Plant Archives Vol. 22, No. 2, 2022 pp. 152-157 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2022.v22.no2.027

OCCURRENCE AND ANTIBIOTIC SUSCEPTIBILITY OF *ENTEROBACTERIACEAE* ISOLATED FROM SELECTED FRESH GREEN LEAFY VEGETABLES

Shreyasi Dubey, Pinki Saini*, Mazia Ahmed and Unaiza Iqbal
Centre of Food Technology, University of Allahabad, Prayagraj, India
*Email: pspinki55@gmail.com; Ph: 08299208092
(Date of Receiving : 02-12-2021; Date of Acceptance : 14-07-2022)

ABSTRACT

The study aims at accounting the prevalence of pathogenic bacteria within the family *Enterobacteriaceae* in green leafy vegetable samples. Eighty samples were collected from eight different locations of Prayagraj region. Isolation was done using selective plating according to ISO 21528-1:2004. Differentiation and characterization of isolates was based on their growth characteristics on specific culture media, their biochemical confirmatory tests and gram staining reactions. Total soluble proteins of the isolates were estimated by Biuret method. Antibiotic susceptibility of the isolates was tested against antibiotics including ampicillin, streptomycin and ciprofloxacin at different concentrations. A total of 54 isolates were obtained and identified as *Escherichia coli*, *Klebsiella planticola*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Yersinia enterocolitica*, *Salmonella typhi.* All the isolates were susceptible to ampicillin, streptomycin and ciprofloxacin at concentrations of 10, 50, 80 and 100 µg/ ml. About 78.5% isolates at 80 µg/ml of Ampicillin and 85.8% isolates at the concentration of 50 µg/ml of Streptomycin showed their zone of inhibition within the range of 10-15 mm diameter, while, 91.6% isolates showed their zone of inhibition within the range of 15-20 mm diameter at 10 µg/ml concentration of ciprofloxacin. The fresh green leafy vegetables sold in the local markets of Prayagraj showed presence of pathogenic bacteria belonging particularly to the family *Enterobacteriaceae*; indicating poor hygienic conditions as well as improper storage environment.
Keywords **:** Antibiotic, susceptibility, *Enterobacteriaceae,* Green leafy vegetables, Isolates Characterization.

Introduction

In the recent past, there has been an increased concern among the consumers for eating a healthy diet. The consumption of fresh vegetables has increased significantly because they are highly nutritious and healthy (Birt, 2017). But due to the consumption of fresh and uncooked foods, the chances of food-borne diseases have also increased many folds as these are considered unsafe to eat. Unsafe food is a major concern for billions of consumers all over the world. Hundreds of thousands of people become sick or even die every year due to the consumption of unsafe food. Therefore, production and distribution of safe food is the biggest challenge nowadays. Safe food ensures the health and well-being of an entire population. The practice to enhance the safe food production helps in improving the economy as well as health rate of the entire region. The supply of safe food depends both on science and impartial law enforcement.

During the last decades, the food-borne outbreaks due to the consumption of fresh produce have increased, most of them associated with bacterial (mainly to species belonging to the *Enterobacteriaceae* family) contamination (Wadamori *et al*., 2017; Martínez-Vaz *et al*., 2014; Lynch *et al*., 2009). Fresh vegetables contain non-pathogenic epiphytic bacteria and can be contaminated with pathogenic and non-pathogenic bacteria from human and animal sources at any step from the farm (growth and harvest) to the consumers (Kaczmarek *et al.,* 2019; Rajwar *et al*., 2016; Olaimat and Holley, 2012; Heaton and Jones 2008). An important source of contamination is the use of manure and irrigation water that can incorporate bacteria from animal and/or human origin to soil and vegetables (Araújo *et al.,* 2017; Marti *et al*., 2013; Chee-Sanford *et al*., 2009; Venglovsky *et al*., 2009).

