 2).

High transfer frequencies of $bla_{CTX\text{-}M\text{-}1}$ gene occurred at 30°C, with the lowest transfer frequencies observed at 42°C for both mating pairs (N13-EF2 and N13-S3) in food matrices such as *P. monodon* and *V. cyprinoides* (Table 2). The transfer frequency was higher from *E. coli* to *S.* Enteritidis (N13-S3) than from *E. coli* to *E. coli* (N13-EF2) in *P. monodon* at all tested temperatures, with values of 1.25×10^{-2} at 24°C, 1.35×10^{-2} at 30°C, 0.85×10^{-2} at 37°C and 0.35×10^{-4} at 42°C ($p<0.05$). In *V. cyprinoides*, the transfer frequency from *E. coli* to *S.* Enteritidis (N13-S3) was also higher than from *E. coli* to *E. coli* (N13-EF2) at all tested temperatures except 42°C, with rates of 1.34×10^{-2} at 24°C, 1.37×10^{-2} at 30°C, 1.12×10^{-2} at 37°C and 0.81×10^{-3} at 42°C ($p<0.05$). Both mating pairs (N13-EF2 and N13-S3) exhibited higher transfer frequencies in *V. cyprinoides* than in *P. monodon*.

Transconjugants (*E. coli* to *E. coli*) retained the antibiotic-resistant phenotype of their recipient strain (EF2). These transconjugants exhibited resistance to CTX, CPD, CAZ, CTR, and CXM, but not to CFX. Our results indicate that resistance to beta-lactams and TET was co-transferred with CAZ resistance. Similarly, transconjugants (*E. coli* to *S.* Enteritidis) retained the antibiotic-resistant phenotype of their recipient strain (S3). In these transconjugants, resistance to CPD, CTR, and CXM was co-transferred with CAZ resistance, while they remained susceptible to CTX and CFX. Phenotypic tests demonstrated that both transconjugants (*E. coli* and *S.* Enteritidis) were resistant to CAZ, with an MIC greater than 128 μg/mL, which was the same level of resistance shown by the donor. PCR screening revealed the presence of the CAZ resistance gene $bla_{CTX\text{-}M\text{-}1}$ in transconjugants (Fig. 1).

The ERIC fingerprints of the donor (N13), recipient

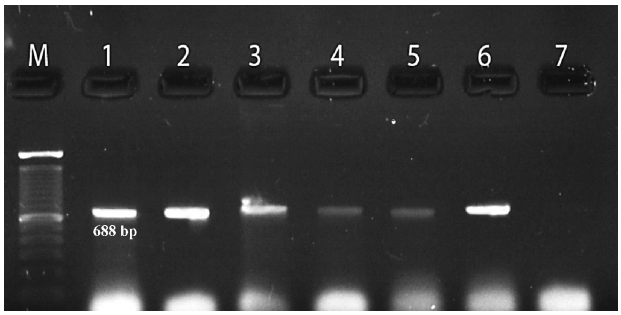

Fig. 1. Gel image showing PCR amplification of $bla_{CTX\text{-}M\text{-}1}$ gene in transconjugants. Lanes: M: 100 bp marker (New England Biolabs; Lane 1-7, Transconjugants).

Table 2. Transfer frequency of $bla_{CTX\text{-}M\text{-}1}$ gene between donor and recipient bacterial strains across different food matrices at various temperatures.

Matrix	(Donor-Recipient)	Transfer frequency at			
		24°C	30°C	37°C	42°C
LB broth	*E. coli* N13-*E. coli* EF2	1.08×10^{-1}	1.29×10^{-1}	1.31×10^{-1}	0.90×10^{-2}
	E. coli N13-*S.* enteritidisS3	1.58×10^{-5}	1.17×10^{-4}	1.25×10^{-4}	0.77×10^{-4}
Penaeus monodon	*E. coli* N13-*E. coli* EF2	0.57×10^{-3}	1.04×10^{-2}	0.66×10^{-3}	0.25×10^{-4}
	E. coli N13-*S.* enteritidisS3	1.25×10^{-2}	1.35×10^{-2}	0.82×10^{-2}	0.35×10^{-4}
Villorita cyprinoides	*E. coli* N13-*E. coli* EF2	1.27×10^{-2}	1.31×10^{-2}	2.43×10^{-3}	0.87×10^{-3}
	E. coli N13-*S.* enteritidisS3	1.34×10^{-2}	1.37×10^{-2}	1.12×10^{-2}	0.81×10^{-3}

