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Escherichia coli - occurrence in the meat of shrimp, fish, chicken and mutton and its antibiotic resistance

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ABSTRACT

The incidence of Escherichia coli in the meat samples of shrimp, fresh and marine fish, chicken and mutton at Visakhapatnam, Andhra Pradesh and its resistance to antibiotics - ampicillin, chloramphenicol, ciprofloxacin, co-trimoxazole, gentamicin, nalidixic acid, nitrofurantoin, penicillin-G, norfloxacin and tetracycline. The bacterial load was higher in chicken and mutton than the shrimp and fish meat. The antibiotic resistance of E. coli was 100% towards pencillin-G, 90% to tetracycline and ampicillin, 40% to nitrofurantoin and chloramphenicol, 20% to gentamicin, norfloxacin, nalidixic acid and ciprofloxacin. It was only 10% resistance to co-trimoxazole. The resistance of E. coli in mutton sample was higher than the remaining samples. Chicken stood next followed by fresh water fish and shrimp.

Key words: *E. coli*, Occurrence, Shrimp and Fish muscle, Chicken, Mutton, Antibiotic resistance

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INTRODUCTION

Foods being organic with sufficient water content are excellent media for rapid growth of microorganisms and the extent of microbial spoilage of food depends primarily on its physical and chemical properties and innate resistance [1]. Food industry recognizes three groups of foods – highly perishable foods include poultry, eggs, meat, most fruits and vegetables and dairy products etc., semi-perishable foods consisting of potatoes, apples, nutmeats etc., and nonperishable foods composed of cereals, rice, flour, nuts, sugar etc and the meat comes under highly perishable food [2]. The common species of bacteria occurring in fresh meat are *Pseudomonads, Staphylococci, Micrococci, Enterococci, Coliforms* etc., and are largely a reflection of the microbial quality of the environment from where they have been isolated [3].

Dines [4] has reported that occurrence of *E. coli* in various sea food samples. According to Chen and Hsing-Chen [5] the major sea food bacteria are coliforms belonging to *Enterobacteriaceae*. Laura Webster *et al* [6] have analyzed the antibiotic resistance of *E. coli* in the surface waters of both developed and undeveloped water-sheds in coastal South Calorina. Lim *et al* [7] have studied the enteric bacteria from waters of Mhlathuze River. Bandekar and Kamat [8] have screened bacteria from prawns and rohu fish processing factories of Kakinada and Mumbai. Falcao *et al* [1] have made prospective epidemiological studies on possible health effects from sea bathing at seven

popular bathing beaches in New Zealand. Cagney *et al* [9] have determined the incidence of *E. coli* in minced beef and beef burgers (frozen and fresh) in Irish butcher and supermarket outlets.

Dhanashree and Shrikar Mallya [10] have isolated Shiga-Toxigenic *E. coli* (STEC) from diarrhoeagenic stool samples of patients and beef samples marketed through retail outlets in Mangalore. Bindu Kiranmayi and Krishnaiah [11] have detected the presence of *E. coli* O157:H7 in freshly dressed and washed animal carcasses at slaughter houses and markets in Hyderabad. Immaculate Jeyasanta *et al* [12] have estimated the presence of *E. coli* in sea foods at landing centers, inter tidal areas and fish markets of Tuticorin and tested its antibiotic resistance to 15 antibiotics. Isaac *et al* [13] have investigated the contamination of meat from cattle and chicken with *E. coli* 0157:H7 at the metropolitan abattoirs and slaughtered slabs of selected poultry farms in Lagos and Ibadan, Nigeria. Momtaz *et al* [14] have found antibiotic-resistant genes in *E. coli* isolates from slaughtered commercial chickens in Iran. Zende *et al* [15] have studied virulent genes in the strains of *E. coli* isolated from chicken meat from retail shops in Mumbai city.

