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## Detection and Antimicrobial Resistance Profiling of Coliforms Isolated from Fresh Vegetables in Lahore, Pakistan

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**ABSTRACT:** *Vegetables are among the finest sources of high-quality nutrients including, carbohydrates, vitamins, fibers and minerals, having beneficial health effects. However consuming raw vegetables obtained from retailer shops renders them a reservoir of pathogenic bacteria due to unhygienic production, storage, and handling practices, and could be a major route of antibiotics resistance dissemination. The present work aimed at the evaluation of fresh vegetables for total microbial load and coliforms presence and determination antibiotic susceptibility of the isolated strains. The selective media were used for the targeted isolation of coliforms and antimicrobial resistance profiling of the isolates was performed using disc diffusion assay to determine the current resistance patterns of the bacterial isolates. Escherichia coli and Salmonella were the top isolated strains from the vegetable and salad samples. The antimicrobial resistance patterns showed an increased resistance to most of the tested antibiotics highlighting the critical issue of the dissemination of antibiotics resistance through common food items. The frequent presence of coliforms in the fresh vegetables and salads indicate suitable environmental conditions in the area for opportunistic pathogens and other harmful microorganisms to grow and dwell in the frequently consumed food items. The study highlighted the importance and need for proper cooking practices for vegetables before consumption and emphasizes the minimal use of partially cooked or uncooked vegetables*

**Keywords:** AMR dissemination; Antibiotics resistance; Susceptibility testing

## INTRODUCTION

Vegetables such as cucumbers, spinach cabbage, mint and lettuce have been historically associated with health benefits such as positive effects on the health of diabetics and anemics etc. However contamination risks and unhealthy handlings of the vegetables may also make it a source of infection and hence may cause variety of health concerns. There is a chain of events such as production, transportation and making these vegetables to the table are prone to every possible of contamination if not taken care with properly, hence ensuring microbiological surveillance during the entire process is highly required for safe consumption (Henson and Reardon, 2005). In most of the Asian countries sewage or wastewater is added into the rivers with or without proper management in some cases. Farmers mostly rely on river water for the water demand of the crop and unstoppable flow of nutrients which is highly required to combat the ever-growing demand of food for the increasing population (Aminharati et al., 2019). This practice of using river water containing waste water is much more common in developing

countries due to unavailability of water treatment resources, hence rendering this supply much more risky for the potential pathogenic microbes which can certainly affect the quality of food. Variety of food borne pathogens including bacteria and viruses can be linked with this supply system and hence may lead to a food borne epidemic in a given area (Callejón et al., 2015). The Enterobacteriaceae is a group of Gram-negative rod shaped bacteria (bacilli) most of which are the common commensals of humans and other animals gut also known as the normal microbiota of the gut. Members of this family are generally motile excluding *Shigella* and *Klebsiella*. Other properties of this group include the division of its members into two sub-groups such as those which can ferment lactose (lactose-fermenters) also known as coliforms and those which cannot (non-lactose fermenters) such as *Salmonella*, *Shigella*, *Yersinia* and *Proteus*. Coliforms can be aerobic or facultatively anaerobic and contains some of the famous foodborne pathogens such as *Enterobacter*, *Escherichia coli* (*E.coli*), *Citrobacter* and *Klebsiella*, all of these commonly

produce and ferment indole (Martin et al., 2016).

Many factors can contaminate the fresh produce of vegetables such as humans, soil and animals and the can contaminants can survive the processes of transportation and storage etc. “Indicator organisms” are those organisms which can reflect the general microbiological quality of the food. Coliforms are often referred as indicator organisms which can depict the general microbiological quality of a given water and food sample. They are often taken as a positive sewage or fecal contamination of any water or food sample hence highlighting the unhygienic and unsanitary processing in a given farm, field area or city (Jin et al., 2004). Recently there have been some serious findings regarding the level of detection of certain coliforms such as *Salmonella* in various food samples such as meat (Fatima et al., 2023; Tagar and Qambrani, 2023). Total coliforms are somehow abundantly present in the environment and its detection is normal however specifically the detection of *E.coli* is enough to ring the alarm bells as they are specifically associated with fecal contamination and hence can describe the poor and

unhealthy microbiological quality of a given food and of vegetables in this case. Moreover the presence of *E.coli* can also be presented as an evidence of fecal contamination in routine microbiological procedures and there have been many instances of foodborne disease outbreaks involving *Salmonella* and *E.coli* after fresh vegetable produce consumption (Berger et al., 2010). Some mammals have *E.coli* other than the normal microbiota which can be diarrheagenic and hence can serve as a contaminant of fresh produce (Franz and Bruggen, 2008). Thus vegetables especially raw leafy vegetables can contain variety of containments such as *E.coli*, *Salmonella*, and other members of the family Enterobacteriaceae (Richter et al., 2019).

