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Prevalence of Antibiotic Resistant *Escherichia coli* in Sea Foods of Tuticorin Coast, Southeastern India

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Abstract: Among the 168 samples of 22 sea foods, 128 were positive for the presence of faecal coliforms and of that 91 were positive for *E. coli*. The antibiotic resistance of *E. coli* isolates was tested with 15 different antibiotics. The isolates were highly resistant to four antibiotics, intermediate and least resistant to five antibiotics and highly sensitive to one antibiotic. Among the total isolates eleven showed multiple resistance to four antibiotics and of which one had multiple resistance to five antibiotics. Present study revealed that Amikacin, ciprofloxacin and Chloramphenicol are the best antibiotics to treat *E. coli* infection. Antibiotic susceptibility studies revealed that sea foods from Tuticorin contains antibiotic resistant *E. coli* strains which may serve as a reservoir for antibiotic resistant genes in the seafood environment.

Key words: *E. coli* · Faecal Coliforms · Sea Foods · Antibiotic Resistance

INTRODUCTION

Microbiological quality and safety of foods has gained great attention among the present day consumers, food processors and regulatory agencies and this continues to increase day by day. It is more pronounced in sea foods because of the apparent distinction from other foods in terms of the extreme perishable nature and short shelf life [1]. Seafood is one of the best supplies of protein, vitamins and minerals and essential nutrients required for supplementing both infant and adult diets [2]. Sea foods are susceptible to a wide variety of potentially pathogenic bacteria [3]. Seafood is a major vehicle for transmission of several bacterial diseases [4]. Estuaries and coastal water bodies are the major sources of sea foods in India and are often contaminated by the activities of adjoining population and partially treated or untreated sewage released in to these water bodies. Sea foods harvested from such areas often contain pathogenic microorganisms. In addition poor sanitation in landing center and the open fish markets exacerbates the situation [5]. It has been reported that quality of fish sold in domestic market in India is poor compared to that of export trade and are mostly contaminated with pathogenic microorganisms [6]. The bacteriological quality of freshly landed as well as retail market fish and other seafood in different parts of the country have been studied by many workers [7-9].

Most of these studies have established that a sizeable portion of the fish available in the market for consumption is not meeting the quality criteria prescribed by Indian standards. Human infections due to many pathogenic bacteria are reported to have been transmitted through fin fish, shell fish and other sea food products [10]. Fish is highly perishable and should be handled with great care to preserve the natural attributes of fish and prevent microbial proliferation. One of the major factors contributing to poor quality of the fish in retail trade is unhygienic handling and storage leading to off-smell, physical damage, building up of bacterial load and contamination with dirt and objectionable microorganisms [11].

In food microbiology as well as water microbiology *E. coli* is considered primarily as an index organism. . The faecal coliform test often leads to the estimation of the contamination in tropical seafood [12]; it remains the most convenient method in the absence of any other reliable indicator organism. *E. coli* has been traditionally recognized as an indicator organism of faecal contamination of water and sea foods [12]. Testing of seafood for the presence of *E. coli* is still a gold standard used to assess the faecal contamination of seafood processing plants in India and else where [13].

E. coli, the most common flora of the gastrointestinal tract may become pathogenic and cause gastrointestinal tract infection and blood stream infection to living beings

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[14, 15]. Human pathogenic *E. coli* has evolved as a causative agent of a broad range of human diseases compared to any other pathogenic bacteria [16, 17].

Although most isolates of *E. coli* are non pathogenic, they are considered as indicator or faecal contamination in seafood and about 10 -15% of intestinal coli forms are opportunistic and pathogenic sero types [18] and cause a disease to the reservoir. Among the diseases; some are often severe and some times lethal infection such as meningitis, endocarditis, bloody/ mucoid diarrhea, urinary tract infection, septicemia and epidemic diarrhea of adults and children can occur [19]. During the past two decades, severe outbreaks of gastrointestinal illness have been caused by food borne pathogenic *E. coli* [20].