Jack Millman *et al* [16] have compared four major types of poultry chicken - conventional, kosher, organic, and those raised without antibiotics (RWA) to assess the contamination with antibiotic resistant *E. coli* in greater New York City area. Abdellah *et al* [17] have surveyed the microbiological quality of turkey meat sold in various outlets in Meknes city of Morocco to examine the antimicrobial resistance of *E. coli* strains. Ali Akbar *et al* [18] have analysed the presence of *E. coli* and its pathogenic strain O157 in retail poultry meat at different open and super markets of greater Bangkok. Farhan *et al* [19] have evaluated the prevalence of *E. coli* in minced meat, raw milk, kareesh cheese and in fecal samples of children. Sami Abdaslam *et al* [20] have characterized food borne bacteria *Staphylococcus aureus, E. coli, Salmonella and Klebsilla* isolated from animal food products to determine the antibiotic susceptibility to phenolic compounds.

An attempt is made in the present study to assess the incidence of *E. coli* in the meat of shrimp, fresh water and marine fish, chicken and mutton and its antibiotic resistance has been tested with 10 antibiotics (Ampicillin, Chloramphenicol, Ciproflaxin, Cotrimoxazole, Gentamicin, Nalidixixc acid, Nitrofurantoin, Penicillin-G, Norflaxacin and Tetracycline).

MATERIALS AND METHODS

The meat samples used were shrimp (*Metapenaeus dobsoni*) (Marine), fresh water fish (*Catla catla*), marine fish (*Thunnus albacares*), chicken and mutton (Fig.1 A to E). Shrimp and fish samples were collected from Visakhapatnam Fishing Harbour (Fig. 2 A) and chicken and mutton from the slaughter house near Town Kotha Road, Visakhapatnam (Fig. 2 B & C). The samples were brought to the lab and analyzed immediately. The total coliforms, fecal coliforms and *E. coli* were analyzed by the Most Probable Number (MPN) methods of Suredran *et al* [21]. *E. coli* was confirmed by Gram's staining and biochemical tests [21].

The disc plate technique was used for determining susceptibility of microorganisms to antibiotic resistance [3, 22] against 10 antibiotics Ampicillin, Chloramphenicol, Ciprofloxacin, Cotrimoxazole, Gentamicin, Nalidixixc acid, Nitrofurantoin, Penicillin-G, Norflaxacin and Tetracycline. The cultures of *E. coli* were spread on the Nutrient agar plate and small antibiotic paper discs with known amounts were placed on surface of inoculums. The plates were incubated at 37^{o}C for 24 hrs. The zone of inhibition surrounding the discs was observed and measured after the incubation following the standard "Zone size Interpretative Chart" and categorized into Resistant, Intermediate and Sensitive.

RESULTS

Most probable number (MPN) values

The MPN values for presumptive total coliforms was 140+ per gram in all samples studied whereas confirmed total coliforms were 25 in fresh water fish, 2.5 in marine fish, 140+ in chicken and 140+ in mutton. The fecal coliforms were 25, 0.4, 9.5, 140+ and 140+ in fresh water fish, shrimp, marine fish, chicken and mutton respectively (Table 1).

E. coli

The *E. coli* was confirmed by Gram's staining as gram negative rods or bacillus (bacilli). Isolated colonies with greenish metallic sheen and dark purple centered colonies were observed (Fig. 3). Biochemically *E. coli* was positive to indole and methyl red tests and negative to Voges – Proskauer test and citrate utilization tests.

Antibiotic resistance

In shrimps *E. coli* showed 100% resistance towards penicillin and tetracycline; 50% resistance to gentamicin, nitrofurantoin and ampicillin and was sensitive to the remaining antibiotics. The percentage of antibiotic resistance of *E. coli* in fresh water fish meat was 100% towards penicillin, tetracycline and ampicillin; 50% resistance to nitrofurantoin and was sensitive to the remaining antibiotics (Fig. 4a). It was 100% in marine fish towards penicillin and ampicillin; 50% resistance towards tetracycline, gentamicin, nitrofurantoin and chloramphenicol and was sensitive to the remaining antibiotics (Fig. 4b). It was 100% in chicken towards penicillin, tetracycline and ampicillin; 50% to nitrofurantoin, chloramphenicol, norflaxacin, nalidixic acid and ciprofloxacin and was sensitive to co-trimoxazole and gentamicin. Mutton showed 100% resistance towards penicillin, tetracycline, chloramphenicol and ampicillin; 50% resistance to co-trimoxazole, norflaxacin, nalidixic acid and ciproflaxacin and was sensitive to gentamicin and nitrofurantoin (Table 2 & Fig. 5).