It was previously described that fresh vegetables marketed in Valencia (Spain) harbor high-frequency non-pathogenic *Enterobacteriaceae* species belonging to genera *Enterobacter* (mainly *E. cloacae*) and *Klebsiella* and that *Enterobacteriaceae* species are also found in samples of organic produce (Falomir *et al.,* 2014; Falomir *et al.,* 2013; Rico *et al.,* 2013). They isolated and compared 195 bacterial cultures obtained from 230 fresh vegetable samples. Different kinds of vegetables carry different bacterial pathogens with the potential of carrying at least one resistant variety in all types of vegetables (depending on the type of vegetable, up to 3, 4, or 5 antibiotics). The results demonstrated the most frequent (60-77%) occurrence of *Enterobacter* (particularly *E. cloacae*) and *Klebsiella* (*K. pneumoniae* and *K. oxytoca*) spp. on freshly-cut vegetables, prepared-salads, and conventional food products. On the other hand, the most frequent bacterial species in organic products were *Pantoea agglomerans* (24%), followed by *Serratia marcescens* (about 16%), and 13% *E. cloacae*.

Liu *et al*. (2019) carried out a study on 528 vegetable samples, collected from the 53 different supermarkets present in 23 cities of China. The analysis and characterization of these vegetable samples were done for detecting *mcr* positive *Enterobacteriaceae*. They found that 19 samples (about

3.6%) were *mcr* positive and the highest rate of detection was found in pak choi, green pepper, and carrot. The main objective of this study therefore was to determine the prevalence and antibiotics resistance of *Enterobacteriaceae* spp. Isolated from fresh leafy vegetables being commercialized in Prayagraj district of Uttar Pradesh, India.

Materials and Methods

Sample Collection: Eighty vegetable samples (including cabbage, coriander leaves, dill, spinach, fenugreek leaves, and radish leaves) were collected from different localities (Civil lines, Katra, Teliarganj, Jhunsi, Colonelganj, Mumfordganj, Daraganj, and Allahpur) of Allahabad to conduct the study. The collected samples were immediately transported to the microbiology laboratory of the centre in ice-boxes.

Chemicals and Media: All chemicals, antibiotics, and bacteriological media were purchased from Hi-Media Laboratories, India. The procurement of reference cultures of *Salmonella enterica* (MTCC 3224), *E.coli* (MTCC 3221), and *Shigella flexenerii* (MTCC 1457) was done from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh, India.

Isolation of *Enterobacteriaceae* sp.: The enteric pathogens were isolated from the samples according to the ISO Standard; ISO 21528-1:2004. To isolate the *Enterobacteriaceae,* firstly the serial dilution of the sample was done and then it was pour-plated into Violet Red Bile Glucose Agar (VRBGA) medium. The petri dishes were kept at 37º C for 24 hours. Typical red to purple colonies were observed which were then purified by streaking on Nutrient Agar (NA) media.

Biochemical Characterization of Isolates: After sub-culturing the isolated colonies on NA media, various biochemical tests were performed for confirming the bacteria. The following tests were performed: Catalase test, Indole test, Lysine decarboxylation test, Nitrate reduction test, Vogues-Prauskauer test, Methyl red test, Citrate utilization test, Gelatin liquefaction test, Motility test, Triple sugar iron (TSI) agar test, Urease test, and Malonate utilization test (Hemraj *et al.,* 2013). All the biochemical tests were performed according to the procedures mentioned in Bergy's manual of detection (Holt *et al.,* 1994).

Carbohydrate Fermentation Test: The carbohydrate fermentation test was done on all the selected bacterial isolates. The fermentation pattern of different carbohydrates such as lactose, glucose, sucrose, mannitol, raffinose, xylose, and sorbitol was checked for identifying the isolates (Holt *et al.,* 1994; Bhardwaj *et al.,* 2012).

