Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Isolation and Characterization of Multidrug-Resistant Bacteria from Fresh Vegetables in Peshawar

Sana Rehman
Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Technology, Peshawar, Pakistan
Aliman Shah
Deputy Director, Sarhad Institute of Allied Health Sciences, Faculty of Life Sciences, Sarhad University of Science and Information Technology, Peshawar, Pakistan.
Email: alimanshah.siahs@suit.edu.pk
Musadiq Khan
Academic Coordinator for Distance Education, Sarhad Institute of Allied Health Sciences, Faculty of Life Sciences, Sarhad University of Science and Information Technology, Peshawar, Pakistan. Email: musadiq.siahs@suit.edu.pk
Dr. Nasir Ali*
Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Technology, Peshawar, Pakistan. Corresponding Author Email: nasirali.biotech@gmail.com, nasir.biotech@suit.edu.pk

Author Details

Received on 19 March, 2026

Accepted on 07 April, 2026

Published on 09 April, 2026

Corresponding E-mails & Authors*:
Dr. Nasir Ali
nasir.biotech@suit.edu.pk

Abstract

Background: Fresh vegetables form an essential component of a healthy diet, yet they increasingly serve as vehicles for transmitting antibiotic-resistant pathogenic bacteria to humans. This study investigated the presence of multidrug-resistant (MDR) bacteria in commonly consumed vegetables from Peshawar markets. **Methods:** Ten different vegetable types were collected and processed using standard microbiological techniques. Bacterial isolates were identified through colony morphology, Gram staining, and biochemical profiling. Antibiotic susceptibility testing was performed using the disc diffusion method following CLSI 2022 guidelines. Biofilm formation was assessed using the microtiter plate method, while extended-spectrum beta-lactamase (ESBL) production was detected through the double disc synergy test. **Results:** Five bacterial genera were isolated, including *Clostridium* spp., *Vibrio* spp., *Yersinia pseudotuberculosis*, *Streptococcus* spp., and *Staphylococcus aureus*. Antibiotic susceptibility testing revealed concerning resistance patterns, with most isolates showing resistance to multiple antibiotics including Augmentin and

ceftazidime. *Staphylococcus aureus* isolated from apple gourd exhibited methicillin resistance (MRSA). All tested isolates demonstrated biofilm-forming capabilities, with relative biofilm formation values ranging from 0.07 to 0.13. ESBL production was detected in *Streptococcus* spp., *Vibrio* spp., *Clostridium* spp., and *Yersinia pseudotuberculosis*, while S. *aureus* was ESBL-negative. **Conclusion:** The presence of MDR and ESBL-producing bacteria in fresh vegetables represents a significant public health concern. These findings underscore the urgent need for improved agricultural practices, enhanced surveillance systems, and public awareness campaigns regarding vegetable safety in Peshawar.

Keywords: Multidrug-resistant bacteria, fresh vegetables, ESBL, biofilm, food safety, Peshawar

1. Introduction

Fresh vegetables occupy a fundamental position in balanced human nutrition, supplying essential minerals, dietary fibers, and vitamins that collectively support human health. Public health organizations worldwide advocate for daily vegetable consumption, recommending at least five portions daily to protect against various malignancies and cardiovascular conditions (Callejon et al., 2015). The World Health Organization and Food and Agriculture Organization jointly launched a global initiative in 2003 promoting vegetable intake, establishing a daily recommendation of 400 grams (Holzel et al., 2018; WHO, 2003).

The growing awareness of these health benefits has driven increasing demand for fresh vegetables. However, contemporary lifestyle changes have paradoxically reduced time spent on meal preparation (Zekar et al., 2017), leading to greater consumption of raw or minimally processed vegetables. This trend raises significant safety concerns, as raw vegetables may harbor pathogenic microorganisms (Amaechi et al., 2016). Poor hygiene practices during production and post-harvest handling contribute substantially to contamination, with international trade facilitating the global dissemination of pathogenic bacteria (Akoachere et al., 2018; Punsawad et al., 2019; Han et al., 2021).

Vegetables such as tomatoes, capsicum, and potatoes are particularly susceptible to microbial contamination. Tomatoes, the world's second most cultivated vegetable, provide valuable nutrients including vitamins A, C, E, folate, and lycopene (Shah et al., 2021; Ramya and Patel, 2019). Yet they remain vulnerable to over 200 diseases caused by various pathogens. While chemical pesticides offer some protection, their frequent application adversely affects nutritional content, soil quality, and productivity (Singh and Sahareen, 2017).

The emergence of antibiotic-resistant bacteria in vegetables represents an escalating global health challenge. Contamination can occur throughout production cycles through direct antibiotic application, contaminated irrigation water, or fertilizer use (Latif et al., 2018). Insects may serve as vectors for resistant bacteria (Bengtsson et al., 2018), while enterococci from warm-blooded

animals enter environments through fecal contamination, eventually colonizing plants (Mogren et al., 2018). Multiple studies have documented vegetable contamination by *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella* species, and other pathogens (Ilyas et al., 2016; Mir et al., 2018; Elenwo and Imade, 2020).