Table 1: MPN values of Presumptive Total Coliforms, Confirmed Total Coliforms and Fecal Coliforms in different meats of shrimp, Fresh water and marine fish, chicken nd mutton

	Fresh water fish	Marine fish	Shrimp	Chicken	Mutton
Presumptive total Coliforms	140+	140+	140+	140+	140+
Confirmed total Coliforms	25.0	2.5	25.0	140+	140
Fecal Coliforms	25.0	0.4	9.5	140+	140

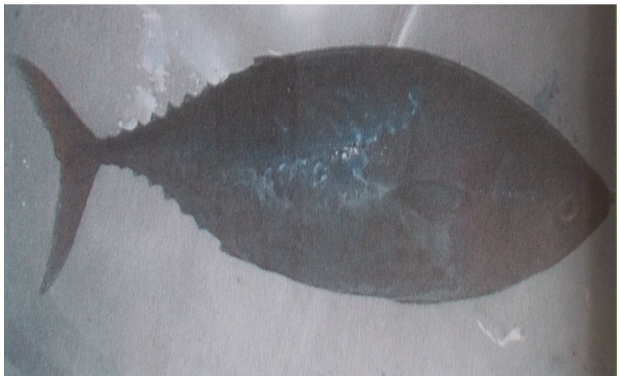
Table 2 Antibiotic resistance of *E. coli* isolated from different meat source to antibiotics.

Antibiotics	Sample									
	Freshwater fish		Marine fish		Shrimps		Chicken		Mutton	
	1	2	1	2	1	2	1	2	1	2
Penicillin	R	R	R	R	R	R	R	R	R	R
Tetracyclin	R	R	S	R	R	R	R	R	R	R
Co-trimoxazole	I	S	S	S	S	S	S	S	R	S
Gentamycin	I	S	R	S	R	S	S	S	S	S
Nitrofurantoin	R	S	S	R	R	I	R	S	I	I
Chloramphenicol	S	S	S	R	S	S	S	R	R	R
Norfloxacin	I	S	S	S	I	S	I	R	R	S
Nalidixic acid	S	S	S	S	S	S	S	R	R	S
Ampicillin	R	R	R	R	I	R	R	R	R	R
Ciprofloxacin	S	S	I	S	S	S	S	R	R	S

S : sensitive *I : Intermediate* *R : Resistant*

A: Shrimp (*Metapenaeus dobsoni*)

B: Fresh water fish (*Catla catla*)



C: Marine fish (*Thunnus albacares*)

