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Isolation of culturable, extended spectrum beta lactamase producing *Escherichia coli* from fish and fish waste from local fish market

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Abstract

Faecal indicator patterns of antibiotic susceptibility of *Escherichia coli* was investigated in fresh fish purchased at retail market. The phenotypic characteristics of antibiotic resistance were investigated in 430 fresh seafood samples of ESBL *E. coli* (fresh fish, fish waste, and associated environment). Sixty ESBL-positive *E. coli* were recovered from them. A significant number of isolates were resistant to amoxicillin (98.4%), cefepime (91.9%), and both cefepime and ceftazidime (91.1%). Colistin (100%) was shown to have a relatively higher susceptibility than cefotaxime-clavulanic acid (85.5%) or amoxyclav. Over three antibiotics were resistant to all 60 isolates. The EC01 and EC59 isolates were resistant to 14 different antibiotics. Cefotaxime had MIC values of 1024g/ml for 87% of the isolates. Twenty isolates tested positive for resistance to multienzyme MIC™ strips.

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Introduction

The main therapeutic use of antibiotics is the management of infectious diseases in both people and animals. However, the usage of antibiotics in the food production industry has increased significantly, mostly as growth promoters or stimulants (Founou *et al*., 2016). Antibiotic-resistant bacteria have emerged as a result of the indiscriminate and careless use of antibiotics and the absence of strict antibiotic regimes (Mazel and Davies 1999). These microorganisms can contaminate surface water, groundwater, and estuaries when they enter the environment through untreated hospital and industrial effluents (Sharma *et al*., 2010; Akin, 2016). In densely populated nations like India, *E.coli* contamination of seafood occurs often as a result of home sewage contamination of coastal waters (Kumar *et al*., 2004). Numerous illnesses, including gastroenteritis, wound infections, septicaemia, urinary tract infections etc. can be brought on by such pathogenic *E. coli* (Gomes *et al*., 2016). The effectiveness of antibiotic chemotherapy has been seriously hampered by *E. coli*'s resistance to numerous, therapeutically important antibiotics. Multidrug resistant *E.coli* (MDR) is not just found in clinical settings, but is frequently discovered in seafood because humans and animals are causes of contamination of aquatic ecosystems. Since MDR *E.coli* can be transported by seafood to far-off geographic places where such resistance patterns were previously unknown, this is a severe concern (Lekshmietal., 2017). Beta-lactam antibiotics, which also include penicillins, cephalosporins, cephamycins, and carbapenems, are among the most frequently prescribed medicines worldwide. Production of one or more forms of β-lactamases that hydrolyze β-lactam antibiotics is a significant mechanism of resistance in *E.coli* and a number of other Enterobacteriaceae members (Pitout., 2008; Sheng., 2013). Third generation cephalosporins and monobactams are among those that can be hydrolyzed by extended-spectrum β-lactamases (ESBLs), which can give resistance to bacteria against these antibiotics (McDanel., 2017). The main cause of *E.coli* infection in seafood is faecal contamination of coastal waters. In fish landing facilities and marketplaces, seafood can get contaminated with *E.coli* after harvest. Diverse *E. coli* pathogroups have been isolated from seafood in previous Indian research (Sanath *et al*., 2001; Sehgal *et al*., 2008). In this study we investigated the prevalance of antibiotic resistance in *E.coli* isolated from seafood. The study emphasizes the widespread presence of multidrug-resistant *E.coli* in seafood, which poses a health risk to both seafood handlers and consumers.

Materials and methods

Samples were collected from December 2020 to February 2021. The samples were gathered in Kalamboli, Navi Mumbai, at a local fish market. Fresh fish, fish wastes, and its surrounding were taken into account in this study since these are the foods most likely to cause food-borne diseases (Magiorakos *et al*., 2012). Samples were collected for 3 months (n=430) were gathered in total.

