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Prevalence of extended-spectrum β-lactamase producing *Escherichia coli* in seafood from the retail fishery outlets of Veraval, Gujarat, India

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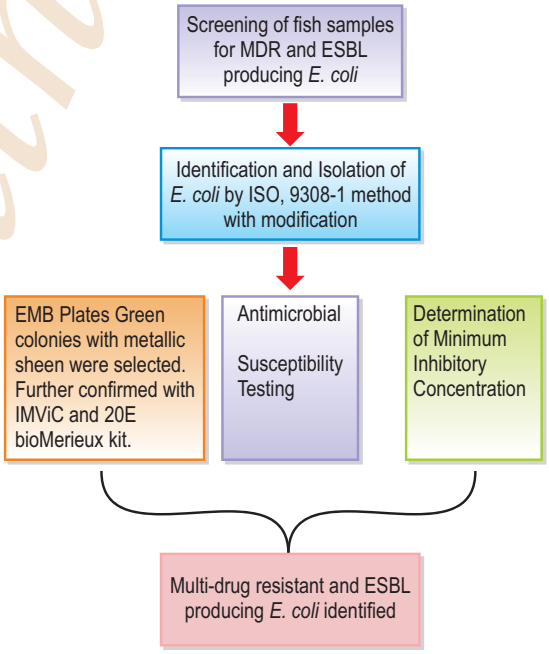
Abstract

Aim: Profound and unregulated utilization of beta-lactam antibiotics for the treatment of various infections has lead to various bacteria being resistant against these antibiotics. *E. coli* is well known to produce extended, spectrum *β*- lactamase (ESBL) type of *β*- lactamase, which has the ability to hydrolyse the entire group of *β*- lactam antibiotics. Currently, reports on the occurrence of ESBL producing *E. coli* in seafood is meagre. Therefore, the objective of this study was to monitor the prevalence of multiple drug resistant (MDR) and ESBL producing *E. coli* in seafood in Veraval region of Gujarat.

Methodology: Identification and isolation of *E. coli* was carried out using ISO, 9308-1 method with minor modification. A 0.5 ml of serially diluted fish sample was spread on Tergitol-7 media (Oxoid, CM0793) and incubated at 35°C for 24 hrs. Typical flat, dry and yellow colonies with or without red tinge were further streaked on EMB plates and then subjected to IMViC and 20E BioMerieux identification kit. All the confirmed *E. coli* isolates were subjected to antimicrobial susceptibility testing, and Minimum Inhibitory Concentration (MIC) was determined.

Results: A total of 28 number of *E. coli* (approx 12%) isolates were identified from 238 fish samples. All these isolates were subjected to antimicrobial susceptibility testing and a higher rate of resistance was found with ampicillin (39.29%), trimethoprim/ sulfamethoxizole (32.14%), ciprofloxacin (28.57%) cefepime (17.86%) and cefuroxime (17.86%). All these isolates were sensitive to carbapenems and beta-lactam, beta-lactam inhibitors and aminoglucosides. Out of total 28, *E. coli* isolated (15.71%) eleven isolates were found to be multi-drug resistant and four (14.29%) isolates were ESBL producers.

Interpretation: This study confirms the presence of hazardous MDR and ESBL producing *E. coli* in fish and fishery products in Veraval, Gujarat.



Introduction

Beta-lactam antibiotics are most by preferred for the treatment of various infectious diseases in human therapies, farm animals and aquaculture now a days. β- Lactamase is an enzyme produced by particular type of bacteria to counterattack the β-lactam antibiotics. The term Extended Spectrum β-lactamase (ESBL) refers to a special type of β-lactamase, which can hydrolyse the entire group of β- lactam antibiotics starting from narrow spectrum to broad spectrum. The widespread and indiscriminate use of antibiotics coupled with transmissibility of resistance leads to the emergence new ESBL producing *E. coli* (Cantos *et al.,* 2016). A person infected with ESBL producing *E. coli* is difficult to treat (Bradford, 2001). Since, *E. coli* are the most common commensal bacteria existing in human and animal intestinal tract; it can become opportunistic and obligate pathogens when coexists with pathogenic strains (Bush and Jacoby, 2010). *E.coli* strains that produce ESBL are also becoming Multi drug resistant (MDR) and are considered to be one of the emerging pathogens worldwide (Cheng *et al.,* 2014). Recently, ESBL producing *E. coli* have been reported in most of the food product such as meat, chicken, raw milk, fish and food products (CLSi, 2006; Egea *et al.,* 2012; FSSAI, 2012; Elhadi and Alsamman, 2015). But, scanty reports are available regarding the occurrence of *E. coli* in seafood. Since, there is no data available about ESBL-producing bacteria in food and aquaculture products and food of animal's origin in Gujarat, the present study was carried out to monitor the presence of multiple drug resistant (MDR) and ESBL producing *E. coli* in seafood sampled during 2011 to 2015 from retail fishery outlets in Veraval, Gujarat, India.