Antibiotic Susceptibility Test: The antibiotic susceptibility test was performed for determining the sensitivity of the selected isolates against the commonly used antibiotics. Kirby Bauer's method of disk diffusion was used for the analysis (Manikandan and Amsath, 2013). The selected isolates were first grown for about 12 hours in nutrient broth media at 37º C. The isolates were adjusted according to 0.5 McFarland standards, which is equal to $1.5x10^8$ CFU/ml. Different antibiotics such as ampicillin, ciprofloxacin, and streptomycin were used, each at a concentration of 100 µg/ml, 80 µg/ml, 50 µg/ml, and 10 µg/ml for the study. The grown isolates were taken from the nutrient broth and swabbed on the already-prepared nutrient agar petri plates. The discs prepared by using Whatman filter paper No. 42 were placed on these petri plates and different antibiotic solutions were spread on them. The Petri plates containing antibiotic-soaked filter paper were incubated for 24 hours at 37º C. When the incubation period was over, triplicate reading of the zone of inhibition was taken from each plate.

Results and Discussion

Isolation of *Enterobacteriaceae* sp. from green leafy vegetables: Out of 80 samples of green leafy vegetables including (cabbage, coriander leaves, dill, spinach, fenugreek leaves, and radish leaves) about 54 (67.5%), colonies were isolated (Table 1). Maximum percentages of isolates were found from samples of Jhunsi (22.22%) followed by Katra (20.37%), Teliyarganj (18.51%), Allahapur (11.11%), Colonelganj and Mumfordganj (9.26%), Daraganj (7.41%), and minimum percentage of isolates were found from Civil lines (1.851%) area.

Al-Holy *et al.* 2013, investigated the presence of *Enterobacteriaceae* in different vegetables such as lettuce, leek, coriander, dill, rocket leaves, parsley, and green onions purchased from the regional market of KSA (Saudi Arabia). They found the *Enterobacteriaceae* count in lettuce to be 5.72 $\log_{10}$ CFU/g and in leek, it was found to be 7.06 $\log_{10}$ CFU/g. They identified *Escherichia coli* as the main pathogenic bacteria in both rocket leaves as well as green onions. Mritunjay and Kumar (2015) had also reported that lettuce, cucumber, spinach, cabbage, radish can become a harbor of food-borne pathogenic microbes like *Listeria monocytogenes, Salmonella spp., Staphylococcus aureus, Clostridium botulinum,* and *E. coli O157: H7.*

Table 1 : Isolates of *Enterobacteriaceae* obtained from Green Leafy Vegetables

Locations	Total no. of sample	Total no. of isolates	Overall % of isolates
Katra	10	11 (D1, D2, CA1, CA2, CA3, CA4, R1, R2, R3, FE1, FE2,)	20.37%
Teliyargunj	10	10 (R4, R5, R6, CO1, CO2, CO3, FE3, FE4, FE5, FE6)	18.51%
Colonelgunj	10	5 (D3, D4, S1, D8, S3)	9.259%
Mumfordgunj	10	5 (CA5, FE7,CO4, D5, S4)	9.259%
Allahpur	10	6 (D6, D7, S5, S6, S7, S8)	11.111%
Civil Lines	10	1 (S9)	1.851%
Daragunj	10	4 (S10, CA6, R7, R8)	7.407%
Jhunsi	10	12 (CO5, S2, CO6, CO7, D9, R9, R10, S11, CA7, CA8, CA9, CA10)	22.22%
TOTAL	**80**	**54**	**67.5%**

(*D- Dill leaves, R- Radish, FE- Fenugreek, CO- Coriander, CA- Cabbage, S- Spinach)

Colonial Morphology and Gram Staining of the Isolates: The basis for the selection of all the 54 bacterial strains was colony morphology i.e., color, size, margin, shape, reaction to Gram's stain, and opacity. About 11.11% of isolates showed pink, circular, smooth, and compact colonies, and 16.67% isolates showed circular, mucoid, pink to red colony with bile precipitate on MacConkey agar (MCA) whereas opaque, creamy white, and moist colonies on Nutrient agar (NA) (Table 2). Gram-negative bacteria usually show good growth on the media and are distinguished from one another by their lactose fermentation ability. The species that can ferment the lactose usually grow as pink or red colonies and sometimes show a region of acid precipitated bile around itself. The development of red or pink color is because of the acid production resulting from lactose fermentation as the neutral red color gets absorbed with the subsequent change in the color of the dye when the pH of the media drops down to 6.8 or below.