For the purpose of sample collection, gut swabs from a variety of fish species, including *Pangasiusbocourti* (Basa fish), *Anguilliformes* (Eel fish), *Harpadonnehereus* (Bombayduck), *Xiphiasgladius* (Tar masa), *Oncorhynchusgorbuscha* (Pink salmon), *Parupeneusindicus* (Goat fish), *Labeocatla* (Bengal carp), *Pampusargnteus* (Pomfret), *Aphanopuscarbo* (Black scabbard fish), *Platybeloneargealus* (Needle fish), *Coregonusclupeaformis* (Lake whitefish), *Labeobata* (Bata or bhatya fish) etc., as well as swabs from fish-related environments, including water samples, sterile culture collecting swabs were used to collect samples (HiMedia, Mumbai, India). Swabs were taken under sterile circumstances, transferred to the lab in a cold chain, and processed there the same day in preparation for further isolation. Swabs were incubated for 24 hours at 37°C in 5ml of sterile Brain Heart Infusion Broth (BHI). HiCrome agar (HiMedia, India) supplemented with HiCrome ESBL agar supplement (HiMedia, India) was employed for the isolation of ESBL *E.coli*. Ceftazidime, Cefotaxime, Ceftriazone, Aztreonam, and Fluconazole are all included in the supplement. On HiCrome agar, enriched BHI broth was streaked. Colonies with pinkish-purple colouring were chosen for additional research.

On MacConkey (MAC) and Eosin Methylene Blue (EMB) agar, a few chosen colonies were streaked. *E. coli* colonies on EMB agar display a green metallic shine, while pink colonies are visible on MAC agar (Boeckel *et al.*, 2019). Gram staining, catalase and oxidase tests, motility at 20-25°C, MR-VP, nitrate reduction, and sugar fermentation (Sorbitol, Fructose, Mannitol, And Dextrose) are used to visually and biochemically confirm typical bacterial colonies (Bush *et al.*, 2011., Olaitan *et al.*, 2015, Baron *et al.*, 2016).

Phenotypic Antibiogram of ESBL E.coli Isolates

The Clinical Laboratory Standard Institute Guidelines standard disc diffusion method was used to analyse the antibiogram pattern of phenotypically proven ESBL *E. coli* strains (CLSI, 2018). The usage of fifteen various antibiotic discs (HiMedia, Mumbai, India) representing nine various classes (Table.1). On Muller Hinton (MH) agar, antibiotic susceptibility testing (AST) was carried out using the Kirby Bauer method.

Table 1. List of antibiotics used.

Class	Antibiotics
Aminoglycosides	Amikacin (30mcg)
	Gentamycin (30mcg)
Carbapenems	Imipenem (10mcg)
	Meropenem (10mcg)
Cephalosporins (Third Generation)	Cefotaxime (10mcg)
	Ceftazidime (30mcg)
	Cefepime (30mcg)
Polypeptide	Colistin (10mcg)
Penicillin	Amoxycillin (25mcg)
Quinolones / Fluoroquinolones	Ofloxacin (30mcg)
	Nalidixic acid (30mcg)
Tetracycline	Tetracycline (30mcg)
Beta Lactamase inhibitor Antibiotics	Cefotaxime / Clavulanic acid (30/10mcg)
	Ceftazidime / Avibactam) (30/10mcg)
	Amoxyclav (Amoxycillin / Clavulanic acid) (20/10 mcg)

Results

Sample collection, Isolation and Identification of E. coli

A total of (n=430) samples were gathered in three months. Sixty samples out of 430 tested positive (13.95%) for ESBL-producing *E. coli*. When compared to fresh fish samples and the surrounding environment, it was clear that more ESBL *E. coli* were isolated from fish waste (Table 2).

On the basis of cultural morphology on (differential) MAC and (selective medium) EMB agar plates, *E.coli* were isolated and identified. Dark colonies with a green metallic sheen on EMB medium and pink lactose-fermenting colonies on MAC plates are characteristics of *E.coli*. The isolates were motile, catalase, indole, and methyl red tests were positive, they reduced nitrates, and they fermented sorbitol, fructose, mannitol, and dextrose sugars. The isolates were therefore identified as *E.coli* (Fig. 1a & b).

Table 2. Sample-wise distribution of *E. coli* showing ESBL activity.