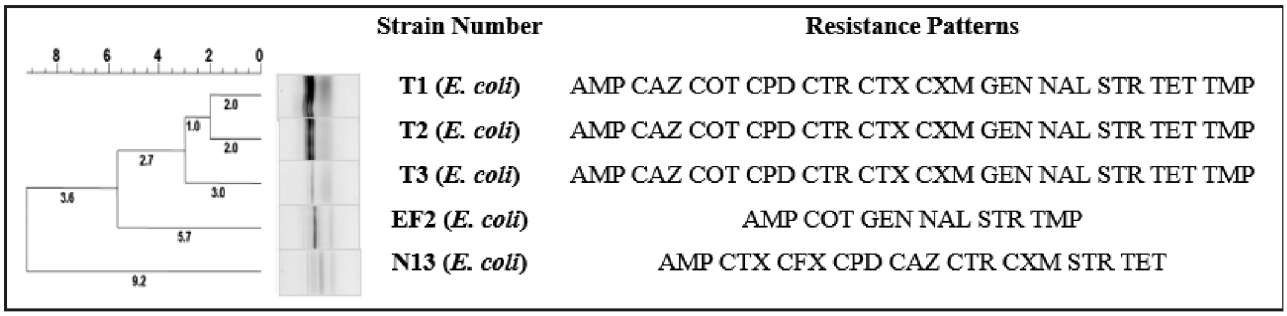

Fig. 2A. ERIC-PCR profiles of Donor (N13), recipient (EF2), and transconjugants (T1-T3); AMP (Ampicillin); CTX (Cefotaxime); CFX (Cefoxitin); CPD (Cefpodoxime); CAZ (Ceftazidime); CTR (Ceftriaxone); CXM (Cefuroxime); COT (Co-trimoxazole); GEN (Gentamicin); NAL (Nalidixic acid); STR (Streptomycin); TET (Tetracycline); TMP (Trimethoprim).

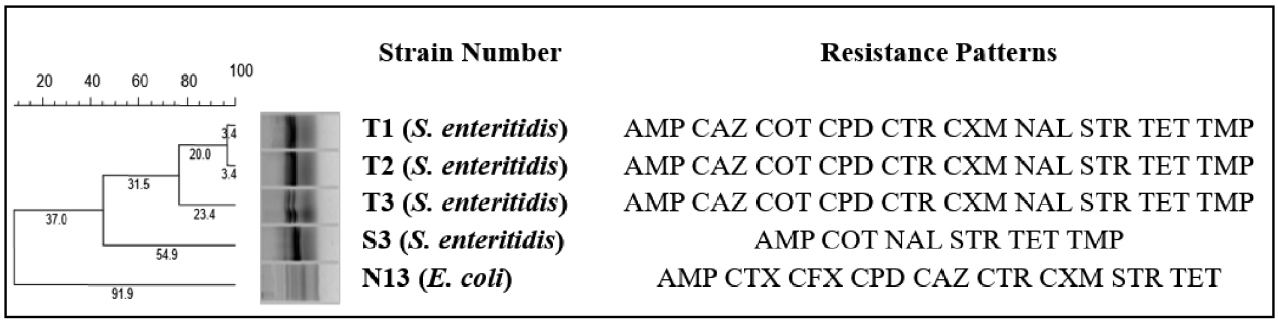

Fig. 2B. ERIC-PCR profiles of Donor (N13-*Escherichia coli*), recipient (S3- *S. enterica* ser. Enteritidis), and transconjugants (T1-T3); AMP (Ampicillin); CTX (Cefotaxime); CFX (Cefoxitin); CPD (Cefpodoxime); CAZ (Ceftazidime); CTR (Ceftriaxone); CXM (Cefuroxime); COT (Co-trimoxazole); GEN (Gentamicin); NAL (Nalidixic acid); STR (Streptomycin); TET (Tetracycline); TMP (Trimethoprim).

(EF2) and transconjugant (T1, T2, T3) strains are shown in Fig. 2A. ERIC fingerprint analysis allowed the differentiation between transconjugants and recipients. *E. coli* (EF2) and the transconjugants formed a distinct cluster with approximately 60% similarity, while the donor (N13) was categorized in a separate cluster (Fig. 2B). Similarly, the ERIC fingerprints of the donor (N13), recipient (S3) and transconjugant (T1, T2, T3) strains are presented in Fig 2B. *S.* Enteritidis (S3) and the transconjugants also formed a cluster with 60% similarity, while the donor (N13) remained in an independent cluster (Fig. 2B).

Antibiotic resistance in the seafood industry and aquaculture presents a critical public health concern due to the widespread use of antibiotics in these sectors (Sun et al., 2024). ARB from various sources enter the aquatic ecosystem, creating an ideal setting for HGT and the dissemination of ARB among aquatic animals (Chi et al., 2024). ARB can transfer resistance genes through HGT mechanisms such as conjugation, transformation and transduction, as well as through vertical gene transfer (Wang et al., 2021; Amirfard et al., 2024; Chi et al., 2024). While previous studies have documented conjugation in fresh produce and dairy products, there has been limited focus on HGT among foodborne pathogens in seafood matrices (Balbuena-Alonso et al., 2023; Zhai et al., 2023). This is the first study to investigate the transfer of the $bla_{CTX-M-1}$ gene in seafood, specifically in *V. cyprinoides* and *P. monodon*.

