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Assessment of microbial quality of fish processing industrial effluent in bar-mouth at Bhidia landing site, Veraval, Gujarat, India

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Abstract

The present study was carried out to assess the microbial quality of fish processing industries effluent at Bhidia bar- mouth, Veraval, Gujarat during April, 2012 to March 2013. The total viable bacterial count (TVBC), total *Enterobacteriaceae count, E.coli count (*EC), *Staphylococcus aureus and* Fecal Streptococcal count *in* effluent ranged from 3.0×10^{1} to 6.8×10^{6}, 9.0×10^{1} to 2.9×10^{4}, 0 to 0.5×10^{4}, 0 to 0.4×10^{2} and 0.3×10^{1} to 0.1×10^{4} cfu.ml^{-1}, respectively. Significantly higher load of TEC*, E.coli*, *S.aureus*, Fecal Streptococci, Total coliforms and Fecal coliforms were higher during summer whereas, TVBC was higher in the month of Sept.-Oct. Furthermore, the total coliform and fecal coliform counts were found to be higher with 1400+ /100ml MPN value throughout the year of the study, except in the month of August. Overall occurrence of pathogenic strains *of E.coli*, *S.aureus* and Fecal streptococci were 41.67%, 25.00% and 66.67% respectively during this period. The antibiogram of the isolated *E.coli* isolates show that almost 50% were resistant to Cefazidime/ Clavulanic acid (CAC), Amoxyclav (AMC), Ciprofloxacin (CIF) and Ampicillin (AMP). The present study indicated that the effluent of fish processing industry was heavily contaminated with *E.coli*, *S. aureus and* Fecal Streptococci which confirmed improper treatment of fish processing effluent. Moreover, the precedence of antibiotic resistant *E.coli* may pose threat to public health safety.

Key words

E.coli, Fish processing industry, Industrial effluent, Microbial quality

Introduction

Increasing demand of processed seafood product in international market, there is an ever mounting pressure on processing industry of Gujarat to increase the processing volume of seafood which in turn leads to increased effluent discharge to nearby area/ water bodies. Several workers have reported the presence microbial contamination such as total aerobic counts, *E.coli*, *Staphylococci*, *Streptococci, Salmonella, Listeria* and *Vibriosis* in raw fish, processed fish products, utensils, workers hand, ice and water used in fish processing industries (Cann *et al*., 1981; Zuberi *et al*., 1983; Karunasagar and Karunasagar, 2000; Rashid *et al*., 2000, Bagge-Ravn *et al*., 2003; Hossain *et al*., 2010 and Sivaraman *et al*., 2012). So the fish processing industrial effluents are likely to produce adverse effects on the receiving coastal and marine environments.

Moreover, the effluent generated from fish processing plants is directly discharge in to the bar-mouth and the effect of effluent in the marine aquatic environment has not received much attention so far. The principal components of the effluent of fish processing unit usually contain water, ice, fish muscle pieces, skin, scales, fins, fat, oil, protein, head, tail, shells, inedible parts, chlorine, calcium hypochlorite, potassium permanganate and soap. Thus, the fish processing effluent contains sufficient quantity of organic material which acts as a very good source of nutrients

for growth of microbes and affects sustainability of fishery resources (Bonsdorf *et al.,* 1997) and potential hazards to the ecosystem (Islam *et al.*, 2004). Moreover, few information on the microbial quality of fish processing industrial effluent waters in India (Sivaraman *et al.,* 2012). Keeping all these in consideration a preliminary survey was conducted to monitor the microbial quality of fish processing industrial effluent in Veraval, Gujarat which would provide the possible source of microbiological quality of fish processing industrial effluents.

Materials and Methods

Effluent water sampling : Sampling was done on monthly basis from April 2012 to March 2013 (n= 12) at the effluent discharge point near bar-mouth site, Bhidia, Veraval, and not from the open sea. 250- 500ml of water samples were collected in pre-sterilized containers, kept at < -4°C in ice storage box and immediately transported to the laboratory within an hour.

Microbiological examination of effluent water : Total Viable Bacterial Count (TVBC), Total Enterobacteriaceae, *E.coli*, *S.aureus*, *F.streptococci* count, *Salmonella*, *V.cholera*, *V.parahemolyticus*, *Listeria* and MPN for coliforms were tested as per the standard procedures of Food and drug Administration (FDA), Bacteriological Analytical Manual (BAM), (2012). For TVBC, total enterobacteriacea and Fecal Streptococi water samples were serially diluted and 1ml each of the appropriate dilutions was pipetted and pour plated on to total plate count agar, violet red bile glucose agar and Kennel fecal streptococcal agar (Oxoid, UK) with the corresponding medium for triplicate in and duplicate plates for *Enterobateriaceae and Fecal streptococci*.

