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RESEARCH ARTICLE

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Microbiological Evaluation and Antibiotic Susceptibility Pattern of Bacterial Isolates Associated with Some Contaminated Edible Fruits and Vegetables

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ABSTRACT

Background: The present study was undertaken to study the percentage of pathogenic bacteria present in different fruit and vegetable samples available in the market in and around Kalaburagi, Karnataka, South India.

Methods: A total of 940 different samples were collected from Kalaburagi, out of which 390 (23.84%) were fruits and 550 (35.27%) were vegetables. The pathogenic bacteria were isolated, by enrichment culture method using peptone water. The bacterial isolates were identified by convention microbial identification procedures.

Result: Antibiotic resistant testing by disc diffusion method performed for *E.coli, Salmonella* and *Shigella.* Among the pathogens, *E. coli* (86.50%) of the isolates were resistant to Nalidixic acid while Imipenem and Trimethoprim-Sulfamethoxazole has the lowest resistance (19.84%), *Salmonella* (86.66%) isolates were resistant to Ciprofloxacin while Norfloxacin has the lowest resistance (4.76%) and *Shigella* (80.35%) isolates were resistant to Vancomycin while Amoxicillin has the lowest resistant (3.57%). Multiple drug resistance (MDR) was seen in *E. coli* at (38.88%)*, Salmonella* at (26.66%) and *Shigella* at (10.71%) accordingly. The study therefore shown that *E.coli, Salmonella* and *Shigella* occur in Fruits and vegetables which collected from market place in Kalaburagi, Karnataka India, As per the results, adequate precaution should be taken while handles these fruits and vegetables. The antimicrobial resistance pattern shown by the isolates is an indication that adequate measurement needs to be taken to regulate the drug use in both humans and animals in order to minimize the risk of increasing antimicrobial resistance.

Key words: Antibiogram fruits and vegetables*, E.coli, Salmonella, Shigella*.

INTRODUCTION

Fresh fruits and vegetables provide mankind with an abundance of benefits. They give the necessary vitamins, fats, minerals and oil to the body in the right proportion for human growth and development. These play an important role in health and have the ability to prevent many diseases such as heart disease, cancers and diabetes (Liu, 2003; Pandey and Rizvi 2009). Fruits and vegetables play a major role worldwide and have more attention in Kalaburagi of south India as a major diet probably due to its health benefits, low cost availability and active promotion of fresh Fruits and vegetables as a part of a healthy diet (Mallikarjun and Gaddad, 2016; Mallikarjun and Gaddad, 2016).

Over the years, there has been increase in the need to identify and isolate the microorganisms associated with the spoilage as a way of finding a means of controlling it (Akinyele and Akinkunmi, 2012). Fresh fruits and vegetables are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with this consumption. Despite the benefits derived from fruits and vegetables, they are major sources of food borne diseases (Robinson *et al.,* 2000).

Recent improvement of the standard of life in India is associated with diversification of food needs including availability of fresh food producedeverywhere throughoutthe year. All these require proper transport and storage facility.

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India is the second largest producer of fruits and vegetables in the world and accounts for about 15% of the world's total production. Fruits and vegetables plays key role in growth and development of body and in prevention of many diseases due to high nutritive value. The majority of diseases associated with fresh fruits and vegetables are primarily those transmitted by the faecal-oral route and therefore, a result of contamination at some point in the process (De Rover, 1988).

Incidences of disease associated with bacteria have been attributed mainly to *Escherichia coli*, *Salmonella*, *Listeria*, *Shigella*, *Bacillus*, *Aeromonas*, *Clostridium* and *Campylobacter* (Beuchat, 1995). Fruits and vegetable samples examined in Kalaburagi were contaminated with *Salmonella, shigella and E. coli* (Mallikarjun and Gaddad, 2016). A Study conducted in India on salad vegetables, revealed the presence of *E. coli, Enterobacteraerogenes, Pseudomonas* spp., *S. aureus, Salmonella* spp. and *Shigella* spp., (Fain, 1996). Further, WHO reports that leafy green vegetables are of great concern in terms of microbiological hazards (WHO, 2008).