In this study, N= 40 samples of different fresh vegetables and salads such as cucumber, mint, coriander, cabbage, spinach, lettuce, fenugreek etc. were collected from various areas of Lahore. The samples were processed for selective isolation of coliforms followed by their, morphological and biochemical identification. The Antimicrobial resistance profiles of the isolates

was also determined by using antibiotics discs of different classes.

## MATERIALS AND METHODS

### Sample Collection and Microbiological Analy

The vegetable samples N=40 including coriander, cabbage, spinach, lettuce fenugreek cucumber, mint and salad were collected from various areas in Lahore and were subsequently processed from Jan 2022 to mid of April 2022. The samples were assessed for different microbiological aspects such as presence of coliforms, CFU/ml and detection of *E.coli* and *Salmonella* etc. the samples were purchased from different areas in Lahore city which included, Faisal town, Johar town, Barkat Market, Guru mangat road, Makkah colony, Muslim Town Block B, Sabzi Mandi, Kamran Block, Allama Iqbal Town, Multan Chungi, Najaf Colony Hunza block, Kashmir Block, Ghari Shahu and Zaraar Shaheed Road. These places were chosen as a part of random pool sampling and because these are some of highly populated areas of the city and hence present a better general picture of the situation. The

samples were transported in air tight sterilized plastic bags and were processed with in 24 h after the transportation. The 25g of each sample was weighed which was then chopped and mixed in 225 ml of sterile peptone water (1:10). N-agar was chosen for the preliminary isolation of the bacteria as the sample mixtures were serially diluted up to 6 times and 20 µl of each dilution was spread onto N-agar plates followed by the overnight incubation at 37°C.

CFU/ml was calculated by the formula:

$$CFU/ml = (no. of colonies \times dilution factor) / volume of culture plate$$

### Selective Isolation, Biochemical Characterization and Identification of the Isolates

Initial isolation resulted in rich primary culture having diverse microbial growth on N-agar plate. The morphologically different colonies were picked and streaked onto various selective media such as MacConkey and EMB agar followed by overnight incubation at 37°C. Following the incubation, various characteristics of colonies were taken into consideration such as color, margins, elevation, shape and size etc. Gram-staining

procedure was done for every isolate followed by biochemical characterization that included, Oxidase test, Methyl Red test, MRVP test, Indole test, Catalase test, Original VP test, Barrett's method, Citrate Utilization test, Motility test, Triple Sugar Iron test and Urease test.

### Antibiotics Susceptibility Testing (AST) of the Selected Isolates

After initial identification of the selected isolates, isolates of interests were spread plated onto Muller-Hinton agar. The AST was measured using famous disc diffusion method developed by Kirby-Bauer. Discs already impregnated with antibiotics were applied onto the plates containing lawn of isolates of interests and were incubated overnight at 37 °C. Zone of inhibitions (ZOIs) were measured in accordance with CLSI standards in which all plates were incubated at 37C for 18 to 24

hrs. Results were recorded by measuring the diameter of zones of inhibition (ZOIs) in millimeters and activity was interpreted as resistant, sensitive or intermediate according to CLSI 2020 criteria for each antibiotic class used in this assay.

## RESULTS AND DISCUSSION

### CFU/mL Count and Colony Morphology

Cabbage and Spinach produced higher number of CFU/mL. CFU/ml for each vegetable sample was reported with Cabbage having the highest average CFU/ml of 8.51e8 following spinach, cucumber, mint, coriander and lettuce, respectively. The isolates were assessed for variety of colony features such as, elevation, texture and other features on respective selective media, such as McConkey agar and details of which are given in the table 1 and 2.

