Bacterial Resistance to Antimicrobial Agents and Microbiological Quality among Escherichia coli Isolated from Dry Fishes in Southeast Coast of India

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Abstract

Escherichia coli is one of the most common causes of bloodstream infections associated with high mortality. The quality deterioration of foods during processing, storage and distribution is mainly caused by microorganisms. The kind of microorganisms present in foods is closely connected to the microflora of the environment. Dry fishes used for the present study were Sardinella gibbosa, Terapon theraps, Terapon sp., S.longiceps, Sphyraena sp., Sardinella fimbriata, Upeneus sp., Thryssa setirostris, Lutjanus vitta, Sillago siham, Gerres filamentosus, Stolephorus japonicus, Lethrinus sp., Sardinella albella and Mugil cephalus. It was also observed that the E.coli strains isolated from all the fishes were found to be resistant to the three antibiotics, Vancomycin, Bacitracin and Penicillin G. The study also reveals that the E.coli strains isolated from at least seven of the fish samples were found to be sensitive to Neomycin, Streptomycin and Chloramphenicol. So the present work reveals that the salt-dried fishes sold in Tuticorin fish markets are contaminated with fungi and pathogenic bacteria like E.coli. The E.coli strains has developed a high resistance pattern to a few of the antibiotic tested. So public awareness (fisherman, fish workers and vendors) on sanitary and hygienic practices to stress the importance of quality is of utmost importance. Landing sites should be maintained clean.

Keywords: Dry fishes, *E.coli*, microbiological quality, seafood, antibiotic resistance.

Introduction

Escherichia coli is one of the most common causes of bloodstream infections associated with high mortality. *E.coli* produces toxins that interfere with our intestine's ability to absorb water, thus causing diarrhea. Salting and sun drying of fish is a traditional method of food processing adopted by most of the countries and in India, the cured fishes are popular in the local markets. Some commercially important species are exported to other countries. But in recent years, the export of cured fish products has declined due to their poor quality (21). Fungal contaminations are a common problem and it adversely affects the quality of cured fishes. The presence of fungi in dry fish along the West Coast of India is prevalent (7).

Fish is a reservoir of large number of microorganism. Some are inherent, coming from where the fish is caught, and others are to contaminations at various stages of handling, from the time of catch till it reaches the consumer. Majority of these microorganisms are non-pathogenic, causing only spoilage of the fish, but there are some, which are pathogenic causing food poisoning. Quality standards have been prescribed for fish and fishery products meant for export and they are being monitored strictly. No such control exits for the retail

trade of fish and fishery products (24). The qualities of fishes sold in the retail markets of Bombay and the quality of commercially frozen boiled clam meat have been reported by (25). There are reports available on the incidence of some pathogens in fishes available in the markets (20 & 11). The incidence of *Salmonella* and some faecal indicator bacteria in fishes sold in the retail markets in Cochin also has been reported (15).

The quality deterioration of foods during processing, storage and distribution is mainly caused by microorganisms. The kind of microorganisms present in foods is closely connected to the microflora of the environment. Population densities of bacteria in seawater are ranging from 10^3 to 10^6 cells in 1 ml depending on the environmental conditions. In general, seawater in coastal areas contains more bacterial cells than in open sea. Microflora of fish and shellfish are closely connected to these of the water and sediment (13).

The use of antimicrobial drugs to control infectious diseases must be among the greatest achievements of medicine in the century. After many clinically useful antibiotics like streptomycin, chloramphenicol, chlortetracycline, neomycin, oxytetracycline, erythromycin etc. were discovered and there by most bacterial infection seemed to be conquered. However about 10 years after the spread of antibiotic therapy a number of species of *Staphylococcus, Mycobacterium* and Gram negative enteric bacteria had developed resistant to antibiotics. Infectious organisms often develop forms resistant to specific antibiotics and hence newer antibiotics must be found. There are many factors responsible for the development of resistance of microorganism to antimicrobial agents. The emergence of drug resistance among the food-borne pathogens like *Escherichia coli* has become an important issue in the food safety. So, proper antimicrobial drugs should be selected to combat with these disease producers (18).