As seen above *Salmonella* and *Shigella* species have been associated with food borne diseases with significant morbidity and mortality rate in people of all ages. In the U.S. between 1995 and 1998, there were 9 outbreaks of food borne illnesses caused by *Salmonella, E. coli* 0157:H7 due to consumption of fresh vegetable sprouts (Martin *et al*., 2003). There have been an increasing number of outbreaks of food poisoning linked to the consumption of vegetables and fruit in Westernised countries. The number of reported outbreaks in the USA more than doubled from the period 1973-1987 to the period 1988-1991 (Tauxe *et al*., 1997). Recently, association between fresh vegetables and outbreaks of food borne infections has led to a greater concern about contamination of vegetables with a pathogenic bacterium like *E. coli, Vibrio, Salmonella,* Norovirus, *Shigella, Listeria etc* (Mahima *et al.*, 2013).

These fruits and vegetables can also get contaminated with pathogenic microbes during harvesting, transportation, storage and retailers handling. These are the wide range sources of microbial contamination to different varieties of fruits and vegetables (Ray and Bhunia*,* 2007).

Moreover, the availability of potable water for proper washing of fruits and vegetables is also lacking in different areas. As a result of which dirty or contaminated water is used for washing, which could lead to further increasing the microbial load on these vegetables which some people buy and eat without further washing. Previous report revealed that *Listeria monocytogenes* and *Salmonella* spp. have been isolated from raw vegetables making them potential threat to consumers (Biniam and Moggessie 2010).

Most of the reported outbreaks of gastrointestinal disease linked to the fresh produce have been associated with bacterial contamination, particularly with members of the *Enterobacteriaceae*family (Hamilton *et al*., 2006). Occasional reports of multistate outbreaks of *Salmonellosis* in the United States associated with contaminated fresh fruits and vegetables have coincided with increased consumption of fresh produce in recent years due to changing consumer preferences, greater selections, wider distribution and year-round availability (Hedberg *et al.,* 1994).

Excessive and misuse of antimicrobials to control pathogens in animals and crops led to antibiotic resistance and transfer to human through contaminated food. These are consistent with earlier reports which shown that antibiotic resistant bacteria also may be ingested with vegetables (Kilonzo *et al.,* 2009).Vegetables such as corn, green onion and cabbage absorb antibiotics when grown in soil fertilized with livestock antibiotics contaminated manure (Kumar *et al.,* 2005).

Microbiological studies from many developing countries carried out on street vended food articles. These studies have revealed that a high bacteria count on the edible fruits and vegetables. *Salmonella* species, *S.aureus* and members of the family, Enterobacteriaceae were common pathogens found in such food items (Bryan *et al.,* 1997). Enteric pathogens such as *E. coli* and *Salmonella* are among the greatest concerns during food related outbreaks (Buck *et al.*, 2003).

Food contamination with antibiotic resistant bacteria can be a major threat to public health, as 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 pathogens has increased during recent decades (Davis *et al.,* 1999). Thus the present study was under taken for antibiotic susceptibility of pathogenic bacteria such as *E.coli spp.*, *Salmonella spp.* and *Shigella spp.,* from fruits and vegetables from vending market at Kalaburagi (Karnataka, India).

MATERIALS AND METHODS

Sample collection

A total 940 mixed fruits and vegetable were collected irrespective of verity from different super market in Kalaburagi, Kanni markets and Timmapuri (Karnataka) during October 2013- November 2015.Among the 940 samples, 390 were belongs to fruits and 550 were belongs to vegetables. The fruits and vegetables samples were collected in sterile polythene bags and transported to the laboratory within 2 hours of collection under aseptic conditions.

Bacterial isolation and characterization

From each collected vegetable and fruit sample, 0.5g was aseptically weighed and 5ml of peptone water was added and keep it for 6hours in incubator shaker at 37^0C. After 6 hour, samples were enriched and then streaked on specific media like XLD, MacConkey and EMBagar the isolates were further identified on the basis of cultural, morphological and biochemical tests (Whitman, 2015, Mallikarjun and Gundappa 2016). The dehydrated readymade media were obtained from Himedia Limited, Mumbai India. The various morphological characteristics of isolates *viz*., colony morphological (Colour, Shape,Arrangement and Gram staining) were observed and analysed. The various biochemical tests like Indole test, Methyl Red, Voges-Proskauer test, Citrate Utilization test and triple sugar iron test (TSI) were done, for identification of isolates as per the procedure described earlier (Poonam, 2013).

