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Original Research Article

Multi-drug resistance in indicator bacteria: *Coliforms* and *Escherichia coli* isolated from ready to eat food samples

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A B S T R A C T

This study investigated the levels and patterns of antibiotic resistance in indicator bacteria: *Coli forms* and *Escherichia coli* isolated from ready to eat food. Isolates were obtained from food samples collected from local markets of India and tested against selected panels of antibiotics. High resistance was observed for both *E. coli* and *Coliform*s species. Both isolates showed high resistance Overall, high resistance was observed for *Coliforms* against Impenem (90%), Cephalothin (90%), Amoxycillin (89%)and Azithromycin (88%)and Sulphafurazole (88%). For *E. coli* the highest resistance was seen against Cephalothin (89%),Nalidixic acid (87%), Ciprofloxacin,(85%),Neomycin and Cotrimoxazole (83%), with *Coliforms* isolates showing a generally higher trend. However, *E. coli* species showed more resistance against Oxacillin, Cotrimoxazole and Kanamycin than coliforms isolates. Both E. coli and *Coliforms* isolates showed more than 70% resistance all selected antibiotics and none were sensitive to all antibiotics. Comparison of resistance between *Coliforms* and *E.coli* isolates from the same food category for different antibiotics indicated a significant difference ($p < 0.05$) in the resistance was observed for all antibiotics. Overall 97 and 83 percent of *Coliform*s and *E. coli* were resistant to twenty antibiotics, respectively. The high level of multi- drug resistance to clinically important antibiotics in commensal indicator bacteria indicated that bacteria of food origin can be significant reservoir of resistant gene pool that may spread resistance to pathogens in humans and has public health implications.

Keywords

Antimicrobial resistance; Indicator bacteria; Multi- drug.

Introduction

Multidrug resistance is a natural biological phenomenon that often results from antibiotic use pressure in humans, yield improvement of crops, preservation of foods and the widespread use of disinfectants in farming. There is an increasing concern for the antimicrobial resistance problem. When it gets amplified many times, it results in serious public health concerns and long term shifts in resistance levels (Houndt and Ochman, 2000).Microorganisms are designed to be highly adaptable so they can take advantage of changes in their environment and pass those changes onto

other organisms to promote the survival of the species as a whole. Resistance to antibiotics has increased dramatically over the past few years and has now reached a level that places future patients in real danger. Several studies have demonstrated that animals and humans share several ecological systems including microorganisms in those environments and they spread easily globally facilitated by globalization and the current ease of travel across the world Developing countries in particular have received limited attention regarding this problem (Okeke et al., 2007). Indeed antimicrobial agents are grossly misused in many developing countries leading to high selective pressure on microorganisms (Byarugaba, 2005). Microorganisms are designed to be highly adaptable so they can take advantage of changes in their environment and pass those changes onto other organisms to promote the survival of the species as a whole. Multi-drug resistance is a serious challenge to disease treatment with possibility of a complete treatment failure occurring and is occurring with much more frequency (Besser et al., 2000). This phenomenon has been reported in other bacteria and is more of a rule rather an exception (Spera and Farber, 1994; Savage 2001; Lee et al., 2002; Dargatz and Traub-Dargatz, 2004;) A number of *E. coli* strains are recognised as important pathogens of Colibacillosis in poultry and some of them can cause severe human diseases such as haemorrhagic colitis and haemolytic uremic syndrome(Riley et al. 1983; Chansiripornchai 2009; Ferens and Hovde 2011). There is a comparatively higher use of antimicrobials in developed countries than in developing countries both for prophylaxis and therapy (Mitema et al., 2001) although there is higher therapeutic use in developing countries than prophylactic use. Antibiotics are often used in rearing animals for food and this use among others leads to the creation of resistant strains of bacteria. In some countries antibiotics are sold over the counter without a prescription which also leads to the creation of a pool of resistant organisms both commensals and pathogenic. Administration of antimicrobial agents affects both targeted pathogenic organisms as well as non-target commensals. Thus frequent antimicrobial use creates a pool of resistant commensal bacteria that contribute to the general increase and dissemination of bacterial resistance worldwide, and can be a source of resistance genes for pathogens (Andremont, 2003). Microorganisms such as *Escherichia.coli* and *Klebsiella pneumoniae*, which are commensal and pathogens for humans and animals, have become increasingly resistant to third-generation cephalosporins. Moreover, in certain countries, they are also resistant to carbapenems and therefore susceptible only to tigecycline and colistin. Many different bacteria now exhibit multidrug resistance, including *staphylococci, enterococci, gonococci, streptococci, salmonella, Mycobacterium tuberculosis* and others. The causes are numerous, but the role of the overuse of antibiotics in both humans and animals is essential, as well as the transmission of these bacteria in both the hospital and the zxcommunity, notably via the food, contaminated hands, and between animals and humans. Most studies on antimicrobial resistance of bacteria of animal origin in developing countries have mostly examined pathogenic bacteria (Byarugaba, 2004). Food of animal origin has been demonstrated to be a source of a majority of food-borne bacterial infections caused by *Campylobacter, Yersinia,E.coli*-0157,non-typhoid *Salmonella,* and other

pathogens (Threlfall et al., 2003; Padungtod et al., 2006; Miles et al., 2006; Meyer et al., 2008). Resistance mechanisms acquired by bacteria typically occur at the genetic level. Chromosomal mutations resulting in an alteration in the target of an antimicrobial drug can lead to drug resistance. Bacteria have developed different mechanisms to develop resistance to the antibiotics used against them. Particular antibiotic resistance genes first described in human specific bacteria have also been found in animal-specific species of microorganisms and vice versa, suggesting that those bacterial populations can share and exchange these genes (O'Brien, 2002). Resistance is primarily attributed to the production of beta-lactamase genes located on mobile genetic elements, which facilitate their transfer between different species. Some resistant bacteria are able to transfer copies of DNA that codes for a mechanism of resistance to other bacteria, thereby conferring resistance to their neighbors, which then are also able to pass on the resistant gene. It is critical to understand the gravity of the problem and this requires that information about the levels and patterns, as well as the mechanisms of resistance to specific antibiotics of bacteria, be available**.** A few systematic investigations have been conducted in using the commensal indicator bacteria that can be used to estimate the exact resistance problem. Monitoring antimicrobial resistance using indicator bacteria avoids overestimating resistance levels by use of pathogenic bacteria, which is less accurate as the resistance patterns of pathogenic strains can be influenced by the preceding antimicrobial treatment. The objective of the present investigation was to investigate the occurrence of multidrug resistance bacteria in ready to eat food samples collected from local markets in india and furthermore, to evaluate multidrug resistance by Kirby-Bauer Disk Diffusion method for detection.

In the present study indicator bacteria such as *Coliforms* and *E.coli* were selected as their isolation, identification and confirmation is easier and they fulfilled the required determination of multidrug resistance in short duration. This study investigated the current levels and patterns of bacterial resistance in food to common antibiotics, using standard resistance indicator bacteria as a first step towards containment of antimicrobial resistance.

