

The higher weight gain and specific growth rate in shrimp head waste feed observed in the trial might be on account of the higher protein content

and better feed attraction and subsequent higher level of feed consumption.

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## Prevalence of extended spectrum beta lactamase (ESBL) *E. coli* in fishes from the retail markets of Guwahati, Assam.

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It is reported that 90-95% of the population of Assam depend on fish as their main protein diet with per capita consumption of 9.00 kg (Bhuyan *et al.*, 2017). Fishes being a major food in a state like Assam and reports direct the possible transmission of antimicrobial resistance through fish, it is impending to conduct a surveillance to estimate the prevalence of antimicrobial resistance pathogen. The impact of drug resistant bacterial transmission from livestock and aquaculture resources/seafood to humans is poorly understood. Antimicrobial resistance is a global issue as indicated by the rapid increase in cephalosporin and carbapenem resistance in Enterobacteriaceae, leading to limitations in treatment options for infections caused by these bacteria (Bush *et al.*, 2011). Extended- Spectrum Beta Lactamase (ESBLs) are considered as a major source of resistance in Enterobacteriaceae towards oxyimino- cephalosporins (Bradford, 2001). The present study highlights the prevalence of ESBL *E. coli* in fishes sold in the selected retail markets of Guwahati, Assam. The microbiological identification and antibiotic susceptibility test (AST) profile of the isolates were carried out

using BD Phoenix™ M50 automated system (BD Diagnostics, USA).

A total of 79 fish samples were collected from the retail markets situated in three selected locations (Silagant, Garchuk and North Guwahati Town Committee region) in Assam in the month of August, 2019. The samples include the most common food fishes silver carp (*Hypophthalmichthys molitrix*), rohu (*Labeo rohita*), red belly piranha (*Pygocentrus nattereri*), catla (*Catla catla*) and native fish varieties caught from either river or beel (lake) namely singara (*Mystus tengara*), singhi (*Heteropneustes fossilis*), kawoi (*Anabas testudines*), puthi (*Puntius spp.*), bata (*Labeo bata*), aree (*Sperata seenghala*) etc.

Fish samples were collected in sterile polythene bags and transported (maintaining cold chain) to the laboratory in National Research Centre on Pig, Rani, Guwahati for initial processing. Samples were processed within 3-4 hrs of initial procurement. The gut portion of the fish were taken for the isolation of *E. coli*. Macerated fish samples were aseptically transferred to modified form of Brilliant Green Bile Lactose

Broth, EE broth Mbsel enrichment media (pH 7.2) and were incubated for 18-24 h at 35- 37° C. A loopful of the enriched culture was streaked onto MacConkey agar plates supplemented with 1 µg/ml cefotaxime and incubated for 18-24 hrs at 35-37 °C. Typical pink colored colonies, indicative of lactose-fermenting characteristics of *E. coli* were picked and further streaked onto Eosin-Methylene Blue agar. Presumptive *E. coli* colonies with typical purple colour with or without dark purple center or with green metallic sheen were picked. Non duplicate isolates were inoculated further on to tryptic soy agar plates for identification and AST.

The BD Phoenix™ M50 automated system is specifically used for the identification and antibiotic susceptibility test of the isolates. NMC/ID55 ID-AST combo panel used in this study of Gram-negative bacteria has a distinct 'BD Phoenix ESBL screening test' which is based on the growth response to selected second or third generation cephalosporins in the presence or absence of a beta-lactamase inhibitor, clavulanic acid. This feature enables to categorize an isolate as ESBL if it is resistant to the following antibiotics: cefotaxime/ clavulanate, ceftazidime/ clavulanate, cefpodoxime-proxetil, ceftazidime and ceftriaxone/ clavulanate. Other than these, the panel consists of 19 different antibiotic wells representing 13 different antimicrobial categories: amikacin (AN), amoxicillin/ clavulanate (AMC), ampicillin (AM), aztreonam (ATM), cefazolin (CZ), cefepime (CPM), cefotaxime (CTX), ceftazidime (CAZ), chloramphenicol (C), ciprofloxacin (CIP), gentamicin (GN), imipenem (IPM), levofloxacin (LVX), meropenem (MEM), piperacillin (PIP), piperacillin/ tazobactam (TZP), tetracycline (TE) and trimethoprim/ sulfamethoxazole (SXT). Isolates were loaded to individual panels and procedures were followed according to manufacturer's instructions. Quality control using the reference strains *E. coli* ATCC 25922 was also performed. Susceptibility patterns

were interpreted strictly adhering to CLSI, 2020. Multiple antibiotic resistance (MAR) index for *E. coli* was calculated based on the number of antibiotics to which the isolates showed resistance to the total number of antibiotics to which isolates were exposed (Christopher *et al.*, 2013).