On Xylose Lysine Deoxycholate Agar (XLDA), 22.22% isolates showed circular smooth black colonies (Table 2), 16.67% isolates showed reddish black color colonies, and 16.67% isolates showed yellow colonies (Figure 1). The color of the phenol red indicator changes to yellow when sucrose, lactose, and xylose present in XLD agar get degraded to acids. The decarboxylation of lysine into cadaverine by some bacteria can be identified by the emergence of red color surrounding the culture colonies because of the increased pH. The progression of these reactions can be simultaneous or successive, causing the pH indicator to display various colors. On delayed incubation, the color may change from yellow to red.

Fig. 1 : Growth of GLV isolates on selective media (1) XLDA, (2) MCA, (3) DCA

About 17% of the isolates showed black, condensed, smooth, compact, and irregular colonies on Deoxycholate Citrate Agar (Figure 1). This particular media is employed for isolating the intestinal pathogens and their maximum recovery. These intestinal bacteria belong to *Shigella* and *Salmonella* groups as obtained from food products. When the ferric citrate reduces to iron sulfide, it gives the symptomatic manifestation of black-centered colonies. Citrate salts (already added in the DCA media) inhibits the proliferation of gram-positive bacteria and all the other intestinal microbes.

On gram-staining, all 54 isolates were found to be pink, small or large rods and cocci (Table 2); because it does not retain the crystal violet during gram staining due to the cell wall of phospholipids and lipopolysaccharides. Hence, all isolates were gram-negative rods as examined under the microscope. After confirmation of gram-negative reaction, each isolate was further characterized biochemically and confirmed at the molecular level.

Table 2 : Morphological characteristics of GLV isolates on selective media

Isolates * (54)	% isolates	Selective Media	Color	Shape	Gram - reactions
D1, R2, CO1, FE6, FE7, S5, S6, S7, S8, S9,S10, CA6	(12) 22.22%	XLDA	Black colonies changing the media color to red	Circular, smooth and large	(–)ve rods
D2, D3, CA1, R3, FE1, CA2, R1, CA3, CA4	(9) 16.67%	XLDA	Red to black	Circular, smooth and compact	(–)ve rods
FE2, R4, R5, R6, CO2, CO3, FE3, FE4, FE5	(9) 16.67%	XLDA	Yellow colonies	Large, flat and mucoid	(–)ve rods
D4, S1, S2, S3, CA5, CO4, CO6, CO7, D5	(9) 16.67%	DCA	Black condensed Colonies	Smooth, compact and irregular	(–)ve rods
S4, D6, D7, R7, R8, CO5	(6) 11.11%	MCA	Pink colonies	Circular, smooth and compact	(–)ve rods
D8, D9, R9, R10, S11, CA7, CA8, CA9, CA10	(9) 16.67%	MCA	Pink to red with bile precipitate	Circular and mucoid	(–)ve rods

(*D- Dill leaves, R- Radish, FE- Fenugreek, CO- Coriander, CA- Cabbage, S- Spinach)

Biochemical characterization of GLV isolates

Each isolate demonstrated effervescence when subjected to H_2O_2; thus, it can be said that all the 54 isolates of green leafy vegetables were positive for the catalase test (Table 3). Fifty nine percent of the isolates were found to be non-motile (Table 3). However, there was turbidity in some test tubes and thus about 41% of isolates were found to be motile with full growth in the whole test tube. For methyl red test, about 63% of isolates were found to be positive (formation of a red ring), and the rest of 37% isolates, gave brown rings demonstrating a negative methyl red test (Table 3). Approximately 78% of isolates showed positive Voge's Proskauer's test by forming pinkish-red color within 5-10 minutes while 22% isolates showed no change in color indicating a negative test result. Out of 54 isolates, 33% were found to be positive for Simmon's citrate test and 36 isolates (67%) were negative (Table 3). About 74% of the isolates were positive for Indole test, while 26% of isolates showed negative results. As far as Malonate test is concerned, 46% of the isolates showed negative results. In Nitrate Reduction test, 57 % of total isolates showed negative results (Table 3). All the test results were in agreement with the characteristic tests of *Enterobacteriaceae* family.