Biofilm formation further complicates this picture, as bacterial communities embedded in extracellular matrices resist sanitizers and disinfectants (Ng et al., 2018). These structured communities survive unfavorable conditions, including nutrient deprivation and temperature extremes, making their elimination from food processing environments exceptionally challenging (Giaouris et al., 2015). The 2017 vegetable recalls in Canada underscore the practical implications of these concerns (Herod et al., 2019).

Extended-spectrum beta-lactamase (ESBL) producing bacteria have attracted particular attention as one of the world's six major antibiotic resistance-related health threats (Richter et al., 2019). Their ability to inactivate broad-spectrum cephalosporins severely limits therapeutic options when infections occur (Freitag et al., 2018). Fresh vegetables commonly harbor these organisms (Ye et al., 2017), raising questions about their role in transmitting resistant determinants to humans.

This study aimed to enhance existing knowledge regarding vegetable contamination levels and characterize antibiotic-resistant microbes in fruits and vegetables from Peshawar markets. Specific objectives included isolating and identifying MDR bacteria, performing antibiotic susceptibility testing, investigating biofilm formation capacity, and detecting beta-lactamase production among selected isolates.

2. Materials and Methods

2.1 Study Duration and Location

This research was conducted from August 2020 to August 2021 in the Microbiology Laboratory of the Institute of Biological Sciences, Sarhad University of Science and Technology, Peshawar, Pakistan.

2.2 Sample Collection and Processing

Ten vegetable types were purchased from various markets in Peshawar: radish (*Raphanus sativus*), carrot (*Daucus carota*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), bitter gourd (*Momordica charantia*), capsicum (*Capsicum annuum*), cabbage (*Brassica oleracea*), turnip (*Brassica rapa*), salad leaves (*Lactuca sativa*), and apple gourd (*Praecitrullus fistulosus*).

Samples were placed in sterile zip-locked polythene bags and transported to the laboratory under cold chain conditions (4°C) for bacteriological analysis (Sarker et al., 2018). Each sample was rinsed with 20 mL phosphate-buffered saline, transferred to sterile plastic bags, and aseptically cut and ground using sterile blades. Samples underwent ten-fold serial dilution in nutrient broth (Sarker et al., 2018).

2.3 Bacterial Isolation

Nutrient broth served as the initial isolation medium. Inoculated broths were incubated overnight at 37°C, then streaked onto MacConkey agar prepared according to manufacturer specifications. Single isolated colonies were subcultured on MacConkey agar to obtain pure cultures (Sarker et al., 2018).

2.4 Identification of Bacterial Isolates

Isolate identification relied on colony morphology, Gram staining, and biochemical profiling (Siddique et al., 2018). Colony characteristics were observed on nutrient agar, MacConkey agar, mannitol salt agar, and blood agar. Gram staining was performed using standard procedures with crystal violet, iodine, alcohol decolorizer, and safranin counterstain (Seth et al., 2017).

Biochemical tests included catalase, triple sugar iron (TSI), indole, oxidase, urease, carbohydrate utilization, hemolysis, and spore formation tests, performed according to established protocols (Islam et al., 2020; Holt et al., 2013; Opara, 2020).

2.5 Antibiotic Susceptibility Testing

The Kirby-Bauer disc diffusion assay evaluated antibiotic susceptibility following CLSI 2022 guidelines. Bacterial suspensions were adjusted to 0.5 McFarland standard turbidity (1.5×10^8 CFU/mL) prepared by mixing 0.05 mL barium chloride with 9.95 mL sulfuric acid (Ghasemi et al., 2020).

Test bacteria were inoculated in nutrient broth, incubated overnight, and subcultured under identical conditions. Suspensions were swabbed onto cation-adjusted Mueller-Hinton agar plates. Antibiotic discs (Musa Ji Adams and Sons, Peshawar) included: imipenem (10 µg), ceftazidime (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), piperacillin-tazobactam (110 µg), moxifloxacin (30 µg), cefoxitin (30 µg), tigecycline (15 µg), linezolid (30 µg), chloramphenicol (30 µg), sulfamethoxazole-trimethoprim (25 µg), and augmentin (3 µg). Plates were incubated overnight at 37°C, and inhibition zone diameters were measured in millimeters. Isolates were classified as susceptible, intermediate, or resistant according to CLSI interpretive criteria (CLSI, 2022).

2.6 Biofilm Formation Assay

Biofilm-forming capacity was assessed using the microtiter plate method (Kim et al., 2018). Test isolates were inoculated in trypticase soy broth and incubated overnight. Cultures were diluted 100-fold in fresh TSB, and aliquots were added to 96-well polystyrene microtiter plates. After 48-hour incubation at 37°C, plates were rinsed with phosphate-buffered saline to remove non-adherent cells, then stained with 0.1% crystal violet. Following drying and gentle washing with distilled water, ethanol was added to each well, and absorbance was measured at 600 nm using an ELISA reader. Each test was performed in triplicate with uninoculated culture medium as control.

Biofilm-forming ability was categorized as strong, moderate, or weak based on relative biofilm formation calculated as:

Relative biofilm formation = (A_x - A_0) / A_0

where A_x represents isolate absorbance and A_0 represents blank absorbance.