**Table 1.** Colony characteristics of isolates on MacConkey agar

Isolates	Shape	Margin	Texture	Color	Elevation	Size
Sa2	Circular-c	Entire-e	Smooth-s	Purple	Raised-r	Small-s
C6	Circular-c	Entire-e	Mucoid-m	Dark purple	Raised-r	Small-s
CU1	Circular-c	Entire-e	Smooth-s	Purple /dark spots	Raised-r	Small-s
CU3	Circular-c	Entire-e	Slimy	Purple /dark center	Raised-r	Small-s
M8	Circular-c	Entire-e	Slimy	Purple /green	Raised-r	Small-s

*AMR Profiling of Coliforms in Fresh Vegetables*

				sheen			
S4	Circular-c	Entire-e	Smooth-s	Purple sheen	/green	Raised-r	Small-s
S3	Circular-c	Entire-e	Smooth-s	Yellow		Raised-r	Small-s
S1	Circular-c	Entire-e	Mucoid-m	Light purple		Raised-r	Small-s
D4E	Circular-c	Entire-e	Smooth-s	Purple		Raised-r	Small-s
Sa3	Circular-c	Entire-e	Smooth-s	Dark purple		Raised-r	Small-s
Sa1	Circular-c	Entire-e	Smooth-s	Purple		Raised-r	Pinpointed
CO13	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
D4	Circular-c	Entire-e	Smooth-s	Purple		Raised-r	Small-s
C7	Circular-c	Entire-e	Smooth-s	Purple		Raised-r	Small-s
S2	Circular-c	Entire-e	Mucoid-m	Purple		Raised-r	Small-s
L13	Circular-c	Entire-e	Smooth-s	Purple		Raised-r	Small-s
CO11	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
CO14	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
M31	Circular-c	Entire-e	Slimy	Purple		Raised-r	Small-s
M40	Circular-c	Entire-e	Smooth-s	Purple		Raised-r	Pinpointed
M2	Circular-c	Entire-e	Smooth-s	purple		Raised-r	Small-s
C1F4	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
M22	Circular-c	Entire-e	Smooth-s	Dark purple		Raised-r	Small-s
L1	Circular-c	Entire-e	Smooth-s	Light purple		Raised-r	Pinpointed
L3	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
CO22	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Pinpointed
L4	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Pinpointed
C1F1	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
C1F2	Circular-c	Entire-e	Smooth-s	Light Pink-p		Raised-r	Small-s
C1F3	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
CO21	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s
CH13	Circular-c	Entire-e	Smooth-s	Colorless		Raised-r	Small-s

*AMR Profiling of Coliforms in Fresh Vegetables*

FG2	Circular-c	Entire-e	Smooth-s	Colorless	Raised-r	Small-s
SP2	Circular-c	Entire-e	Smooth-s	colorless	Raised-r	Pinpointed
CL13	Circular-c	Entire-e	Smooth-s	Colorless	Raised-r	Small-s
SP1	Circular-c	Entire-e	Smooth-s	Light purple	Raised-r	Small-s

**Table 2.** Morphological details of the isolates on EMB Agar

<b>Isolates</b>	<b>Elevation</b>	<b>Color</b>	<b>Margin</b>	<b>Texture</b>	<b>Shape</b>	<b>Size</b>
S4	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
C6	Raised-r	Pink-p	Entire-e	Mucoid-m	Circular-c	Small-s
CU1	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
CU3	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
M8	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Pinpointed
S3	Raised-r	Reddish	Entire-e	Smooth-s	Circular-c	Small-s
S2	Raised-r	Pink-p	Entire-e	Mucoid-m	Circular-c	Small-s
D4E	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
S1	Raised-r	Pink-p	Entire-e	Slimy	Circular-c	Small-s
Sa3	Raised-r	Pink-p	Entire-e	Mucoid-m	Circular-c	Small-s
Sa2	Raised-r	Light Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
Sa1	Raised-r	Light Pink-p	Entire-e	Smooth-s	Circular-c	Pinpointed
C7	Raised-r	Pink-p	Entire-e	Slimy	Circular-c	Small-s
L1	Raised-r	Colorless	Entire-e	Smooth-s	Circular-c	Pinpointed
M31	Raised-r	Pink-p	Entire-e	Slimy	Circular-c	Small-s
M22	Raised-r	Light Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
CO11	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
CO13	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
D4	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Pinpointed
CO14	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
M40	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Small-s

