Extended-Spectrum Beta-Iactamase (ESBL) genotypes among *Escherichia coli* and *Klebsiella pneumoniae* isolates recovered from shrimp aquaculture farms

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In recent years, aquaculture has gained much attention as an environmental gateway to the development and spread of antimicrobial resistance (AMR); however, studies pertaining to AMR in aquaculture are scanty from India. The prevalence and molecular features of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae* recovered from shrimp aquaculture farms in Kerala are addressed in this communication. ESBLs are enzymes capable of hydrolyzing oxymino-cephalosporins and monobactam antimicrobials and are generally found in Enterobacteriaceae and *Pseudomonas aeruginosa*. ESBL renders penicillins, first-, second- and third-generation cephalosporins, and aztreonam ineffective. Apart from their widespread occurrence in clinical settings, ESBL-producing bacteria are increasingly being reported in livestock, companion animals and various environmental settings (Hordijk *et al.,* 2019; Toombs-Ruane *et al.,* 2020).

In the present study, a total of 261 samples comprising shrimp (n=77), water (n=92) and sediment (n=92), collected from shrimp aquaculture farms (n=37) in Kodungallur and Thuravoor- two major shrimp farming zones in Kerala were screened for the presence of ESBL-*E. coli* and *K. pneumoniae* (Sivaraman *et al.,* 2021). Briefly, samples (10 ml of water; 10 g each of sediment and shrimp) were incubated in 90 ml of EE broth Mossel (BD Difco, pH 7.2) overnight at 37 °C, and a loopful of the enriched culture was streaked onto MacConkey agar plates supplemented with 1 µg/ml cefotaxime (Sigma Aldrich, USA) and incubated for 18-24 h at 37 °C. Bacterial identification and susceptibility testing were performed using BD Phoenix™ M50 automated system (BD Diagnostics, USA), with the ID-AST combo panel, NMIC/ID55. A total of 32 and 15 isolates were identified as ESBL-producing *E. coli* and *K. pneumoniae*, respectively. All of them were cefotaxime-resistant with minimal inhibitory concentration (MIC) ≥32 µg/ml. The percentage of resistance among the *E. coli* isolates towards other antibiotics was as follows: tetracycline (40.6%), trimethoprim-sulfamethoxazole (34.4%), ciprofloxacin & levofloxacin (34.4%), chloramphenicol & gentamicin (6.3%). Among the isolates of *K. pneumoniae,* 13 (86.7%) showed simultaneous resistance to tetracycline, ciprofloxacin and trimethoprim/sulfamethoxazole. Based on

phenotypic resistance patterns, PCRs were performed to identify various ESBL genes and other resistance determinants among the isolates. This included detection of genes conferring resistance to cephems ($bla_{CTX-M,}$ $bla_{TEM,}$$bla_{SHV}$), tetracycline (tetA and tetB), chloramphenicol (cmlA and catA), fluoroquinolones (qnrA, qnrB, qnrS, qepA, oqxA, oqxB and aac (6')-Ib-cr), aminoglycosides (aadA1, strA and strB) and sulfonamides (sul1 and sul2). Among the ESBL-genes screened CTX-M group 1 ($bla_{CTX-M-15}$) was found to be the predominant type, with 23 *E. coli* (71.9%) and 15 *K. pneumoniae* (100%) isolates testing positive for the same. CTX-M group 9 was detected in 9 (28.1%) isolates of *E. coli,* but not in any of the *K. pneumoniae* isolates. Concerning TEM and SHV-type ESBL determinants, 11 isolates (73.3%) of *K. pneumoniae* harboured both types. CTX-M-15, reported for the first time in India in 2011, has now become a widespread ESBL genotype and has been reported in humans, livestock and fishes (Palmeira and Ferreira, 2020). Screening for other AMR genes identified tetA & tetB (13, 40.6%), sul1 (11, 34.4%), sul2 (9, 28.1%), catA & cmlA (11, 34.4%), qepA & aac (6')-Ib-cr (9, 28.1%) and strAB & aadA1 (2, 6.3%) in *E. coli*; and qnrB (13, 86.7%), qnrS (3, 20%), oqxB (13, 86.7%), tetA (13, 86.7%) and sul2 (13, 86.7%) in *K. pneumoniae* isolates.