Isolation and identification of indicator bacteria : For *E.coli* and *Staphylococci*, 0.5 ml of serial diluted samples was spread on to the T-7 and Baird parker agar plate in duplicate of each dilution. The Tergitol-7 (T-7) and VRBGA plates were incubated at 37°C for 18- 24 hrs and TVBC, BP and KF were incubated at 37°C for 48 hr. The plate containing 25 to 250 colonies were determined as count.

Microbiological analysis: Isolation and purification of *E.coli was perf*ormed on T- 7 agar (lime yellow colored colony with deep yellow centre and yellow halo around) and TSA- *S.aureus* on Baird Parker Agar (black colored colony with thin white margin and a zone of clearance around), Fecal streptococci on Kennel Fecal Streptococcocal Agar (red/ pink colored colonies with or without halo zone around) and for *Salmonella* spp, *Vibrio cholerae*, *V.parahemolyticus, Listeria monocytogenes* and *Pseudomonas* spp were performed using the pre and selective enrichment and selective plating and then confirmed with necessary biochemical tests (FDA, BAM, 2012). Strains were identified by Gram staining, motility, catalase, oxidase, glucose fermentation, pigment production, glucose utilization and penicillin sensitivity test. Purification and confirmation of isolated strains were done on to the specific selective media: *E.coli* on T7 and confirmed on EMB agar (metallic green sheen with purple to black colored colony and dark centre) and IMViC tests (++- -); *S.aureus* on BP agar and confirmed on Mannitol Salt Agar (yellow colored colonies surrounded by bright opaque zone) and rabbit coagulase plasma (coagulation / clot formation) and *Fecal streptococci* on KF agar with catalase test (negative) (Surendran *et al*., 2013). The confirmed isolates from each sample was taken for calculation of occurrence of bacterial pathogen. 5 tube MPN method was carried out for total coliforms, fecal coliforms and *E.coli*.

Antimicrobial susceptibility testing : Antimicrobial resistance pattern of isolated *E.coli (n*=43) from effluent water samples was carried out to a panel of twenty four antimicrobial agents (HiMedia, Mumbai) by disc diffusion method of Bauer *et al*. (1966) on Mueller-Hinton agar. A loopful of growth from slant was inoculated in Brain Heart Infusion (BHI) broth and incubated at 37 °C for 3 to 5 hr. The opacity of broth tube was matched with McFarland's tube No 0.5 (1.5×10^8 org ml^{-1}). A sterile cotton swab was dipped into the broth culture; excess of bacterial suspension was removed by pressing and rotating the swab against the inner walls of the test tube. Streaks were applied across the entire agar surface of Petriplate with swab, three times, the plate being turned for 60° between each streaking. The plates were incubated overnight at 37 °C. The results were interpreted with CLSI standards, (2014).

Results and Discussion

The waste water discharge from the fish processing industries at Bhidia Bar- mouth site, Veraval, Gujarat was found to harbour *Enterobacteriaceae, Staphylococcus, Vibrios* and *Pseudomonas*. Total viable bacterial count (TVBC), total enterobacteriaceae count (TE), *E.coli* count (EC), *S.aureus* (SA) and Fecal Streptococcal count (FS) in the effluent ranged from 3.0×10^1 to 6.8×10^6, 9.0×10^1 to 2.9×10^4, 0 to 0.5×10^4, 0 to 0.4×10^2 and 0.3×10^1 to 0.1×10^4 cfu 100 ml^{-1}, respectively (Table 1). TVBC is one of the important microbial parameters for determining the quality of environmental pollution of natural water bodies as it act as a good indicator in monitoring microbial pollution in association with the presence of huge amount of organic pollutants (Das *et al.*, 2010).

The present findings revealed that higher level of TVBC was found in September to October. Whereas, Das *et*

Table 1 : Microbial load of fish processing industrial effluent during April 2012 to March 2013

