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Antibiotic Resistance Patterns of *Escherichia coli* and *Salmonella* from Environmental Samples of Thoothukudi Coast

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The incidence and antibiotic resistance pattern of *E. coli* and *Salmonella* from water, beach sand and fish collected from four fish landing centres of Thoothukudi were studied. Presence of *E. coli* was confirmed in 90%, 92% and 79% of water, beach sand and fish samples respectively, while *Salmonella* was detected in 25% of water and sand samples and 17% of fish samples. The isolates were tested against fifteen antibiotics by disc diffusion method. While all the *E. coli* isolates were resistant to bacitracin, erythromycin and rifampicin, none of the isolates was resistant to ampicillin, chloramphenicol and sulphafurazole. Among *Salmonella* isolates, all were resistant to bacitracin and most were resistant to ampicillin, colistin, erythromycin and rifampicin. None of the *Salmonella* isolates was resistant to chloramphenicol, ciprofloxacin and norfloxacin. Multiple antibiotic resistance (MAR) index was higher than 0.2 in all strains of *E. coli* and *Salmonella* revealing that they might have originated from high-risk sources of contamination such as humans.

Key words : Antibiotic resistance, *E. coli*, *Salmonella*, fish landing centre, Thoothukudi.

Antibiotics are used as therapeutic and prophylactic measures to combat infections caused by microorganisms. Prolonged use of antibiotics results in the emergence of resistant strains of bacteria, some of which are human pathogens like *Salmonella*. Marine ecosystem is totally free from antibiotics. However, antibiotic resistant strains of bacteria enter the aquatic environment when untreated domestic sewage is let into the coastal water that carries along hospital wastes and antibiotics from other sources. Studies have documented the distribution of antibiotic resistant bacteria in aquatic environment (Koditschek & Guyre, 1974; Goyal *et al.*, 1979). Incidence of drug resistant coliform in Cochin backwaters was reported by Pradeep & Lakshmanaperumalsamy (1986). Resistance of *E. coli* to antibiotics is a matter of concern since they posses threat to human health through transferable resistance determinant (R-factor) to enteric flora or other enterobacteria in man through direct colonization with resistant bacteria (Sarma & Joshi, 1987). Drug resistance among bacteria depends on the amount and kind of drugs used in that particular geographical area. The present study was undertaken to study the incidence of *E. coli* and *Salmonella* in water, beach sand and fish samples along Thoothukudi coast and to find out the antibiotic resistance patterns of *E. coli* and *Salmonella* isolated.

Materials and Methods

Surf beaten sea water was collected directly in sterile glass bottle. Sand samples from the inter-tidal zone were collected in sterile polythene bags using sterile spatula. Sardines (*Sardinella* sp.) were procured from

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the four fish landing centres of Thoothukudi, placed in sterile polythene bags, kept in insulated ice boxes and brought to the laboratory for analysis. *E. coli* was isolated by MPN method using lauryl sulphate tryptose broth (LSTB). After 24 h of incubation at 37°C a loopful of culture from the positive tubes of LSTB was transferred into EC broth. After 24h of incubation at 44.5 ± 0.5°C a loopful of culture from the positive tubes of EC broth was streaked on eosine methylene blue (EMB) agar. Colonies showing metallic sheen were confirmed as *E. coli* using indole, methyl red, vogues – proskaur and citrate (IMViC) tests (Speck, 1976). *Salmonella* was detected by pre-enrichment in lactose broth (LB) at 37°C for 24 h and selective enrichment in tetrathionate broth (TTB) and selenite cysteine broth (SCB) for 24 h at 37°C. A loopful of culture from TTB and SCB was streaked on bismuth sulphite agar (BSA) and xylose-lysine deoxycholate agar (XLD) and incubated at 37°C for 48 h. Typical colonies were purified on trypticase soya agar (TSA) plates and subjected to biochemical tests. Colonies showing typical reactions were further confirmed as *Salmonella* by serological test using Polyvalent 'O' antiserum (AOAC, 1998).

