Bacteriological Analysis of Vegetables found in Kathmandu Valley

Mina Kumari Gurung[1], Priyanka Bhandari[1], Aarju Bhandari[1], Samjhana Yadav[1], Saroj Paudel[2], Jeena Amatya[1]

[1]Tri-Chandra Multiple Campus, Kathmandu, Nepal
[2]Nepalese Farming Institute, Kathmandu, Nepal

***Corresponding author**: Mina Kumari Gurung, Department of Microbiology, Tri-Chandra Multiple Campus, Kathmandu, Nepal; E-mail: mina.gurumg1700@gmail.com

ABSTRACT

Objectives: The study was conducted to determine the bacteriological analysis of vegetables by comparing the number of bacterial count between farm and market along with antibiotic susceptibility pattern of the isolates and evaluate the status of MDR and MRSA.

Methods: The cross-sectional study was done from February to July of 2023. A total of 60 samples were gathered from three distinct districts: Kathmandu, Bhaktapur, and Lalitpur, consisting of 10 samples from each farm and market and the research was carried out at the Nepalese Farming Institute, Kathmandu. The bacterial count was compared and identified by standard microbiological approaches such as serial dilution, spread plate method, and other biochemical tests. The antimicrobial profile was carried out by the Kirby-Bauer disc diffusion method to analyze the status of MDR and MRSA.

Results: From all 60 samples the highest total plate count and total coliform count (mean) was found in spinach (9.3×10^6) and cabbage (4.9×10^6) from market. A total of 35 bacteria were identified i.e. 16 (46%) from farms including *E. coli*, *Klebsiella* spp., *Pseudomonas* spp. and *Staphylococcus aureus* and 19(54%) from markets such as *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Serratia* spp. and *Staphylococcus aureus*. Antibiotic susceptibility pattern shown that Tetracycline was the most effective antibiotic as 80% *Enterobacteriaceace* isolates were sensitive but contradicted to *Serratia marcescens* and *S. aureus* was 100% susceptible to Linezolid, Cotrimoxazole followed by Gentamicin and Tetracycline. Out of 35 isolates, 14 showed MDR Strains i.e., *Enterobacteriaceace* (n=7; 35%) and *S. aureus* (n=7; 46.67%) and 3 isolates were screened as suspected ESBL producers but none were confirmed. A total of 15 *S. aureus* were isolated; among which 12 exhibited methicillin resistance (MRSA).

Conclusion: The present study shows that the vegetables from the studied area contain a wide variety of bacteria raising significant public health concerns. It highlights the need for improved protocols and practices throughout the entire process of vegetable production, distribution and consumption.

Keywords: Seasonal vegetables, bacterial count, antibiotic susceptibility, MDR, MRSA

INTRODUCTION

Vegetables provides outstanding wealth of vitamins, minerals, dietary fiber, and antioxidants (Salvin and Lloyd, 2012). Numerous countries have established dietary guidelines that advocate for vegetable consumption, typically recommending at least three servings of vegetables per day for adults (Wallace et al 2020). Additionally, the Food and Agriculture Organization (FAO) of the United Nations advises a daily intake of at least

Date of Submission: September 7, 2024
Date of Acceptance: December 17, 2024
Published Online: December 31, 2024
DOI: https://doi.org/10.3126/tujm.v11i1.82074

400 grams of fruits and vegetables per day (FAO, 2015).

In the Kathmandu Valley, vegetable sellers depend on various farms, making it essential to study whether spoilage occurs at the farm due to farmer practices or at the market due to retailer handling (Adhikari and GC, 2021). Vegetables can become contaminated at any stage of the production chain, with contamination sources classified into two main categories: pre-harvest and post-harvest (Jung, 2014). The use of pond and river water for washing vegetables increases contamination risk, as these water sources often harbor pathogenic microbes (Steele and Odumeru, 2004). In Nepal, irrigation during the dry season is common, with many farmers using dirty or wastewater for crop watering (Chand, 2018). The rivers flowing through the Kathmandu Valley are heavily polluted with untreated sewage further exacerbating the risk of contamination. (Shrestha et al., 2017).

Microbial food spoilage is a global issue, resulting in significant food waste and consumer dissatisfaction (Alegbeleye, 2022). Microbial contamination in vegetables poses significant health risks, leading to foodborne illnesses and other health complications. (Balali et al., 2020).

In various study areas, certain vegetable vendors utilize a single source of water for washing all their produce. When this water is not adequately sanitized and is repeatedly used, it can result in the transmission of pathogens from contaminated to otherwise uncontaminated vegetables. (Gombas et al., 2017). During the purchasing process, consumers frequently handle vegetables to evaluate their freshness. This interaction poses a risk of cross-contamination if the consumer carries pathogens due to insufficient hygiene practices. (James, 2006).

METHODS

Study design, study site and sample size

The study will employ by a cross-sectional design for the microbial analysis of seasonal vegetables found in Kathmandu valley. Samples of vegetables were gathered from various farms and markets across three districts: Kathmandu, Bhaktapur and Lalitpur. Sample were processed in the laboratory of Nepalese Farming Institute, Kathmandu. The surface of each sample was carefully sliced into small pieces using a sanitized knife. Ten grams of each sample were then measured and enclosed in aluminum foil.

Microbial Analysis

The measured samples underwent a series of dilutions, reaching up to 10^5, utilizing sterile water as the diluent. The process involved mixing 10 grams of vegetable sample with 90 ml of water to achieve a 10^5 dilution, followed by transferring 1 ml of this mixture into a new tube containing 9 ml of diluent, resulting in a 10^{-2} dilution. This dilution process continued in stages until reaching 10^{-5} (Acharya T, 2022). Additionally, dilutions of 10^{-3} and 10^{-5} were used for further analysis.