Carbohydrate fermentation pattern of the GLV isolates: This sugar utilization test was performed for checking the ability of suspected enteric bacteria to ferment a particular carbohydrate when combined with basal media. This results in the production of acid along with gas or only acid. Total, 8 sugars were selected for performing this confirmational test of enteric bacteria such as galactose, sucrose, maltose, fructose, lactose, xylose, ribose, mannitol. The different pattern of sugar utilization was observed by different isolates. Some isolate-containing test tubes turned yellow and produce gases while other remain orange in color indicating positive and negative test respectively for sugar fermentation (Table 4).

Table 3 : Biochemical characterization of GLV isolates

Isolate (54)	Catalase	Lysine	Indole	Malonate	MR	VP	Citrate	Nitrate	Motility	Urease
D1, R2, CO1, FE6, FE7, S5, S6, S7, S8, CA6,	+	+	+	+	+	+	+	+	+	+
D2, D3, CA1, R3, FE1, CA2, R1, S1, S2,S9, S10, D7, FE2, CA3, CA4	+	+	+	+	+	+	-	-	-	+
R4, R5, R6, CO2, CO3, FE3, FE4, FE5	+	-	+	-	-	+	+	+	-	+
D4,, S3, CA5, CO4, CO6, CO7, D5	+	+	+	-	-	-	-	-	+	+
S4, D6, R7, R8, CO5	+	-	-	-	-	-	-	+	+	-
D8, D9, R9, R10, S11, CA7, CA8, CA9, CA10	+	-	-	-	+	+	-	-	-	+

(*D- Dill leaves, R- Radish, FE- Fenugreek, CO- Coriander, CA- Cabbage, S- Spinach
(+) = Positive and (-) = Negative)

Table 4 : Sugar utilization pattern of the GLV isolates

Isolates (54)	Galactose	fructose	Lactose	Sucrose	Ribose	Mannitol	Maltose	Xylose	Probable identified Organism	% isolates
D1, R2, CO1, FE6, FE7, S5, S6, S7, S8, CA6,	+		+	+	+	+	+	+	*Klebsiella planticola*	18.52%
S1, S2, S9, S10, FE2, D2, D3, CA1, R3, FE1, CA2, R1, CA3, CA4, D7	-	+	+	-	+	-	+	+	*Escherichia coli*	27.78%
R4, R5, R6, CO2, CO3, FE3, FE4, FE5	+	+	+	+	+	+	+	+	*Klebsiella oxytoca*	14.81%
D4, S3, CA5, CO4, CO6, CO7, D5	+	-	-	-	+	+	+	-	*Yersinia enterocoltica*	12.96%
S4, D6, R7, R8, CO5	+	-	-	-	-	+	+	+	*Salmonella Typhi*	9.26%
D8, D9, R9, R10, S11, CA7, CA8, CA9, CA10	-	+	+	-	+	+	-	+	*Klebsiella pneumonia*	16.67%

(*D- Dill leaves, R- Radish, FE- Fenugreek, CO- Coriander, CA- Cabbage, S- Spinach)
(+) = Positive and (-) = Negative

Probable isolated organisms from GLV samples

When these sugar fermentation and biochemical results were compared with PIBWIN software (Bryant, 2004), different bacterial isolates such as *Escherichia coli* (27.78%), *Klebsiella planticola* (18.52%), *Klebsiella pneumonia* (16.67%), *Klebsiella oxytoca* (14.81%), *Yersinia enterocolitica* (12.96%), *Salmonella typhi* (9.26%) were identified.