Confirmed *E. coli* and *Salmonella* isolates were screened for resistance against the following antibiotics by standard disc assay technique (Barry & Thornsberry, 1991): ampicillin (10µg), bacitracin (10 IU), chloramphenicol (30µg), ciprofloxacin (5µg), colistin (10µg), erythromycin (5µg), (10µg), kanamycin (30µg), nalidixic acid (30µg), norfloxacin (10µg), rifampicin (5µg), streptomycin (10µg), sulphafurazole (300µg), tetracycline (30µg), trimethoprim (5µg). Young cultures in tryptic soya broth (TSB) were seeded over Muellor-Hinton agar plates using sterile cotton swabs. Antibiotic impregnated discs (Hi media, Mumbai) were placed on the surface of the agar plates after drying it for a few minutes. Plates were incubated at 37°C for 24h and the diameter of the zone of inhibition was measured. Based on the diameter of the zone, the isolates were classified as resistant, intermediate or sensitive to the particular antibiotic. The multiple antibiotic resistance (MAR) index was calculated by dividing the number of antibiotics to which the isolate was resistant to the total number of antibiotics tested (Krumperman, 1983).

Results and Discussion

Presence of *E. coli* was confirmed in 90%, 92% and 79% of water, beach sand and fish samples respectively (Table 1). However, the incidence of *E.* coli was confirmed in 44% of water and 100% of sand samples from Cherai beach, Kerala (Raveendran *et al.*, 1978) and 90% of water and 79% of sand samples from Cochin backwaters (Gore *et al.*, 1979). Occurrence of *E. coli* in fish samples along Thoothukudi coast was found to be much higher than those reported from other coasts (Rao & Gupta, 1978; Lakshmanan *et al.*, 1984). The antibiotic resistance pattern of sixteen strains of *E. coli* isolated from various sources is given in Table 2. All strains were resistant to bacitracin, erythromycin and rifampicin, none of the strains was resistant to ampicillin, chloramphenicol and sulphafurazole. None of the isolates from water and sand samples except ETS_3 was resistant to , similar to that reported from Cochin backwaters (Pradeep & Lakshmanaperumalsamy, 1986). The strain (ETS_3) isolated from Thirespuram sand showed resistance to eleven out of the fifteen antibiotics tested. Earlier studies from Cochin backwaters (Pradeep & Lakshmanaperumalsamy, 1986), brackish water ponds (Harish *et al.*, 2003) and drinking water sources (Begum *et al.*, 2003) showed resistance of *E. coli* strains against a number

Table 1. Percentage occurrence of *E. coli* and *Salmonella* in environmental samples along Thoothukudi coast.

Sample	Number	% incidence	
		E. coli	*Salmonella*
Water	48	90	25
Sand	48	92	25
Fish	47	79	17

Table 2. Antibiotic resistance pattern of *Escherichia coli* isolated from various sources

Code	Source	Name of antibiotics and sensitivity pattern															
		A	B	C	Cf	Cl	E	G	K	Na	Nx	R	S	Sf	T	Tr	MAR
ETW_5	Thirespuram water	S	R	S	S	S	R	S	IM	S	S	R	IM	S	S	S	0.20
ETS_3	Thirespuram sand	S	R	S	R	S	R	R	R	R	R	R	R	IM	R	R	0.73
ETF_2	Thirespuram fish	S	R	S	S	S	R	IM	IM	S	S	R	S	S	S	S	0.20
EFW_{11}	Fishing harbour water	S	R	S	S	IM	R	S	IM	S	S	R	S	S	IM	S	0.20
EFS_1	Fishing harbour sand	S	R	S	S	IM	R	S	S	S	S	R	IM	S	IM	S	0.20
EFS_5	Fishing harbour sand	S	R	S	S	IM	R	S	S	S	S	R	IM	S	S	S	0.20
EFF_7	Fishing harbour fish	S	R	S	S	R	R	S	S	S	S	R	IM	S	IM	S	0.27
EEF_2	Fishing harbour fish	S	R	S	S	S	R	S	S	S	S	R	IM	S	IM	S	0.20
EEF_6	Fishing harbour fish	S	R	S	S	S	R	S	S	S	S	R	IM	S	S	S	0.20
ESW_5	South landing water	S	R	S	S	S	R	S	S	S	S	R	S	S	S	S	0.20
ESS_{11}	South landing sand	S	R	S	S	S	R	IM	IM	S	S	R	IM	S	IM	S	0.20
ESF_6	South landing fish	S	R	S	S	IM	R	S	IM	S	S	R	IM	S	S	S	0.20
EPW_9	Port beach water	S	R	S	S	S	R	S	S	S	S	R	S	S	S	S	0.20
EPS_4	Port beach sand	S	R	S	S	IM	R	S	S	S	S	R	IM	S	S	S	0.20
EPS_2	Port beach sand	S	R	S	S	IM	R	S	S	S	S	R	IM	S	S	S	0.20
EPF_3	Port beach fish	S	R	S	S	S	R	S	S	S	S	R	IM	S	S	S	0.20

A-Ampicillin; B-Bacitracin; C-Chloramphenicol; Cf-Ciprofloxacin; Cl-Colistin; E-Erythromycin; G-Gentamycin; K- Kanamycin; Na-Nalidixic acid; Nx-Norfloxacin; R-Rifampicin; S-Streptomycin; Sf-Sulphafurazole; T-Tetracycline; Tr-Trimethoprim.