2.7 ESBL Phenotype Detection

The double disc synergy test detected ESBL production (Shallouf, 2018). Mueller-Hinton agar plates were inoculated with test bacteria. Discs containing amoxicillin-clavulanic acid, ceftazidime, cefotaxime, and ceftriaxone were placed 30 mm apart using sterile forceps. Plates were incubated for 24 hours at 37°C, and inhibition zone diameters were measured. Distortion or increased zone diameter toward the amoxicillin-clavulanic acid disc indicated ESBL positivity.

3. Results

3.1 Bacterial Isolates from Vegetables

Five bacterial genera were isolated from the ten vegetable types examined. Table 1 presents the distribution of isolates across vegetable samples.

Table 1: ***Bacterial Isolates from Different Vegetables***

Vegetable	Bacterial Isolate
Radish (*Raphanus sativus*)	*Clostridium* spp.
Carrot (*Daucus carota*)	*Clostridium* spp.
Potato (*Solanum tuberosum*)	*Vibrio* spp.
Tomato (*Solanum lycopersicum*)	*Yersinia pseudotuberculosis*
Bitter gourd (*Momordica charantia*)	*Streptococcus* spp.
Capsicum (*Capsicum annuum*)	*Vibrio* spp.
Cabbage (*Brassica oleracea*)	*Streptococcus* spp.
Turnip (*Brassica rapa*)	*Clostridium* spp.
Salad leaves (*Lactuca sativa*)	*Streptococcus* spp.

Vegetable	Bacterial Isolate
Apple gourd (*Praecitrullus fistulosus*)	*Staphylococcus aureus*

3.2 Colony Morphology and Gram Staining Characteristics

Colonial morphology varied among isolates on different media (Table 2). Gram staining revealed *Clostridium* spp. as purple-staining rods, *Vibrio* spp. as pink rods, *Yersinia pseudotuberculosis* as pink rods, *Streptococcus* spp. as purple cocci, and *Staphylococcus aureus* as purple round colonies.

Table 2: ***Colony Morphology of Bacterial Isolates***

Isolate	Nutrient Agar	MacConkey Agar	MSA	Blood Agar
Clostridium spp.	Whitish opaque colonies	Pinkish colonies	,	,
Vibrio spp.	Opaque colonies	Pinkish colonies	,	,
Y. pseudotuberculosis	Whitish colonies	Pinkish spots	,	,
Streptococcus spp.	Dome-shaped colorless colonies	,	,	Grayish/black smooth colonies
S. aureus	Golden-yellowish colonies	,	Yellow colonies	Golden colonies

3.3 Biochemical Characterization

Biochemical test results confirmed isolate identities (Table 3). Notable findings included catalase positivity for *S. aureus*, oxidase positivity for *Vibrio* spp., urease positivity for *Y. pseudotuberculosis*, and spore formation and strict anaerobe characteristics for *Clostridium* spp.

Table 3: *Biochemical Test Results*

Test	*Clostridium*	*Vibrio*	*Y. pseudotuberculosis*	*Streptococcus*	*S. aureus*
Catalase	-	-	-	-	+
Mannitol fermentation	-	-	-	-	+
Indole	-	-	-	-	-
Oxidase	-	+	-	-	-
Urease	-	-	+	-	-
Carbohydrate utilization	-	+	-	-	+
Hemolysis	-	-	-	+	-
Spore forming	+	-	-	-	-
Motility	-	-	-	-	-
Strict anaerobes	+	-	-	-	-

+ = positive, - = negative

3.4 Antibiotic Susceptibility Patterns

Antibiotic susceptibility testing revealed concerning resistance profiles across isolates. *Clostridium* species from various vegetables demonstrated consistent resistance to augmentin (3 µg) with zone diameters of 0 mm (Tables 4-6). Ceftazidime resistance was also common, with some isolates showing complete resistance (0 mm zones).

Table 4: *Antibiotic Susceptibility of Clostridium spp. from Turnip*

Antibiotic	Concentration	Zone Diameter (mm)
Gentamicin	30 µg	15
Augmentin	3 µg	0
Ciprofloxacin	5 µg	20
Imipenem	10 µg	15
Piperacillin/tazobactam	110 µg	16
Ceftazidime	30 µg	12

Table 5: *Antibiotic Susceptibility of Clostridium spp. from Carrot*

Antibiotic	Concentration	Zone Diameter (mm)
Gentamicin	30 µg	20
Augmentin	3 µg	0

Ciprofloxacin	5 µg	22
Imipenem	10 µg	19
Amikacin	110 µg	16
Piperacillin/tazobactam	30 µg	12
Ceftazidime	30 µg	11

Table 6: ***Antibiotic Susceptibility of Clostridium spp. from Radish***

Antibiotic	Concentration	Zone Diameter (mm)
Augmentin	3 µg	0
Ceftazidime	30 µg	0
Imipenem	10 µg	15
Gentamicin	30 µg	16
Amikacin	30 µg	17
Ciprofloxacin	5 µg	22
Piperacillin/tazobactam	30 µg	14

Vibrio species from capsicum and potato exhibited similar resistance patterns (Tables 7-8), with augmentin and ceftazidime resistance (0 mm zones) and reduced susceptibility to piperacillin-tazobactam (2 mm zones).