Materials and Methods

Sample collection : A total of 238 fish samples consisting of Pomfret (4), Horse Mackerel (19), Indian Mackerel (18), Tuna (05), Ribbon fish (32), Seer fish (08), Croaker (19), Ghol (05), Dhoma (11), Sardine (06), Prawns (19), Shark (4), Ray fish (5), Dried fishes (23), cuttle fishes (29), cephalopods (18) and surimi (13) were collected from retail fishery outlets in and around Veraval region (Gujarat) and brought to laboratory immediately replace with in suitable sterile polythene bags for enumeration of *E. coli*.

Enumeration and identification of *E. coli*: For enumeration of *E. coli* was carried out by ISO, 9308-1, 2014 method with slight modification *i.e.*, 0.5 ml from the serially diluted fish sample is transferred into Modified Tergitol-7 (Oxoid, CM0793) plates supplemented with 0.25ml of 1% Triphenyl Tetrazolium Chloride (Himedia, FD057) and spread over the surface; then, all the plates were incubated at 35°C for 24 hrs. Flat dry yellow colonies with or without red tinge were further streaked over Eosin Methylene Blue (EMB) agar plates; after incubation, colonies showing greenish metallic sheen were further subjected IMViC test (Montenegro *et al.,* 1990).

Antibiotic patterns of the isolates: Twenty four antimicrobial agents were tested by disk diffusion method (Kang *et al.,* 2005) on MuKeller-Hinton agar (MHA) in accordance with CLSI guidelines (Koo and Woo, 2011) of antimicrobial concentration and the detailed procedure given by Sivaraman *et al.,* 2016. Around 0.5 Mc Farland concentration cultures of the isolate were taken in a sterile cotton swab and smeared over 200mm (Himedia, Mumbai) size petri plates already filled with MHA. Then the readymade antibiotics disc Dodecca Enterobacteriaceae- 1 (# DE053, Himedia, Mumbai) with Ampicillin-10µg, gentamicin- 10µg, amikacin-30 µg, ciprofloxacin-5µg, ofloxacin-5µg, Co-trimoxazole-25µg, amoxyclav- 30µg, cefuroxime-30µg, ceftazidime-30µg, ceftazidime/clavulanic acid 30/10µg, cefepime-30µg and imipenem-10µg and Dodecca enterobacteriaceae-2 with cefotaxime-30µg, ceftriaxone-30µg, cefoxitin- 30µg, meropenem-10µg, piperacillin/ tazobactum – 100/10µg, aztreonam-75µg, gatifloxacin-5µg, ampicillin/ sulbactum- 10/10 µg, cefoperazone- 75µg, levofloxacin- 5µg, ceftizoxime- 30µg and ticarcillin/ clavulanic acid- 75/ 10µg (#DE054, Himedia, Mumbai) was kept over the surface and incubated for 16 – 24 hrs in inverted position for the antibacterial activity.

Minimum Inhibitory concentration (MIC) : *E. coli* isolate was cultured in Brain heart infusion (BHI) broth and the concentration was adjusted to 0.5Mc Farland, then culture was spread over the preset MHA plates. A MIC detection strip, ESBL and AmpC detection Ezy MIC (#079, Himedia) was placed over the agar. The MIC strip was coated with a mixture of three different antibiotics with and without clavulanic acid on a single strip in a concentration gradient manner. The upper half had Ceftazidime, Cefotaxime and Cefepime (mixture) with highest concentration of clavulanic acid tapering downwards, whereas lower half was similarly coated with Ceftazidime, Cefotaxime and Cefepime (mixture) in a concentration gradient in reverse direction.

Results and Discussion

Out of total 238 fish samples collected, 28 fish samples such as Horse Mackerel (1), Indian Mackerel (2), Ribbon fish (4), Croaker (2), Prawns (4), Dried fish (8), and surmi (7) harboured higher than recommended level of *E. coli*. Food Safety Standard Authority of India has recommended the maximum limit of 20 cfu g^{-1} *E. coli* in fresh and frozen sample. Based on the above standards, Veraval retail fishery outlets were contaminated with higher level of *E. coli* (0 to 2.5 x 10^2) . Total twenty eight number of *E. coli* isolates were randomly collected from the samples, positive for *E. coli* and tested for resistant level and ESBL production. The higher rate of resistant was found with ampicillin (39.29%), trimethoprim/ sulfamethoxizole (32.14%), ciprofloxacin (28.57%) cefepime (17.86%) and cefuroxime (17.86%). Whereas, these isolates were susceptible to carbapenems (imipenem and meropenem), beta-lactam and beta-lactam inhibitors (piperacillin/tazobactum and ampicillin/