D: Chicken

E: Mutton

Fig. 1 Meat samples

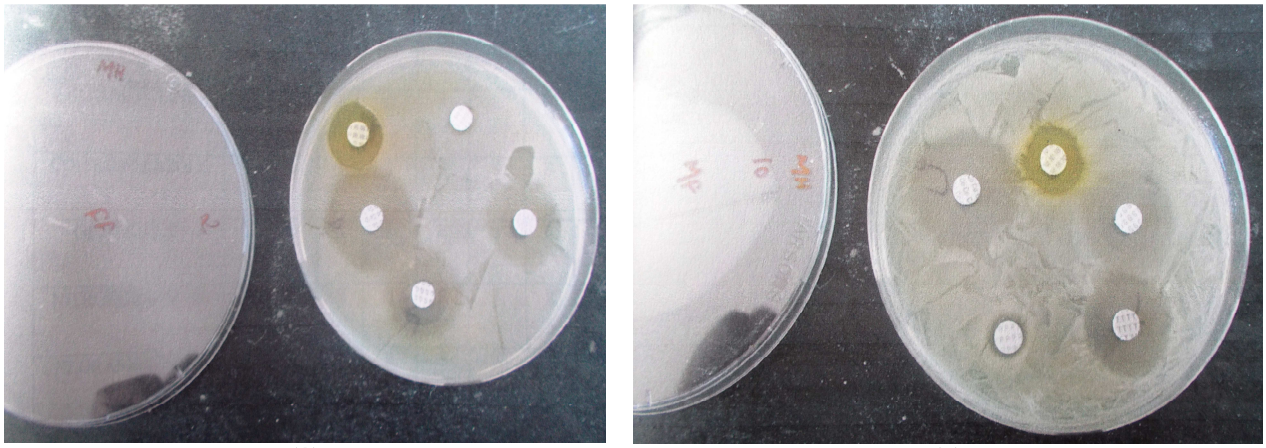
A: Fishing harbour

B: Mutton shop

C: Chicken shop

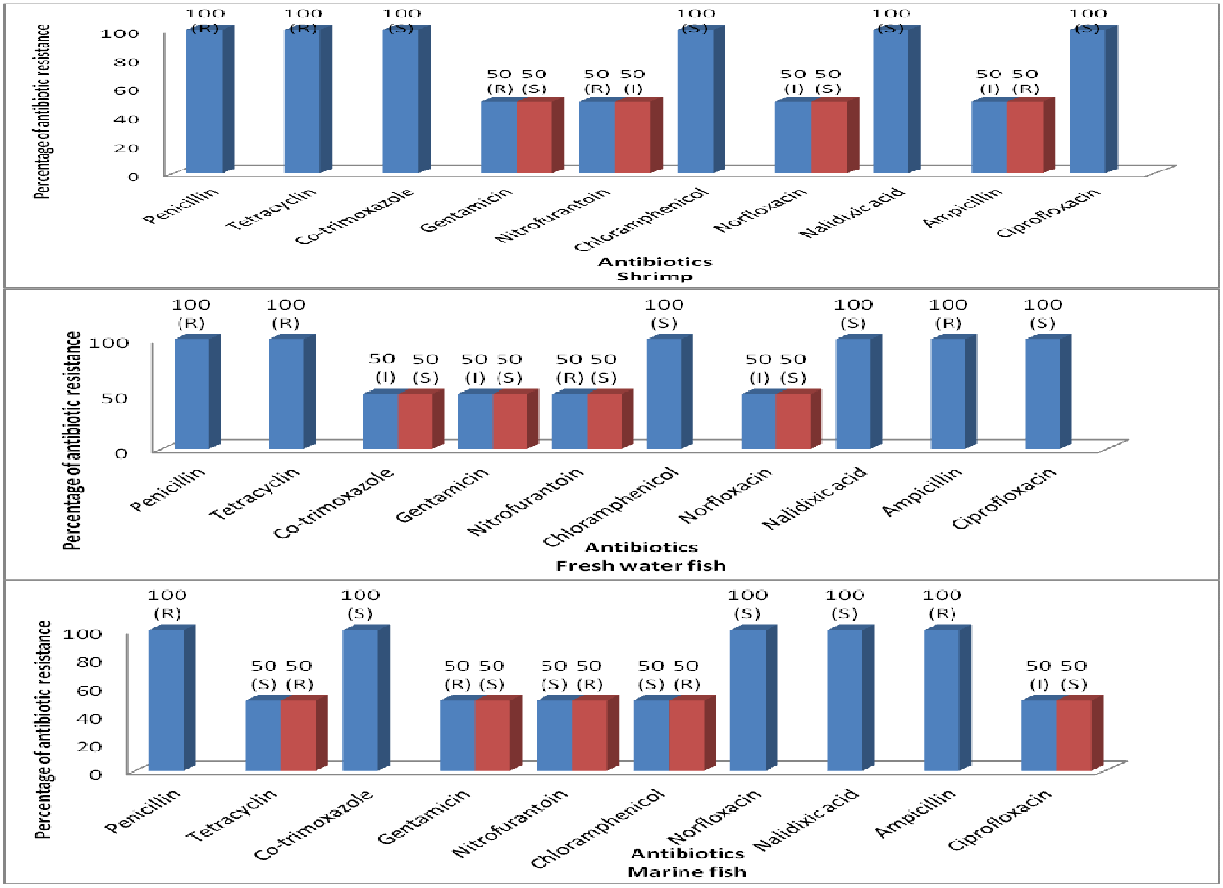
Fig. 2 Sampling stations

Fig. 3 Greenish metallic colonies of *E. coli* on EMB agar plate



(A) **(B)**

Fig. 4 *E. coli* isolated from fresh water fish and marine fish showing antibiotic resistance



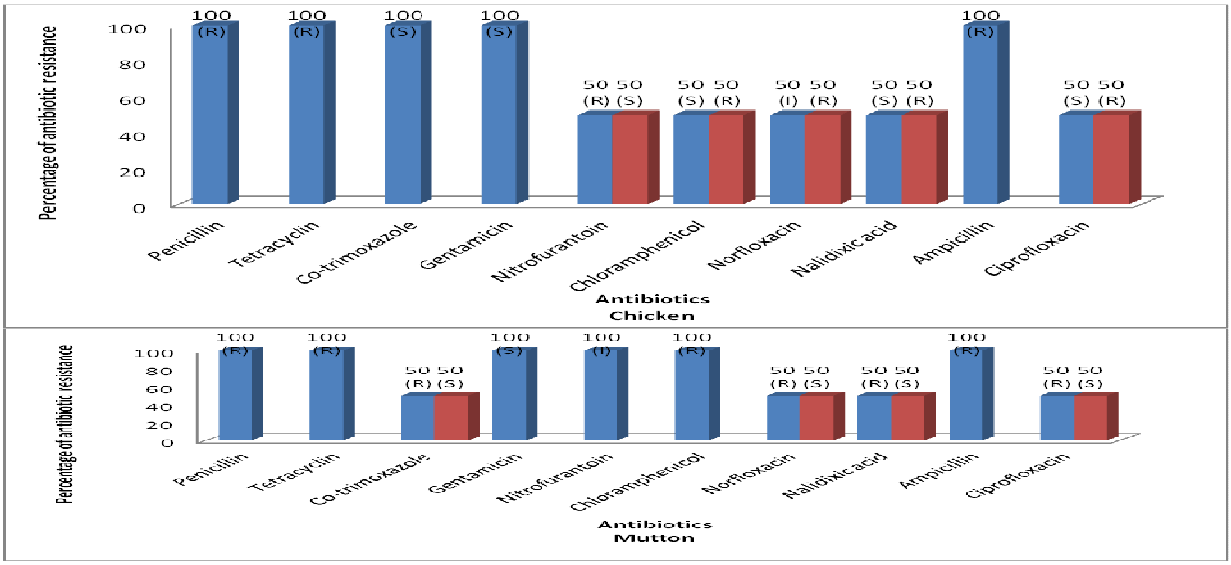


Fig. 5 Antibiotic resistance of *E. coli* in shrimp, fresh water fish, marine fish, chicken and mutton

DISCUSSION

The MPN values of the mutton and chicken have been found to be high when compared to fresh water fish, shrimp and marine fish. The values of fresh water fish and shrimp are found to be between those of other meat foods. The marine fish can be considered to be much safer for consumption than the other meat foods in view of low MPN values. The antibiotic sensitivity of *E. coli* has shown resistance to a wide range of antibiotics and at the same time it has also shown intermediate resistance and sensitivity to certain antibiotics. As far resistance of *E. coli* is concerned chicken stands in the second position followed by fresh water fish and shrimp. The *E. coli* isolated from mutton sample has shown high antibiotic resistance.