Table 7: ***Antibiotic Susceptibility of Vibrio spp. from Capsicum***

Antibiotic	Concentration	Zone Diameter (mm)
Augmentin	3 µg	0
Ceftazidime	30 µg	0
Imipenem	10 µg	12
Gentamicin	30 µg	10
Amikacin	30 µg	16
Ciprofloxacin	5 µg	22
Piperacillin/tazobactam	30 µg	2

Table 8: ***Antibiotic Susceptibility of Vibrio spp. from Potato***

Antibiotic	Concentration	Zone Diameter (mm)
Augmentin	3 µg	0
Ceftazidime	30 µg	0
Imipenem	10 µg	10
Gentamicin	30 µg	10
Amikacin	30 µg	22
Ciprofloxacin	5 µg	16

Piperacillin/tazobactam	30 µg	2

Yersinia pseudotuberculosis from tomato demonstrated the most concerning resistance profile, with small zone diameters across all tested antibiotics (Table 9).

Table 9: ***Antibiotic Susceptibility of Y. pseudotuberculosis from Tomato***

Antibiotic	Concentration	Zone Diameter (mm)
Augmentin	3 µg	0
Ceftazidime	30 µg	0
Imipenem	10 µg	10
Gentamicin	30 µg	8
Amikacin	30 µg	6
Ciprofloxacin	5 µg	7
Piperacillin/tazobactam	30 µg	5

Streptococcus species from bitter gourd, cabbage, and lettuce showed variable but generally reduced susceptibility (Tables 10-12). Complete resistance to augmentin was consistent across isolates.

Table 10: ***Antibiotic Susceptibility of Streptococcus spp. from Bitter Gourd***

Antibiotic	Concentration	Zone Diameter (mm)
Augmentin	3 µg	0
Ceftazidime	30 µg	0
Imipenem	10 µg	10
Gentamicin	30 µg	10
Amikacin	30 µg	10
Ciprofloxacin	5 µg	15
Piperacillin/tazobactam	30 µg	4

Table 11: Antibiotic Susceptibility of *Streptococcus* spp. from Cabbage

Antibiotic	Concentration	Zone Diameter (mm)
Augmentin	3 µg	0
Ceftazidime	30 µg	6
Imipenem	10 µg	12
Gentamicin	30 µg	12
Amikacin	30 µg	16
Ciprofloxacin	5 µg	0
Piperacillin/tazobactam	30 µg	4

Table 12: *Antibiotic Susceptibility of Streptococcus spp. from Lettuce*

Antibiotic	Concentration	Zone Diameter (mm)
Ceftazidime	30 µg	5
Imipenem	10 µg	0
Gentamicin	30 µg	10
Amikacin	30 µg	8
Ciprofloxacin	5 µg	6
Piperacillin/tazobactam	30 µg	2

Staphylococcus aureus from apple gourd demonstrated a distinct susceptibility pattern (Table 13), with large zones for chloramphenicol (31 mm), gentamicin (34 mm), amikacin (33 mm), and linezolid (32 mm). However, cefoxitin resistance (12 mm) indicated methicillin-resistant *S. aureus* (MRSA).

Table 13: *Antibiotic Susceptibility of S. aureus from Apple Gourd*

Antibiotic	Concentration	Zone Diameter (mm)
Sulfamethoxazole-trimethoprim	25 µg	29
Moxifloxacin	5 µg	19
Chloramphenicol	30 µg	31
Cefoxitin	30 µg	12
Gentamicin	30 µg	34
Amikacin	30 µg	33
Linezolid	30 µg	32
Tigecycline	15 µg	22

3.5 Biofilm Formation

All isolates demonstrated biofilm-forming capacity (Figure 1). Relative biofilm formation values ranged from 0.07 to 0.13, with all isolates showing significantly greater biofilm formation than the laboratory control strain *E. coli* ($P < 0.05$).

Figure 1: Biofilm Formation of Isolated Bacteria

[Bar graph showing relative absorbance values:
Clostridium spp.: 0.13
Vibrio spp.: 0.11
Y. pseudotuberculosis: 0.09
Streptococcus spp.: 0.10
S. aureus: 0.07]

3.6 ESBL Production

ESBL production was detected in four of the five bacterial genera examined (Table 14). *Streptococcus* spp., *Vibrio* spp., *Clostridium* spp., and *Yersinia pseudotuberculosis* were ESBL producers, while *Staphylococcus aureus* was negative for ESBL production.

Table 14: ***ESBL Production Among Isolates***

ESBL Producers	ESBL Non-Producers
Streptococcus spp.	*Staphylococcus aureus*
Vibrio spp.	-
Clostridium spp.	-
Yersinia pseudotuberculosis	-

4. Discussion

Fresh vegetables represent a double-edged sword in human nutrition, while providing essential nutrients and protection against chronic diseases, they simultaneously serve as potential vehicles for pathogenic microorganisms. This study's findings reveal alarming levels of multidrug-resistant bacteria contaminating commonly consumed vegetables in Peshawar, aligning with global concerns about foodborne transmission of antibiotic resistance.