R – Resistant ; IM – Intermediate; S – Sensitive MAR – Multiple antibiotic resistance index

of antibiotics. However, the antibiotics were different from those used in the present study. Cent percent resistance was recorded against bacitracin, erythromycin and rifampicin. All the isolates were sensitive to ampicillin and chloramphenicol (Table 4). Least resistance (less than 20%) was noticed against ciprofloxacin, gentamicin, nalidixic acid, norfloxacin, steptomycin, tetracycline and trimethoprim. The antibiotics generally recommended to treat infections caused by *E. coli* are sulfonamide, cephalosporin and ampicillin (Prescott *et al.*, 1996). While 94% of the *E. coli* isolates were sensitive to sulphafurazole, all the isolates were sensitive to ampicillin. The mechanism of action of ampicillin is that they inhibit cell wall synthesis and sulphafurazole inhibits folic acid synthesis by competition with p–aminobenzoic acid (Prescott *et al.*, 1996). In the present study ampicillin and chloramphenicol proved to be the best antibiotics to treat infections caused by *E. coli* since they were fully sensitive. All the isolates in the current study showed multiple antibiotic resistance (MAR) index higher than 0.2. Mean MAR value was 0.24. Thus it is obvious that the study area is exposed to contamination with faecal bacteria of high-risk sources. The results were comparable with the earlier studies in water and sediments of Cochin backwaters (Pradeep & Lakshmanaperumalsamy, 1986), river water (Hatha *et al.*, 1993) and aquaculture pond samples (Harish *et al.*, 2003) where high MAR index values have been reported.

The incidence of *Salmonella* in water, beach sand and fish samples is given in Table 1. *Salmonella* could be detected only in 25% of water and sand samples along Thoothukudi coast while Venkateswaran & Natarajan (1987) confirmed its presence in most water samples of estuarine, lakes and mangrove biotopes of Tamil Nadu. However, the incidence of *Salmonella* varied from 7 – 11% in samples of sand from Mangalore coast (Srikantaiah *et al.*, 1985). *Salmonella* was present in 17% of fish samples of Thoothukudi coast. *Salmonella* was reported in 30%

Table 3. Antibiotic resistance patterns of *Salmonella* isolated from various sources

Code	Source	Name of antibiotics and sensitivity pattern															
		A	B	C	Cf	Cl	E	G	K	Na	Nx	R	S	Sf	T	Tr	MAR
STW_5	Thirespuram water	R	R	S	S	R	S	S	IM	S	S	R	S	S	S	S	0.27
STW_8	Thirespuram water	IM	R	S	IM	R	R	S	S	R	S	R	R	S	IM	R	0.46
STW_9	Thirespuram water	IM	R	S	IM	R	R	S	IM	R	S	R	R	S	R	R	0.53
STW_{11}	Thirespuram water	IM	R	S	S	R	R	S	S	S	S	R	R	S	IM	S	0.33
STW_{12}	Thirespuram water	R	R	R	S	R	R	R	R	IM	S	R	R	R	R	R	0.80
STF_3	Thirespuram fish	R	R	S	S	R	IM	S	S	S	S	R	S	S	S	S	0.27
STF_6	Thirespuram fish	S	R	S	S	R	IM	S	IM	S	S	IM	S	R	IM	IM	0.20
SFF_2	Fishing harbour fish	R	R	S	S	R	S	S	S	S	S	IM	S	IM	S	S	0.20
SFF_4	Fishing harbour fish	S	R	S	S	R	IM	S	S	S	S	R	S	R	S	S	0.27
SFF_7	Fishing harbour fish	R	R	S	S	IM	IM	S	S	S	S	R	S	R	IM	S	0.27
SFF_8	Fishing harbour fish	IM	R	S	S	R	R	S	S	S	S	R	IM	R	IM	S	0.33
SFF_{10}	Fishing harbour fish	R	R	S	IM	R	IM	S	S	S	IM	R	S	R	S	S	0.33
SFF_{11}	Fishing harbour fish	R	R	S	S	IM	R	S	R	S	S	R	R	S	IM	S	0.40
SSW_1	South landing water	R	R	S	S	R	IM	S	IM	S	S	R	S	R	IM	S	0.33
SSW_3	South landing water	R	R	S	S	R	IM	S	S	S	S	R	S	R	S	S	0.33
SSW_9	South landing water	R	R	IM	S	R	R	S	S	IM	S	R	S	S	R	S	0.40
SSS_5	South landing sand	S	R	S	S	R	IM	S	S	S	S	R	S	S	S	S	0.20
SSF_6	South landing fish	R	R	S	S	R	IM	S	IM	S	S	IM	S	R	S	IM	0.27
SPW_6	Port beach water	R	R	S	S	R	R	S	IM	S	S	R	S	R	IM	IM	0.40
SPW_{10}	Port beach water	R	R	S	S	IM	R	S	S	S	S	R	IM	S	IM	S	0.27