In the fish curing yards of Tuticorin coast, different varieties of fishes are washed in the seawater and are immersed in brine of 20% concentration for 16-24 hours and dried for 2-3 days (21) and the same method is being followed till date with little modification. Even though preservation of fish by salt curing has long been practiced in Tuticorin as a traditional technique, the ratios of salt-to-fish are too low to ensure adequate preservation (8). So the present work is aimed at analyzing the microbial load of dry fishes sold in Tuticorin fish markets and the antibiotic susceptibility of *E.coli* is determined.

Materials and Methods

The dry fishes used for the present study were Sardinella gibbosa, Terapon theraps, Terapon sp., S.longiceps, Sphyraena sp., Sardinella fimbriata, Upeneus sp., Thryssa setirostris, Lutjanus vitta, Sillago siham, Gerres filamentosus, Stolephorus japonicus, Lethrinus sp., Sardinella albella and Mugil cephalus. Dry fish samples were brought to the laboratory under aseptic conditions in clean polythene covers and were analysed for microbial quality. Enumeration of the total bacterial load was done using the Plate Count Agar (PCA) (Casein peptone-0.5g, yeast extract-2.5g, Dextrose-1g, Agar-15g in 1 L distilled water) by the conventional pour plate technique (4). Enumeration of the Total Fungal count was done using the Potato Dextrose Agar (PDA), (Hi media, Mumbai, India) by the conventional pour plate technique (3). Plating was done in triplicate. The plates were inverted and incubated at 37°C for 48 hours for enumeration of TPC and 4-5 days for the enumeration of TFC. Plates containing 30 - 300 colonies were counted and expressed as Colony Forming Units/g of the sample (CFU/g). *E.coli* in the dry fish sample were enumerated using standard Most Probable Number (MPN) technique. MPN count / 10 gm of the sample was calculated using the MPN

table (4). The antibiotic sensitivity of the *E.coli* strains isolated from the fish samples was tested using disc diffusion method (5). The following ten clinical antibiotic discs (Hi Media, Mumbai, India) with concentration of the drug per disc as stated in parentheses were used in the test: ampicillin (10 µg), chloramphenicol (30 µg), bacitracin (10 µg), erythromycin (15 µg), gentamycin (10 µg), streptomycin (10 µg), oxytetracycline (30 µg), vancomycin (30 µg), penicillin (10 µg) and neomycin (30 µg). The discs were impregnated on the seeded plate aseptically with centers at least 25 mm apart. After 18 h incubation at 37^{o}C, strains were characterized as susceptible or resistant based on inhibition zone sizes created around the discs. Results were recorded as sensitive, intermediate or resistant. Classification of the degree of susceptibility, intermediate or resistance of the test isolate to each antimicrobial agent tested was based on predetermined guidelines.

Results and Discussion

The results of the bacteriological analysis of the salted-dried fish are presented in Table 1. Higher Total Plate Counts (TPC) of $350x10^3$ (CFU/g) was observed for *Sardinella fimbriata*, $201x10^3$ (CFU/g) for *Sphyraena* sp., $175x10^3$ (CFU/g) for *Sardinella albella*, $148x10^3$ (CFU/g) for *Lethrinus* sp, $119x10^3$ (CFU/g) for *S. longiceps* and $117x10^3$ for *S. albella*. Lower plate counts of $25x10^3$ (CFU/g) was recorded for *Upeneus* spp, $50x10^3$ (CFU/g) for *Thrryssa setirostris*, $55x10^3$ (CFU/g) for *Sillago sihama* and $57x10^3$ (CFU/g) for *Stolephorus japonicus*. However earlier reports states that the Total bacterial count of dried fish collected from Cochin were found to be less than 10^7 g^{-1} and moisture content ranged from 30 to 65% (19). The cured fish collected from Tamil Nadu coast were free from *S. aureus* (12). The mean total bacterial count and staphylococci count of dried beef and dried fish samples collected from Nigerian markets were $1.2x10^8$ g^{-1} and $4.6x10^6$ g^{-1} (1). Fish flesh containing 100 million (10^8) bacteria g^{-1} is considered as unsuitable for food (2) and *Staphylococci* count 10^6 g^{-1} is considered to be hazardous (6).