The bacterial genera isolated, *Clostridium*, *Streptococcus*, *Yersinia*, *Staphylococcus*, and *Vibrio*, reflect those reported in similar investigations worldwide. The predominance of *Clostridium* and *Streptococcus* species parallels findings by Tango et al. (2018), who isolated *Clostridium* from turnip and carrot, and Nithya and Babu (2017), who reported both genera in salad leaves and tomato. The recovery of *Staphylococcus aureus* from apple gourd, cabbage, and lettuce corresponds with Akoachere et al. (2018) and Kwaku et al. (2016), who documented similar contamination patterns. Notably, Khan et al. (2019) previously reported comparable isolates including *Clostridium*, *Bacillus*, *S. aureus*, and *Listeria* species from Peshawar and Mardan, confirming regional contamination patterns.

The presence of *Yersinia pseudotuberculosis* in tomato samples corroborates findings by Verbikova et al. (2018), Chen et al. (2019), and Bintsis (2018). Savin et al. (2022) similarly isolated this pathogen from tomatoes, while Mengal et al. (2019) reported *Yersinia* from salad leaves in Pakistan. These consistent findings suggest that *Yersinia* contamination of fresh produce may be more widespread than previously recognized.

Antibiotic susceptibility testing revealed concerning resistance patterns. The complete resistance to augmentin observed across multiple isolates, particularly *Clostridium* species, mirrors Cole et al.'s (2018) report of ceftazidime-resistant *Clostridium*. The isolation of methicillin-resistant *Staphylococcus aureus* from apple gourd represents a particularly significant finding, given

MRSA's clinical importance. Similar MRSA isolates have been reported from turnip by Weldezgina et al. (2016) and from cabbage by Obajuluwa et al. (2021). However, Wu et al. (2018) described cefoxitin-sensitive *S. aureus*, highlighting geographical variation in resistance patterns.

The classification of all isolates except *S. aureus* as multidrug-resistant aligns with Golly et al. (2016), who documented MDR *Enterobacteriaceae* from cabbage, carrot, and lettuce. This widespread MDR occurrence suggests that vegetable contamination with resistant bacteria represents a systematic problem requiring comprehensive intervention.

Biofilm formation capacity among all tested isolates raises additional concerns for food safety. Relative biofilm formation values ranging from 0.07 to 0.13, while modest, indicate that these organisms can establish surface-associated communities resistant to removal. Kim et al. (2018) similarly reported biofilm-forming *E. coli* from lettuce, while Lin et al. (2019) documented biofilm-forming *S. aureus* from ready-to-eat salads. Verbikova et al. (2018) described biofilm-forming *Yersinia* from fresh lettuce, cabbage, and tomato, and Igbinosa et al. (2019) isolated biofilm-forming *Vibrio* from salads. Chen et al. (2020) reported biofilm-forming *Streptococci* from cabbage, consistent with our findings. Park et al. (2020) isolated biofilm-forming *Bacillus* from lettuce in Korea, demonstrating that this phenomenon extends across bacterial genera and geographical regions.

The detection of ESBL-producing bacteria, *Streptococcus* spp., *Vibrio* spp., *Clostridium* spp., and *Yersinia pseudotuberculosis*, represents perhaps the most clinically significant finding. ESBL production compromises beta-lactam antibiotics, including penicillins and cephalosporins, severely limiting treatment options. Liu et al. (2018) similarly reported ESBL-producing *Clostridium* from potato and cabbage in China. Lopes et al. (2021) and Richter et al. (2019) documented ESBL-producing *Klebsiella pneumoniae*, *E. coli*, and *Enterobacteriaceae* from cabbage, lettuce, and tomato. Daniels et al. (2019) reported ESBL-producing *Streptococci* from ready-to-eat salads, aligning with our observations.

The absence of ESBL production in *S. aureus* isolates, while reassuring, does not diminish concern given the MRSA finding. This pattern suggests that resistance mechanisms vary among bacterial genera, with implications for surveillance and intervention strategies.

Several limitations warrant consideration. The sample size, while adequate for initial characterization, may not fully capture seasonal or geographical variation in contamination patterns. The study did not investigate potential contamination sources, such as irrigation water quality or post-harvest handling practices. Molecular characterization of resistance genes would complement phenotypic resistance profiling and provide insights into resistance mechanisms and potential transmission pathways.

5. Conclusion and Recommendations

5.1 Conclusion

This study demonstrates an alarming prevalence of multidrug-resistant pathogenic bacteria in fresh vegetables from Peshawar markets. The isolation of ESBL-producing organisms, including *Streptococcus* spp., *Vibrio* spp., *Clostridium* spp., and *Yersinia pseudotuberculosis*, alongside methicillin-resistant *Staphylococcus aureus*, highlights the potential role of vegetables as vectors for transmitting clinically significant resistant pathogens. The universal biofilm-forming capacity among isolates further complicates contamination management and elimination. These findings collectively indicate that fresh vegetables represent a significant public health concern requiring immediate attention from regulatory authorities, healthcare providers, and consumers.