Materials and Methods

Collection of samples

Five categories of ready to eat food samples from local market were selected for analysis. These categories were cooked Rice with vegetables, chicken curry, Noodles, Veg- Burger, Veg- Momos etc. A total of hundred samples consisting twenty of each category of food were collected by sterile forceps and tweezers, packed individually in sterile plastic bags. The quantity of sample taken was approximately 100 g for each food samples were collected. All samples of each food categories were processed the following day, i.e.within 24 h of collection, analyzed for isolation of resistant indicator bacteria *Escherichia coli* and *Coliforms.*

Isolation and identification of coliforms

Each 10 g sample was emulsified in peptone water. One ml sample from different dilution was poured in sterile Petri plates (in duplicate for each dilution) and then 15-20ml sterile crystal violet

neutral red bile lactose Agar media (VRBA,Hi-media)) medium was poured. After mixing, plates were allowed to set. Allowed it to solidify. To prevent surface growth and spreading of colonies, overlaid with 5 mL VRBA, inverted the plate after complete solidification and incubated at 37^0C for 24-48h. The number of purplish pink colonies were counted after incubation (colonies having diameter of 0.5mm surrounded by zone of precipitated bile acids). Typical colonies were identified and confirmed by Gram staining and gas production at37°C after 48 hour incubation of inoculated BGLB broth. Confirmed colonies were tested for multidrug resistance. Pure isolates were stored in 30% glycerol at -20°C until further use.

Isolation and identification of *E. coli*

Each 25 g sample was emulsified in peptone water and MPN method was used to confirm the presence of *E. coli*. Gas production in Mac.Conky Broth and BGB(Brilliant green bile lactose broth) at 44^oC after 48 hours incubation confirmed the presence of *E.coli*. Further confirmation of isolates was done by inoculating Eosin Methylene blue lactose Agar (EMB,Hi-media) plate, incubated at37°C for 24 hour and Biochemical analysis. Typical metallic sheen Colonies on EMB plate were confirmed by Gram staining, indole, Methyl Red, Voges-Proskauer, Citrate Utilization and Urease Test. Confirmed colonies were tested for multidrug resistance. Pure isolates were stored in 30% glycerol at -20°C until further use.

Determination of susceptibility to different drugs

Isolates were tested for their susceptibility to twenty four antibiotics with different concentrations whichincludes Amikacin(30µg),Ampicillin(30µg),Amoxicillin(25µg),Azithromycin(15µg),Ciprofloxacin(5µg),Erythromycin(15µg),Clindamycin(2µg),Tetrracycline(30µg),Gentamycin(10µg),oxycillin(1µg),Vancomycin(15µg),ofloxacin(5µg),Sparfloxacin(5µg),Penicillin (1units),Cotrimoxazole(25µg), Kanamycin (30 µg), Levofloxacin(5 µg), Cephalothin(30 µg), Nalidixic Acid(30 µg), Norfloxacin(10 µg), Neomycin(30µg), sulfafurazole(30µg), Trimethoprim (5µg), Impenem(10 µg)The screening for antimicrobial resistant isolates was carried out by the Kirby-Bauer Disk Diffusion method using Muller Hinton agar(Hi-media). The inoculum prepared in a saline suspension from pure isolated bacterial colony at a turbidity equivalent to a 0.5 McFarland standard. A sterile cotton swab was placed in that suspension and excess fluid was removed by pressing and rotating the cotton against the inside of the tube. Each swab was surface spread uniformly onto Mueller Hinton agar (Hi-media) plate to yield uniform growth. Antimicrobial paper disks (Hi-media) were then applied to the surface of the plate. After incubation at 37°C for 18 to 24 h, plates were inspected for growth and inhibition. The presence of a growth inhibition zone larger than the break point diameter as defined by standard procedures in the NCCLS M31A Manual (NCCLS, 1999) was considered to indicate susceptibility to the agent. The presence of a growth inhibition zone smaller than the break point diameter, the absence of any inhibition zone or the presence of isolated colonies growing inside an inhibition zone of any size were considered indicative of resistance as defined (NCCLS, 1999). In general all susceptibility testing and the interpretation of data were performed according to standard procedures as described in the NCCLS M31A Manual for MIC

determination (NCCLS, 1999). The recommended reference strains E. coli of MTCC were used for quality control.

Data management and analysis

The data were entered into a computer using Microsoft Excel. The descriptive statistics were computed for frequency counts, relative cumulative frequency or presented graphically. The phenotypic resistance was presented as the percentage of the total isolates tested that was resistant. Multi-drug resistance was defined as one isolate being resistant to twenty out of twenty four antibiotics tested. The proportions of the resistant isolates to the various antibiotics were compared between species of bacteria isolated and between the different food items from which the bacteria were isolated, by using chi-square tests at a 5% level of significance.

Results and Discussion

A total number of 100 isolates of coli forms and 100 of E. coli respectively were isolated from the five food items as shown in Table-1, for susceptibility testing. Overall, high resistance was observe for coliforms against Impenem (90%), cephalothin(90%), amoxycillin(89%) and Azithromycin (88%) and Sulphafurazole (88%). For *E. coli* the highest resistance was seen against Cephalothin (89%), Nalidixic acid (87%), Ciprofloxacin,(85%), Neomycin and cotrimoxazole(83%), while *coliforms* isolates showing a generally higher trend (Table 2).

However, *E. coli* species showed more resistance to oxacillin, co-trimoxazole and kanamycin than to coliforms isolates. Both *E. coli* and *Coliforms* isolates showed more than 70% resistance all selected antibiotics and none were sensitive to all antibiotics. *Coliforms* and *E. coli* isolates were tested for susceptibility against Amikacin, Ampicillin, Amoxicillin, Azithromycin, Ciprofloxacin, Erythromycin, Clindamycin, Tetrracyclin, Gentamycin, oxycillin, Vancomycin, ofloxacin, Sparfloxacin, Penicillin, Cotrimoxazole, Kanamycin, Levofloxacin, Cephalothin, Nalidixic Acid, Norfloxacin, Neomycin, sulfafurazole, Trimethoprim, Impenem). Figure 1 shows the levels and patterns of the resistance among the isolates from the different food Categories. Generally there was highest resistance observed for isolates against Cephalothin, Impenem followed by Amoxycillin and Azithromycin ,the lowest resistance was observed in isolates against Levofloxacin. Compared with those from other food categories, isolates from Veg-momos were observed to be generally more resistant, with least resistance observed from isolates obtained from noodles, although the highest resistance was observed against Cephalothin, Impenem (95%) in isolates of noodles.

Analysis was also made for multi-drug resistance among the *Coliforms* isolates. It was observed that 97% of the isolates were multi-drug resistant (Table 3). Moreover 80% of the isolates were resistant to selected all twenty four drugs and about 85% were resistant to 23 antibiotics.

When statistical comparison of the proportions of resistance was made on the isolates from the different food categories for each antibiotic, a significant difference ($p < 0.05$) in the resistance was observed for all antibiotics (Table 4). The higher resistance (90%) being observed in all food categories for Amoxicillin, Amikacin,Ampicillin,Azithromycin,Ciprof loxacin,Erythromycin,Tetrracycline,Genta mycin,oxycillin, Vancomycin, ofloxacin, Penicillin, Cotrimoxazole, Cephalothin,

Nalidixic Acid, Norfloxacin, Neomycin, sulfafurazole (18 out of 24 antibiotics) while the highest resistance (95%) among all food categories being observed for Amoxicillin, Azithromycin,Ciprofloxacin, sulfafurazole, Trimethoprim and Impenem(7 out of 24 antibiotics).Lower resistance (65%)being observed in isolates from noodles, veg burger, cooked rice for Kanamycin, Levofloxacin, Nalidixic acid and Penicillin-G respectively while the lowest resistance(60%) was observed in two isolates, one from cooked rice and one from veg burger for Amikacin and Gentamycin respectively. In the present study not a single isolates from all food categories was sensitive to anyone of the antibiotics. All isolates of all food categories were resistant.

