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Isolation and Identification of Escherichia coli from various foodstuffs and their Resistance against clinically significant antibiotics

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Abstract:

A total of fifty-seven ready-to-eat food samples were collected from different locations of NCR, India to explore the prevalence of *Escherichia coli* in some commonly offered foodstuffs and to assess the antibiotic resistance and susceptibility profile of these isolated bacterial contaminants. These samples were characterized microbiologically for the presence of *Escherichia coli.* Out of fifty-seven collected samples, only twenty-four were found to have *Escherichia coli*. These isolates were morphologically and biochemically identified and were then evaluated for their resistance and susceptibility profile against twenty antibiotics for several clinical implications. This study shows the clear picture of prevalence of resistance among *Escherichia coli* isolates. Isolates were found to have variable susceptibilities against the antibiotics used in the study. Vancomycin was the drug of concern because of its non inhibitory nature. Antibiotics evaluated in this study were shown to have mild-to-moderate inhibitory activity. Also determined the multidrug resistance among *Escherichia coli* isolates. It has been observed that all the isolates were having a sort of susceptibility on the scale of 0-1. A number of strains isolated demonstrated the variable nature of MAR against the evaluated antibiotics.

Keywords: Escherichia coli; Food; Antibiotics; Resistance; MAR Index.

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Introduction:

E. coli is a Gram-negative, non-spore forming, lactose-fermenting, thermo-tolerant, bacteria and belongs to family Enterobacteriaceae. It is an extensively studied microbe throughout the world and is known for the production of various industrial important metabolites such as vitamins, endotoxins, enzymes, recombinant proteins etc. E. coli is commensal to human intestine, thus refer as enteric bacteria. It usually passes through faeces and also called as an 'indicator organism' for faecal contamination of water bodies. Some strains are highly pathogenic. The presence of this bacterium also indicates the possible presence of other enteric pathogens as well; therefore, it is mandatory to detect its presence in Food samples intended for human consumption.

Antimicrobial resistance is an escalating scientific crisis and is a recognized public health hazard. Among human pathogens, Escherichia coli is known for its high prevalence and multidrug resistance. Because of its relatively impermeable outer membrane, it is naturally resistant to many antibiotics and it can also effortlessly attain resistance, creating challenging therapeutic scenarios. Because of this efflux pump system of expelling out the antibiotic, Escherichia coli can be resistant to antibiotics such as penicillin, cephalosporin, tetracycline and many other clinically significant antimicrobials (Livermore et al., 1994). Therefore, infections due to this creature are difficult to treat because of the possible coexistence of several mechanisms of resistance in the same strain; its capacity to produce a variety of virulence factors and the relatively limited choice of effective anti-pseudomonal antibiotics. The expansion of resistance to available antibiotics in pathogenic organisms then precludes the effectiveness of any antibiotic treatment. In vitro susceptibility of commonly used antibiotics against clinical isolates of Escherichia coli provides estimations of the rate of MDRPA infections (Karlowsky et.al, 2003). Because antimicrobial resistance patterns are continually evolving and resistant Escherichia coli undergo progressive antimicrobial resistance, continuously updated data on antimicrobial susceptibility profiles is essential to ensure the provision of safe and effective empiric therapies (Oteo et al., 2002).

Escherichia coli is a very common water and soil microflora; hence it can be easily transmitted to the variety of foodstuffs leading to spoilage. Furthermore, food can also be spoiled due to cross contamination from various sources such as utensils, knives, raw foodstuffs, flies that are sporadically landing on the foods, by vendors' bare hand serving occasionally, food handling by consumers (Gorris, 2005). Ready-to-eat street foods are processed (peeled, squeezed, cut up and/or cooked) and are readily available for purchase and consumption; however, these foods are an easy objects for contamination due to improper holding temperature, poor personal hygiene of food handlers, hence transmission of food-borne diseases (Fang et al., 2003). Therefore, food-borne illness is a major international health problem with respect to reduced economic growth and a major cause of deaths in developing Countries (WHO, 2002 a; b).

This problem poses a major threat to public health due to enhanced contamination of foodstuffs with antibiotic resistant bacteria. Moreover, the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food-borne pathogens has increased dramatically during recent decades (Threlfall et al., 2000; White et al., 2002). Recently many investigators have also wondered to know that many of commensal bacteria may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens (Levy and Salyers, 2002) and are thus very important in our understanding of maintenance of antibiotic resistance genes and spreading through bacterial populations (Levy and Miller, 1989). Such reservoir organisms could possibly be found in various foods and food products containing high densities of non-pathogenic bacteria as a result of their natural production process (Threfall et al., 2000; Chui et al., 2002).