A-Ampicillin; B-Bacitracin; C-Chloramphenicol; Cf-Ciprofloxacin; Cl-Colistin; E-Erythromycin; G-Gentamycin; K- Kanamycin; Na-Nalidixic acid; Nx-Norfloxacin; R-Rifampicin; S-Streptomycin; Sf-Sulphafurazole; T-Tetracycline; Tr- Trimethoprim.
R – Resistant; IM – Intermediate; S – Sensitive MAR – Multiple antibiotic resistance index

Table 4. Incidence of antibiotic resistant *E. coli* and *Salmonella* isolates isolated from various sources

Antibiotics	Antibiotic resistance %					
	E. coli (16 isolates)			*Salmonella* (20 isolates)		
	R	IM	S	R	IM	S
Ampicillin	0	0	100	65	20	15
Bacitracin	100	0	0	100	0	0
Chloramphenicol	0	0	100	5	5	90
Ciprofloxacin	6	0	94	0	15	85
Colistin	38	6	56	85	15	0
Erythromycin	100	0	0	45	45	10
Gentamycin	6	13	81	5	0	95
Kanamycin	6	31	63	10	30	60
Nalidixic acid	6	0	94	10	10	80
Norfloxacin	6	0	94	0	5	95
Rifampicin	100	0	0	85	15	0
Streptomycin	6	69	25	30	5	65
Sulphafurazole	0	6	94	50	5	45
Tetracycline	6	31	63	15	45	40
Trimethoprim	6	0	94	15	15	70

R – Resistance; IM – Intermediate;S - Sensitive

(Kumar *et al.*, 2003) and 14.25% of finfishes (Hatha & Lakshmanaperumalsamy, 1997) from fresh fish markets of Mangalore and Coimbatore respectively. The antibiotic resistance pattern of twenty strains of *Salmonella* towards various antibiotics is shown in Table 3. Many strains of *Salmonella* were sensitive to chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid and norfloxacin. None of the strains was sensitive to bacitracin, colistin and rifampicin. Few strains were resistant to ampicillin, erythromycin and streptomycin. The isolates showed considerable levels of resistance against ampicillin (65%), colistin (85%), erythromycin (45%), rifampicin (85%) and sulphafurazole (50%) (Table 4). Least resistance (less than 20%) was observed with chloramphenicol, gentamicin, kanamycin, nalidixic acid, tetracycline and trimethoprim. The drugs of choice to treat *Salmonella* infections are ampicillin and chloramphenicol (Prescott *et al.*, 1996). However in the present study, 65% of the isolates showed resistance against ampicillin. Mean multiple antibiotic resistance (MAR) index of *Salmonella* was 0.34. Generally bacteria of environmental origin are more susceptible to antibiotics than those from hospital or clinical sources that possess plasmid mediated resistance. However, the present study revealed many strains of *Salmonella* from environmental origin with resistance to several antibiotics causing great concern. Further, a strain isolated from Thirespuram water (STW_{12}) recorded a MAR value of 0.80 which may be due to the reason that domestic sewage from Thoothukudi town is released into the sea right on the sampling site which may carry along with hospital wastes. Further, the fishermen on reaching the shore have the habit of washing their catch in the beach water, thus providing a way for contamination of the catch landed.

Multiple antibiotic resistance (MAR) index was greater than 0.2 in isolates of fish samples from all four landing centres. Thus consumption of fishes from these landing centres has the potential to affect human health. Illness caused by isolates of *Salmonella* is more difficult to be treated because of their antibiotic resistance profile as they do not respond to the therapeutic antibiotics normally used or might respond only at higher dosage, thus causing concern.

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