E.coli isolates were tested for susceptibility against Amikacin, Ampicillin, Amoxicillin, Azithromycin, Ciprofloxacin, Erythromycin, Clindamycin, Tetrracycline, Gentamycin, oxycillin, Vancomycin, ofloxacin, Sparfloxacin, Penicillin, Cotrimoxazole, Kanamycin, Levofloxacin, Cephalothin, Nalidixic Acid, Norfloxacin, Neomycin, sulfafurazole, Trimethoprim, Impenem with same concentration of antibiotics as were used for testing the susceptibility of coliforms. Figure 2 shows the levels and patterns of the resistance among the isolates from the different food categories.

Analysis for multidrug resistance among *E.coli* isolates as defined indicated 83% of the isolates were multi-drug resistant (Table-5).Moreover 78% of the isolates were resistant to 21, 72% of isolates were resistant to 23 and 70% of isolates were resistant to all 24 drugs. All isolates were resistant to maximum 16 antibiotics. In the present study not a single isolates of *E.coli* from all food categories was sensitive to anyone of the antibiotics

A statistical comparison of the proportions of resistance of was made on the isolates of *E.coli* from the different food categories species for each antibiotic, a significant difference ($p < 0.05$) in the resistance was observed for all antibiotics (Table 6).

Generally there was highest resistance(95%) observed for isolates against oxycillin, Cotrimoxazole, Cephalothin, Nalidixic Acid and Neomycin from all four food categories i.e cookedrice,chickencurry,vegburger,veg.momosfollowedby(90%)resistanceagainstAmpicillin,Azithromycin,Ciprofloxacin,Erythromycin,Clindamycin,Tetrracycline,Gentamyci,oxycillin, Kanamycin, Cotrimoxazole, Cephalothin, Nalidixic Acid, Neomycin, sulfafurazole from all five food categories. Isolates from cooked rice, chicken curry, veg burger were observed. 95% resistance was observed for 5 out of 24 antibiotics and 90% resistance was observed for 14 out of 24 antibiotics . (60%) resistance was observed for Clindamycin, Azithromycin, Ofloxacin and Amoxicillin

It is indicated from the data that multidrug resistance percentage was higher in *Coliforms* than *E.coli.* It was 97% in *Coliforms* and 83% in *E.coli.* Resistance was also compared between *Coliforms* and *E. coli* isolates from the same food category for different antibiotics. As from figures 3.1-3.24, it was found that resistance was generally higher in *Coliforms* than *E. coli* isolates for Ampicillin, Amoxicillin, Azithromycin, Tetrracycline, Gentamycin, Vancomycin, ofloxacin, Penicillin, sulfafurazole, Trimethoprim and Impenem. The resistance was nearly equal in *Coliforms*

Table1. Distribution of bacteria isolated from the different food animal species.

Food categories	**Isolates**	
	Coliforms	***E.coli***
Cooked Rice with vegetables	20	20
Chicken curry	**20**	**20**
Noodles	**20**	**20**
Veg- Burger	**20**	**20**
Veg- Momos	**20**	**20**
Total	**100**	**100**

Table 2.Overall resistance of *Coliforms* and *E. coli* from all the food samples against different antibiotics

Antibiotics	**Percentage resistance to Coliforms (n=100)**	Percentage resistance to E.coli (n=100)
Amikacin	80	72
Ampicillin	85*****	78
Amoxicillin/	89*****	71
Azithromycin	88*****	80
Ciprofloxacin	87*****	85*****
Erythromycin	79	82*****
Clindamycin	81*****	80
Tetrracycline	82*****	75
Gentamycin	80	74
oxycillin	78	83*****
Vancomycin	82*****	81*****
ofloxacin	79	70
Sparfloxacin	81*****	75
Penicillin	80	76
Cotrimoxazole	78	83*****
Kanamycin	76	82*****
Levofloxacin	72	72
Cephalothin	90*****	89*****
Nalidixic Acid	80	87*****
Norfloxacin	82*****	81*****
Neomycin	85*****	83*****
sulfafurazole	88*****	79
Trimethoprim	87*****	78
Imipenem	90*****	81*****

Table3. Multi-drug resistance among the *Coliforms* isolates.

Number of drugs to which isolates were resistant	Frequency	Cumulative frequency	Cumulative percentage
24	80	80	80
23	5	85	85
22	5	90	90
21	5	95	95
20	2	97	97
19	2	99	99
18	1	100	100
17	0		

Table4. Comparison of resistance proportions to antibiotics for *Coliforms* isolated from the different food samples

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Antibiotics	Proportion of resistance for isolates obtained from different food samples					P-values
	Cooked Rice with vegetables	Chicken curry	Noodles	Veg Burger,	Veg Momos.	
Amikacin	60	90	75	90	85	0.00000
Ampicillin	85	90	85	75	90	0.00000
Amoxicillin/	80	90	85	95	95	0.00000
Azithromycin	80	85	90	90	95	0.00000
Ciprofloxacin	85	75	90	95	90	0.00000
Erythromycin	75	80	70	90	80	0.00000
Clindamycin	80	70	75	85	85	0.00000
Tetrracycline	90	90	70	80	80	0.00000
Gentamycin	90	75	90	85	60	0.00000
Oxycillin	80	75	90	70	75	0.00000
Vancomycin	80	75	85	90	80	0.00000
ofloxacin	90	70	80	75	80	0.00000
Sparfloxacin	85	70	80	75	85	0.00000
Penicillin	65	90	75	80	90	0.00000
Cotrimoxazole	90	70	80	75	75	0.00000
Kanamycin	75	80	65	75	85	0.00000
Levofloxacin	75	70	75	65	75	0.00000
Cephalothin	90	85	95	90	90	0.00000
Nalidixic Acid	75	90	80	65	90	0.00000
Norfloxacin	90	80	75	85	80	0.00000

Table.5 Multi-drug resistance among the *E. coli* isolates

Number of drugs to which isolates were resistant	Frequency	Cumulative frequency	Cumulative percentage
24	**70**	**70**	**70**
23	**2**	**72**	**72**
22	**3**	**75**	**75**
21	**3**	**78**	**78**
20	**5**	**83**	**83**
19	**7**	**91**	**91**
18	**4**	**95**	**95**
17	**3**	**98**	**98**
16	**2**	**100**	**100**
15	**0**		

Figure.1 Levels and patterns of resistance among *Coliforms* isolated from different food samples