5.2 Recommendations

Based on these findings, the following measures are recommended:

Regulatory and Policy Interventions: Strict quality control measures should be implemented throughout the vegetable supply chain, from production through retail. National antibiotic treatment guidelines must be established to preserve antimicrobial effectiveness, and empirical antibiotic therapy should be avoided given the unusual resistance profiles documented in this study.

Agricultural Practices: Farmers require education regarding contamination sources and prevention strategies. Training programs should address safe irrigation practices, proper fertilizer use, and appropriate post-harvest handling. The use of untreated wastewater for irrigation must be prohibited, and composting practices should ensure pathogen elimination.

Surveillance and Monitoring: Comprehensive surveillance systems tracking antibiotic-resistant bacteria in food products should be established. Healthcare facilities must implement complete infection control policies and antimicrobial stewardship programs. Regular monitoring of high-risk vegetables should inform timely interventions.

Public Awareness: Consumer education campaigns should communicate risks associated with contaminated vegetables and promote safe handling practices, including thorough washing and proper storage. The public must understand that appearance alone does not indicate safety.

Future Research: Investigations should identify specific contamination sources along production chains and characterize resistance genes at the molecular level. Studies examining seasonal variation in contamination patterns and evaluating intervention effectiveness would guide evidence-based policies. Research exploring alternatives to antibiotics in agriculture, including biocontrol agents and plant-based antimicrobials, may offer sustainable solutions.

6. References

Akoachere, J.F.T.K., B.F. Tatsinkou and J.M. Nkengfack. 2018. Bacterial and parasitic contaminants of salad vegetables sold in markets in Fako Division, Cameroon and evaluation of hygiene and handling practices of vendors. BMC Research Notes, 11(1): 1-7.

Amaechi, E.C., C.C. Ohaeri, O.M. Ukpai and R.A. Adegbite. 2016. Prevalence of parasitic contamination of salad vegetables in Ilorin, North Central Nigeria. Momona Ethiopian Journal of Science, 8(2): 136-145.

Bengtsson, P.J., E. Kristiansson and D. Larsson. 2018. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiology Reviews, 42(1): 1-13.

Bintsis, T. 2018. Microbial pollution and food safety. AIMS Microbiology, 4(3): 377-396.

Callejon, R.M., M.R. Naranjo, C. Ubeda, R.H. Ortega, M.C.G. Parrilla and A.M. Troncoso. 2015. Reported foodborne outbreaks due to produce in the United States and European Union: trends and causes. Foodborne Pathogens and Disease, 12(1): 32-38.

Chen, C., X. Yang and X. Shen. 2019. Confirmed and potential roles of bacterial T6SSs in the intestinal ecosystem. Frontiers in Microbiology, 10(1): 1-11.

Chen, X., E.B.M. Daliri, R. Chelliah and D.H. Oh. 2020. Isolation and identification of potentially pathogenic microorganisms associated with dental caries in human teeth biofilms. Microorganisms, 8(10): 1-12.

CLSI. 2022. Performance standards for antimicrobial susceptibility testing. Thirty-second informational supplement, CLSI document M100-Ed32. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

Cole, M.L. and O.V. Singh. 2018. Microbial occurrence and antibiotic resistance in ready-to-go food items. Journal of Food Science and Technology, 55(7): 2600-2609.

Daniels, A.O., O. Jolapamo, T. Temikotan and A.O. Akinkugbe. 2019. Resistance profile of contaminating organisms isolated from ready-to-eat vegetable salad from selected eateries in a university setting. International Journal of Development Research, 9(10): 1-5.

Elenwo, E.J. and O.S. Imade. 2020. Ready-to-eat vegetable salads served in Nigerian restaurants: a potential source of multidrug-resistant bacteria. Journal of Applied Microbiology, 129(5): 1402-1409.

Freitag, C., G.B. Michael, L. Jun, K. Kadlec, W. Yang, M. Hassel and S. Schwarz. 2018. Occurrence and characterization of ESBL-encoding plasmids among Escherichia coli isolates from fresh vegetables. Veterinary Microbiology, 219: 63-69.

Ghasemi, S., M.R. Bari, S. Pirsa and S. Amiri. 2020. Use of bacterial cellulose film modified by polypyrrole/TiO2-Ag nanocomposite for detecting and measuring the growth of pathogenic bacteria. Carbohydrate Polymers, 232: 1-13.

Giaouris, E., M. Heir, M. Desvaux, T. Hebraud, S. Moretro, A. Langsrud, G.J. Doulgeraki, M. Nychas, K. Kacaniova, H. Czaczyk, H. Olmez and M. Simoes. 2015. Intra and inter-species interactions within biofilms of important foodborne bacterial pathogens. Frontiers in Microbiology, 6(841): 1-26.

Golly, M.K., S.P. Salifu and F.C.M. Robertson. 2016. Resistance of bacteria isolates from cabbage (Brassica oleracea), carrots (Daucus carota) and lettuce (Lactuca sativa) in the Kumasi Metropolis of Ghana. Research in Agriculture and Food, 4(11): 1-14.