Isolate code	Source
EC1	*Pangasiusbocourti* (Basa fish)
EC2	*Anguilliformes* (Eel fish)
EC3	*Harpadonnehereus*(Bombay duck)
EC4	*Xiphiasgladius* (Tar masa)
EC5	*Oncorhynchusgorbuscha*(Pink salmon)
EC7	*Parupeneusindicus*(Goat fish)
EC8	*Labeocatla*(Bengal carp)
EC9	*Pampusargnteus* (Pomfret)
EC10	*Aphanopuscarbo*(Black scabbard fish)
EC6, EC11 TO EC18, EC 20 to EC 35	*Platybeloneargealus*(Needle fish)
EC19	*Coregonusclupeaformis*(Lake whitefish)
EC36 and EC 37	*Labeobata* (Bata or bhatya fish)
EC38 TO 60	Fish waste

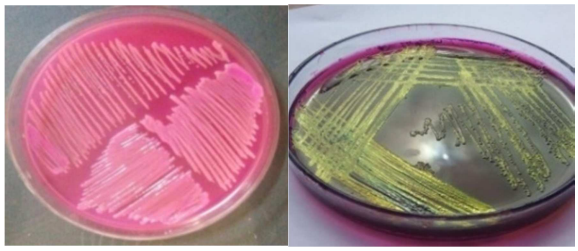

Fig.1a. *E.coli* on MAC agar b. *E. coli* on EMB agar.

Phenotypic Antibiogram of ESBL E. coli Isolates

All of the isolates underwent phenotypic antibiogram analysis using the Kirby Bauer method. The results of the isolate EC01 antibiotic susceptibility tests, which revealed resistance to 14 antibiotics, are given (Fig.2). Based on the results of the CLSI's recommendations, sixty *E. coli* isolates were classified as sensitive, intermediately resistant, and resistant (CLSI, 2018) (Table-3). The antibiotic resistance profiles of 60 ESBL-positive isolates are shown (Fig. 3), with amoxicillin resistance accounting for the highest

number of isolates (98.4%), while colistin susceptibility was observed in all isolates (Table.4). Only cefotaxime (10 mcg) of the three cephalosporins was chosen for additional MIC and investigations since it only showed 21.0% resistance, whereas ceftazidime (30 mcg) and cefepime (30 mcg) showed 91.1% and 91.9% resistance, respectively.

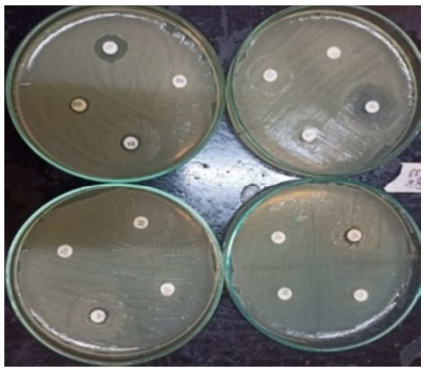

Fig. 2. Antibiotic susceptibility testing of the isolate ECo1.

Discussion

From fresh seafood, Singh *et al.* (2020) identified 475 *E. coli*, of which 71.58% were ESBL. All ESBL producers were discovered to be imipenem-sensitive (Aruna and Mobashsher, 2012). Our data showed 100% sensitivity to colistin and greater than 90% antibiotic resistance. Similar findings were made by Tamta *et al* in 2020, who found that *E. coli* resistance to essential antibiotics cephalosporins compromises the ability to effectively prevent and cure a variety of bacterial infections. Singh *et al.* (2020) identified 79 (52.66%) ESBL strains from shellfish and 261 (80.30%) ESBL strains from fish. The need for treating effluents from fish processing facilities was highlighted by the discovery of ESBL strains in Mangalore's fish processing industry effluents (Divyashree *et al.*, 2019).

Conclusion

Studies on the transmission of antimicrobial resistance genes from animals to humans through close interaction with animal reservoirs have attracted particular attention. However, there are currently no such extensive cohort studies being conducted in India. Due to concerns about the environment and public health, such investigations are urgently needed. Pathogen resistance to newer cephalosporins could be quite problematic. In such a situation, effective management techniques must be used to stop the spread of antibiotic resistance in coastal waterways. These results emphasise the need for a comprehensive examination of the use of antibiotics and growth promoters throughout the food and animal production chains.

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