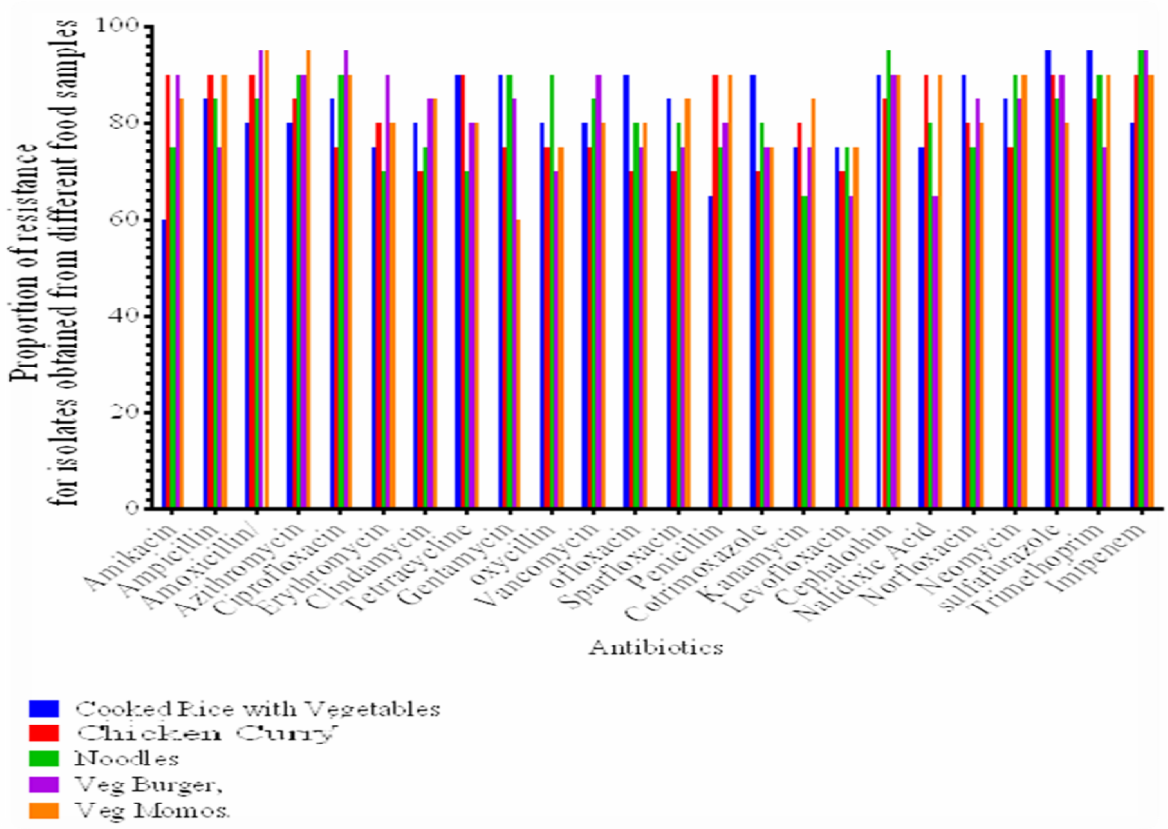

Table.6 Comparison of resistance proportions to antibiotics for *E. coli* isolated from the different food samples

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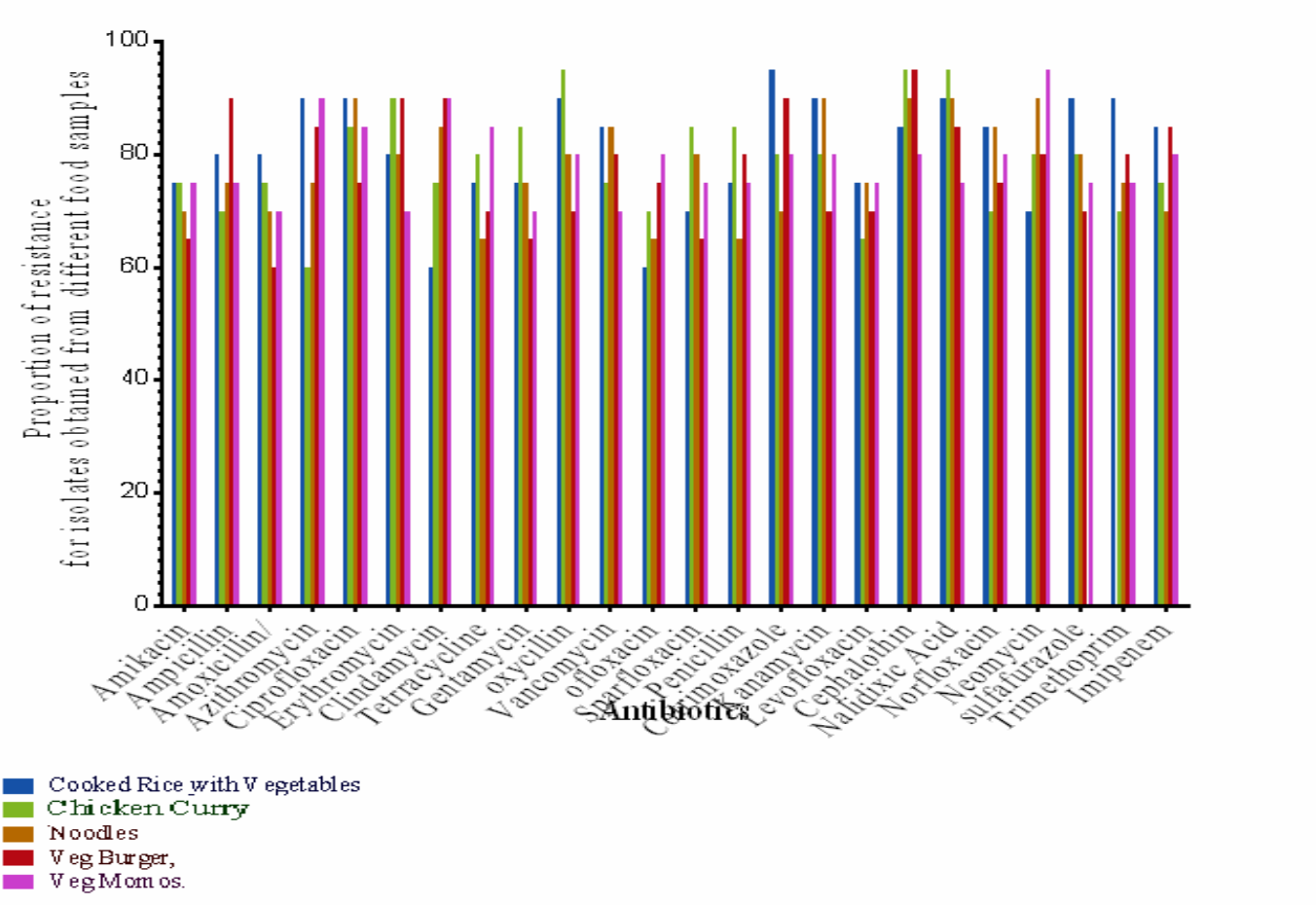
Antibiotics	**Proportion of resistance for isolates obtained from different food samples**					P-values
	cooked Rice with vegetables	**chicken curry**	**Noodles**	**Veg. Burger**	**Veg. Momos.**	
Amikacin	75	75	70	65	75	0.00000
Ampicillin	80	70	75	90	75	0.00000
Amoxicillin/	80	75	70	60	70	0.00000
Azithromycin	90	60	75	85	90	0.00000
Ciprofloxacin	90	85	90	75	85	0.00000
Erythromycin	80	90	80	90	70	0.00000
Clindamycin	60	75	85	90	90	0.00000
Tetrracycline	75	80	65	70	85	0.00000
Gentamycin	75	85	75	65	70	0.00000
oxycillin	90	95	80	70	80	0.00000
Vancomycin	85	75	85	80	70	0.00000
ofloxacin	60	70	65	75	80	0.00000
Sparfloxacin	70	85	80	65	75	0.00000
Penicillin	75	85	65	80	75	0.00000
Cotrimoxazole	95	80	70	90	80	0.00000
Kanamycin	90	80	90	70	80	0.00000
Levofloxacin	75	65	75	70	75	0.00000
Cephalothin	85	95	90	95	80	0.00000
Nalidixic Acid	90	95	90	85	75	0.00000
Norfloxacin	85	70	85	75	80	0.00000
Neomycin	70	80	90	80	95	0.00000
sulfafurazole	90	80	80	70	75	0.00000
Trimethoprim	90	70	75	80	75	0.00000
Imipenem	85	75	70	85	80	0.00000

Figure2. Levels and patterns of resistance among *E. coli* spp. isolated from different food samples



and *E.coli* isolates for Erythromycin, Ciprofloxacin, Sparfloxacin, Levofloxacin, Cephalothin and norfloxacin. The resistance was generally higher in *E. coli* than *Coliforms* isolates for Clindamycin, Kanamycin, Neomycin, Cotrimoxazole, Nalidixic Acidandoxycillin.