Thus, the present study is aimed to evaluate the prevalence and antibiotic resistance of various strains of Escherichia coli isolated from different ready-to-eat foodstuffs. This kind of the study is novel in its terms having the following objectives: (a) Collection of food samples from different locations of NCR, India; (b) Isolation and identification of Escherichia coli strains; (c) Determination of susceptibility and resistance pattern against different antibiotics; (d) Determination of multiple antibiotic resistance (MAR); and (e) Interpretation of the data generated which will have a greater impact in determining the pervasiveness of resistance among microorganisms isolated from foodstuffs.

Materials and Methods

Chemicals, Reagent and Bacteriological Media: In this study, media and chemicals relevant to isolation and identification of Escherichia coli and for antimicrobial evaluation were of analytical grade and procured from Hi-Media and Sigma Laboratories, India. Some of the reagents used during the study were as follows: Mueller Hinton Agar (MHA), Nutrient Agar (NA), MacConkey agar, MacConkey Broth, Eosin Methylene Blue (EMB)Agar, Peptone water, Kovac's Reagent, Motility agar medium, Simmon's Citrate Agar, ONPG, Discs, Nitrate Broth, Alpha-Naphthylamine, Sulphanilic Acid Medium, Gram Staining Kit, Carbohydrate fermentation media, normal saline, Hydrogen peroxide, Ethanol etc.

Collection of food samples: Gamma sterilized wide mouth PET jars were used for sampling of different foodstuffs. Samples were collected in jars using appropriate sampling procedure and then were kept in an ice pack to prevent any changes in the microbial flora of the samples. The samples were then transported to the Microbiology lab while maintaining the temperature 1-4°C with ice pack. Microbiological analysis was started within 6 hrs of collection.

Sterilization of Media: All the media were sterilized in an autoclave at 121°C for 15 minutes. After sterilization, Media were taken out by using asbestos gloves and kept at appropriate condition till the analysis.

Homogenization of Food sample: Surface of container/packaging of sample was cleaned with the help of 70% Isopropyl alcohol. Food samples were transferred in a sterilized stomacher bag and subjected it for homogenization. While homogizing the sample, uniformity of sample was ensured.

Methodology for Isolation and Identification: After homogenization of samples, following procedure as per Indian standard: 5887 was followed:

1. Approximately 25 gram of the sample was weighed in 225 ml of Nutrient Broth and then Incubated at $37^0 \pm 1^0$Cfor 24 hr.
2. After incubation, 1ml from flask was inoculated to 10 ml MacConkey broth and Incubated the tube at $37^0 \pm 1^0$Cfor 48 hr.
3. After 48 hrs. of incubation, tubes were Observed for acid (yellow colour in tube) and gas production (bubble in Durham's tube).
4. A loopful of inoculum was streak on Eosien Methylene Blue and MacConkey agar plates and Incubated the plates at $37^0 \pm 1^0$Cfor 24 hrs.
5. Plates were observed for characteristic green metallic sheen colonies and pink – red colonies on Eosien Methylene Blue and MacConkey agar plates respectively.
6. Five Characteristic colonies were taken by using loop and then streaked it on nutrient agar slant.
7. After Incubation at $37^0 \pm 1^0$C for 24 hrs. Following biochemical test were performed for Identification of *E.coli* strains

Table-1: Biochemical tests for further Identification

S.No.	Name of test	Response of E.coli
1.	Gram's Staining	Gram Negative Rods
2.	Motility	Motile
3.	Indole production	Positive
4.	Citrate utilization	Negative
5.	Glucorinidase	Positive
6.	Nitrate reduction	Positive
7.	ONPG	Negative
8.	Lysine utilization	Positive
9.	Lactose	Positive
10.	Glucose	Positive
11.	Sucrose	Positive
12.	Sorbitol	Positive

Interpretation: On the basis of characteristics colonies and biochemical characteristics of bacteria results are recorded as E.coli Present or Absent/25 g of food sample.

Quality Control: During the experiment, quality control is achieved by running simultaneously, E. coli as 'Positive Control' and S. aureus as 'Negative Control'.