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolated *E.coli, Salmonella* and *Shigella* against the antimicrobials was determined by Kirby-Bauer disc diffusion method in Mueller-Hinton agar (Bauer *et al*., 1996). The inoculums were prepared at a density adjusted to a 0.5 McFarland turbidity standard solution.Three to five well isolated colonies of isolated bacteriawere transferred into 5mL Brain Heart Infusion broth (BHIB, Oxoid) and incubated at 37°C for 18 to 24 hr. The overnight broth culture was diluted using sterile distilled water to a turbidity equivalent to 0.5 McFarland standard (approximately 10^8 cfu/ml) and inoculated onto the entire surface of a dried Mueller-Hinton Agar (MHA, Oxoid) plate creating a lawn of the culture. The inoculated Muller Hinton Agar plates were allowed to dry at room temperature before placing the antibiotic discs followed by incubation. After incubation for 24 hours at 37°C, the diameter (in mm) of the zone around each disk was measured and interpreted in accordance with the Clinical and Laboratory Standards guidelines as described previously (CLSI, 2011).

The antibiotic resistant of bacterial isolates were assessed against the following antiboitucs, Ampicillin (10µg), Gentamicin (10µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Norfloxacin(30µg), Imipenem (30µg), Vancomycin (30µg), Trimethoprim-Sulfamethoxazole (25µg), Piperacillin (10µg), Amoxicillin (10µg), Chloramphenicol (10µg), Nalidixic acid (30µg).

Minimum inhibition concentration (MIC) assay

Minimum concentration of each antibiotic inhibitory to the growth of 50 per cent (MIC_{50}) and 90 per cent (MIC_{90}) of each isolates were determined on MHA in a 90 mm plate. The agar contained concentration ranges of the antibiotics prepared by two-fold serial dilution according to the National Committee for Clinical Laboratory Standards (NCCLS, 1999).

RESULTS AND DISCUSSION

A total 940 samples (both fruits and vegetables) were collected from supermarket in and aroundKalaburagi (Karnataka, India) of which 390 were (23.84%) fruits of different verities and 550 (35.27%) were mixture of different types of vegetables. Among 390 fruitsand 550 vegetable samples, 93 (23.84%) and 194 (35.27%) were showed cultural positivity for the pathogenic bacteria respectively (Table 1). The number of isolated microbial colonies from different fruits and vegetables were represented in (Table 1) and (Fig 1 and 2).

Further, out of 93 positive samples, it is identified that 36.56% (34 out of 93) of *Salmonella spp.,* 7.53% (7 out of 93) of *Shigella* spp. *and* 55.91% (52 out of 93) of *E. coli* were isolated from fruit samples. Similarly, 35.60% of *Salmonella spp.,* 25.26% of *Shigella spp. and* 38.14% *E. coli* was isolated from vegetable samples (Table 1). This clearly indicates that high level of pathogenic bacteria contamination found in food samples.Among the three pathogenic bacteria, the *E. coli* was found to be more prevalent (13.49%) when compared to that of *Salmonella*(11.24%) and *Shigella* (5.99%)in the present study.

The antibiotic sensitivity for the three bacterial isolates was assessed using different antibiotics. High resistance for *E. coli* spp. *Salmonella* spp. and *Shigella* spp. to nalidixic acid (86.50%), ciprofloxacin (86.66%) and vancomycin (80.35%) was observed respectively . However, respond *E. coli, Salmonella* and *Shigella* spp., showed a resistance to

Fig 1: Plate showing the Bacterial isolates (A, *E.coli,* B, *Salmonella*andC, *Shigellaspp.,* on XLD agar media).