In all, 66 non duplicate isolates from a total of 79 fish samples were subjected to identification in BD Phoenix™ M50, out of which 54 isolates were detected as ESBL *E. coli*. These were further confirmed at molecular level by using CTX-M genes (Sivaraman *et al.*, 2020). It is seen that 100% of the *E. coli* isolates showed resistance to beta-lactam antibiotics such as ampicillin, cefazolin, cefotaxime and piperacillin. Though 100% of the *E. coli* isolates showed resistance to cefotaxime, only 6% were resistant to ceftazidime, another third generation cephalosporin. It could be seen that 85% of the isolates showed resistance to fourth generation cefepime and 15% were categorized as susceptible-dose-dependent (SDD). Only two isolates were resistant to ceftazidime. Also 96% of the ESBL *E. coli* isolates were susceptible to -lactam combination agents-amoxicillin/clavulanate and piperacillin/tazobactam. Among the non -lactam group of antibiotics, 33% of the isolates were resistant to ciprofloxacin and <33% to levofloxacin. Tetracycline resistance was significantly less. MAR index showed a range of 0.26-0.63 in ESBL *E. coli* isolates with 48% of the isolates with an index value of 0.31. In the present study, all the ESBL isolates showed resistance to more than three antimicrobial categories and hence were considered as multi drug resistant (MDR). 67% of the *E. coli* were found to have resistance to 4 antimicrobial classes whereas, two *E. coli* isolates were resistant to a maximum of 8 antimicrobial classes. The study highlights the prevalence of extended spectrum -lactamase producing *E. coli* in the fish samples sold for consumption in retail markets. The prevalence of *E. coli* in environment is considered as a contaminant

and any pathogenic strain can present several challenges in human health sector.

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## References

- Bhuyan P.C., Goswami C. and Kakati B.K. (2017) Study of Fish Consumption Patterns in Assam for Development of Market-Driven Strategies. *Res J. Chem. Environ. Sci.* 5 42-52
- Bradford P.A. (2001) Extended-spectrum -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol r*(2001). *Rev.* 14(4) 933-951.
- Bush K. K, Courvalin P., Dantas G., Davies J., Eisenstein B., Huovinen P., Jacoby G.A, Kishony R., Kreiswirth B.N., Kutter E. and Lerner S.A. (2011) Tackling antibiotic resistance. *Nat. Rev. Microbiol.* 9, 894-896.
- Christopher A.F., Hora S. and Ali Z. (2013) Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India, *Ann. Trop. Med. Pub. Health*, 6, 285.
- CLSI (2020) Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing; 30th edition, document M100 (2020). CLSI, Wayne, PA.
- Sivaraman G.K., Sudha S., Muneeb K. H., Shome B., Holmes M and Cole J. (2020) Molecular assessment of antimicrobial resistance and virulence in multi drug resistant ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* from food fishes, Assam, India. *Microb Pathog.* doi: 10.1016/j.micpath.2020.104581.

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## “ Caring the crafts” : off season maintenance of fishing boats of Gujarat coast

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There are number of factors which influence the operation and maintenance of the fishing systems. The coastal districts of Saurashtra and Kutch region of Gujarat, are well-known for skill and craftsmanship for construction of highly efficient fishing and cargo vessels (Prajith et al 2017). The fishery of Gujarat is mainly contributed by the mechanised fishing vessels

(6.96 lakh t) followed by motorized fishing vessels (0.84 lakh t) and non-motorized fishing vessels (397 t). The catches from the mechanized sector are mostly contributed by multiday trawlers (CMFRI, 2019). Veraval, Mangrol, Okha and Porbandar are the major fish landing centres of the state. In Veraval coast, trawling is done with wooden and FRP vessels (only one steel vessel