Standard Antibiotics: A total of twenty antibiotics i.e. azithromycin, norfloxacin, ciprofloxacin, ofloxacin, amplicillin, amoxicillin, streptomycin, cefixime, tetracycline, gentamycin, meropenem, metronidazole, cloxacillin, doxycillin, vancomycin, rifampicin, chloramphenicol, leavofloxacin, gatifloxacin, and erythromycin were used as standard antibiotics for evaluation and comparative analysis of susceptibility and resistance patterns of E.coli isolates. These antibiotics are clinically significant and were obtained from local pharmacy store.

Preparation of Antibiotic Solution: Average weights of all antibiotics were calculated and accordingly stock solution of 1mg/ml was prepared using the appropriate solvent depending on the solubility of antibiotics. Further 1 ml from

stock solution was transferred in 100 ml volumetric flask and make-up the volume to obtain the final concentration of 10 µg/ml that was used for the evaluation of antibacterial susceptibility pattern.

Inoculum Preparation: E.coli isolates, thus obtained from food samples, were sub- cultured on non-selective nutrient agar slants followed by incubation at 37°C for 24h. Furthermore, densitometry was used to adjust 0.5 McFarland density (corresponding to 1.0 x 108 cfu/ml) of bacterial isolates.

Antibacterial susceptibility and Resistance Test (Zone of Inhibition Evaluation): Agar well diffusion assay (Perez et al., 1990; Chauhan et al., 2010) was the key methodology to evaluate the antibiotic susceptibility and resistance profile of isolated strains. 100µl of each of the adjusted cultures were mixed into separate 100 ml of sterile, molten, cool MHA, mixed well and poured into sterile petri plates. Plates were allowed to solidify; marked appropriately for each individual E.coli isolates and then punched to make wells of 6 mm diameter with the help of sterile cork borer. 100 µl of respective antibiotic solutions were pipetted into the well in assay plates. Plates were incubated overnight at 37oC. Following incubation, petri-plates were observed for the inhibition zones, diameters of which were measured by using Vernier Calipers.

Determination of Multiple Antibiotic Resistance Index (MAR Index): The multiple antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics against which the isolate showed resistant over the total number of antibiotics tested. MAR index higher than 0.3 indicates wide use of this antibiotic in the originating environment of this isolate (Paul et al, 1997). Following formula is being used to calculate the MAR Index:

$$\text{MAR Index} = \frac{\text{Number of antibiotics against which isolate showed resistance}}{\text{Total number of antibiotics evaluated}}$$

Results

Present study was aimed to evaluate the susceptibility and resistance patterns of E.coli isolates against selected antibiotics as shown in Table 2. A total of fifty-seven ready-to-eat food samples were collected from different locations of NCR, India. These samples were characterized microbiologically for the presence of E.coli. Out of fifty-seven collected samples, only twenty-four were found to have E.coli These isolates were morphologically and biochemically identified (Figure-1) and were then evaluated for their resistance and susceptibility profile against twenty antibiotics for several clinical implications.

This study shows the clear picture of prevalence of resistance among E.coli isolates as shown in Table-1. Isolates were found to have variable susceptibilities against the antibiotics used in the study. Susceptibility patterns of these isolates against evaluated antibiotics have been shown in Figure 2. Several antibiotics such as Metronidazole, Cloxacillin, Vancomycin and Rifampicin which are commonly prescribed are found to be completely ineffective with 100% resistance shown by E.coli isolates. Vancomycin was the drug of concern because of its non inhibitory nature as VRE (Vancomycin Resistant Enterococci) is of major threat in hospital acquired infections. Broad spectrum antibiotics such as Chloramphenicol and Azithromycin were also found to be of major concern due to their relatively very less inhibitory activity with only 16.67% and 12.5% susceptibility among E.coli isolates. Some of the clinically significant antibiotics evaluated in this particular study were shown to have mild- to- moderate inhibitory activity as shown in Table-2. Meropenem, a modern day antibiotic, was found highly promising as twenty-two E.coli isolates (i.e. 91.67%) were found to be susceptible against this particular antibiotic (Figure-3).