Fig 2: Plate showing the differential Biochemical test (Triple Sugar Iron test) for Bacterial isolates (*E.coli, Salmonella* and *Shigellaspp.,*)

Table 1: Pathogenic bacteria isolates from fruits and vegetables.

Samples	Samples collected	Bacterial isolates (+ culture)	Number of positive samples			
			Salmonella spp.	*Shigella* spp.	*E. coli* spp.	Total
Fruits	390	93	34/93(36.55%)	7/93(7.52%)	52/93(55.91%)	93/390(23.84%)
Vegetables	550	194	71/550(36.59%)	49/550(25.25%)	74/550(38.14%)	194/550(35.27%)
Total	940	287	105/940(36.58%)	56/940(19.51%)	126/940(43.90%)	287/940(30.53%)

Table 2: Antibiogram and prevalence of resistance of *E.coli, Salmonella* and*Shigella* isolates isolated from fruits and vegetables samples.

Antibiotics	*E. coli* spp.			*Salmonella* spp.			*Shigella* spp.		
	R	S	I	R	S	I	R	S	I
AMP-10µg	58 (46.03%)	42(33.33%)	26(20.63%)	78(74.28%)	15(14.28%)	12(11.42%)	34(60.71%)	19(33.92%)	3(5.35%)
GEN-10µg	41 (32.53%)	39(30.95%)	46(12.69%)	10(9.52%)	53(50.47%)	42(40%)	2(3.57%)	30(53.57%)	24(42.85%)
CEF-30µg	87 (69.04%)	10(7.93%)	29(22.48%)	47(44.76%)	25(23.80%)	33(31.42%)	7(12.5%)	29(51.78%)	20(35.71%)
CIP-5µg	68 (53.93%)	35(27.77%)	23(1825%)	91(86.66%)	15(14.28%)	-	10(17.85%)	35(62.5%)	8(35%)
NB-30µg	46 (36.50%)	60(47.61%)	20(15.87%)	5(4.76%)	48(45.71%)	52(49.52%)	15(26.78%)	27(48.21%)	14(25%)
IPM- 30µg	25 (19.84%)	18(14.28%)	83(65.87%)	41(39.04%)	53(50.47%)	11(10.47%)	3(5.35%)	42(75%)	11(19.64%)
VAN-30µg	36 (28.57%)	45(35.71%)	45(35.71%)	28(26.66%)	39(37.14%)	38(36.19%)	45(80.35%)	7(12.5%)	4(7.14%)
TMP/SLU-25µg	25 (19.84%)	13(10.31%)	88(69.84%)	9(8.57%)	63(60%)	33(31.42%)	4(7.14%)	42(75%)	10(17.85%)
PIP- 10µg	65 (51.58%)	35(27.77%)	26(20.63)	15(14.28%)	46(43.80%)	44(41.90%)	0(%)	56(100%)	-
AMO-10µg	45 (35.71%)	27(21.42%)	54(42.85%)	4(3.80%)	75(71.42%)	26(24.76%)	2(3.57%)	48(85.71%)	6(10.71%)
CHL-10µg	30 (23.80%)	55(43.65%)	41(32.53%)	83(79.04%)	13(12.38%)	9(8.57%)	0(%)	56(100%)	-
NALI-30µg	109 (86.50%)	17(13.49%)	-	26(24.76%)	62(62%)	17(16.19%)	8(14.28%)	48(85.71%)	-

R-resistance, S-susceptible, I-intermediate.

(AMP-Ampicillin, GEN-Gentamycin, CEF-Ceftriaxone, CIP-Ciprofloxacin, NB-Norfloxacin, IPM-Imipenem, VAN-Vancomycin, TMP/SLU-Trimethoprim-Sulfamethoxazole, PIP- Piperacillin, AMO-Amoxicillin, CHL-Chloramphenicol, NALI-Nalidixic acid).

Fig 3a: Antibiotic Sensitivity Pattern of Bacterial isolates.