The high prevalence against most antibiotics tested observed in this study suggests that bacteria of food origin can be a significant reservoir of resistant bacteria as has been suggested in other studies (Harada et al., 2007; Young et al., 2009). Commensal bacteria have also been reported to play a role in transmission of resistance (Andremont, 2003). Particularly, the observed resistance to Impenem, Trimethoprim, Sulfafurazole and other antibiotics that are not commonly used, indicates that there is a possibility of exchange of resistant bacteria between humans and food. A similar phenomenon has also been reported in studies (Srinivasan et al., 2007). Transmission of resistant bacteria has been confirmed between bacteria of human origin and food preparation environment. Bacteria became resistant due to over use of these antibiotics in treatment of common diseases and it is possible that the resistance to Impenem, Trimethoprim and Sulfafurazole as seen in this study could have originated from

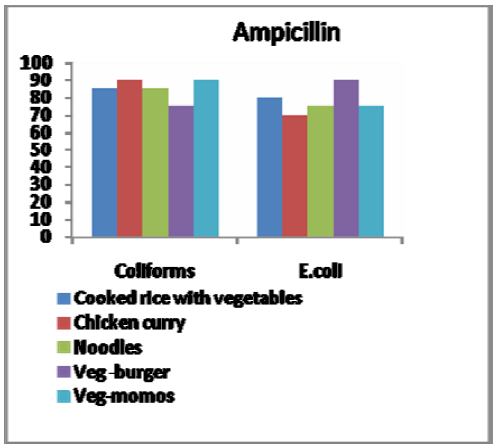


Figure.3.1 Comparison of resistance between *Coliforms* and *E.coli* for Ampicillin

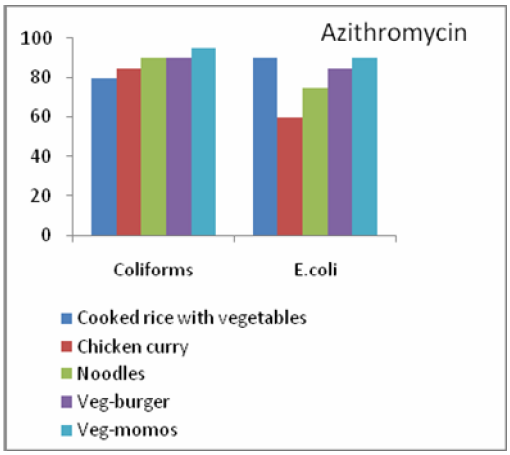


Figure.3.1 Comparison of resistance between *Coliforms* and *E.coli* for Azithromycin

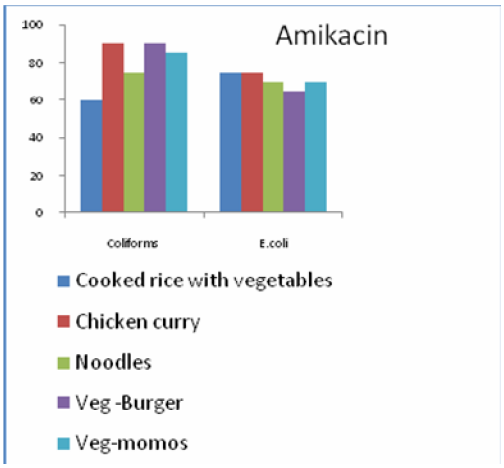


Figure.3.2 Comparison of resistance between *Coliforms* and *E.coli* for Amikacin

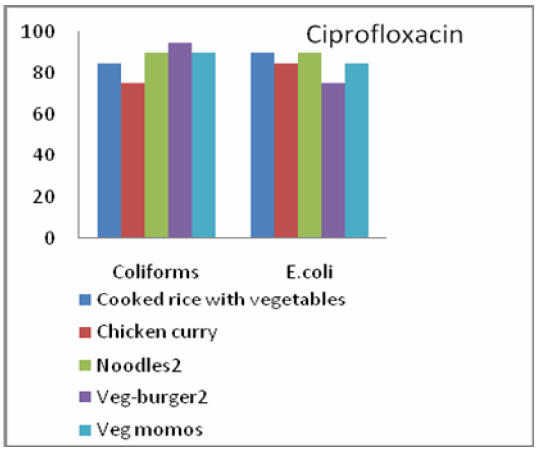


Figure.3.2 Comparison of resistance between *Coliforms* and *E.coli* for Ciprofloxacin

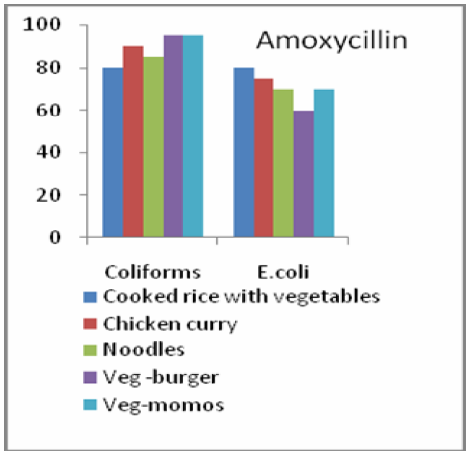


Figure.3.3 Comparison of resistance between *Coliforms* and *E.coli* for Amoxycillin

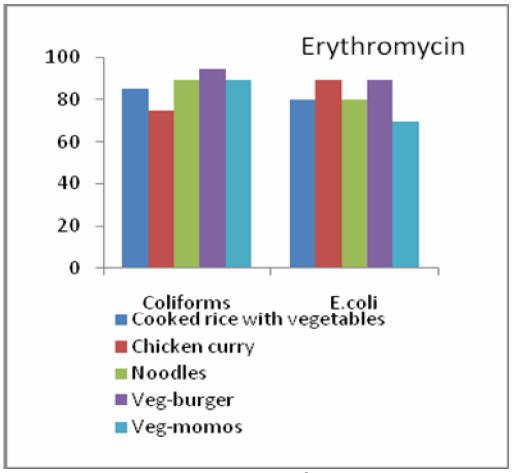


Figure.3.3 Comparison of resistance between *Coliforms* and *E.coli* for Erythromycin

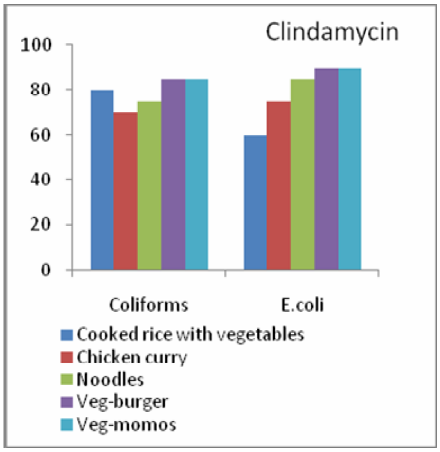


Figure.3.4 Comparison of resistance between Coliforms and *E.coli* for Clindamycin

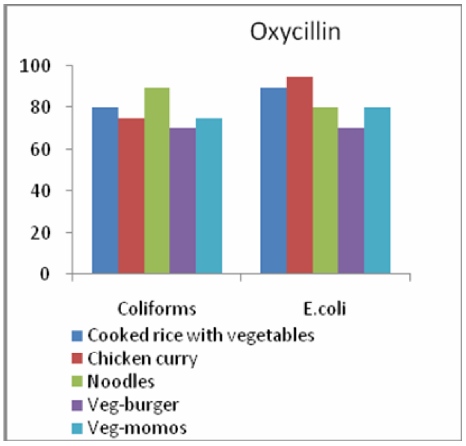


Figure.3.7 Comparison of resistance between *Coliforms* and *E.coli* for Oxycillin