This study was also aimed to determine the multidrug resistance among E.coli isolates and this was done by calculating the Multiple antibiotic resistances (MAR) index which is the indicator of multidrug resistance. MAR's were calculated on the basis of susceptibility and resistance patterns of bacterial isolates and were shown in Table 3. It has been observed that all the isolates were having a sort of susceptibility on the scale of 0-1 and only one of E.coli isolates i.e. S-21 was found to have MAR Index of 1.0 (100% resistant) against all the evaluated antibiotics (Figure-4). S-17 was another isolate showing the highest resistance with MAR Index 0.95. A number of strains isolated demonstrated the variable nature of MAR; however, the least value was observed in case of S-18 (MAR 0.25) showing the maximum susceptibility against the evaluated antibiotics.

Discussion

Extensive use of broad spectrum antibiotics in hospitals exerts selective pressure on bacteria, thereby increasing the resistance phenomenon among microbial pathogens, hence, promoting severe and life threatening infections by multi-drug resistant strains. This problem is further enhanced due to the contamination of these multidrug-resistant pathogens to common food entities which are consumed on the daily basis. Therefore, the current study was performed and the results of this study put forward that a lot of activities including environmental, industrial and human parameters have an undesirable impact on the emergence of antibiotic resistance among the microorganisms pertaining to food, water and other human-related commodities (Bhunia, 2008).

Determination of the antibiotic resistance patterns of food-borne microbes is highly recommended as it is the part of microbial monitoring process of the food and water. E.coli is now-a-days among the major problems throughout the world

due to rapid evolution of its resistance against available antibiotics. This bacterium is also known for its nosocomial infections and multidrug resistance. Therefore, the current study was conducted to determine the resistance profile of E.coli isolates in foodstuffs and it exhibits the fact that the food samples meant for human consumptions were contaminated by a major bacterium i.e. E.coli which has been associated with the food-borne illnesses and if ingested, may cause deleterious effects to consumers' health.

This is widely known fact that the occurrence of resistance among microbial pathogens isolated from different food commodity has significantly risen during last decade and continuous research is being carried out to evaluate the bacterial contamination of food commodities and isolation of resistant microorganisms from different environments which may affect the human health (Chui et al., 2002). A major factor which has been attributed for this alarming consequence is the selection pressure created by the inappropriate and indiscriminate use of antimicrobials in food-producing animals (Teuber, 2001; Bywater, 2004) that leads to emergence of multi-drug resistant microbial strains. It is, therefore, indispensable to forfeit supplementary attentiveness to food hygiene practices to reduce or eliminate the risk from antibiotic resistance and pathogenic bacteria originating from food.

This study is highly prolific and exemplifies the extent of antibiotic resistance in all the isolated E.coli spp. Results were pinpointing the requirement to expend more awareness to Good Hygiene Practices (GHP) for the production of various food commodities, particularly street foods, in order to reduce or eliminate the risk due to pathogenic microorganisms isolated from these food resources. A strict implementation of Sanitary and Phytosanitary (SPS) actions should be applicable for street food vendors in order to make the safe food for human consumption. Therefore, it is the duty of public health authorities to scrutinize and implement the conditions of cleanliness. Food safety education is another vital component of the overall tactics to diminish the occurrence of food-borne infirmities and harmonize authoritarian and other possible actions.

