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Research Note

Antibiotic Resistance of *Escherichia coli* isolated from Seafood of Veraval, Gujarat to third Generation Cephalosporins

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The occurrence of pathogenic *Escherichia coli* in seafood is due to faecal contamination and infected handlers during pre and post harvest stages. Presently, a large number of antibiotics are being used in human therapy, farm animals and aquaculture (Cheong et al., 2014) which leads to increased incidence of antimicrobial resistant bacterial strains (Aarestrup, 2006; Sapkota et al., 2008). The 3rdgeneration cephalosporin antibiotics are broad-spectrum drugs with high intrinsic activity against Gram negative bacteria. The widespread and indiscriminate use of antibiotics coupled with the transmissibility of resistance could lead to the emergence of antibiotic resistant *E. coli* (Kang et al., 2005). Several studies demonstrated the prevalence of resistance in enterobacteriaceae in food products such as meat, chicken, raw milk and fish (Koo & Woo, 2011; Ryu et al., 2012; Ojer-Usoz et al., 2013), food products (Schmid et al., 2013) and environment (Meda et al., 2006). World Health Organization recently reported that the increased incidence of bacteria acquiring resistance to critically important antimicrobials (CIM) is a worldwide phenomenon. The isolates showing resistance to 3rd and 4th generation antibiotics indicates the presence of Extended-Spectrum Beta-lactamase (ESBL) producing *E. coli.* The microbiological safety of fish and fishery products is an important public health concern throughout the world (FAO, 2010). Recently, several studies have shown transmission of resistance genes into food chain via direct contact with humans and animals (Egea et al., 2012). There is no data available on the resistance to 3rd generation cephalosporin in *E. coli* isolated from seafood in Gujarat, India. In view of this, a preliminary study was carried out to monitor the prevalence of resistance to 3rd generation cephalosporin *E. coli* in seafood.

A total of 238 seafood samples were collected from fish market and fish processing industries of Veraval region, Gujarat during 2012 to 2015. *E. coli* isolates were identified as described by Surendran et al. (2013). Ten gram of the fish sample was aseptically cut and transferred to sterile normal saline (NS) in stomacher bag and blended in stomacher at 150 rpm for 2 min. Serial dilutions of fish in sterile NS was spread on Tergitol-7 agar supplemented with 0.25 ml of 1% solution of sterile triphenyltetrazolium chloride in duplicate. Yellow colony with deepened yellow centre and yellow halo are considered as presumptive *E. coli*. 5-6 colonies from each plate was subcultured on TGA slant. The confirmation of the E. coli isolates was done by streaking on EMB agar (Eosine Methylelne Blue Agar) and IMViC test. Four 3rd generation cephalosporins; Ceftazidime (CAZ) 30 µg, Ceftizoxime (CZX) 30 µg, Cefotaxime (CTX) 30 µg and Ceftriazone (CTR) 30 µg (HiMedia, Mumbai) were tested by the disk diffusion method (Bauer et al., 1966) on Mueller-Hinton agar (Oxoid, UK) with 0.1 ml of *E. coli* equivalent to 0.5 McFarland standards in accordance with CLSI

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Table 1. Percentage of antibiotic resistance in *E.coli strains* to third generation cephalosporins

S. No.	Name of the antibiotics	No. of resistant isolates	% of resistant isolates	No. of intermediate isolates	% of intermediate isolates	No. of susceptible isolates	% of susceptible isolates
1	Ceftazidime (CAZ)	4	14.29	1	3.57	23	82.14
2	Ceftizoxime (CZX)	2	7.14	1	3.57	25	89.29
3	Cefotaxime (CTX)	3	10.71	2	7.14	23	82.14
4	Ceftriazone (CTR)	3	10.71	2	7.14	23	82.14

guidelines (CLSI, 2014) of antimicrobial concentration and incubated for 18-24 h at 37°C. The results were interpreted as described by CLSI, (2014). The *E. coli* isolates ATCC 25922 was used as control.

E. coli was detected in 11.76% of the samples with counts ranging from 10^1 to $2x10^2$ cfu g^{-1}. Similar to the present findings, Sivaraman et al. (2014) reported high count of *E. coli* in seafood from Gujarat ranging from 0.5 to 2.0 x 10^3 $cfug^{-1}$ with the incidence rate of 53.19% and suggested, the possibility of faecalor sewage as the source of contamination. Whereas, Jeyasanta et al. (2012) found a very higher percentage (71.2%) of *E. coli* in seafoods in Tuticorin, Tamil Nadu. However, Surendraraj et al. (2005) found varied *E. coli* count from 2 log $cfug^{-1}$ to 5.5 log $cfug^{-1}$ in different fish samples in retail outlets in and around Cochin, Kerala. The lowest incidence rate of 15.0 and 16.0% were observed by Kumar et al. (2005) from the fish market and landing centre of Mangalore, Karnataka, respectively.

Antimicrobial resistance pattern of 27 *E. coli* strains to isolated from seafood to third generation cephalosporins are shown in Table 1 and 2. The resistance to third generation cephalosporins was 14.29, 10.71, 10.71 and 7.14% with ceftazidime, cefotaxime, ceftriaxone and ceftozoxime, respectively. Intermediate resistance was found to CAZ and CZX in 3.57% and CTX and CTR in 7.14% respectively of the *E. coli* strains. Two *E. coli* isolates consistently showed resistance patterns to 3rd and 4th generation antimicrobial agents and were from the Ribbon fish samples. The present study clearly demonstrated the presence of 3rd generation cephalosporin resistant *E. coli* isolates from seafood samples and pose serious public health concern among the seafood consumers. High percentage of resistance was also observed by Elhadi & Alsamman (2015) from imported mackerel fish in Saudi Arabi with 93.3% to both cefotaxime and ceftriaxone in *E. coli*. Further Elhadi & Alsamman (2015) and Heuer et al. (2009) suggested that a wide range of antibiotic classes used extensively in aquaculture and in human therapy lead to the incidence of resistance *E. coli* strains to higher generation antimicrobials. With the consumption of such contaminated fish products, these resistant strains are transferred to human gut and enter the food cycle. But, Hleba et al. (2013) did not find any resistant *E. coli* isolates in *Cyprinus caprio* to third generation cephalosporin like ceftriaxone in Slovokia. The results of the present study reveal that the presence of third generation cephalosporin resistant *E. coli* strains in Gujarat is currently not very high. However, it may go high in future and may become

Table 2. Antimicrobial resistance patterns of *E. coli* isolate to third generation cephalosporins

Resistant type	No. of resistant strains
CAZ	4
CZX	2
CTX	3
CTR	3
CPM	5
CAZ - CTR	3
CAZ- CTX	3
CAZ- CZX	2
CTR- CTX	3
CTR- CZX	2
CTX- CZX	2
CAZ - CTR- CTX	3
CAZ- CTX- CZX	2
CTR- CTX-CZX	2
CAZ - CTR- CTX- CZX	2
CAZ - CTR- CTX- CZX- CPM	2

a very serious public health concern among the seafood consumers, if not controlled. The constant use of third generation cephalosporins in the treatment of infections is probably the reason for these incidences and the spread of highly resistant *E. coli* strains. It further suggested that strict hygienic practices such Hazard Analysis Critical Control Point (HACCP), Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP) are to be followed during the entire chain of seafood processing in order to produce seafood that is free form MDR bacteria.

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