Table 1. Microbiological quality of dry fishes from Tuticorin fish markets

Microbiological Quality of Dry fishes		
Fishes	Total Plate Count (TPC) (CFU/g)	Total Fungal Count (TFC) (CFU/g)
Sardinella gibbosa	$112x10^3$	$9x10^3$
Terapon theraps	$99x10^3$	$3x\ 10^3$
Terapon spp	$113x10^3$	$1x10^3$
Sardinella longiceps	$119x10^3$	-
Sphyraena sp.,	$201x10^3$	$1x10^3$
Sardinella fimbriata	$350x10^3$	$3x10^3$
Upeneus sp.,	$25x10^3$	$15x10^3$
Thryssa setirostris	$50x10^3$	$8x10^3$
Lutjanus vitta	$78x10^3$	$5x10^3$
Sillago sihama	$55x10^3$	$3x10^3$
Gerres filamentosus	$117x10^3$	$4x10^3$
Stolephorus japonicus	$57x10^3$	$1x10^{3+}$
Lethrinus sp.,	$148x10^3$	$1x10^3$
Sardinella albella	$175x10^3$	$4x10^3$
Mugil cephalus	$109\ x10^3$	$3\ x10^3$

A higher fungal count was noted for the fishes *Upeneus* sp ($15x10^3$ CFU/g), *S. gibbosa* ($9x10^3$ CFU/g), *T. setirostris* ($8x10^3$ CFU/g) followed by *Sphyraena* spp, *Stolephorus japonicus and Lethrinus sp*., with a TFC count of $1x10^3$ CFU/g. It was also noted that, *fungi did not contaminate Sardinella longiceps*. The quality of dry salted fish is adversely affected by the occurrence of fungi. Presence of different types of fungi in dried fish has been reported by several workers (7, 9, 10, 16). Moisture level of fish also plays an important role in the spoilage. Lowering of moisture retards the spoilage. Smoked fish products deteriorate by the growth of mould if the water content is approximately 15 %.

The dominant fungi in dried salted fish vary with the place. The commonly occurring fungi in the west coast of India are *Aspergillus* sp., including myctoxin producing *A. flavus* and *A. ochraceus*, *Fusarium* sp., *Rhizopus* and *Mucor*. Apart from contaminated salt and fish, other common sources of fungi are air and dust in and around fish processing plants, store rooms (9), contaminated coastal water and unhygienic onboard handling practices (17).

Maximum *E.coli* count of >2,400 were observed for the fishes, *Sardinella albella* and *Upeneus* sp., and somewhat lower counts for *S. longiceps* (2 MPN/10 g) and *Sphyraena* sp (3 MPN/10 g). It was found that the fishes, *Sardinella gibbosa*, *Terapon* sp., *T.theraps, Thryssa setriostris* and *Lutjanus vitta* were not contaminated with *E.coli*. Similar studies in Chennai beach also indicated the fecal pollution. However the fecal pollution at Bhavanagar coast was reported to be of human origin based on the fecal index (23). Sewage imparts considerably to the fecal microorganisms, which are considered a good indicator of the extent of fecal pollution in an environment. Total coliform bacteria were high in all samples, and it was at the maximum detectable limit of more than 1, 400 per 100ml in most samples (22). MPN *E.coli* count showed more variation between samples collected at the same time and at different seasons, which ranged from 9 / 100 ml to over 1400 / 100ml (22).