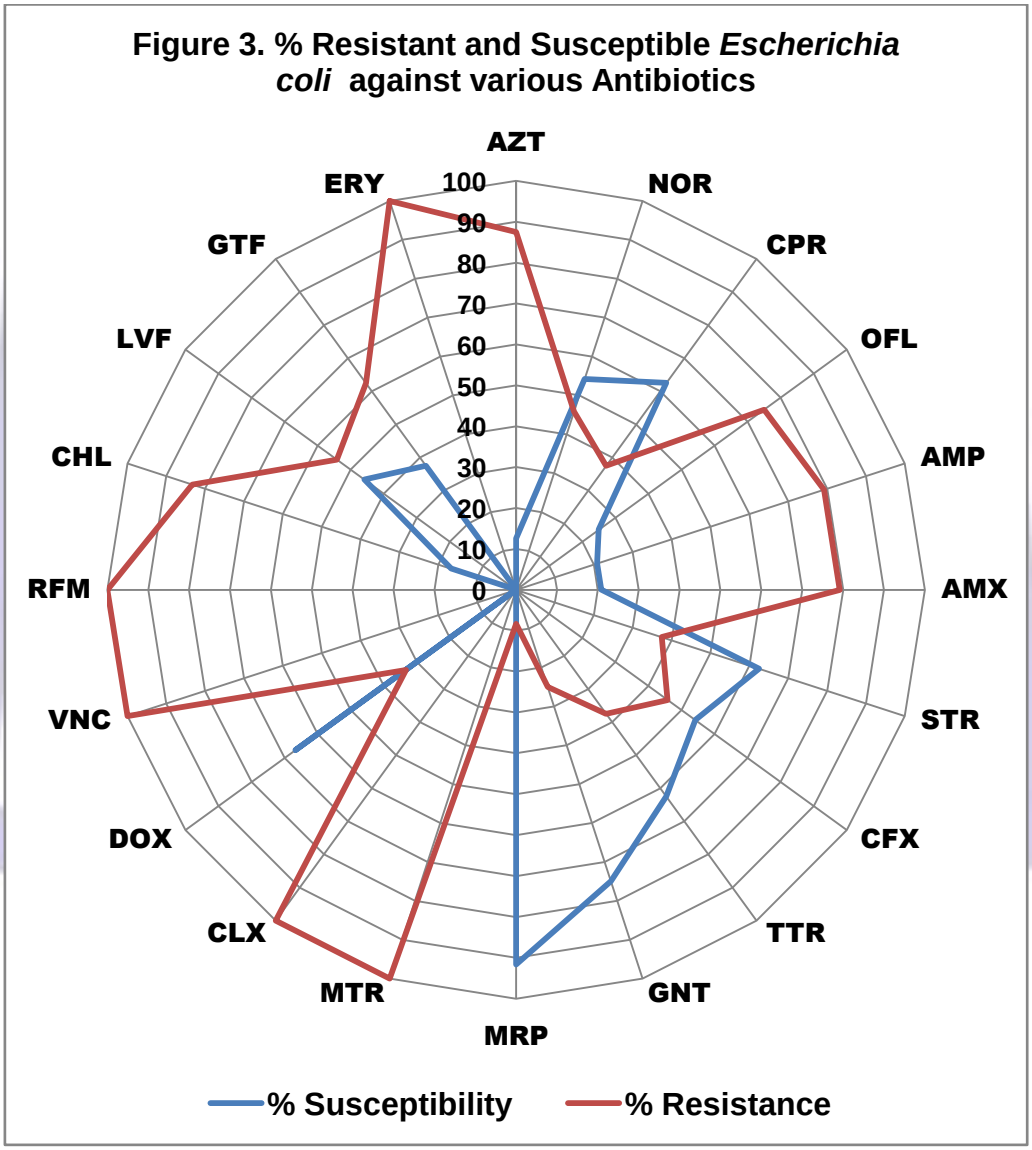
More so, further extensive work should be done to ascertain the extent of these consequences of drug resistant E.coli in our environment. Moreover, results obtained from this study must be used to implement prevention programs and policy decisions to prevent emergence and spread of antimicrobial resistance. Indeed, the problem of antibiotic resistance is global. This will greatly help to improve all steps towards the prevention and control of drug resistant organisms in our community.

References

1. Geyik, M.F., M. Aldemir, S. Hosoglu, H.I. Tacyildiz. 2003. Epidemiology of burn unit infections in children. American Journal Infected Control 6: 342-6.
2. Livermore, D.M., X.Z. Li, H. Nikaido. 1994. Role of efflux pumps in intrinsic resistance of *P. aeruginosa*-Resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrobial Agents and Chemotherapy. 38: (8) 1732-1741.
3. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and Acinetobacter baumannii from hospitalized patients in the United States, 1998 to 2001. Antimicrob Agents Chemother 2003; 47:1681-8.
4. Oteo J, Campos J, Baquero F. 2002. Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System. J. Antimicrob. Chemother., 50: 945-952.
5. Gorris, L.G.M., 2005. Food safety objective: an integral part of food chain management. Food Centr., 16: 801-809.
6. Fang, T.J., Q.K. Wei, C.W. Liao, M.J. Hang and T.H. Wang, 2003. Microbiological quality of 18 degrees ready-to-eat food products sold in Taiwan. Int. J. Food Microbiol., 80: 241-250.
7. World Health Organization (WHO), 2002a. WHO global strategy for food safety food for better health. World Health Organization, Geneva, Switzerland.
8. World Health Organization (WHO), 2002b. Food safety and foodborne illness. Fact Sheet, nE237, pp: 7.
9. Threfall, E.J., Ward, L.R., Frost, J.A., Willshaw, G.A., 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. Int. J. Food Microbiol. 62, 1 –5.
10. White, D.G., Zhao, S., Simjee, S., Wagner, D.D., McDermott, P.F., 2002. Antimicrobial resistance of foodborne pathogens. Microbes Infect. 4, 405– 412.
11. Levy, S.B., Salyers, A.A., 2002. Reservoirs of antibiotic resistance (ROAR) Network. http://www.healthsci.tufts.edu/apua/Roar/roarhome.htm.
12. Levy, S.B., Miller, R.V., 1989. Horizontal gene transfer in relation to environmental release of genetically engineered microorganisms. Gene Transfer in the Environment. McGraw-Hill Publishing Company, New York, pp. 405– 420.
13. Chui, C.H., T.L. Wu, L.H. Su, C. Chu, J.H. Chia, A.J. Kuo,M.S. Chien and T.Y. Lin, 2002. The emergence in Taiwan of fluoroquinelone resistance in *Salmonella enterica* serotype cholerasuls. N. Engl. J. Med., 346: 416-419.