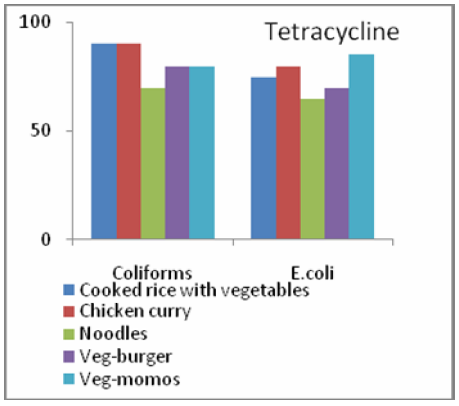


Figure.3.5 Comparison of resistance between *Coliforms* and *E.coli* for Tetracycline

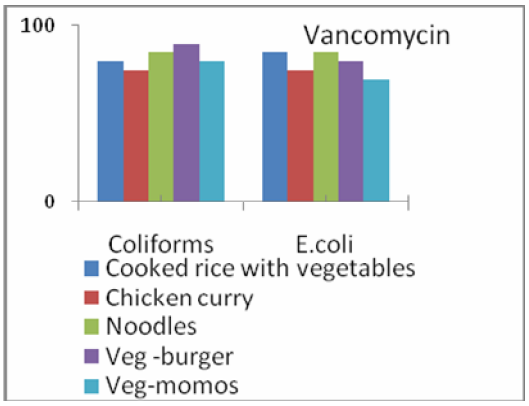


Figure.3.11 Comparison of resistance between *Coliforms* and *E.coli* for Vancomycin

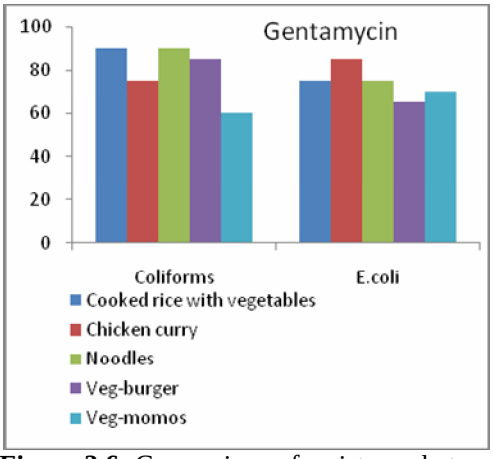


Figure 3.6: Comparison of resistance between *Coliforms* and *E.coli* for Gentamycin

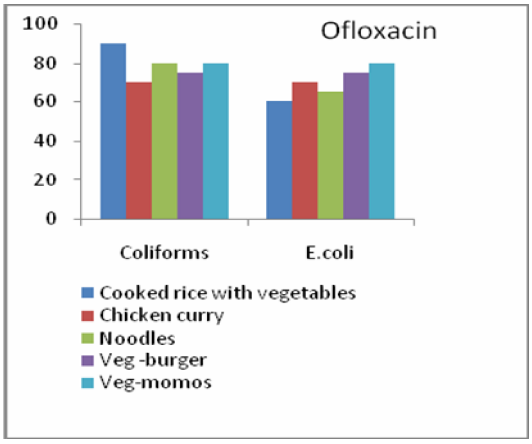


Figure 3.12: Comparison of resistance between *Coliforms* and *E.coli* for Ofloxacin

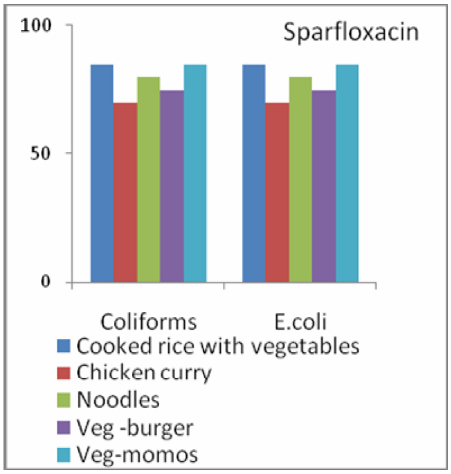


Figure 3.8 Comparison of resistance between *Coliforms* and *E.coli* for Sparfloxacin

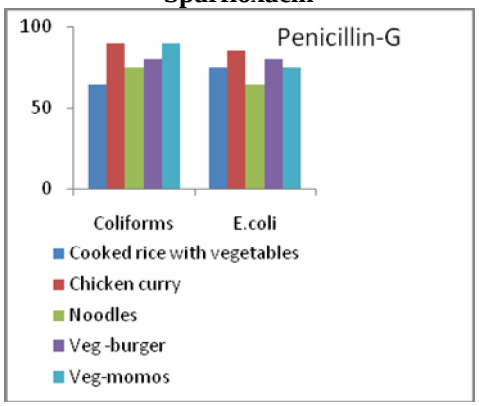


Figure 3.9 Comparison of resistance between *Coliforms* and *E.coli* for Penicillin-G

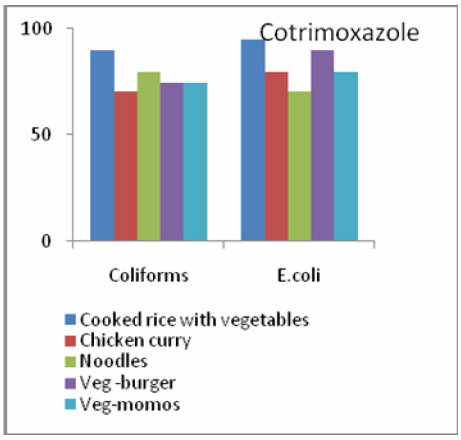


Figure3.10: Comparison of resistance between *Coli forms* and *E.coli* for Cotrimoxazole

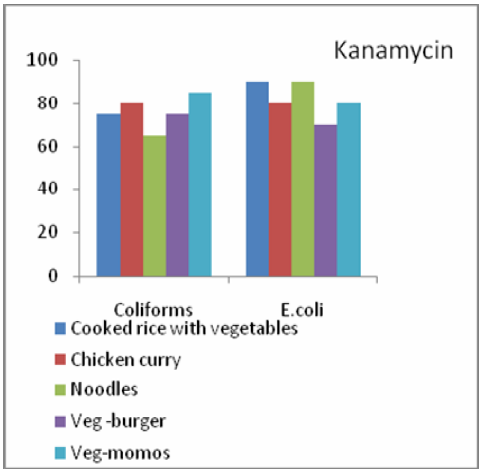


Figure 3.11: Comparison of resistance between *Coliforms* and *E.coli* for Kanamycin

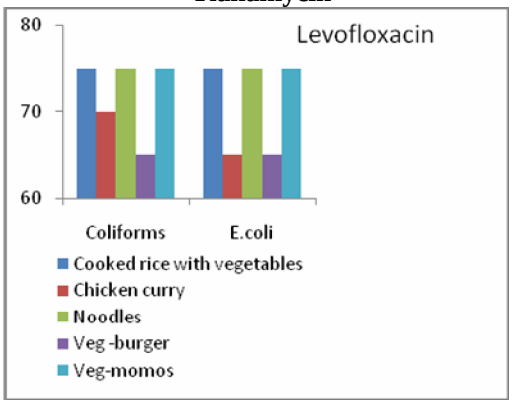


Figure 3.12 Comparison of resistance between *Coliforms* and *E.coli* for Levofloxacin