Table. 2 shows Antibiotic susceptibility patterns of the *Escherichia coli* strains isolated from dry fishes. *E.coli* strains isolated from the fishes (*Sardinella albella*, *S. longiceps*, *Sphyranea* sp., *Gerres* sp., *Upeneus* sp., *Lethrinus* sp., *Sillago sihama*, *Stolephorus japonicus*)) were found to be resistant to Vancomycin, Ampicillin, Bacitracin, Erythromycin and Penicillin G used as compared with the Zone size interpretative chart provided in Table 3. It was also observed that the *E.coli* strains isolated from all the fishes were found to be resistant to the three antibiotics, Vancomycin, Bacitracin and Penicillin G. The study also reveals that the *E.coli* strains isolated from at least seven of the fish samples were found to be sensitive to Neomycin, Streptomycin and Chloramphenicol. Unlike *Salmonella*, the antibiotic resistance has been slow in its development in *E.coli*. *E.coli* infection is an important cause of illness and death in infants in developing countries (14).

Table 2. Antibiotic susceptibility pattern of the *Escherichia coli* strains isolated from salt-dried fishes of Tuticorin fish markets

Fish	N	G	V	A	B	E	P	S	C
	Zone of Inhibition (mm)								
Sardinella longiceps	18	15	--	7	--	8	--	18	14
Sphyranea *sp.*,	21	12	7	--	7	--	--	16	22
Sardinella fimbriata	16	14	8	8	10	7	--	17	21
Upeneus *sp.*,	18	15	--	7	8	8	--	12	22
Sillago sihama	16	10	9	--	--	--	--	9	21
Gerres *sp.*,	20	22	--	7	8	--	--	15	16
Stolephorus japonicus	22	28	--	8	--	12	--	16	32

Lethrinus sp.,	22	26	7	--	--	16	--	16	36
Sardinella albella	7	16	--	16	--	22	--	26	36

N-Neomycin; G-Gentamicin; V-Vancomycin; A-Ampicillin; B-Bacitracin; E-Erythromycin; P-Penicillin G, S-Streptomycin; C-Chloramphenicol

Table 3. Zone size interpretative chart (Hi Media)

Antibiotics	Disc content	Diameter of zone of inhibition (mm)		
		Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Neomycin	30 mcg	12	13-16	17
Gentamicin	10 mcg	12	13-14	15
Vancomycin	30 mcg	14	15-16	17
Ampicillin	10 mcg	13	14-16	17
Bacitracin	10 units	8	9-12	13
Erythromycin	15 mcg	13	14-22	23
Penicillin G	10 units	14	--	15
Streptomycin	10 mcg	11	12-14	15
Chloramphenicol	30 mcg	12	13-17	18

Thus, the current work reveals that the salt-dried fishes sold in Tuticorin fish markets are contaminated with fungi and pathogenic bacteria like *E.coli*. The *E.coli* strains has developed a high resistance pattern to a few of the antibiotic tested. So, public awareness (fisherman, fish workers and vendors) on sanitary and hygienic practices to stress the importance of quality is of utmost importance. Landing sites should be maintained clean. The waste waters such as domestic sewage and agricultural run of which flow into the sea could be at least partially can be treated before discharge to avoid hazards to marine biotopes.