Han, J., X. Zhang, S. He and P. Jia. 2021. Can the coronavirus disease be transmitted from food? A review of evidence, risks, policies and knowledge gaps. Environmental Chemistry Letters, 19(1): 5-16.

Herod, A., L. Goodridge and J. Rohde. 2019. Recalls of foods due to microbial contamination classified by the Canadian Food Inspection Agency, 2000 to 2017. Journal of Food Protection, 82(11): 1901-1908.

Holt, J.G., N.R. Krie, P.H.A. Sneath, J.T. Stately and S.T. Williams. 2013. Bergey's Manual of Determinative Bacteriology. 9th Edition. Williams and Wilkins, Baltimore, USA.

Holzel, C.S., J.L. Tetens and K. Schwaiger. 2018. Unraveling the role of vegetables in spreading antimicrobial-resistant bacteria: a need for quantitative risk assessment. Foodborne Pathogens and Disease, 15(11): 671-688.

Igbinosa, E.O. and E.E. Odjadjare. 2019. Characterization of antibiogram susceptibility profile of Vibrio species isolated from fresh vegetables. African Scientist, 17(2): 147-152.

Ilyas, S., M.U. Qamar, M.H. Rasool, N. Abdulhaq and Z. Nawaz. 2016. Multidrug-resistant pathogens isolated from ready-to-eat salads available at a local market in Pakistan. British Food Journal, 118(9): 2068-2075.

Islam, R., M.N. Hossain, M.K. Alam, M.E. Uddin, M.H. Rony, M.A.S. Imran and M.F. Alam. 2020. Antibacterial activity of lactic acid bacteria and extraction of bacteriocin protein. Advances in Bioscience and Biotechnology, 11(2): 49-59.

Khan, H., S. Ullah, M. Salman, F. Hussain, Y. Anwar, L. Ullah and M. Shuaib. 2019. Microbiological safety and antibiogram analysis of selected food products obtained in the marketplace of Peshawar and Mardan, KPK, Pakistan. Polish Journal of Environmental Studies, 28(6): 4239-4245.

Kim, H.J., T. Oh and S.Y. Baek. 2018. Multidrug resistance, biofilm formation, and virulence of Escherichia coli isolates from commercial meat and vegetable products. Foodborne Pathogens and Disease, 15(12): 782-789.

Kwaku, G.M., S.P. Samson and M.R.F. Charles. 2016. Resistance of bacteria isolates from cabbage (Brassica oleracea), carrots (Daucus carota) and lettuce (Lactuca sativa) in the Kumasi Metropolis of Ghana. International Journal of Nutrition and Food Sciences, 5(4): 297-303.

Latif, A., M. Bilal, W. Asghar, M. Azeem, M.I. Ahmad, A. Abbas and T. Shahzad. 2018. Heavy metal accumulation in vegetables and assessment of their potential health risk. Journal of Environmental and Analytical Chemistry, 5(1): 1-7.

Lin, Q., H. Sun, K. Yao, J. Cai, Y. Ren and Y. Chi. 2019. The prevalence, antibiotic resistance and biofilm formation of Staphylococcus aureus in bulk ready-to-eat foods. Biomolecules, 9(10): 524-532.

Liu, B.T., X.Y. Zhang, S.W. Wan, J.J. Hao, R. Jiang and F.J.D. Song. 2018. Characteristics of carbapenem-resistant Enterobacteriaceae in ready-to-eat vegetables in China. Frontiers in Microbiology, 9: 1-12.

Lopes, R., D.F. Castillo, H. Fontana, L. Rodrigues, K. Dantas, L. Cerdeira and N. Lincopan. 2021. Endophytic lifestyle of global clones of extended-spectrum β-lactamase-producing priority pathogens in fresh vegetables: a trojan horse strategy favoring human colonization. mSystems, 6(1): 1-20.

Mengal, H., A. Samad, M.T. Asmat, M.Z. Mustafa, F. Abbas, N. Sajjad and D. Ghilzai. 2019. The study to evaluate the prevalence of Yersinia species in salad sold and water supplied in Quetta city, Pakistan. Pure and Applied Biology, 8(3): 1902-1908.

Mir, S.A., M.A. Shah, M.M. Mir, B.N. Dar, R. Greiner and S. Roohinejad. 2018. Microbiological contamination of ready-to-eat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens. Food Control, 85: 235-244.

Mogren, L., S. Windstam, S. Boqvist, I. Vagsholm, S.K. Oderqvist and A.K. Rosberg. 2018. The hurdle approach-A holistic concept for controlling food safety risks associated with pathogenic bacterial contamination of leafy green vegetables. Frontiers in Microbiology, 9: 1-20.

Ng, C.G., M.F. Loke, K.L. Goh, J. Vadivelu and B. Ho. 2017. Biofilm formation enhances Helicobacter pylori survivability in vegetables. Food Microbiology, 62: 68-76.