Antibiotics once effective at controlling *E. coli* infections are now ineffective due to bacterium's acquired resistance to these compounds. The resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human beings [21, 22]. The use of antimicrobial drugs to control infectious diseases must be among the greatest achievements of medicine in the century. The disease threat from antibiotic resistant strains has increased in recent years [23]. The occurrence of multiple antibiotic resistances among the enteric bacterial species could be a problem associated with transfer of resistance to human beings [24]. The multiple antibiotic resistance index is used for differentiating the source of pollution [25]. Transmission of resistant clones and resistance plasmids of *E. coli* from sea foods to human commonly occurs [26]. However about 10 years after the spread of antibiotic therapy a number of species of *Staphylococcus, Mycobacterium* and Gram negative enteric bacteria have developed resistance to antibiotics. This antimicrobial resistance can spread through horizontal transfer of resistance genes from one type of bacteria to another. The presence of resistance together with the acquisition of virulence genes can lead to clonal expansion and spread of particular disease causing agent [27]. Hence it is considered important to study the antimicrobial resistance in pathogenic as well as indicator bacteria associated with sea foods [28]. This study was intended to estimate the presence of *E. coli* in sea foods in the landing centers and local market and their antibiotic resistance was tested with 15 antibiotics for the benefit of the consumers.

MATERIALS AND METHODS

Twenty two seafood samples such as fin fishes, crustaceans, mollusks and sun dried fishes were randomly collected in triplicate from the main landing centers of Tutucorin coast, inter tidal areas and fish markets of Tuticorin for the study. They were stored in sterile polythene bags and brought to the laboratory under aseptic condition and processed immediately.

Isolation of faecal coli forms and *E. coli*: The technique used to determine the presence of faecal coli forms is the 3 tube MPN method [29]. The samples were homogenized in a sterile blender and inoculated into lauryl sulfate tryptose broth (LSTB) and incubated at 37°C for 24-48 hours. The LSTB tubes showing turbidity and gas in Durham tubes were recorded as positive. Two loops full of culture from positive LSTB tubes were inoculated into corresponding labeled tubes with 5ml of EC broth medium. EC broth tubes exhibiting turbidity and gas production following 24 hours of incubation at 45°C in a water bath were considered positive for the presence of faecal coli forms. For the isolation of *E. coli* two loopfuls of culture from the positive EC broth tubes were streaked on to eosin methylene blue (EMB) agar plates. A minimum of five typical colonies were picked up and purified on tryptone soya agar (TSA) plates.

Identification of *E. coli*: Identification of typical colonies of *E. coli* on EMB agar plates was done according to Buchanan and Gibbons [30] following a series of biochemical tests.

Drug Sensitivity Test: Single disc diffusion method [31] was used to examine bacterial susceptibility to antimicrobial agents. A total of 15 antibiotics discs (Hi media, Mumbai) of ampicillin (10 mcg), Gentamycin (10 mcg), Chloramphenicol (30 mcg), Amikacin (30 mcg), Penicillin G (10 µg), Streptomycin (10 mcg), Tetracycline (30 mcg), Kanamycin (30 mcg), Vancomycin (30 mcg), Erythromycin (15 mcg), Neomycin (30 mcg), Ciprofloxacin (5 mcg), Amoxycillin (30 mcg), Piperacillin (5 µg) and Nalidixic acid (30 mcg) were used. The plates were examined and the diameter of the zones of complete inhibition to the nearest whole millimeter was measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the zone interpretation table of the Hi media, Mumbai.