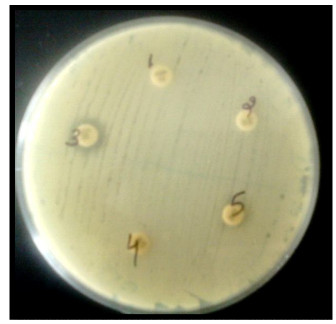

Fig 3b: Antibiotic Resistance Pattern of Bacterial isolates.

different antibiotics at different percentage level (Table 2, 3) and(Fig 3a and b).

We further evaluated the MIC valuesfor *E. coli, Salmonella* and *Shigella* isolates. Among the three isolates, *E.coli* has shown highestMIC value to gentamicin (4-16µg/ml). Astonishingly, *Salmonella* has the higher MIC value toceftriaxone (8-64µg/ml) andimipenem,(4-16µg/ml). Similarly, *Shigella* isolates were shownhighest toampicillin (8-32µg/ml) and nalidixic acid (13-32 µg/ml),when compare to that of other antibiotics (Table 4).

This study provides clear evidence of contamination of fresh Fruits and vegetables from common food-borne pathogens including *E.coli, Salmonella* and *Shigella* spp *are* important food-borne pathogen and its prevalence in fresh food poses a threat to human. The increase in demand and consumption of fruits and vegetables has resulted in a rise in food-borne related illnesses and outbreaks. Fresh fruits and vegetable have been reported to anchor potential food-borne pathogens including *E.coli*, *Salmonella and Shigella* spp., (Mallikarjun and Gundappa, 2016; Harris *et al*., 2003; CDC, 2009). *E. coli* has showed (54.55%) to Nalidixic acid, similar Ampicillin and gentamicin (36.36 %,) Amoxicillin and ciprofloxacin were only (9.09%) [29], *Salmonella* Showed (42%) to Ampicillin and *Shigella (*79%) (Biniam and Mogessie, 2010). The previous study was conducted on microbiological contamination in the Dhaka metropolis Bangladesh mainly for*E.coli*. it was observed that the % of ABR for trimethoprim-sulfamethoxazole combination as (46.03%) compared to the past results of % ABR for same combination on *E.coli*. Similarly, it was found a steep decline in % of ABR for amoxicillin from (46.2%) (past research) to (35.71%) (as per our results) similarly, indicating rise in microbial resistance to this antibiotic. However, there is a

Table 3: Multiple drug resistance of *E.coli*, *Salmonella* and *Shigella isolates* isolated from fruits and vegetables.

Number of resistant *E. coli* isolates(n=49)	**Drugs resisted**
7	AMP/CEF/IPM
3	AMP/ NB/VAN/ AMO/
9	AMP/GEN/CEF/NALI
4	AMP/CEF/NB/TMP-SLU/AMO
6	AMP/CIP/NB/VAN/PIP/NALI
8	IPM/VAN/TMP-SLU/PIP/CHL/NALI
12	AMP/GEN/CEF/CIP/VAN/PIP/CHL
Number of resistant *Salmonella* isolates(n=28)	**Drugs resisted**
8	AMP/CIP/ AMO
1	CIP/ NB/TMP-SLU
4	GEN/CIP/IPM/CHL
2	GEN/CIP/CHL/NALI
6	AMP/GEN/CIP/PIP /NALI
7	AMP/CEF/CIP/IPM/VAN/CHL
Number of resistant *Shigella* isolates(n=6)	**Drugs resisted**
1	CIP/VAN/NALI
1	AMP/CEF/VAN
1	AMP/VAN/NALI
1	AMP/NB/ IPM/VAN
2	AMP/ GEN/CIP/ NB/VAN/NALI

Multiple drug resistance (MDR) was seen in *E. coli* 49 (38.88%), *Salmonella* 28 (26.66%)and*Shigella* 6 (10.71%), isolates respectively.

Table 4: Minimum inhibitory concentrations (MICs) of antimicrobial agents for *E.coli, Salmonella* and *Shigella*spp.