According to Dines [4] the prevalence of *E. coli* in various sea foods may be due to fecal contamination and improper handling. The contamination may also be due to water quality, fishing method and storage [5]. Bacteriological screening of 48 samples comprising 18 samples of prawns, 24 samples of processed rohu and 6 samples of whole rohu obtained from fish processing factories of Kakinada and Mumbai, India have been found to contain *E. coli* 16% samples. None of the *E. coli* strains has been found to be resistant against amikacin, chloramphenicol, streptomycin and trimethoprim, but showed resistance against ampicillin, erythromycin, pencillin-G and vancomycin [8]. Gastrointestinal or respiratory symptoms or other infection with human or animal fecal contamination of beach water by *E. coli* has been observed in seven sea bathing centres of New Zealand [1].

Cagney *et al* [9] have observed a total of 43 (2.80%) out of the 1533 minced beef samples have been positive to *E. coli*. Dhanashree and Shrikar Mallya [10] have found a low incidence of Shiga-toxigenic Escherichia coli (STEC) and high prevalence of *E. coli* in meat samples. Bindu Kiranmayi and Krishnaiah [11] have found 27 samples out of 250 samples have shown presence of *E. coli* O157:H7 (5 beef, 6 beef swabs, 2 mutton, 12 mutton swabs and 2 chicken out of 50 samples each) whereas only 11 samples (one beef, 2 beef swabs, 1 mutton, 6 mutton swabs and one chicken sample) have been found to be positive by culture. According to them the sensitivity of PCR for *E. coli* O157:H7 has been 1.7cfu. Immaculate Jeyasanta *et al* [12] have identified that among the 168 samples of 22 sea foods, 128 are positive for the presence of faecal coliforms and 91 are for *E. coli*. Amikacin, ciprofloxacin and Chloramphenicol according to them are the best antibiotics to treat *E. coli* infection.

Isaac *et al* [13] have found the prevalence of *E. coli* O157:H7 in beef from Ibadan and Lagos as 28.5% and 11.0% respectively that in chicken from Ibadan is 13.0% and in Lagos it is 14.0%, and from farms of Ibadan and Lagos it has been 18.0% and 13.0% respectively. According to them the prevalence of *E. coli* O157 is significantly high in beef than chicken ($p<0.05$) and highest resistance of 91.1% has been observed to tetracycline. Momtaz *et al* [14] have observed that nine strains (15.78%) of *E. coli* has been found to be resistant to a single antimicrobial agent (tetracycline) and 11 strains (19.29%) resistance to two antimicrobial agents (sulfonamides and erythromycin) and

64.91% has been multi-resistant. Zende *et al* [15] have found different sereotypes of *E. coli* in chicken meat samples. Jack Millman *et al* [16] have observed the incidence of *E. coli* in raw chicken obtained from markets of organic, Kosher and those raised without antibiotics (RWA) and found the strains of *E. coli* isolated from Kosher chicken are more resistant to drugs compared to the other categories. Abdellah *et al* [17] have found that the level of contamination of *E. coli* has been 95.8% at supermarket, 33.3% at chicken shop, 41.6% at artisanal slaughter houses and 41.6% at popular market outlets of Turkey meat in Morocco. According to them the isolates recovered from the retail Turkey meat is resistant to different antibiotics.

Ali Akbar *et al* [18] have recorded that 25% of the meat samples has been contaminated with *E. coli* (O157) and 92% of the strain is resistance to ampicillin and tetracycline, 15% kanamycin and 23.7% to streptomycin. Farhan *et al* [19] have recovered 115 isolates of *E. coli* of which 35 (30.43%) isolate from meat, 21 (18.26%) from raw milk, 31 (26.96%) from cheese and 28 (24.35%) from children stool. Sami Abdaslam *et al* [20] have identified that out of the 45 *E. coli* isolates, 11.1% are *E. coli* O157 and all are sensitive to commercial antibiotics. According to them methanol extracted cloves has significant ($P<0.05$) activity than the ethanol extract.

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