14. IS 5887-1976.Reaff: 2005.Indian Standards Methods for detection of bacteria responsible for food poisoning..
15. Perez, C. Pauli, M. Bazerque, P. 1990. An antibiotic assay by the agar-well diffusion method. Acta Biologiae at Medecine Experimentalis.15:113-5.
16. Chauhan A, Pandey V, Chacko KM, Khandal RK, Antibacterial activity of raw and processed honey, E J Bio, 2010, 6(3), 58-66.
17. Paul, S., R.L. Bezbarauh, M.K. Roy and A.C. Ghosh, 1997. Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa, Letter of Appl. Microbiol., 24: 169-171.
18. Teuber, M., 2001. Veterinary use and antibiotic resistance. Curr. Opin. Microbiol., 4: 493-499.
19. Bywater, R.J., 2004. Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. J. Vet. Med. B, 51: 361-363.
20. Bhunia, A. K. 2008. Foodborne Microbial Pathogens: Mechanisms and Pathogenesis. Springer, West Lafayette, IN.

Figure 3. % Resistant and Susceptible *Escherichia coli* against various Antibiotics



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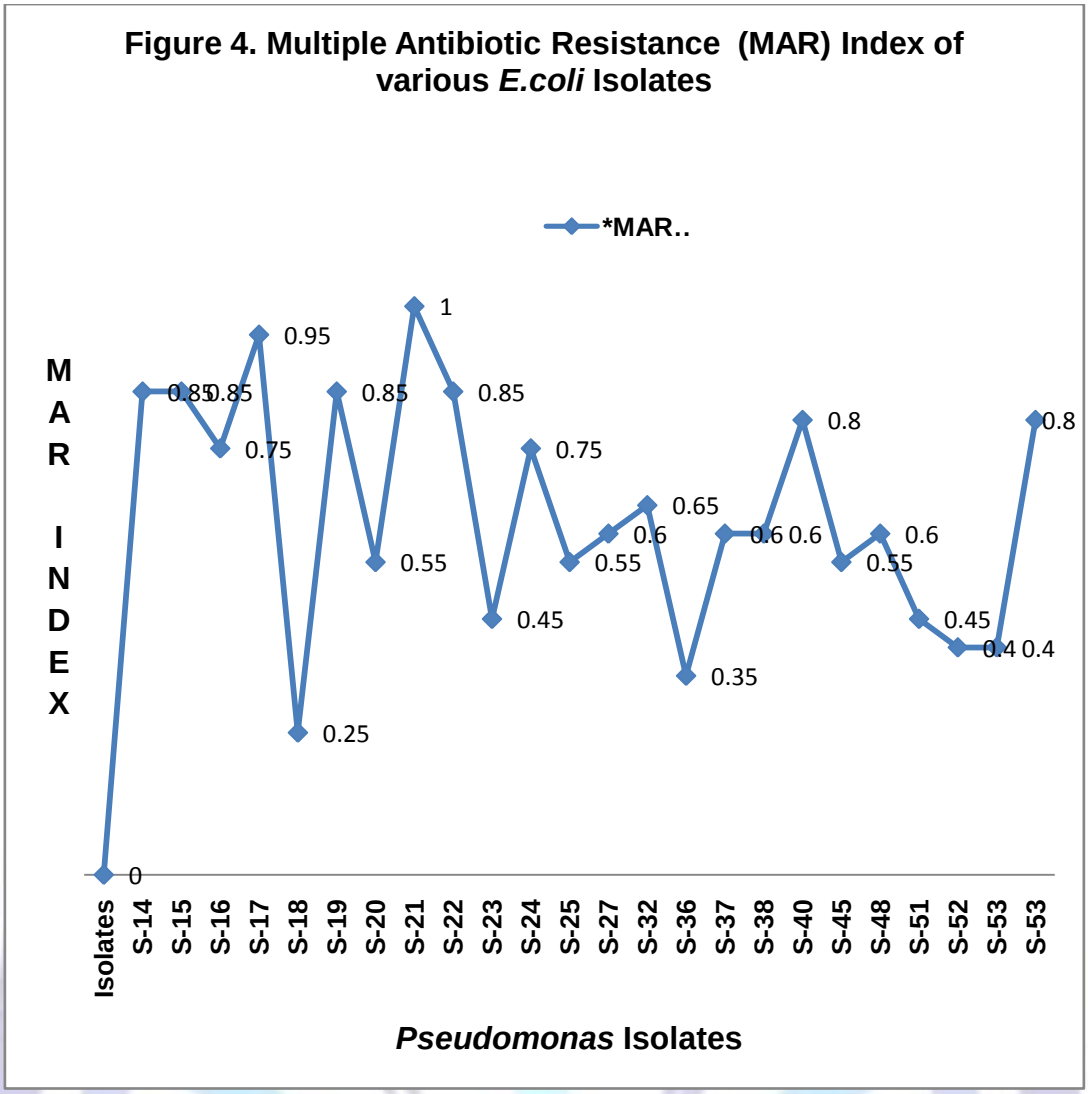