Antibiotic Susceptibility of GLV Isolates

The antibiotic susceptibility test was done to check the resistance of selected isolates with respect to certain antibiotics. The Kirby Bauer disc diffusion method was used for conducting this test by using a wide range of antibiotics such as Ampicillin, Ciprofloxacin, and Streptomycin, each at a concentration of 10µg, 50µg, 80µg, and 100µg. Table 5 showed the inhibition zones as demonstrated by selected isolates at different antibiotic concentrations. Greater the zone of inhibition more will be the resistant power of antibiotics.

It was observed that the maximum isolates obtained from green leafy vegetables have their zone of inhibition within the range of 10-15 mm diameter. About 78.5% isolates at 80 µg/ml concentration of ampicillin and 85.8% isolates at the concentration of 50 µg/ml of streptomycin showed their zone of inhibition within the range of 10-15 mm diameter, while, 91.6% isolates showed their zone of inhibition within the range of 15-20 mm diameter at 10 µg/ml concentration of ciprofloxacin (Figure 2). None of the isolates showed a zone of inhibition more than 20 mm whereas only 3.9% showed an inhibition zone of less than 5 mm against 100 µg/ml ampicillin.

According to various reported studies, *E. coli* isolates in cabbage and green leaf lettuce were found susceptible to multiple antibiotics. These isolates were found to be more susceptible to ampicillin and amoxicillin (92.6%), followed by tetracycline (70.4%) (Chanseyha *et al*., 2018). Adzitey (2018) had also reported that *E. coli* obtained from cabbage samples were 100% resistant to ofloxacin, followed by ampicillin (90.01%) and then erythromycin (81.82%). The overall susceptibility, intermediate, and resistance of *E. coli* obtained from lettuce were 37.04%, 10.37%, and 52.59% respectively. The selected isolates were also found to be 100% resistant against ofloxacin, 93.33% against erythromycin, and 86.67% against ampicillin.