*AMR Profiling of Coliforms in Fresh Vegetables*

L13	Raised-r	Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
M2	Raised-r	Colorless	Entire-e	Smooth-s	Circular-c	Pinpointed
L3	Raised-r	Light Pink-p	Entire-e	Slimy	Circular-c	Pinpointed
CO21	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
L4	Raised-r	Light Pink-p	Entire-e	Slimy	Circular-c	Pinpointed
C1F1	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
C1F3	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
C1F4	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
C1F2	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Pinpointed
CO22	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Small-s
FG2	Raised-r	Yellow	Entire-e	Mucoid-m	Circular-c	Small-s
SP1	Raised-r	Yellow	Entire-e	Smooth-s	Circular-c	Pinpointed
SP2	Raised-r	Light Pink-p	Entire-e	Smooth-s	Circular-c	Small-s
CL13	Raised-r	Colorless	Entire-e	Mucoid-m	Circular-c	Small-s
CH13	Raised-r	Colorless	Entire-e	Mucoid-m	Circular-c	Small-s

Selective colonies were picked from N-agar and streaked on MacConkey agar. This agar is a selective and differential media at the same time as it can not only inhibit the growth of gram positive bacteria but can also differentiate between coliforms and non-coliforms, with the former give a pink color and the latter being colorless.

Colonies N-agar were streaked into EMB agar plates which serves

not only as a selective media but also as a differential media between lactose fermenters and non-fermenter with lactose fermenters forming Pink-p colonies while non-lactose fermenters being colorless colonies due to increasing pH in light of deamination of proteins and Table 3.

**Table 3: Biochemical Characteristics of the Isolated Strains**

Strains	Citrate	Indole	Urease	Oxidase	Catalase	VP	MR	TSI	SIM	Identified strain
CU1	+	-	-	-	+	-	+	A/A and H2S (+)	+	<i>Citrobacter</i>
C7	-	+	-	-	+	-	+	A/A and H2S -	+	<i>E.coli</i>
C6	+	-	+	-	+	+	-	A/A and H2S -	-	<i>Klebsiella</i>
S4	-	+	-	-	+	-	+	A/A and H2S -	+	<i>E.coli</i>
S3	+	-	-	-	+	-	+	A/A and H2S -	+	<i>Enterobacter</i>
Sa3	+	-	-	-	+	+	-	A/A and H2S -	+	<i>Enterobacter</i>
CU3	-	+	-	-	+	-	+	A/A and H2S (+)	+	<i>E.coli</i>
CO14	+	-	-	+	+	+	-	K/K and H2S +	+	<i>Pseudomonas</i>
S2	+	-	-	-	+	-	+	A/A and H2S -	+	<i>Enterobacter</i>
S1	+	-	-	-	+	-	+	K/A and H2S (-)	+	<i>Salmonella</i>
Sa2	+	-	-	-	+	-	+	A/A and H2S (+)	+	<i>Citrobacter</i>
CO11	+	-	+	-	+	-	+	K/A and H2S +	+	<i>Proteus</i>
M8	-	+	-	-	+	-	+	A/A and H2S -	+	<i>E.coli</i>
Sa1	+	-	-	-	+	+	-	A/A and H2S -	+	<i>Enterobacter</i>
M22	+	-	-	-	+	+	-	A/A and H2S -	+	<i>Enterobacter</i>
D4	-	+	-	-	+	-	+	A/A and H2S -	+	<i>E.coli</i>
D4E	-	+	-	-	+	-	+	A/A and H2S -	+	<i>E.coli</i>
M31	+	-	-	-	+	-	+	A/A and H2S (+)	+	<i>Citrobacter</i>
CO13	+	-	-	-	+	-	+	K/A and H2S +	+	<i>Salmonella</i>
L13	-	+	-	-	+	-	+	A/A and H2S -	+	<i>E.coli</i>
M40	-	+	-	-	+	-	+	A/A and H2S -	+	<i>E.coli</i>
L3	+	-	-	-	+	-	+	A/A and H2S (+)	+	<i>Salmonella</i>
L4	+	-	-	-	+	-	+	K/A and H2S (-)	-	<i>Shigella</i>
FG2	+	-	+	-	+	-	+	K/A and H2S +	+	<i>Proteus</i>
C1F1	+	-	-	-	+	-	+	K/A and H2S +	+	<i>Salmonella</i>
CH13	+	-	-	-	+	-	+	A/A and H2S (+)	+	<i>Citrobacter</i>
L1	+	-	-	-	+	-	+	K/A and H2S +	+	<i>Salmonella</i>