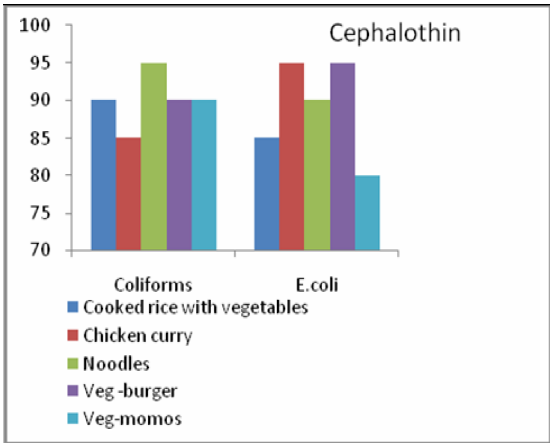


Figure3.13: Comparison of resistance between *Coli forms* and *E.coli* for Cephalothin

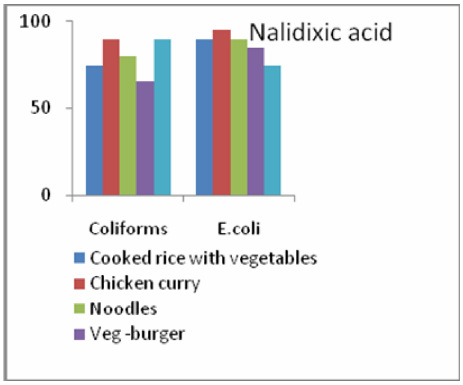


Figure 3.14: Comparison of resistance between *Coliforms* and *E.coli* for Nalidixic acid

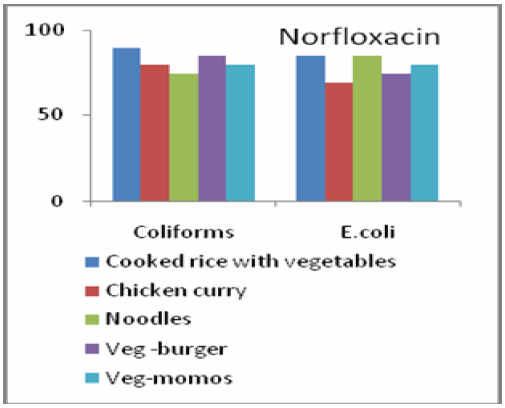


Figure3.15: Comparison of resistance between *Coliforms* and *E.coli* for Norfloxacin

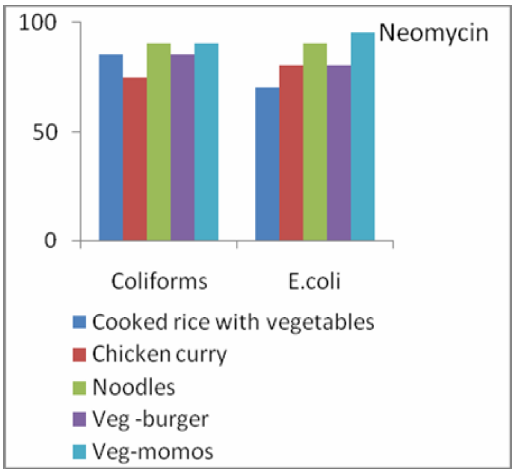


Figure 3.16: Comparison of resistance between *Coliforms* and *E.coli* for Neomycin

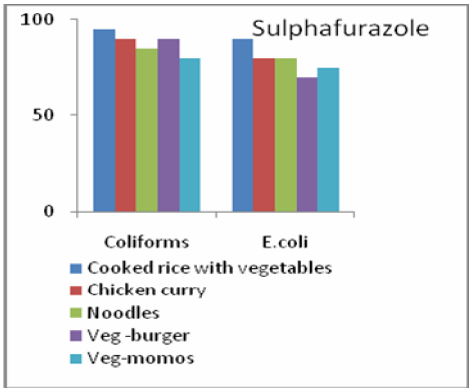


Figure 3.17: Comparison of resistance between *Coliforms* and *E.coli* for Sulphafurazole

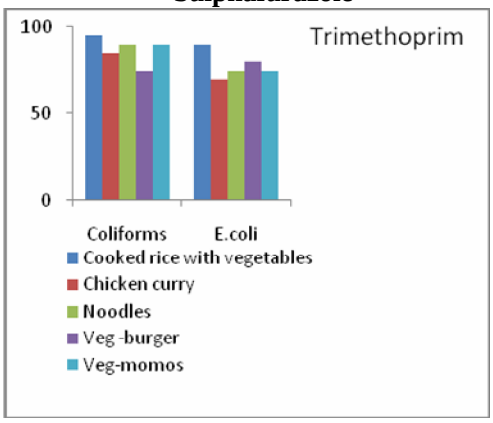


Figure3.18: Comparison of resistance between *Coliforms* and *E.coli* for Trimethoprim

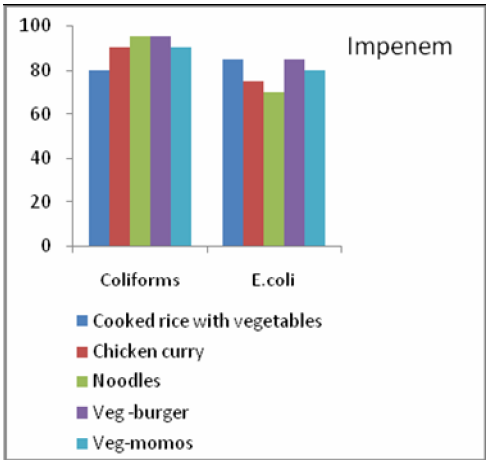


Figure 3.19: Comparison of resistance between *Coliforms* and *E.coli* for Impenem

Table7. A comparison of resistances levels to antibiotics between *Coliforms* and *E. coli* spp. isolated from different food categories.

Antibiotics	Food Item	*Coliforms*	No. of samples	*E.coli*	No. of samples	P-values
Amikacin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	60 90 75 90 85	20 20 20 20 20	75 75 70 65 70	20 20 20 20 20	0.00000
Ampicillin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	85 90 85 75 90	20 20 20 20 20	80 70 75 90 75	20 20 20 20 20	0.00000
Amoxicillin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	80 90 85 95 95	20 20 20 20 20	80 75 70 60 70	20 20 20 20 20	0.00000
Azithromycin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	80 85 90 90 95	20 20 20 20 20	90 60 75 85 90	20 20 20 20 20	0.00000
Ciprofloxacin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	85 75 90 95 90	20 20 20 20 20	90 85 90 75 85	20 20 20 20 20	0.00000
Erythromycin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	75 80 70 90 80	20 20 20 20 20	80 90 80 90 70	20 20 20 20 20	0.00000
Clindamycin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	80 70 75 85 85	20 20 20 20 20	60 75 85 90 90	20 20 20 20 20	0.00000
Tetrracycline	cooked Rice with vegetables chicken curry Noodles	90 90 70 80	20 20 20 20	75 80 65 70	20 20 20 20	0.00000

	Veg Burger Veg Momos	80	20	85	20	
Gentamycin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	90 75 90 85 60	20 20 20 20 20	75 85 75 65 70	20 20 20 20 20	0.00000
Oxycillin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	80 75 90 70 75	20 20 20 20 20	90 95 80 70 80	20 20 20 20 20	0.00000
Vancomycin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	80 75 85 90 80	20 20 20 20 20	85 75 85 80 70	20 20 20 20 20	0.00000
Oloxacin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	90 70 80 75 80	20 20 20 20 20	60 70 65 75 80	20 20 20 20 20	0.00000
Sparfloxacin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	85 70 80 75 85	20 20 20 20 20	70 85 80 65 75	20 20 20 20 20	0.00000
Penicillin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	65 90 75 80 90	20 20 20 20 20	75 85 65 80 75	20 20 20 20 20	0.00000
Cotrimoxazole	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	90 70 80 75 75	20 20 20 20 20	95 80 70 90 80	20 20 20 20 20	0.00000
Kanamycin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	75 80 65 75 85	20 20 20 20 20	90 80 90 70 80	20 20 20 20 20	0.00000