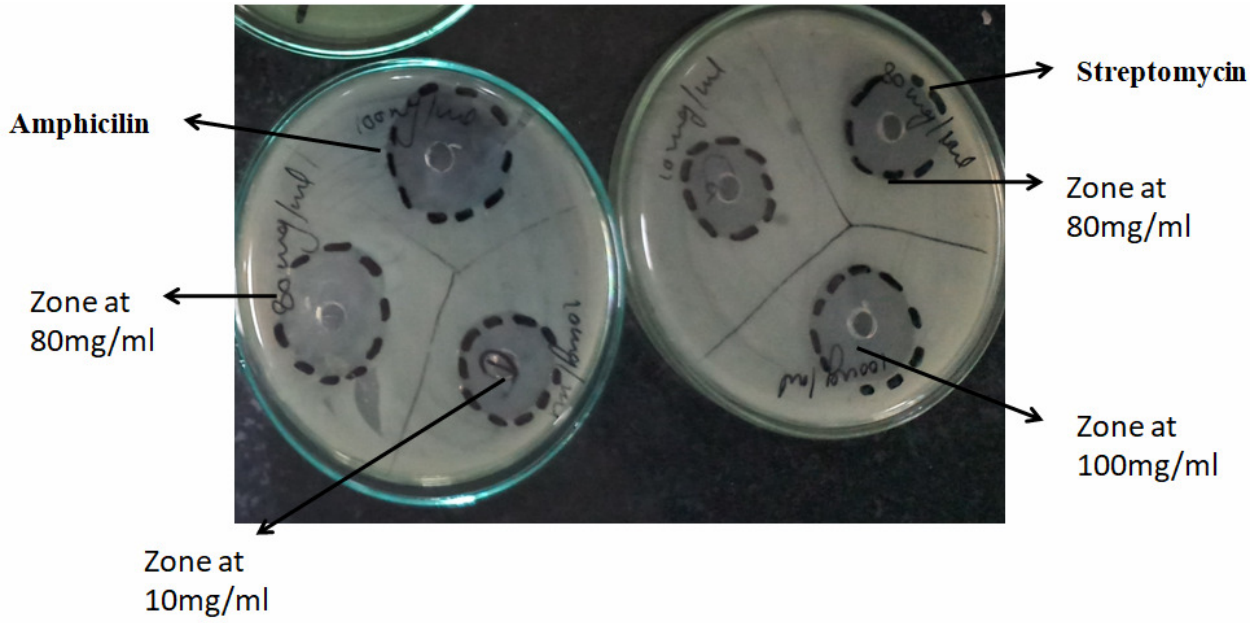

Fig. 2 : Antibiotic susceptibility test result of GLV isolates

Table 5 : Antibiotic Susceptibility Test of Green Leafy vegetable isolates

Antibiotics*	% of Isolates in different ranges of Diameter of inhibition zones (mm)				
	< 5 mm	5- 10 mm	10-15 mm	15-20 mm	> 20 mm
Amp (10 µg/ml)	-	10.1%	68.1%	15.0%	-
Amp (50 µg/ml)	-	14.5%	71.0%	4.7%	-
Amp(80 µg/ml)	-	-	78.5%	21.4%	-
Amp (100 µg/ml)	3.9%	23.2%	35.7%	-	-
Strepto (10 µg/ml)	-	17.2%	72.6%	7.2%	-
Strepto (50 µg/ml)	-	-	80.1%	7.5%	-
strepto (80 µg/ml)	-	-	8.7%	72.7%	-
Strepto (100 µg/ml)	-	-	11.5%	70.9%	-
Cipro (10 µg/ml)	-	-	7.4%	92.6%	-
Cipro (50 µg/ml)	-	-	11.5%	81.3%	-
Cipro (80 µg/ml)	-	-	51.3%	8.4%	-
Cipro (100 µg/ml)	-	-	-	72.0%	-

(* Amp= Ampicillin, Strepto= Streptomycin, and Cipro= Ciprofloxacin)

Conclusion

The vegetables sold in the local markets of Allahabad showed presence of pathogenic bacteria, particularly of the family *Enterobacteriaceae*, indicating poor hygienic conditions as well as improper storage environment. The biological quality of the samples obtained from different locations was found to be unacceptable. Presence of *Escherichia coli, Klebsiella planticola, Klebsiella pneumoniae, Klebsiella oxytoca, Yersinia enterocolitica*, and Salmonella *typhi* was confirmed in green leafy vegetable samples. Amongst them, *E. coli* (27.78%) was found to be highly prevalent whereas the least prevalent species was *S. typhi* (9.26%). In spite of a small sample size, the study positively indicates the presence of pathogenic bacteria in fresh green leafy vegetables in the local markets of Allahabad region. Future surveys are needed with a larger sample size and for a longer duration to know the occurrence of these pathogenic bacteria. The fresh vegetables must be handled with care to avoid the cross-contamination. For human safety, food laws should be set up in India for regulating and inhibiting the occurrence of pathogenic bacteria in ready-to-eat fresh vegetables.

Acknowledgements

The authors would like to express their sincere gratitude to the Centre of Food Technology, University of Allahabad, Prayagraj for providing all the essential facilities and support.

Conflict of interest

The authors declare no conflict of interest.

References

Adzitey, F. (2018). Antibiotic resistance of *Escherichia coli* and *Salmonella enterica* isolated from cabbage and lettuce samples in Tamale metropolis of Ghana. *Int J Food Contam*., 5(1): 1-7.

Al-Holy, M.; Osaili, T. and El-Sayed, S. (2013). Microbiological quality of leafy green vegetables sold in the local market of saudi arabia. *Ital J Food Sci*., 25(4).

Araújo, S.; Silva, I.; Tacão, M. and Patinha, C. (2017). Characterization of antibiotic resistant and pathogenic *Escherichia coli* in irrigation water and vegetables in household farms. *Int J Food Microbiol*., 257: 192–200.

Bhardwaj, A.; Puniya, M.; Sangu, K.P.S.; Kumar, S. and Dhewa, T. (2012). Isolation and Biochemical Characterization of *Lactobacillus* species Isolated from Dahi. *J Diary Sci Technol*., 1: 18-31.

Birt, C.A. (2017). Healthy diets and a healthy planet. *Eur J Public Health*. 27: 790–791.