Table 1 : Antibiotic resistance of *E.coli* isolated from the seafood

Antibiotics	Antibiotic group	No. of resistant isolates	% of resistant isolates	No. of intermediate isolates	% of intermediate isolates
Ceftazidime (CAZ)	Cephems	4	14.29	1	3.57
Cefepime (CPM)		5	17.86	1	3.57
Cefuroxime (CXM)		5	17.86	1	3.57
Ceftizoxime (CZX)		2	7.14	1	3.57
Cefoperazone (CPZ)		3	10.71	2	7.14
Cefotaxime (CTX)		3	10.71	2	7.14
Ceftriaxone (CTR)		3	10.71	2	7.14
Cefoxitin (CX)		2	7.14	0	0
Amoxyclav (AMC)	Beta- Lactam and	3	10.71	2	7.14
Ticarcillin/ Clavulanicacid (TCC)	Beta- Lactamase Inhibitors	2	7.14	5	17.86
Piperacillin/ Tazobactum (PIT)		0	0	3	10.71
Ampicillin/Sulbactum (A-S)		0	0	3	10.71
Imipenem (IPM)	Carbapenems	0	0	0	0
Meropenem (MRP)		0	0	0	0
Ofloxacin (OF)	Fluoroquinolones	1	3.57	5	17.86
Ciprofloxacin (CIP)		8	28.57	2	7.14
Gatifloxacin (GAT)		1	3.57	2	7.14
Levofloxacin (LE)		0	0	0	0
Ampicillin (AMP)	Penicillin	11	39.29	2	7.14
Trimethoprim/ Sulfamethoxazole (COT)	Sulfonamides	9	32.14	1	3.57
Amikacin (AK)	Aminoglycosides	0	0	0	0
Gentamicin (GEN)		0	0	0	0
Aztrenam (AT)	Monobactum	3	10.71	3	10.71
Ceftazidime/ Clavulanicacid (CAC)	Cephems/ Beta-Lactam Inhibitor	2	7.14	2	7.14

sulbactum) and aminoglycosides (amikacin and gentamicin) shown in Table. 1.

In the present study, eleven (15.71%) *E. coli* isolates mainly from Ribbon fish and dried fish were found to be multidrug resistant multidrug resistant (MDR). As per report, these isolates were resistant to more than three types of antibiotics and were considered as MDR isolate. The presence of hazardous nature of MDR bacteria in the fish indicates poor hygienic status of the fishery outlets.

The ESBL producing *E. coli* are more dangerous than the MDR *E. coli*. In the present study, four (14.29%) of *E. coli* isolates out of twenty eight confirmed *E. coli* were ESBL producers. Triple ESBL detection Ezy MIC Strip was used to identify the ESBL strain. The upper half had Ceftazidime, Cefotaxime and Cefepime (Mixture) with Clavulanic acid with highest concentration tapering downwards, where as lower half was similarly coated with Ceftazidime, Cefotaxime and Cefepime (Mixture) without Clavulanic acid in a concentration gradient in reverse direction. Based on the kit protocol, it was found that four out of twenty eight isolates were ESBL producing *E. coli*. All the ESBL producers were further analyzed in the antibiogram with twenty four antibiotics and found that all the ESBL producers were ESBL-Amp C positive, multidrug resistant and showed reduced susceptibility to ampicillin.

Numerous reports are available for the presence of ESBL producing *Enterobacteriaceae* in food products such as meat, chicken, raw milk and fish (CLSi, 2006; Egea *et al.,* 2012 and Elhadi and Alsamman, 2015). The present study also supported the previous report and indicated the widespread prevalence of ESBLs and MDR *E. coli* in seafood of Veraval region, Gujarat. So, the fish retail market may act as a possible reservoir for multiplication and spread of bacteria from fish to handlers/ consumers that become a serious threat. ESBL enzymes carrying *E. coli* confer resistance to most beta-lactam antibiotics mainly of extended- spectrum cephalosporins (Ojer-Usoz *et al.,* 2013). The ESBL genes enter and disseminate through the food chain *via* direct contact with humans and animals and can contribute to the spread of these strains (Ozcakar *et al.,* 2011) Moreover, the genes responsible for enzymes are easily transfered to other enterobacterial species when encounter it (Perez *et al.,* 2007). ESBL gene are encoded on plasmids that hydrolyze all groups of beta lactum antibiotics, including new generation group of cephalosporins mainly cefotaxime and cefriaxone (Ryu *et al.,* 2012 and Schmid *et al.,* 2013). *E. coli* strains producing ESBLs are MDR and are considered to be one of the emerging pathogen world-wide (Cheng *et al.,* 2014).

The presence of ESBL producing *E. coli* in the fish and fishery products may be due to post harvest contamination such as infected handlers, uncleaned vessels and repeated use of contaminated water in the fishery outlets.

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