Months	TVBC	TE	Staphylococci	Fecal Streptococci	Total Coliforms 100ml^{-1}	Fecal Coliforms 100ml^{-1}
April-12	3.1 x 10^{5}	2.0 x 10^{4}	4.0 x 10^{1}	3.6 x 10^{3}	1400	1400
May-12	4.25 x 0^{6}	2.9 x 10^{5}	3.2 x 10^{1}	1.0 x10^{5}	1400	1400
June-12	1.0 x 10^{7}	6.2 x 10^{3}	0	2.0 x 10^{2}	1400	1400
July-12	1.0 x 10^{7}	5.0 x 10^{4}	3.0 x 10^{2}	5.7 x 10^{3}	1400	1400
August-12	2. 4 x 10^{3}	1.5 x 10^{2}	0	2.4 x 10^{1}	250	250
October-12	6.8 x10^{6}	9.0 x 10^{2}	3.0 x 10^{1}	3.0 x 10^{4}	1400	1400
November-12	2.9 x 10^{4}	2.9 x 10^{4}	0	2.0 x 10^{3}	1400	1400
December-12	2.2 x 10^{5}	8.8 x 10^{3}	0	0.3 x 10^{1}	1400	1400
January-13	6.0 x 10^{4}	6.5 x 10^{3}	1.2 x 10^{2}	2.2 x 10^{3}	1400	1400
February-13	9.0 x 10^{1}	3.0 x 10^{1}	0	5.0 x 10^{1}	1400	1400
March-13	1.0 x 10^{6}	1.1 x 10^{5}	4.0 x 10^{2}	7.0 x 10^{3}	1400	1400
Minimum	3. 0 x 10 ^{1}	9.0 x 10^{1}	0	0.3 x 10^{1}	250	250
Maximum	6.8 x 10^{6}	2.9 x 10^{5}	4.0 x 10^{2}	1.0 x 10^{5}	1400	1400

TVBC, TE, EC, SC, FSC= cfu 100ml^{-1}, TE= Total Enterobacteriaceae

al. (2010) also observed that microbial load of tannery and textile effluent at water receiving rivers of Dhaka that higher TVBC of 7.2 x 10^{6} cfu ml^{-1} during dry season as well as in rainy season. High microbial TVBC in the present study indicated that the fish processing industrial effluent is of poor quality and is not treated effectively before discharge it. Moreover, TVBC was more than ISO (2012) microbial permissible limit of 1.0 x 10^{6} cfu ml^{-1} either in fish and its products or the processing waste water and contaminates coastal environment to greater extent and is also comparable with the recommended limit of ICMSF (2011), plate count of 1 x 10^{6} cfu.g^{-1}. Similarly Hasan *et al*. (2006); Neboh *et al*., (2013); Verla *et al*., (2014); Ibe *et al*., (2014) and Porwal *et al*., (2015) also observed higher number mean viable bacteria in Buriganga River, abattoirs effluent, palm oil mill effluent, cassava mill effluent and dairy effluent respectively and they suggested that effluents avert potential problems to the environment due to indiscriminate dumping without treatment. Further, it was suggested by Islam *et al*. (2004) and Bagge- Ravn *et al*. (2003) that microflora of raw fish and shrimp material, preservation condition and processing equipment of food industries also act as a source of microbial ecosystem, whereas, Zuberi *et al*. (1983) reported average TVBC of 9.4 x 10^{6} cfu g^{-1}, total coliforms MPN count of 94 g^{-1} and MPN fecal coliforms of 41 g^{-1} in frozen shrimp.

Similar to the present investigation, Das *et al*. (2010) also observed that the Staphylococci present in the industrial effluent water throughout the year. However, the presence of coagulase positive *Staphylococci* during the month of July, January and March were more than the ISO (2012) permissible limit of 100 numbers 100ml^{-1} (FDA, BAM, 2012). This finding clearly indicates that waste water from fish processing industry of Bhidia, Veraval discharged at Bar-mouth Bhidia was highly contaminated.

E.coli and coliform is an effective indicator of fecal contamination. The present findings revealed that highly significant level (P<0.01) of *E.coli* occurred in the month of summer than those of rainy and winter season with 5.0 x 10^{5} cfu 100ml^{-1} and the bacterial counts were more than the permissible limit of ISO (2012) in all the month of study except in August and February (FDA, BAM, 2012). Similar to the present findings, Das et al., 2010 also found that higher number of E.coli counts with 2.4 x 10^{3} cfu / 100 ml in tannery and textile waste water. The total coliform and fecal coliform groups were found significantly high in all the months, except in August with 250 100 ml^{-1} which might be associated with the rainy season and also the sampling site was not directly contaminated with domestic sewage. Hasan et al. (2006) also found that the coliform count was higher (2.4 x 10^{3} cfu / 100 ml) in sewage lagoon and the E.coli count varied from 1.1 x 10^{3} to 2.4 x 10^{3}/ 100 ml at different part of Buriganga River. The present study revealed that the total coliforms and fecal coliforms was higher than the recommended standards, which clearly indicates higher level of contamination from the fish processing industries. However, pathogenic bacteria such as *V. cholera*, *V.parahemolytica*, *Salmonella* and *Listeria* were not detected from the fish processing industrial effluent. Whereas, Karunasagar and Karunasagar (2000) reported the occurrence of food-borne human pathogens such *as Listeria* spp. from fresh and processed fishery products and Rashid *et al.* (2000) identified different source of microbial contamination apart from raw material like ice (703- 2500 cfu ml^{-1}), water (970- 1800 cfu ml^{-1}) contact surface utensils (450- 3570 cfu ml^{-1}) and worker hands (330- 1985 cfu ml^{-1}) and suggested the possible source of microbial contamination in the environment.