Chanseyha, C.; Sadiq, M.B. and Cho, T.Z. (2018). Prevalence and analysis of antibiotic resistant genes in *Escherichia coli* and *Salmonella* isolates from green leaf lettuce. *Chiang Mai J Sci.,* 45(3): 1274-1286.

Chee-Sanford, L.C.; Mackie, R.I. and Koike, S. (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J Environ Qual*. 38: 1086–1108.

Falomir, M.P.; González, P. and Rico, H. (2014) Resistances to chemotherapeutic agents in *Enterobacteriaceae* isolates from organic fresh vegetables marketed in Valencia (Spain*). Int. J. Food. Nutr. Saf*., 5: 39–49.

Falomir, M.P.; Rico, H.; Gozalbo, D. (2013) *Enterobacter* and *Klebsiella* species isolated from fresh vegetables marketed in Valencia (Spain) and their clinically relevant resistances to chemotherapeutic agents. *Foodborne Pathog Dis*., 10: 1002–1007.

Heaton, J.C. and Jones, K. (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. *J Appl Microbiol*., 104: 613–626.

Hemraj, V.; Sharma, D. and Gupta, A. (2013). A review on commonly used biochemical test for bacteria. *Innovare J Life Sci*., 1(1): 1-7.

Holt, J.C.; Krieg, N.R.; Sneath, P.H.A. and Stanley, J.Y. (1994). Bergey's manual of determinative bacteriology. Springer. USA. pp 764-799.

Kaczmarek, M.; Avery, S.V. and Singleton, I. (2019). Microbes associated with fresh produce: sources, types and methods to reduce spoilage and contamination. *Adv Appl Microbiol*., 107: 29–82.

Liu, B.T.; Li, X.; Zhang, Q.; Shan, H.; Zou, M. and Song, F.J. (2019). Colistin-resistant *mcr*-positive *Enterobacteriaceae* in fresh vegetables, an increasing infectious threat in China. *Int. J. Antimicrob. Agents*., 54(1): 89-94.

Lynch, M.F.; Tauxe, R.V. and Hedberg, C.W. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol Infect*., 137: 307–315.

Manikandan, C. and Amsath, A. (2013). Antibiotic susceptibility of bacterial strains isolated from wound infection patients in Pattukkottai, Tamilnadu, India. *Int. J. Curr. Microbiol. App. Sci*., 2(6): 195-203.

Marti, R.; Scott, A. and Tien, Y.C. (2013). Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. *Appl Environ Microbiol*. 79: 5701–5709.

Martínez-Vaz, B.M.; Fink, R.C. and Diez-González, F. (2014). Enteric pathogen-plant interactions: Molecular connections leading to colonization and growth and implications for food safety. *Microbes Environ*., 29: 123–135.

Mritunjay, S.K. and Kumar, V. (2015). Fresh farm produce as a source of pathogens: a review. *Res J Environ Toxicol*., 9(2): 59-70.

Olaimat, A.N. and Holley, R.A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiol*., 32: 1–19.

Rajwar, A.; Srivastava, P. and Sahgal, M. (2016). Microbiology of fresh produce: route of contamination, detection methods, and remedy. *Crit Rev Food Sci Nutr. Microbial Drug Resistance*, 56: 2383–2390.

Rico, H.; Gozalbo, D. and Sebastiá, C. (2013). *Enterobacter cloacae* in fresh vegetables: A potential carrier of antibiotic resistances to consumers. Food Studies: *Interdiscipl J. Microbial Drug Resistance,* 2: 1–8.

Venglovsky, J.; Sasakova, N. and Placha, I. (2009). Pathogens and antibiotic residues in animal manures and hygienic and ecological risks related to subsequent land application. *Bioresour Technol*. 100: 5386–5391.

Wadamori, Y.; Gooneratne, R.; and Hussain, M.A. (2017). Outbreaks and factors influencing microbiological contamination of fresh produce. *J Sci Food Agric*., 97: 1396–1403.