The plasmid incompatibility (InC) types present in the isolates were also analysed in the study using the PCR-based replicon typing (PBRT) as described by Carattoli *et al.* (2005). This revealed the predominance of IncFIA & IncFIB plasmids in *E. coli*; however, in *K. pneumoniae,* the major replicon type detected was IncHI1 (Figure 1). It has been shown previously that resistance genes harboured on the narrow host range. IncF-type plasmids in Enterobacteriaceae spread readily in *E. coli* (Mathers *et al.,* 2015). Moreover, IncF plasmids carrying $bla_{CTX-M-15}$ have been reported in Enterobacteriaceae isolated from clinical, environmental and livestock settings (Zurfluh *et al.*, 2015). This perhaps indicates the role of IncF plasmids in disseminating CTX-M-15 across different settings. IncHI1, the plasmid type found in the *K. pneumoniae* isolates from our study, has a broad host range and has been reported in various environmental Gram-negative species (Villa *et al.,* 2012).

The distribution of phylogroups (A, B1, B2, C, D, E, F and cryptic clade) among the ESBL-producing *E. coli* isolates were analyzed using the PCR method described by Clermont et al. (2013). Phylogenetic groups identified among *E. coli* isolates included B1 (4, 12.5%), B2 (6, 18.8%), C (10, 31.3%), D (3, 9.4%) and E (9, 28.1%). This is a worrisome information from the public health point of view as most of the virulent extra-intestinal strains of *E. coli* are primarily from group B2 and to a lesser extent from group D. Also, it is noteworthy that CTX-M-15-producing isolates from this study were spread over different phylogroups viz., B1, B2, C, D and E, whereas all CTX-M-group 9 isolates belonged to phylo-group C.

In summary, there are the multidrug-resistant isolates of ESBL-producing *E. coli* and *K. pneumoniae,* with resistance mainly towards cephalosporins, fluoroquinolones, tetracycline and trimethoprim-sulfamethoxazole drugs, in samples from various shrimp aquaculture farms in Kerala. To the best of knowledge, this study provides the first data on the molecular features of ESBL-producing isolates prevailing in shrimp aquaculture settings of this region. In the investigated isolates of *E. coli* and *K. pneumoniae,* bla_{CTX-M} was found to be the predominant ESBL genotype. This, along with a high prevalence of tet, suland various plasmid-mediated quinolone resistance genes may be a public health concern. The findings of our study shed light on the importance of monitoring aquaculture settings for the possible emergence of antibiotic-resistant bacteria.

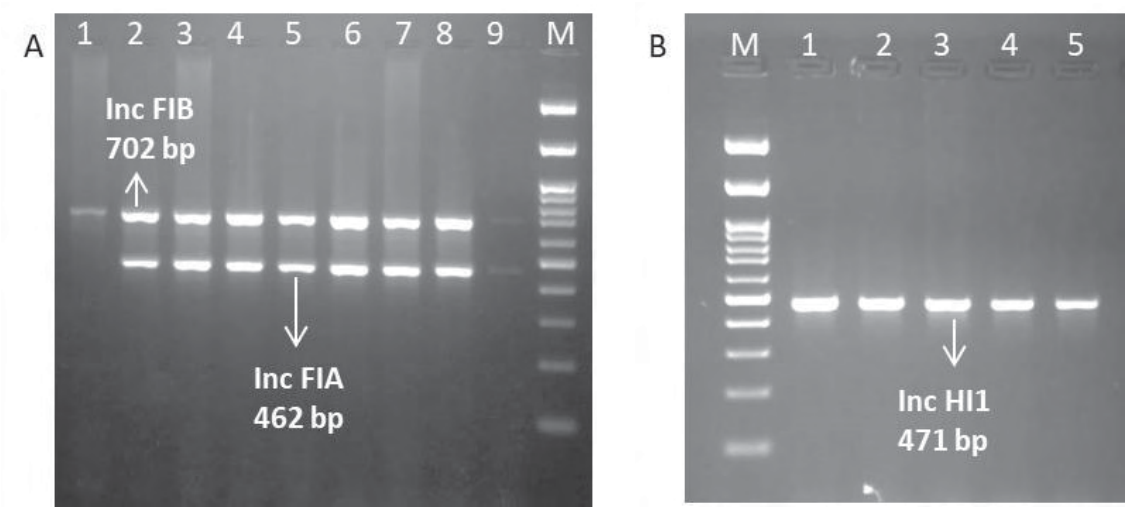

Fig.1 *Gel image showing the amplicons of (A) IncFIA / FIB type plasmids in E. coli and (B) IncHI1 type plasmid in K. pneumoniae.*

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