P. monodon is a crucial species in the global aquaculture industry due to its high growth rate and substantial market demand, making it a significant contributor to seafood exports (Salunke, Kalyankar, Khedkar, Shingare, & Khedkar, 2020; Anh, Nam, Lan, Kurihara, & Hai, 2024). *V. cyprinoides* holds considerable importance in the seafood industry, particularly in India, due to its widespread consumption and role in supporting local economies and livelihoods through both domestic markets and exports (Joy & Chakraborty, 2017).

The $bla_{CTX\text{-}M\text{-}1}$ gene was transferred from the donor to recipients (*E. coli* and *S.* Enteritidis) in both liquid medium and food matrices, including *V. cyprinoides* and *P. monodon.* The tetracycline resistance encoding gene *tet(M)* and the erythromycin resistance encoding gene *ermB* resident on conjugative plasmids in *Enterococci faecalis*, have been shown to facilitate the horizontal transfer of these ARGs (Gazzola, Fontana, Bassi, & Cocconcelli, 2012). These plasmids demonstrated the ability to transfer the ARGs not only to other *Enterococcus* species, but also to different bacterial genera, including *Lactobacillus*, *Staphylococcus* and *Bacillus* via conjugation (Gazzola et al., 2012). *In-vitro* conjugation experiments have shown that the tetracycline resistance determinant *tet* (*M*) present in *Enterococcus faecium* S27, isolated from fermented sausage, could be successfully transferred to clinical isolates of both *E. faecium* and *Enterococcus faecalis* (Jahan, Zhanel, Sparling, & Holley, 2015). Additionally, the streptomycin resistance of *E. faecium* S27 was transferred to the clinical strain *E. faecium* 82916, as confirmed by the presence of the streptomycin resistance encoding gene *aadA* in both the donor and transconjugant strains (Jahan et al., 2015).

We found that the transfer frequency of the $bla_{CTX\text{-}M\text{-}1}$ gene was highest at 30°C for both mating pairs (N13-EF2 and N13-S3) in *V. cyprinoides* and *P. monodon*. A previous study reported that temperature influences conjugation frequency and it is highest at low temperatures (25 to 30 °C) and decreases as the temperature increases (Forns, Baños, Balsalobre, Juárez, & Madrid, 2005). Similarly, the optimal temperature for the transmission of carbapenemase genes in *K. pneumoniae* ranged between 25 to 30°C, suggesting that warmer seasons facilitate the transfer of a higher number of ARGs (Yang, Nam, Lee, & Yoo, 2024).

The frequency of gene transfer from *E. coli* to *S.* Enteritidis (N13-S3) was higher than from *E. coli* to *E. coli* (N13-EF2). Interspecies barriers for gene transfer can be low in complex bacterial communities in natural environments and ARGs are easily disseminated between different species, facilitated by HGT (Dahlberg et al., 1998). Analysis of codon usages indicate that unique genes in *E. coli* and *S.* Enteritidis are more similar to each other than to their vertically inherited genes, suggesting they may originate from a common source within a supraspecies phylogenetic group (Karberg, Olsen, & Davis, 2011). HGT can significantly influence the evolutionary dynamics and genetic diversity of bacterial populations, enabling them to adapt swiftly to environmental fluctuations.

Both conjugation pairs (N13-EF2 and N13-S3) exhibited higher transfer frequencies in *V. cyprinoides* compared to *P. monodon*. *V. cyprinoides* have higher lipid content, more saturated fatty acids, and essential amino acids (Joy & Chakraborty, 2017). The high fat and lipid content in seafood, including fish, has been reported to create a favourable environment for the growth of pathogenic bacteria, potentially leading to higher rates of bacterial contamination in these species (Hatha & Lakshmanaperumalsamy, 1997; Odeyemi et al., 2023). Our study indicated that *V. cyprinoides* may provide a suitable food matrix for supporting ARG transfer. *V. cyprinoides* is the most consumed clam species in India, which contributes about 73.8% of national clam production (Laxmilatha & Appukuttan, 2002). In our study, the donor strain was CAZ resistant, while the recipient strain was NAL resistant. The differing antibiotic resistance profiles of the donor and recipient strains prior to conjugation do not contradict the occurrence of conjugation, as the transconjugants will acquire the resistance characteristics of both strains (Rao, Laidlaw, Li, Young, & Tamber, 2021; Laidlaw et al., 2024). As expected, both transconjugants (N13-EF2, and N13-S3) retained the antibiotic-resistant phenotypes of the recipient strain, exhibiting resistance to NAL, as well as acquiring resistance to CAZ from the donor strain.