Table 2 : Antibiogram pattern of the *E.coli* isolates from the fish processing industrial effluent

Antibiotic discs	Resistance		Intermediate	
	Number of isolates	%	Number of isolates	%
Ampicillin AMP (10mcg)	18	72.00	5	20.00
Genmicin GEN (10µg)	0	0	3	12.00
Amikacin AK (30µg)	14	56.00	11	44.00
Ciprofloxacin CIP (5µg)	19	76.00	5	20.00
Oxfloxacin OF (5µg)	18	72.00	2	0.08
Co-Trimoxazole CoT (25 µg)	0	0	0	0
Amoxyclav AMC (30µg)	23	92.00	2	0.08
Cefuroxime CXM (30µg)	13	52.00	6	24.00
Cefuridine CAZ (30µg)	0	0	12	48.00
Cefazidime/ Clavulanic acid CAC (30 10µg^{-1})	22	88.00	3	12.00
Cefepime CPM (30µg)	6	24.00	11	44.00
Imipenem IPM (10µg)	0	0	0	0
Cefotaxin CTX (30µg)	4	16.00	21	84.00
Ceftriaxone CTR (30µg)	0	0	12	48.00
Cefoxitin CX (30µg)	0	0	0	0
Meropenem MRP(10µg)	12	48.00	0	0
Piperacillin/ Tazobactum PIT (100 10µg^{-1})	6	24.00	13	52.00
Aztreonam AT (75µg)	-		-	
Gatfloxacin GAT (5µg)	-		-	
Ampicillin/ Sulbactum A/S (10 10µg^{-1})	17	68.00	1	0.04
Cefoperazone CPZ (75µg)	0	0	12	48.00
Levofloxacin LE (5 µg)	5	20.00	13	52.00
Ceftizoxime CZX (30µg)	0	0	0	0
Ticarcillin/ Clavulanicacid TCC (75 10 µg^{-1})	21	84.00	4	16.00

Higher microbial load of TVBC, total enterobacteriaceae, *E.coli*, Staphylococci, fecal Streptococci, total coliforms and fecal coliforms were observed during summer than rainy and winter season. The overall microbial quality of water (Total count, total enterobacteriaceae, *E.coli*, Staphylococci, fecal Streptococci and coliforms) was found to be poor. However, these microbial quality parameters varied during different season. Highest total enterobacteriaceae, *E.coli* and fecal Streptococci were found in May and are attributed to effluent concentration at the bar-mouth site. Highest TVBC, fecal coliforms and *E.coli* count were found in almost all the month, except in August, 2012.

Furthermore, the overall percentage of occurrence of *E.coli*, *S.aureus* and fecal Streptococci were 41.67%, 25.00% and 66.67% with microbial load varying from 0.0 to 5.0 x 10^5, 0 to 4.0 x 10^2 and 0.3 x 10^1 to 1.0 x 10^5 cfu ml^{-1}, respectively, during April, 2012 to March 2013. 41.67%, 75%, 25%, 66.67% and 83.33% of water samples had TVBC, *E.coli* count, *Staphylococci*, fecal *Streptococci*, respectively, that exceeded the limit in effluent. The results clearly indicate that the fish processing industrial effluent was highly contaminated with microbes and therefore, the effluent was not treated effectively in effluent treatment plant. The antibiotic resistant pattern of isolated *E.coli* isolates show that more than 50% of *E.coli* isolates were resistant to Cefazidime/ Clavulanic acid, Amoxyclav, Ciprofloxacin and Ampicillin (Table 2) indicating that the fish processing effluent was highly contaminated with multi drug resistant *E.coli* strains. Moreover the precedence of antibiotic resistant bacteria might pose threat to public health safety due to the presence of multi drug resistant *E.coli* isolates.

The present findings highlights the presence of microbial contamination in the fish processing industrial effluent with *E.coli*, *S. aureus and* Fecal Streptococci and also with antibiotic resistant *E.coli* may pose threat to public health safety.

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