CIF2	+	-	-	-	+	-	+	K/A and H2S +	+	<i>Salmonella</i>
C1F4	+	-	-	+	+	-	-	K/K and H2S +	+	<i>Pseudomonas</i>
CO21	+	-	- a	+	+	-	-	K/K and H2S +	+	<i>Pseudomonas</i>
M2	+	-	+	-	+	-	+	K/A and H2S +	+	<i>Proteus</i>
CO22	+	-	+	-	+	-	+	K/A and H2S +	+	<i>Proteus</i>
C1F3	+	-	-	-	+	-	+	A/A and H2S -	-	<i>Shigella</i>
SP1	+	-	-	+	+	-	-	K/K and H2S -	+	<i>Pseudomonas</i>
SP2	+	-	-	-	+	-	+	K/K and H2S -	-	<i>Shigella</i>
CL13	+	-	-	-	+	-	+	A/A and H2S (+)	+	<i>Citrobacter</i>

Gram staining results are confirming typical Gram-negative pattern.

### Biochemical Identification

Different biochemical tests characterized each strain on the basis of results obtained. A N= 36 strains were characterized on the basis of morphological and biochemical identifications. All biochemical tests were performed within 24 h using fresh cultures on nutrient agar plates. Overall biochemical results are shown in the following table. The isolates C7, CU3, M8, S4, S3 D4, D4E, L13 and M40 were confirmed as *E.coli*. The isolate S3, S2, Sa3, Sa1 and M22 were detected as *Enterobacter*. Similarly isolates S1, L1, L3, C1F1 and CIF2 were confirmed as *Salmonella*. The results depict a typical coliform biochemistry hence morphological identification of the strains followed by the growth on

selective media were reconfirmed by the biochemical identification scheme (Table 3). In total 19.4% isolates belonged to *Salmonella* and *E.coli* respectively followed by *Citrobacter* and *Enterobacter* with 11.3 % respectively (Fig. 1).

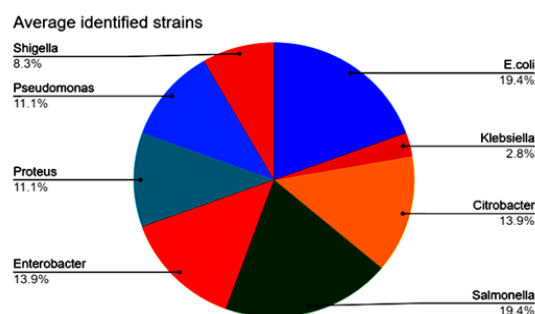


Fig. 1. Percentage of isolated strains from sample

### Antibiotic Sensitivity Pattern of the Recovered Isolates

The AST shows that, among all the antibiotics applied, Cefazolin, Cefepime, Polymyxin B and Erythromycin were among the least active against the isolates showing total resistance of 97%, 100%, 97% and 100 %

respectively. Alternatively it also shows the recovered isolates are highly resistant against these antibiotics which could pose a major concern for the respective

authorities dealing with food with food hygiene such as Punjab food authority. Rest of the details are given in the following table 4.

**Table 4. AST results of the identified strains**

Antibiotics	Intermediate	Sensitive	Resistant	Total	Resistance %
Ampicillin	6	5	25	36	69.40
Cefazolin	1	0	35	36	97.20
Streptomycin	15	14	7	36	19.40
Cefepime	0	0	36	36	100
Ceftriaxone	6	5	25	36	69.40
Imipenem	6	29	1	36	2.77
Polymyxin B	0	0	36	36	100
Ciprofloxacin	10	20	6	36	16.60
Levofloxacin	0	34	2	36	5.55
Chloramphenicol	10	15	11	36	30.50
Gentamicin	2	33	1	36	2.77
Tetracycline	0	11	25	36	69.40
Tobramycin	6	27	3	36	8.33
Amikacin	2	34	0	36	0
Azithromycin	0	28	8	36	22.20
Erythromycin	1	2	33	36	91.60