RESULTS AND DISCUSSION

Incidence of Faecal Coliforms and *E. Coli* in Sea Foods: The prevalence of Faecal coli forms and *E. coli* in sea foods collected from different sources of Tuticorin coast is presented in Table 1. From the 22 seafood species, 168

Table 1: Prevalence of Faecal coli forms and E. coli in sea foods collected from different sources

Ssea food samples	Sources	No. of samples	No.. of samples positive for faecal coli forms	No. of samples positive for *E. coli*
Fin fishes				
Sardinella fimbriata	Fish market	10	8	8
Leiognathus splendens	Fish market	10	10	6
Liza parsia	Landing centre	10	10	6
Sardinella gibbosa	Landing centre	10	10	7
Lethrinus nebulosus	Fish market	3	1	-
Stolephorus japonicus	Fish market	10	6	6
Lutjanus vitta	Fish market	5	5	2
Upeneus sundaicus	Landing centre	10	10	6
Katsuwonus pelamis	Fish market	5	3	1
Sphyraena barracuda	Landing centre	5	3	2
Shrimps	Landing centre	10	4	2
Penaeus monodon				
Penaus indicus	Fish market	10	3	1
Crabs	Landing centre	6	5	4
Portunus pelagicus				
Scylla serrata	Fish market	5	4	3
Squid				
Loligo duvauceli	Fish market	4	1	-
Cuttle fish				
Sepia aculeata	Fish market	5	3	3
Octopus				
Octopus Vulgaris	Landing centre	10	9	6
Dried fishes	Dryfish market	5	4	3
Carangoides Sp..				
Sardinella sp..	Dryfish market	5	2	2
Gastropod				
Meurex meurex	Inter tidal area	10	9	9
Bivalves				
Pinctada radiata	Estuary	10	10	10
Saccostrea cuculata	Landing centre	10	8	5
Total		168	128	92

microbial cultures were made and among that 128 cultures were positive for faecal coli forms (76.1%). Among the 128 faecal coliform positive cultures 92 cultures were positive for *E. coli* (71.8%) . Our results strengthen the earlier observations of Kumar *et al*. [4] and Jeyasekaran *et al*. [13]. Quality of sea foods depends on the quality of water from where the fishes are caught and the sanitary conditions of the landing centers. Sanitation and infrastructure facilities at the retail markets play an important role in the overall quality of the fish. Even if the fish catch is landed in prime condition, possible contamination at poor landing sites and contact with untreated sewage cause faecal contamination [32].

It is of significance that some fin fishes from fresh fish market had a high prevalence of *E. coli* (69%). In the case fin fishes collected from the landing center 60.% had *E. coli* prevalence. Our results showed that fishes in the market had poor quality and this might be due to the poor facilities at the marketing sites like improper roofing, unavailability of water, ice, insulated boxes and near by waste disposal site. Untreated sewage let into the sea near the landing site was an important source of contamination of fish.

E. coli does not survive in the marine environment for long and therefore this organism cannot be expected to occur in fish harvested from the sea. However in the present study detection of *E. coli* in some marine fishes might represent post harvest contamination such as in the landing center and fish market [33].

The shrimp samples collected from fish market had (30%) incidence of faecal coliforms but the prevalence of *E. coli* in these samples was just (10%). In the case of shrimp samples collected from landing center of Tuticorin 40% had faecal coliforms and 20% of the samples contained *E. coli*. Fresh shrimp such as *P.monodon* collected from landing center and *P.indicus* collected from fish market showed contamination by *E. coli* due to poor handling and sanitary status. In view of this high market value these items should be carefully handled to prevent any contamination [4].

Among the five crabs samples collected from the market four were positive for the presence of faecal coliforms and three were positive for *E. coli*. In the case of six crab samples collected from landing center, five were positive for the presence of faecal coli forms and 4 were positive for *E. coli*. Seasonal variation of faecal indicator bacteria in fish and coastal waters has already been reported to be high along Tuticorin fish landing centers [34, 32]. Based on the present study most of the local market samples were brought from Thirespuram fish landing center which is contaminated by the disposal of sewage. Extensive contamination of coastal water along Thirespuram has already been reported [32, 35]. All samples were having coliform bacteria in considerable numbers. The *E. coli* levels were within acceptable limits of 20 per g, except in few samples collected from Therespuram.