Antimicrobial	*E.coli*			*Salmonella*			*Shigella*		
drugs	Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Ampicillin	8-32	16	32	8-32	16	32	8-32	16	64
Gentamicin	4-16	8	64	4-16	8	32	4-16	8	32
Ceftriaxone	8-16	16	32	8-64	16	64	8-64	16	32
Ciprofloxacin	1-2	2	4	1-4	3	9	1-4	4	8
Norfloxacin	4-8	8	16	8-16	16	32	8-16	5	8
Imipenem	1-4	2	4	4-16	16	64	4-16	8	16
Vancomycin	4-32	8	16	2-16	4	8	2-16	4	16
Trimethoprim-Sulfamethoxazole	2/38	4/76	8/156	2/38-4/76	4/76	8/152	2/38-4/76	4/76	8/152
Piperacillin	4-16	8	16	4-16	16	21	8-16	16	32
Amoxycillin	4-8	8/16	32	13-32	16	32	16-32	-	-
Chloramphenicol	2-8	4	8	8-16	16	32	8-16	6	8
Nalidixic acid	8-32	16	32	13-32	8	16	13-32	16	64

'steep rise' in % of ABR for antibiotics such as impenem (from 0% to 19.84%), gentamicin (from 0% to 32.53%) and ceftriaxone (from 0% to 69.04%) confirmed by these results. Therefore, our results indicating that the microbe *i.e*. E coli have acquired 'less antibiotic resistance to the above series of antibiotics' (Khatib *et al*., 2015).

A number of surveys have attempted to detect *E. coli* O157:H7 in fresh fruits and vegetables, in a study that included 3,200 vegetables, no O157:H7 positive sample was detected and in another survey of 890 fruits and vegetables, this pathogen could not be found either (Johannessen *et al.,* 2000). *Salmonella* resistance against ampicillin (55.5%-31.4%), gentamicin (0%-%), ciprofloxacin (6.5%-13.7%), vancomycin (100%-100%), trimethoprim-sulfamethoxazole (51%-51%), chloramphenicol (3.2%-0%) and nalidixic acid (0%-5.9%), against isolates from cabbage and spinach respectively (Isoken, 2015). *Salmonella*spp., showed resistance against ampicillin (100%), gentamicin (0%), ciprofloxacin (50%), trimethoprim-sulfamethoxazole (6.3%), Amoxycillin (81.3%), chloramphenicol (6.3%) and nalidixic acid (12.5%) (Najwa *et al.,* 2015). *E.coli, Salmonella* and *Shigella* spp., showed resistance against, gentamicin (60%), ciprofloxacin (0%), Norfloxacin (10%), amoxicillin (70%), chloramphenicol (30%), *Salmonella* gentamicin (7.7%), ciprofloxacin (0%), Norfloxacin (0%), amoxicillin (77%), chloramphenicol (92.3%) and *Shigella* showed resistance against gentamicin (0%), ciprofloxacin (0%), Norfloxacin (0%), amoxicillin (30%), chloramphenicol (0%), (Poonam, 2013). *E.coli, Salmonella* and *Shigella* spp., showed resistance against ampicillin (25%), ceftriaxone (67%), ciprofloxacin (60%), trimethoprim-sulfamethoxazole (20%), piperacillin (30%), amoxicillin (33%), chloramphenicol (45%), nalidixic acid (85%), *Salmonella* ampicillin (100%), gentamicin (0%), ceftriaxone (0%), ciprofloxacin (100%), trimethoprim-sulfamethoxazole(0%), amoxicillin (0%), chloramphenicol (0%), nalidixic acid (0%),*Shigella* ampicillin (100%), gentamicin (0%), ceftriaxone (0%), ciprofloxacin (0%), vancomycin (100%),trimethoprim-sulfamethoxazole (0%),amoxicillin (0%), chloramphenicol (100%), nalidixic acid (100%), (Nour *et al.,* 2013). *E. coli* has been used as the reference indicator for faecal contamination and a number of surveys have reported its isolation from fresh fruits and vegetables (Jay, 2000). However, in the present study *E. coli* was found in (26.8%) of conventional fresh vegetables in Lebanon, this result is consistent with the prevalence of (25%) this bacterium in ready-to-use lettuce (Soriano *et al.*, 2000).