Levofloxacin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	75 70 75 65 75	20 20 20 20 20	75 65 75 70 75	20 20 20 20 20	0.00000
Cephalothin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	90 85 95 90 90	20 20 20 20 20	85 95 90 95 80	20 20 20 20 20	0.00000
Nalidixic Acid	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	75 90 80 65 90	20 20 20 20 20	90 95 90 85 75	20 20 20 20 20	0.00000
Norfloxacin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	90 80 75 85 90	20 20 20 20 20	85 70 85 75 80	20 20 20 20 20	0.00000
Neomycin	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	85 75 90 85 90	20 20 20 20 20	70 80 90 80 95	20 20 20 20 20	0.00000
sulfafurazole	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	95 90 85 90 80	20 20 20 20 20	90 80 80 70 75	20 20 20 20 20	0.00000
Trimethoprim	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	95 85 90 75 90	20 20 20 20 20	90 70 75 80 75	20 20 20 20 20	0.00000
Impenem	cooked Rice with vegetables chicken curry Noodles Veg Burger Veg Momos	80 90 95 95 90	20 20 20 20 20	85 75 70 85 80	20 20 20 20 20	0.00000

humans. Recent studies have shown that enteric bacteria isolates from humans and animals had the same antimicrobial resistance determinants (Johnson et al.,2007; Skov et al., 2007). It is therefore possible that the high resistance seen in this study is indeed due to the selective pressure exerted by the use of antibiotics in the management of bacterial infections in humans in the country. Significant differences were observed between bacteria isolated from different ready to eat food samples i.e chicken curry, cooked rice,veg-burger, veg-momos etc.collected from local markets of india in the present study. This was seen both for *Coliforms* isolates and *E. coli* isolates. This may be attributed to the poor handling of raw material, poor hygienic conditions of food preparation area, resistant survivors in food and different types of antibiotics used for treatment of different diseases. This correlates with the observation made in this study of high resistance seen in some of the commonly used antibiotics. The use of antibiotics, whether for prophylaxis or chemotherapy, does not only affect the pathogenic bacteria but also the commensal bacteria. This maintains a pool of resistant bacteria with a pool of resistance genes in the population which further contributes to the general increase and dissemination of bacterial resistance and can be a source of resistance genes for pathogens (Abatih et al., 2009; Blake et al., 2003). The comparison of resistance between Gram negative resistance indicator *Coliforms* and *E. coli* further confirmed that all commensal bacteria are affected. When particular organisms become resistant to a particular antimicrobial agent depends on many factors which may include extent of exposure to antibiotics, (Parry, 1989).Today, the emergence of bacterial strains which display resistance to a variety of drugs is a major cause of failure of treatment of infections worldwide and a serious concern to animal and public health (Vergidis and Falagas, 2008). Multi-drug resistance was demonstrated in both *Coliforms* and *E. coli* isolates with 97% and 83%, respectively, in the present study. Moreover, some isolates were resistant to a large number of drugs; for example, Moreover 80% of the *Coliforms* isolates were resistant to selected all twenty four drugs and about 85% were resistant to 23 antibiotics. Moreover 78% of *E. coli* isolates were resistant to 21, 72% were resistant to 23 and 70% were resistant to all 24 drugs. *E.coli* is commonly found in human and animal intestinal tracts and, as a result of fecal contamination or contamination during raw food handling such as raw chicken, poor sanitation, unhygienic preparation, using rotten raw material, is often found in soil, water, and foods. As indicated from the present study the appearance and rise of bacterial resistance to commonly used antibiotics, programs for monitoring resistance should be prepared and implemented in countries for the purpose of protecting the health of humans as well as animals. These programs should monitor indicator bacteria such as *Escherichia coli.* Environmentally, antibiotic resistance spreads as bacteria themselves move from place to place; bacteria can travel via food, water and air as in the present study demonstrated that bacteria might have contact with food via air, Water or even with unwashed hands and utensils. People can pass the resistant bacteria to others; for example, by coughing or contact with unwashed hands.

As in present study of resistant to antibiotic, one part of the problem is that bacteria are remarkably resilient and have developed several ways to resist

antibiotics and other antimicrobial drugs. Another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and medicine and in agriculture. Resistance observed may be likely due to acquisition of already resistant bacteria from the environment, actions should be taken immediately. The high level of multi- drug resistance to clinically important antibiotics in commensal indicator bacteria indicated that bacteria of food origin can be significant reservoir of resistant gene pool that may spread resistance to pathogens in humans and has public health implications.

The present study demonstrated the microorganisms present in food that have become resistant to antimicrobial agents, play an important role in the development and persistence of disease in persons consuming it. Once antimicrobials considered second or third line drugs are no longer able to inhibit or kill their intended targets, pathogens will gain an advantage and will be able to infect multiple hosts free of inhibition. The development of multidrug resistance is a danger to public health especially in the case of immune-compromised individuals, whose only defense against pathogens is provided by antimicrobials.

The present study can help in informing the public of how resistant organisms develop and are transmitted from contaminated food to person is vital to lowering the amount of antimicrobial resistance observed. The purpose of this study was to determine the extent of microbial contamination and antimicrobial resistance throughout a typical local market of ready to eat food. Determining the numbers of antibiotic resistant bacteria in ready to eat food may provide insight into the continued development of antimicrobial resistance, and may lead to changes in consumer health.

An action as simple as hand washing ,proper cleaning of food preparing utensils, hygienic cooking, use of fresh and clean raw material plays a vital role in reducing the transmission of diseases spreading through contaminated food ,which can be helpful in maintaining good health. It is important to inform the public of the pathogens that surround them, the significance of antimicrobial resistance, and how to prevent the development and transmission of resistant pathogens.

Our study has demonstrated that there is a high level of antimicrobial resistance within the market of ready to eat samples and these food can be considered as reservoir for multidrug resistant bacteria. The observation of ciprofloxacin resistance 90% for *coliforms* in case of cooked rice and noodles and 90% for *E.co*li in case of noodles and veg-momos throughout the present study demonstrates a inefficient treatment method for multiple illnesses and it may soon become widely ineffective in the treatment of multiple bacterial pathogens. The high levels of multidrug resistance in isolates of food samples observed in the present study demonstrate that the development of resistance and widespread transmission of resistant pathogens is already occurring. Therefore, there should be increased awareness of the significance of antimicrobial resistance and methods in the prevention of pathogen transmission. The Knowledge of antibiotic resistance of bacteria in food and their resistance mechanisms would provide critical information about antibiotic problems and the information required to formulate strategies for containment of the problem of antimicrobial resistance and food

safety. It suggested that other factors like the density of resistant bacteria and presence of a favorable environment may contribute towards increased resistance. Present study proved that multidrug-resistant bacteria travel not only locally but also globally, with newly introduced resistant strains spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally and they need to be monitored closely because of their implications for public health.

The present study can be proved helpful to find out the reasons of contamination of ready to eat food and to understand the mechanism of multi-drug resistance of isolates of these contaminated foods. The number of microbes present in a manufacture product, or in an environment is often very important. They are considered to relate to the sanitary conditions maintained in the processing area, to the keeping quality of the product

The present study may offer comprehensive solution to the problem of antibiotic resistance development in the ready to eat food. We expect that the results of this study will be helpful in creating basis for further scientific enquiry regarding the presence of emerging multidrug resistant contaminants in food and its impact on public health.

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