Our results showed that the resistance to β-lactam and TET was co-transferred along with CAZ resistance. Resistance to cephalosporins (CPD, CTR, CXM) was also co-transferred with CAZ resistance. The plasmids conferring resistance to CAZ also carried resistance to other antibiotics, including TET and cephalosporins, due to genetic linkage on the plasmid. This linkage enables the simultaneous spread of multiple resistance traits among bacterial populations (de Been et al., 2014; Mahmud et al., 2022). The *CTX-M* genes have become associated with a variety of mobile genetic elements that facilitate the co-transfer of other antibiotic resistance (Botelho et al., 2020). It has also been found that kanamycin resistance was acquired along with the AMP resistance (Bezanson, Macinnis, Potter, & Hughes, 2008).

The donor strain *E. coli* N12 exhibited a broad

resistance spectrum to AMP, CTX, CPD, CAZ, CTR, CXM, STR, TET, and TMP. This extensive resistance profile highlights the significant challenge posed by multidrug-resistant *E. coli* strains in both clinical and aquaculture settings. The high MIC values observed for CAZ in the donor strain and for NAL in the recipient strains emphasize the substantial resistance levels in these bacteria.

The conjugative transfer experiments demonstrated the efficient transferability of the $bla_{CTX-M-1}$ gene from the donor *E. coli* N13 to both *E. coli* N13-EF2 and *S.* Enteritidis S3 recipients. The highest transfer frequency to EF2 and S3 was observed at 37ºC, while the lowest transfer frequency occurred at 42ºC for EF2 and 24ºC for S3. The consistently higher transfer frequencies from *E. coli* to *E. coli* compared to *E. coli* to *S.* Enteritidis across all tested temperatures indicate a species-specific preference in the conjugative transfer process (Table 2).

The transfer of the $bla_{CTX-M-1}$ gene in food matrices such as *V. cyprinoides* and *P. monodon* revealed differential pattern of transfer frequencies. The transfer frequency was higher from *E. coli* to *S.* Enteritidis (N13-S3) than *E. coli* to *E. coli* (N1-EF2) in *P. monodon* across all tested temperatures. In contrast, *V. cyprinoides* exhibited opposite trend at all temperatures except 42ºC, where *E. coli* to *E. coli* transfers occurred more frequently. This suggests that the type of food matrix and the interacting species significantly influence gene transfer efficiency, with *V. cyprinoides* generally facilitating higher frequencies than *P. monodon*. Conjugation has been identified as a mechanism for both intra- and inter-species HGT, significantly contributing to the rapid dissemination of ARGs (Barlow, 2009; Vignaroli, Zandri, Aquilanti, Pasquaroli, & Biavasco, 2011; Ashwini, Ray, Halami, & Sumana, 2022).

Transconjugants retained the antibiotic resistance phenotype of their respective recipient strains, indicating successful gene transfer. *E. coli* to *E. coli* transconjugants exhibited resistance to a range of cephalosporins (except CFX) and co-transferred resistance to beta-lactams and TET along with CAZ resistance. Similarly, *E. coli* to *S.* Enteritidis transconjugants demonstrated resistance to CPD, CTR, and CXM, while remaining susceptible to CTX and CFX. Both types of transconjugants displayed high levels of CAZ resistance, comparable to the donor strain, confirming the effective acquisition of the $bla_{CTX-M-1}$ gene.

ERIC-PCR analysis effectively distinguished between transconjugants and recipients, clustering *E. coli* EF2 and *S.* Enteritidis S3 with their respective transconjugants, separate from the donor strain *E. coli* N13. The ERIC-PCR analysis and resistance patterns indicate that conjugation transfers specific traits, such as antibiotic resistance, without altering the genetic background of the recipient strain (Li, Chang, Zhang, Hu, & Wang, 2019). This genetic profiling underscores the successful transfer and integration of the resistance gene into the recipient strains, establishing a clear genetic distinction between the donor and recipient/transconjugant strains. This study highlights the antibiotic resistance profiles and genetic differences between donor, recipient and transconjugant strains, and details the conjugative transfer frequency of the $bla_{CTX-M-1}$ gene. This underscores the importance of monitoring and controlling the spread of antibiotic resistance genes in both clinical and environmental settings.

This study is the first report of the transfer of the $bla_{CTX-M-1}$ gene in seafood (food matrices), specifically in *V. cyprinoides* and *P. monodon*. The high transfer frequency observed in *V. cyprinoides* is particularly concerning, given its status as the most consumed shellfish in India. The $bla_{CTX-M-1}$ gene has demonstrated HGT between different bacterial species, facilitating its spread across the environment, food, and human populations. Our findings underscore the urgent need for targeted surveillance and stringent regulations to monitor and control the presence of ARB in seafood.

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