The previous microbiological studies have illustrated to evaluate microbiological safety in various regions of Vidarbha through various 'antibiotic-microbial assays'. In the present study, it is been analyzed and compared antibiotic resistance (ABR) property in microbes such as '*E .coli*, *Salmonella* and *Shigella*' using several antibiotics. It was found that the antibiotic resistance of *E.coli* to norfloxacin is almost (36.5%) when compared to the past studies performed by others for studying same antibiotic resistance using same norfloxacin, where it was observed that only(10%)of antibiotic resistance in *E coli*. Similarly, previous reportshave got high antibiotic resistance for amoxicillin (70%)but it was observed the 'antibiotic resistance for same amoxicillin' lesser % of resistance (35.71%). Similarly, the other researchers have observed antibiotic resistance using gentamicin (60%)but we found lesser % of resistance (32.53%), where it was observeda huge variation in this antibiotic-resistance property for gentamicin. However, it was nearly ABR for chloromphenicol is (23.80%) that slightly lesser than other researcher's result for ABR, where other reportsgot (30%) whereas in present studyABR value (53.93%) forciprofloxacin compared to the past research results for same antibiotic, where ABR value was (0%). Here, it was clearly observedmore ABR for ciprofloxacin compared to the past results (Soriano *et al.*, 2000).

It was observed ABR for norfloxacin on *Shigella spp*., approx. (26.78%) compared tothe past results by other researchers where it was observed only 0% of ABR on same species for same antibiotic. Similarly, we have observed lesser percentageof ABR for amoxicillin approx. (3.57%) when compared tothe past researcher's result of ABR, which is nearly (70%) on same *Shigella species*. There is a huge variation in ABR results that could be due to the microbial ability to acquire resistance to amoxicillin antibiotic.It was found that the percentage of ABR for gentamicin increased from 0% (as per past research) to (3.57%) (as per our results) whereas the percentage of ABR is nearly same and unchanged (0% for both past and our present work) for chromamphenicol on same *Shigella species*. However, there is a 'steep rise' in percentage of ABR for ciprofloxacin from percentage (as per past research) to (17.85%) (as per results) (Soriano *et al.*, 2000).

We have significantly observed that the percentage of ABR for norfloxacin on *Salmonella spp*. steeply 'reduced' from 30.8% (past research) to (4.76%) (as per our results) whereas the percentage of ABR for ciprofloxacin steeply 'increased' from percentage (past research) to (44.76%) as per our results. Similarly, it is clearly observed with our results that the % of ABR for amoxicillin is (3.80%), where past researchers have got (69.2%) indicating 'high microbial resistance' to this antibiotic. In the meanwhile, the percentage of ABR results for gentamicin is (9.52%) whereas the past research has illustrated that the percentage ABR for same antibiotic is (84.6%). It was observed that percentage ABR for chloromphenicol on *Salmonella spp*. (79.40%) indicating a steep decline in ABR compared to the past results (92.3%). Therefore, the microbial resistance patterns for this species on these antibiotics are quiet variable (Soriano *et al.*, 2000; Poonam, 2013).

The present study provided valuable information regarding pathogenic bacteria species such as, *E. coli*, *Salmonella* and *Shigella* contamination of fruits or vegetables collected from various markets in Kalaburagi region. This data can facilitate the effective assessment of risk of contamination by *E. coli*, *Salmonella* and *Shigella* spp., in consumable non-processed fruits or vegetables in Kalaburagiregion suggesting potential public health hazard.

CONCLUSION

Antibiotic resistance patterns among the three major Enterobacteriaceae pathogens such as,*E. coli, Salmonella spp*. and *Shigella spp*., isolates from fruits, vegetable samples in Kalaburagi region has been evaluated and analyzed. Multi drug resistance (MDR) status has been found to be more prevalent predominantly among *E. coli* isolates than the other two pathogens thus calls for monitoring hygienic conditions in growing, processing and marketing of the consumable fruits and vegetables to prevent the risks of human health.

Conflict of interest statement

We declare that no conflict of interest.

Authors contribution

First author is responsible for carrying out the research work, data analysis and optimization of experimental work and Corresponding author is responsible for research planning executing and providing valuable inputs and in writing manuscript.

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