Table 2: Zone of Inhibition of different antibiotics against *E.coli* isolated from different food samples

ISOLATES	AZT	NOR	CPR	OFL	AMP	AMX	STR	CFX	TTR	GNT	MRP	MTR	CLX	DOX	VNC	RFM	CHL	LVF	GTF	ERY
S-14	0	0	0	0	0	0	9.01	14.01	0	0	24.6	0	0	0	0	0	0	0	0	0
S-15	0	0	0	0	0	0	0	0	12.12	10.9	0	0	0	18.36	0	0	0	0	0	0
S-16	9.35	0	0	0	0	0	0	17.35	0	8.85	24.61	0	0	10.17	0	0	0	0	0	0
S-17	0	0	0	0	0	0	0	0	0	0	24.37	0	0	0	0	0	0	0	0	0
S-18	13.93	21.31	23.75	21.69	10.61	10.62	12.18	19.74	17.21	9.32	25.27	0	0	14.96	0	0	15.94	15.66	15.09	0
S-19	0	0	0	0	0	0	9.72	10.6	0	0	12.51	0	0	0	0	0	0	0	0	0
S-20	0	14.27	17.33	14.21	0	0	12.98	14.78	17.87	9.82	21.02	0	0	15.5	0	0	0	0	0	0
S-21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S-22	0	0	0	0	0	0	0	9.37	15.43	0	23.55	0	0	0	0	0	0	0	0	0