References

1. ADESIYAN, A. A., & J. S. KAMINJOLO. Susceptibility to antibiotics of *Escherichia coli* strains isolated from diarrhoeic and non-diarrhoeic livestock in Trinidad. *Rev. Elev. Med. Vet. Pays Trop*. 45:260-262. (1992).
2. ALMAS, A.K. *Chemistry and microbiology of fish processing*. Dept. of and Application. Agro Botanica. Bikaner, (India), Pp 650 –652. (1981).
3. APHA. Compendium of methods for the Microbiological Examination of Foods. (Ed.) M.L. Speck, Published by America Public Health Association Inc., New York. 701 p. (1971).
4. APHA. Compendium of methods for the microbiological examination of foods 3rd (VANDERZANT. C & SPLITTSOESSER. D, Eds,), APHA, Washington, DC. (1992).
5. BAUER, H.W., KIRBY, W.M.M., SHERRIS, J.C. & TURCK, M.. *American. J. Clin. Pathol*. 45:493. (1996).
6. BERGDOLL, M.S. *Food borne Infections and Intoxications* (Rieman, H and Bryan, Eds.) p.444 Academic Press, New York. USA (1979).
7. CIFT. *Annual Report 1993-94*, Central Institute of Fisheries Technology, Cochin, India. (1994).
8. FELICIA SHANTHINI & JAMILA PATTERSON. Fungi in salted and dried fishes of Tuticorin, Southeast coast of India, Seafood Safety, Society of Fisheries Technologists (India) Cochin- India. pp. 412-417. (2003).
9. FOOD AND DRUG ADMINISTRATION. *Reference Manual to codes of practices for fish and fishery products*. pp 152, FAO, Rome. (1982).
10. GUPTA, R, & SAMUEL, C, T. *A. niger* in dried fish samples in rainy season in Cochin coast, *Fish. Technol*. 22, 132. (1985).
11. IYER, T.S.G. & SHRIVASTAVA, K.P. *Fish.Technol*. 25:39. (1989).
12. JOSEPH, G. K., MURALEEDHARAN, V. KALAIMANI, N & UNNIKRISHNAN NAIR, T. S. *Fish. Tech.* 23, 63. (1986).

13. KADOTA, H. Spoilage of marine products. In: science of processing marine food products Voi.1. (MOTOHIRO, T, KADOTA, H.,HASHIMOTO, K, KAYAMA, M. AND TOKUNAGA,T., Eds.) Japan Internationals Cooperation Agency, Hyogo Internationals Centre, pp 60-76. (1990).
14. LEVINE, M. M. *E. coli* that causes diarrhea; enterotoxigenic; enteropathogenic; enetroinvasive; enterohemorrhagic and enteroadherent. *J. Infect. Dis.* 15:377-389. (1987).
15. NARAYANAN NAMBIAR, V & SURENDERAN, P, K. Contamination by Pathogenic bacteria during handling and processing of seafoods, Seafood Safety, Society of Fisheries Technologists (India) Cochin-India. pp. 458-465. (2003).
16. PHILLIPS, S & WALLBRIDGE. Fungi in Dried Fish, Proc. Conf. Handling, Processing and marketing of tropical fish, London. (1976).
17. PRABHAKARAN, N. & GUPTA, R. *Fish. Technol*., 27, 69. (1990).
18. PUROHIT. S.S. Antimicrobial Drugs, In: Microbiology: Fundamental and Applications, Sixth edition, (Pb, Agro Botanica) pp, 641-666. (1997).
19. SANJEEV.S. *Studies on* coagulase positive *Staphylococcus* and *Vibrio parahaemolyticus* in selected items of fish, crustaceans and fishery products, Cochin University of Science and Technology, Cochin, India. (1997).
20. SANJEEV, S. *Fish Technol*, 36(1): 13-18. (1999).
21. SUGUMAR G., JAWAHAR ABRAHAM, T. & JEYACHANDRAN P. *Fish. Technol.* 32, 136. (1995).
22. SUGUMAR.G. *Suggestion for improvement*, Microbiology Unit. (2002).
23. VAIDYA S.Y., A.K. VALA AND H.C.DUBE. Bacterial indicators (2001).
24. VALSAN, A. P., NAMBIAR, V. N., DAMLE, S. D., GARG, D. K & IYER, T. S. G. In: Harvest and Post-harvest Technology of fish. Quality of dry non-penaeid prawn of Bombay markets, 661-664. (1985).
25. VARMA, P.R.G., MATHEN, C., MATHEW, A., THOMAS, F. & IYER, H.K. *Fish. Technol*. 23:70-72. (1986).