In the case of mollusks, 2 bivalve species *Saccostrea cuculata* and *Pinctada radiata* were tested. Among ten samples of *Saccostrea cuculata,* 8 had faecal coliforms and five were positive for *E. coli*. All samples of *Pinctada radiata* were positive for faecal coliforms and *E. coli.* Bivalves present in the off shore area are contaminated with faecal matter and our results confirm this statement [4]. The fecal matter in the off shore areas of Tuticorin is mainly from human and animal sources as well as birds. Studies on the faecal pollution in Cochin backwaters with indicator bacteria revealed that the pollution is of non-human type; however a potential health hazard due to consumption of fish was reported [36]. Similar studies in Chennai beach also indicated that the faecal pollution is of non-human sources [37]. However the faecal pollution at Bhavanagar coast was reported to be of human origin based on the faecal index [38]. Among the ten gastropod samples of *Murex murex* nine were positive for the presence of faecal coliforms and *E. coli.* Presence of faecal coliforms and *E. coli* in gastropods was already reported [4].

Sun dried fish samples of *Carangoides species* and *Sardinella* sp five each were collected from dry fish market and among that 4 samples of *Carangoides* and two samples of *Sardinella* were positive for faecal coliform. All the *Sardinella* samples had *E. coli* and only three samples of Carangoides had *E. coli*. *E. coli* count of >2,400 was observed in dried fish from tuticorin dry fish market [39]. Faecal coliforms counts of (> 95 and > 20/g) were observed in sun dried fishes of Tuticorin dry fish market [40].

In the case of cephalopods 4 squids and 5 cuttle fish were collected from the fish market of which one squid and 3 cuttle fish were positive for faecal coliforms, none of the squid samples were positive for *E. coli* and three cuttle fish samples were positive for *E. coli*. Among the ten octopus samples from landing center 9 were positive for faecal coliforms and six were positive for *E. coli.*

In the present study, the fin fishes showed lower *E. coli* contamination than shellfishes. Our results agree with the earlier observations [7, 6 and 41]. Considerable amount of faecal coli forms in coastal aquatic system and fish have been reported from Parangipettai region [42].

Faecal indicator bacteria were also reported from beach sand, seawater and sediments of mahe estuary of Malabar Coast due to the disposal of sewage and land drainage into estuary [43]. High level of faecal indicator bacteria was also reported both in fish and in other samples from Cochin fisheries harbour area and retail markets of Mumbai [44].

Antibiotic Resistance of *E. Coli* Isolated from Seafood: The sensitive, intermediate and resistance patterns of the 92 *E. coli* isolated from different seafood samples against the 15 tested antibiotics were shown in Table 2. One isolate of *E. coli* was resistant to 14 out of 15 antibiotics tested. Resistance spectrum of *E. coli* for 15 antibiotics tested was in descending order; Vancomycin, Penicillin G, Erythromycin, Streptomycin, Amoxicillin, Kanamycin, Nalidixic acid, Ampicillin, Tetracycline, Pipieracillin, Chloramphenicol, Neomycin, Gentamycin and Ciprofloxacin. No strain was found either sensitive to penicillin G, Kanamycin, Vancomycin and Erythromycin or resistant to Amikacin. More over some isolates exhibited intermediate resistance to 13 antibiotics out of 15 tested. Out of the 92 *E. coli* isolates examined in this study, 11 strains showed multiple resistances to 4 antibiotics and one was multiplely resistant to 5 antibiotics.

The bacterial isolates showed considerable levels of resistance against Kanamycin (43%), Amoxicillin (47%), Nalidixic acid (36%), Ampicillin (34%) and Tetracycline (26%). However in the present study, Amikacin, Ciprofloxacin and Chloramphenicol were proved to be the best antibiotics to treat *E. coli* infection since they were highly effective. The results agree with those reported by several investigators [45, 46], who also obtained similar resistant patterns of *E.coli* strains isolated from river and aquaculture water.