S-23	9.83	17.69	21.4	17.88	0	0	13.49	17.42	0	9.09	25.14	0	0	16	0	0	0	10.25	11.63	0
S-24	0	0	17.29	14.15	0	0	0	0	10.92	9.49	24.23	0	0	0	0	0	0	0	0	0
S-25	0	25.71	33.84	20.1	0	0	9.71	20.25	17.3	9.43	24.17	0	0	10.42	0	0	0	0	0	0
S-27	0	17.62	30.82	0	0	0	8.36	0	0	9.62	15.05	0	0	11.8	0	0	0	13.94	12.08	0
S-32	0	0	13.03	0	0	0	11.29	0	12.36	10.07	22.26	0	0	13.55	0	0	0	9.74	0	0
S-36	0	13.58	18.47	0	10.18	11.02	12.23	12.15	11.82	8.76	24.4	0	0	15.32	0	0	10.05	10.86	11.51	0
S-37	0	12.42	14.89	0	0	0	0	0	12.83	10.98	23.03	0	0	11.69	0	0	0	10.4	9.68	0
S-38	0	17.28	14.83	0	0	0	14.17	0	11.92	9.74	23.32	0	0	11.39	0	0	0	9.18	0	0
S-40	0	11.45	14.99	0	0	0	0	0	0	0	20.04	0	0	20.25	0	0	0	0	0	0
S-45	0	0	0	0	10.53	11.14	13.58	14.58	12.59	9.91	23.44	0	0	13.12	0	0	10.93	0	0	0
S-48	0	13.93	17.35	0	0	0	0	0	12.6	10.8	22.01	0	0	12.58	0	0	0	11.59	12.83	0
S-51	0	14.65	23.04	11.73	0	0	13.55	11.21	12.79	11.07	22.53	0	0	12.78	0	0	0	17.72	11.73	0
S-52	0	9.71	17.55	0	11.48	11.49	12.04	14.12	13.39	9.4	24.72	0	0	13.59	0	0	0	10.9	10.75	0
S-53	0	9.38	16.88	0	10.89	11.95	13.63	9.67	12	10.09	22.94	0	0	16.91	0	0	0	10.17	10.81	0
S-55	0	0	0	0	0	0	11.81	0	0	10.02	23.06	0	0	0	0	0	10.7	0	0	0
POSITIVE CONTROL	0	15.85	26.78	17.17	32.88	33.25	20.78	0	25.26	17.27	22.23	0	11.16	29.68	17.07	18.08	18.86	18.1	18.95	0

AZT: AZITHROMYCIN; NOR: NORFLOXACIN; CPR: CIPROFLOXACIN; OFL: OFLOXACIN; AMP: AMPLICILLIN; AMX: AMOXYCILLIN; STR: STREPTOMYCIN; CFX: CEFEXIME; TTR: TETRACYCLIN; GNT: GENTAMYCIN; MRP: MEROPENEM; MTR: METRONIDAZOLE; CLX: CLOXACILLIN; DOX: DOXYCILLIN; VNC: VANCOMYCIN; RFM: RIFAMPICIN; CHL: CHLORAMPHENICOL; LVF: LEAVOFLOXACIN; GTF: GATIFLOXACIN; ERY: ERYTHROMYCIN

Table 3: Percentage resistant and susceptible *E.coli* against various antibiotics

Name of Antibiotics	% Susceptibility	% Resistance	Name of Antibiotics	% Susceptibility	% Resistance
Azithromycin	**12.5 (3)**	**87.5 (21)**	Meropenem	**91.67 (22)**	**8.33 (2)**
Norfloxacin	**54.17 (13)**	**45.83 (11)**	Metronidazole	**0 (0)**	**100 (24)**
Ciprofloxacin	**62.5 (15)**	**37.5 (9)**	Cloxacillin	**0 (0)**	**100 (24)**
Ofloxacin	**25 (6)**	**75 (8)**	Doxycillin	**66.67 (16)**	**33.33 (8)**
Amplicillin	**20.83 (5)**	**79.17 (19)**	Vancomycin	**0 (0)**	**100 (24)**
Amoxicillin	**20.83 (5)**	**79.17 (19)**	Rifampicin	**0 (0)**	**100 (24)**
Streptomycin	**62.5 (15)**	**37.5 (9)**	Chloramphenicol	**16.67 (4)**	**83.33 (20)**
Cefixime	**54.17 (13)**	**45.83 (11)**	Leavofloxacin	**45.83 (11)**	**54.17 (13)**
Tetracycline	**62.5 (15)**	**37.5 (9)**	Gatifloxacin	**37.5 (9)**	**62.5 (15)**
Gentamycin	**75 (18)**	**25 (6)**	Erythromycin	**0 (0)**	**100 (24)**

Table 4. Multiple Antibiotic Resistance (MAR) Index of *E.coli* Isolates

***Pseudomonas* Isolates**	***MAR Value**	***E.coli* Isolates**	***MAR Value**
S-14	0.85	S-27	0.60
S-15	0.85	S-32	0.65
S-16	0.75	S-36	0.35
S-17	0.95	S-37	0.60
S-18	0.25	S-38	0.60
S-19	0.85	S-40	0.80
S-20	0.55	S-45	0.55
S-21	1.00	S-48	0.60
S-22	0.85	S-51	0.45
S-23	0.45	S-52	0.40
S-24	0.75	S-53	0.40
S-25	0.55	S-55	0.80

*MAR refers the Multiple Antibiotic Resistance