There is always a chance of disease transmission with the contaminated irrigation water as it can harbor a variety of pathogens with serious health implications. The level of contamination is linked to the depth of the water as ground water can act as a good

irrigation source, in contrary surface water has poor microbiological quality and is generally not fit for the irrigation purpose (Ungureanu et al., 2020). However among all, the human waste water can be of the poorest quality and it should always be

treated before it can be used for the irrigation processes (Khalid et al., 2018). Different studies have revealed that Salmonella and E.coli etc. can easily colonize plants and may reach to the sub-stomatal cavities (Cooley et al., 2003; Deering et al., 2012; Nicholson et al., 2015). This research was aimed to highlight the potential contamination of fresh vegetables produce due to contaminated water and overall handling and storage process. N= 40 samples were assessed for their microbiological load and coliforms were targeted specifically along with the other members of Enterobacteriaceae family. The polyphasic taxonomic approached applied in the identification contained growth on selective media followed by biochemical identification which showed the presence of coliforms in these vegetables at alarming level. In Nigeria and other developing countries the use of untreated wastewater used for irrigation has resulted in several outbreaks previously (Grivokostopoulos et al., 2022; Du Plessis et al., 2015; Chigor et al., 2020). The contamination rates of different vegetables were almost similar and *E.coli* was found to be

present in higher numbers suggesting fecal contamination of the irrigated water. Factors such as improper handling and storage may also account for this *E.coli* presence. The use of antibiotics in animal husbandry and agriculture has resulted in the evolution of multi drug and extensively drug resistant pathogens which have now become a global concern as these pathogens are unstoppable with the majority of the current lot of antibiotics. In this study the most common coliform was found to be *E. coli* which is a major concern as its CFU/ml count was found to be 8.51 E+08. The higher resistance rates towards notable antibiotics is a grave concern as these pathogens can be a source of foodborne epidemic with some of the isolates showing 100% resistance. A study from US showed that the risk of contamination in fresh produce increases with the gradual steps followed from harvesting to storage and supply (Possas et al., 2023; Alegbeleye et al., 2018). Many factors can contribute in overall cross contamination of the fresh produce and every factor itself can be a sole reason or a contributing factor in the entire scenario. Insufficient knowledge

regrading handling, storage and supply can also be accountable for the growing contamination reports of vegetables (El-Ramady et al., 2015; Gil et al., 2015). Another common practice among the vendors is the continuous sprinkling of water over vegetables to maintain its freshness. Poor microbiological quality of the sprinkled water can also contribute to the contamination of vegetables (Salamandane et al., 2020).

There are various reports that support the fact that the incidences of antimicrobial resistance in the food samples are on the rise and different studies have recently reported the detection of resistant pathogens from fruits (Olanbiwoninu et al., 2024; Brunn et al., 2022; Lima et al., 2019 ), vegetables (Kgoale et al., 2024; Jia et al., 2024; Patra et al., 2024), dairy products (Pires et al., 2024; Khalid et al., 2023) and meat (Algammal et al., 2024; Nastasijevic et al., 2024; Tenea et al., 2023; Martino et al., 2024). Contamination of raw produce with microorganisms have been reported previously in many aspects but detection of coliforms in fresh produce from the second most populated city of Pakistan

should be a concern and this study highlights important aspects of vegetables handling, harvesting and storage. Each step in its own way can contribute to the contamination of the fresh produce but mainly the contaminated irrigation water which contain sewage mixing is the most crucial quality check in this regard. To achieve satisfactory levels of less microbial load as specified by FDA in produce, regular monitoring at various levels is important.

## **CONCLUSION**

Consuming raw vegetables is a public health concern due to their unhygienic production practices and further contamination throughout the processing until brought to the tables. A proper surveillance system is essential to ensure food cleanliness, prevent cross-contamination, and manage water pollution for safe agricultural use. Since irrigation sources greatly impact crop sanitation, water treatment is vital for crop production. Regular check on vendors, determination of random microbiological quality of fresh produce and proper cooking practices are some of the highly required steps to stop dissemination of antibiotics resistance through this route

### CONFLICT OF INTEREST

The authors declare no conflict of interests.

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