Table 2: Antibiotic susceptibility patterns of 91 selected strains of *E. coli* isolated from sea foods

	Resistant		Intermediate		Sensitive	
	% of positive strains	Inhibition zone(mm)	% of positive strains	Inhibition zone(mm)	% of positive strains	Inhibition zone (mm)
Ampicillin (10mcg)	34.06	<14	5.4	15-16	60.43	>17
Gentamycin (10mcg)	2.1	<12	19.78	13-14	78.02	>15
Chloramphenicol (30mcg)	9.8	<12	-	13-17	90.10	>18
Amikacin (30mcg)	-	<14	-	15-16	100	>17
Penicillin G (10 U)	82.41	<14	17.58	-	-	>15
Streptomycin (10 mcg)	58.24	<11	4.3	12-14	37.36	>15
Tetracycline (30 mcg)	26.37	<14	34.06	15-18	39.56	>19
Kanamycin (30 mcg)	43.95	<13	56.04	14-17	-	>18
Vancomycin (30 mcg)	92.30	<14	7.6	15-16	-	>17
Erythromycin (15 mcg)	70.32	<13	26.67	14-22	-	>23
Ciprofloxacin (5 mcg)	1.09	<15	6.5	16-20	91.20	>21
Amoxycillin (30 mcg)	47.25	<13	12.08	14-17	40.65	>18
Piperacillin (5µg)	16.48	<17	8.79	18-20	74.72	>21
Neomycin (30mcg)	6.5	<12	15.38	13-16	78.02	>17
Nalidixic acid (30mcg)	36.26	<13	7.6	14-18	56.04	>19

The presence of multiple antibiotic resistances in *E. coli* isolated from sea foods is of major concern. In Tuticorin untreated or partially treated sewage, industrial effluents and hospital wastes are released in to open and near coastal area and estuaries .These might introduce the resistance strains of *E. coli* in to the coastal environments. Fish or shell fish harvested from such environment would be contaminated with *E. coli*. Other chances for contamination of *E. coli* was areas of seafood harvest, markets, utensils and water used for preserving or washing the fish [4].

In our study 2.1 and 26.37% of the strains were resistant to Gentamycin and Tetracycline respectively. Some isolates of present study exhibited multiple resistances to various antibiotics. Similar findings on multiple drug resistance of *E. coli* strains have been reported from various parts of the world [47-51]. Due to indiscriminate exploitation of antimicrobial agents such high incidence of multidrug resistance might have occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment [52].

In this work *E. coli* isolated from sea foods such as fin fishes, crustaceans and mollusks showed antibiotic resistance, occasional strains being resistant to more than 5 antibiotics. *E. coli* strains isolated from dried fishes were found to be resistant to 3 antibiotics; Vancomycin, Bacitracin and Penicillin G [39]. The susceptibility studies of *E. coli* isolated from seafood samples showed that they were highly resistant to most of the antibiotics tested. Gram negative organisms are more resistant than the gram positive organisms this is expected because of intrinsic nature of Gram negative cell wall. The Gram negative microorganisms isolated belong to the Enterobacteriaceae family; this group of organisms is always resistant to various classes of antibiotics [53].

In the case of transmission of resistant bacteria from infected seafood to human being, the rate of transmission is affected by other factors including where the sea foods are caught, how they are slaughtered, processed and transported [54].

Current work revealed that sea foods sold in Tuticorin was contaminated with *E. coli*. *E. coli* strains have developed a high resistance pattern against the antibiotics tested. Improvement in fresh fish market has to be carefully planned with liberal supply of fresh water, hygienic sewage disposal facilities and power supply. Hygienic and sanitary practices for fisher man fish workers and vendors to stress the importance of quality and possible implications.

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