Nithya, A. and S. Babu. 2017. Prevalence of plant beneficial and human pathogenic bacteria isolated from salad vegetables in India. BMC Microbiology, 17(1): 1-16.

Obajuluwa, A.F., A. Kefas and S.K. Parom. 2021. Antibiotics susceptibility pattern of bacteria isolated from ready-to-eat vegetables sold in Mohammadu Gumi Market, Kaduna, Nigeria. Nigerian Journal of Pharmaceutical Research, 16(2): 131-137.

Opara, A. 2020. Biochemical tests in bacteriology. In: Laboratory Manual for Microbiology. University Press, Nigeria.

Park, K.M., H.J. Kim, M. Jeong and M. Koo. 2020. Enterotoxin genes, antibiotic susceptibility, and biofilm formation of low-temperature-tolerant Bacillus cereus isolated from green leaf lettuce in the cold chain. Foods, 9(3): 1-14.

Punsawad, C., N. Phasuk, K. Thongtup, S. Nagavirochana and P. Viriyavejakul. 2019. Prevalence of parasitic contamination of raw vegetables in Nakhon Si Thammarat province, southern Thailand. BMC Public Health, 19(1): 1-7.

Ramya, V. and P. Patel. 2019. Health benefits of vegetables. International Journal of Chemical Studies, 7(2): 82-87.

Richter, L., E.M.D. Plessis, S. Duvenage and L. Korsten. 2019. Occurrence, identification and antimicrobial resistance profiles of extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae from fresh vegetables retailed in Gauteng Province, South Africa. Foodborne Pathogens and Disease, 16(5): 421-427.

Sarker, A., M. Haque, R. Rifa, F. Ema, A. Islam and M. Khatun. 2018. Isolation and identification of bacteria from fresh guava sold at local markets in Mymensingh and their antibiogram profile. Veterinary World, 11(8): 1145-1149.

Savin, C., A.S.L. Guern, F. Chereau, J. Guglielmini, G. Heuze, C. Demeure and J.P.P. Cerda. 2022. First description of a Yersinia pseudotuberculosis clonal outbreak in France, confirmed using a new core genome multilocus sequence typing method. bioRxiv, 11(1): 1-27.

Seth, R., P.S.R. Murthy, S. Sistla, M. Subramanian and K. Tamilarasu. 2017. Rapid and accurate diagnosis of acute pyogenic meningitis due to Streptococcus pneumoniae, Haemophilus influenzae type b and Neisseria meningitidis using a multiplex PCR assay. Journal of Clinical and Diagnostic Research, 11(9): 1-18.

Shah, K.K., B. Modi, B. Lamsal, J. Shrestha and S.P. Aryal. 2021. Bioactive compounds in tomato and their roles in disease prevention. Fundamental and Applied Agriculture, 6(2): 210-224.

Shallouf, M.A. 2018. Identification and characterization of cephalosporins and carbapenem-resistant Klebsiella pneumoniae isolates from Misrata, Libya. Iranian Journal of Medical Sciences, 7(3): 156-165.

Siddique, M.H., M. Qamar, S. Hayat, B. Aslam, H. Nadeem, S. Hussain and S. Muzammil. 2018. Polymicrobial multidrug-resistant bacteria isolated from street vended fresh fruit juices in Pakistan. British Food Journal, 120(6): 1358-1365.

Singh, M. and T. Sahareen. 2017. Investigation of cellulosic packets impregnated with silver nanoparticles for enhancing shelf-life of vegetables. LWT - Food Science and Technology, 86: 116-122.

Tango, C.N., S. Wei, I. Khan, M.S. Hussain, P.F. Kounkeu, J.H. Park and D.H. Oh. 2018. Microbiological quality and safety of fresh fruits and vegetables at retail levels in Korea. Journal of Food Science, 83(2): 386-392.

Verbikova, V., G. Borilova, V. Babak and M. Moravkova. 2018. Prevalence, characterization and antimicrobial susceptibility of Yersinia enterocolitica and other Yersinia species found in fruits and vegetables from the European Union. Food Control, 85: 161-167.

Weldezgina, D. and D. Muleta. 2016. Bacteriological contaminants of some fresh vegetables irrigated with Awetu River in Jimma Town, Southwestern Ethiopia. Advances in Biology, 11(11): 1-19.

World Health Organization. 2003. Diet, nutrition, and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. WHO Technical Report Series 916. Geneva, Switzerland.

Wu, S., J. Huang, Q. Wu, F. Zhang, J. Zhang, T. Lei and L. Xue. 2018. Prevalence and characterization of Staphylococcus aureus isolated from retail vegetables in China. Frontiers in Microbiology, 6(52): 59-67.

Ye, F., Z. Xie, X. Wu and X. Lin. 2017. Determination of pyrethroid pesticide residues in vegetables by pressurized capillary electrochromatography. Talanta, 69: 97-102.

Zekar, F., S.A. Granier, M. Marault, L. Yaici, B. Gassilloud, C. Manceau and Y. Millemann. 2017. From farms to markets: Gram-negative bacteria resistant to third-generation cephalosporins in fruits and vegetables in a region of North Africa. Frontiers in Microbiology, 8: 1-11.