

Assessment and Identification of Specific Pathogenic *Enterobacteriaceae* in the Flesh and Gut of *Rastrelliger kanagurta* (Indian Mackerel) and the Associated Impact of Different Collection Sources

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Abstract

Seafood is considered a vital source of protein globally. However, industrial or sewage waste discharges can introduce pathogens into the marine environment, making fish highly susceptible to contamination by microorganisms. Improper handling, storage or environmental factors at various collection sources may also contribute to contamination, potentially resulting in human gastrointestinal disease outbreaks. Therefore, the current preliminary study aimed to identify pathogenic *Enterobacteriaceae* such as *Escherichia coli*, *Salmonella* and *Shigella* spp. in *Rastrelliger kanagurta*, and the associated impact of two different collection sources. Five Indian mackerel per location were collected directly from a fishing boat off the coast of Elakanda and the Peliyagoda fish market. The fish were transported to the laboratory immediately after capture in an ice box. Samples were taken from the flesh and gut and were homogenised using 0.9% saline. MacConkey agar was used for selective and differential isolation of *Enterobacteriaceae*. Biochemical characterisation involving Methyl-Red, Voges-Proskauer, Citrate, Sulphur, Indole, and Motility testing was carried out. The isolates were further confirmed by culturing on differential media using Xylose Lysine Deoxycholate (XLD) and chromogenic *E.coli* agar. Suspected isolates were then selected for an optimised boil-cell bacterial DNA extraction and quantified using a NanoDrop spectrophotometer. Polymerase chain reaction targeting pathogenic genes, including *eaeA*, *bfpA*, *invC*, and *ipaH*, was carried out. The results revealed two bands at the expected 619bp for the *ipaH* gene in the marine samples and one band for market samples. No bands were detected for the other genes. This denotes the identification of Enteroinvasive *E.coli* (EIEC), a pathotype of *E.coli* that causes dysentery and infection closely related to Shigellosis. Furthermore, a Chi-squared test indicated no significant association (p-value: 0.923) between the presence of pathogenic *Enterobacteriaceae* and the two collection sources. Based on the EUCAST guidelines, antibiotic susceptibility testing (ABST) revealed that the isolated EIEC was sensitive against Chloramphenicol, Gentamicin, and Tetracycline but completely resistant to Ampicillin and Erythromycin. The isolation of EIEC in this case, combined with its antibiotic susceptibility profile, raises serious concerns related to public health risk, emphasising the significance of stringent waste management and pollution control measures to minimise the risk of microbial contamination in seafood.

Keywords: *Pathogenic Enterobacteriaceae, Seafood, Pathogenic genes, Antibiotic susceptibility tests, public health*