Isolation and identification

The total count of bacteria was conducted by the spread plate technique, using sterile glass rods to spread 10^{-3} and 10^{-5} suspensions onto PCA, VRBA and MSA agar plates. Subsequently, the plates were incubated for 24 hours at 37°C. After the incubation period, colony morphology was observed on all three media: PCA, VRBA, and MSA. Furthermore, colony counting and calculation of colony-forming units (CFU) per milliliter were performed using the CFU formula for VRBA and PCA (Ben-David and Davidson, 2014). Following colony counting, the colonies were transferred onto nutrient agar plates by quadrant streaking method and incubated for 24 hours at 37°C. Subsequently, the colonies were selected from the nutrient agar plates and subjected to a series of tests including, catalase and oxidase tests, as well as gram-staining, biochemical test (IMViC), TSI, OF and urease tests for coliform identification and for *Staphylococcus aureus* identification coagulase, DNAse, oxidase and catalase tests were conducted (Sharma et al., 2023). Based on the results of these tests, the organisms were identified.

Antibiotic susceptibility testing by Disc Diffusion method

The antibiotic susceptibility test was conducted using the Kirby-Bauer Disk Diffusion Technique, following the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI, 2017). The antibiotics included in the test were Cefotaxime 30µg (CTX), Ciprofloxacin 5µg (CIP), Chloramphenicol 30µg (C), Ampicillin 10µg (AMP), Tetracycline 30µg (TE), Cotrimoxazole 25µg (COT), Ceftazidime 30µg (CAZ), Amikacin 30µg (AK), and Gentamicin 10µg (GEN), specifically targeting *Enterobacteriaceace*, *Serrratia* spp. and *Pseudomonas* spp. Additionally, for *Staphylococcus aureus* Linezolid 30µg (LZ), Penicillin 10µg (P), Gentamicin 10µg (GEN), Erythromycin15µg (E), Clindamycin 2µg (CD), Tetracycline 30µg (TE), Cefoxitin 30µg (CX), Ciprofloxacin 30µg (CIP), and Cotrimoxazole 25µg (COT) were used in the test.

MDR and screening of ESBL producing

MDR isolates were identified as exhibiting non-susceptibility to at least one agent in three or more antimicrobial categories (Karumathil et al., 2016). ESBL detection was performed using a combined test method involving Ceftazidime 30 µg alone and in combination wit

h clavulanic acid 10 µg, as recommended by the clinical laboratory standard guidelines (2020). Each isolates were identified as ESBL producers that showed an increased zone inhibition diameter ≥5 mm in combination discs compared to Ceftazidime or Cefotaxime discs alone (Teklu, 2019).

Detection of MRSA

Additionally, all isolates of *Staphylococcus aureus* were screened for methicillin resistance using Ceftazidime 30 µg, and those *S aureus* isolates resistant to Ceftazidime were identified as MRSA (CLSI, 2020).

Statistical analysis

The results were recorded in worksheet and mean of the vegetable samples and standard were calculated.

RESULTS

Comparison of Bacteria found between Farm and Market vegetables

Out of 60 vegetable samples collected from 6 different farms and markets of Kathmandu Valley. The highest total bacterial count (mean) was found in Cabbage (9.12×10^6) from Bhaktapur farm and Spinach (9.3×10^6) from Kathmandu market respectively. (Table 1). The highest total coliform count (mean) was found in ladyfinger (3.105×10^6) from Bhaktapur farm and Cabbage (4.9×10^6) from Lalitpur market respectively (Table 2).

Total number of bacteria identified

A total of 60 vegetable samples collected from 6 different locations of Kathmandu Valley. A total of 35 bacteria were identified, 16 (46%) from local farm and 19 (54%) from local market as shown in Figure 1.

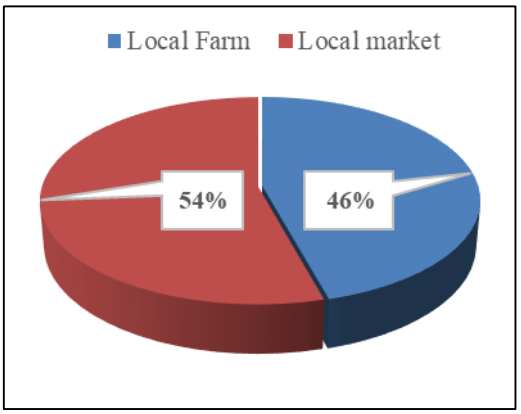

Figure 1: Total number of Bacteria isolated from Local farm and market

Out of 30 vegetable samples from local farms, 16 bacteria were identified from 3 different farms of Kathmandu Valley. Among 16 confirmed bacteria, 8 (49%) were *S. aureus*, 5 (31%) were *Klebsiella* spp., 2 (12%) were *E. coli* and 1 (8%) was *Pseudomonas* spp. respectively (Table 3).
Out of 30 vegetable samples from local market, 19 bacteria were identified from 3 different markets of Kathmandu Valley. Among 19 confirmed bacteria, 7 (37%) were *S. aureus*, 6 (32%) were *Klebsiella* spp., 3 (16%) were *E. coli*, 2 (10%) were *Serratia marcescens* and 1 (5%) was *Pseudomonas* spp. respectively (Table 4).

Table 1: Comparison of total bacterial count (Mean) found between Farm and Market vegetables

S.N	Sample	Kathmandu		Bhaktapur		Lalitpur	
		Farm (1)	Market (2)	Farm (3)	Market (4)	Farm (5)	Market (6)
1	Brinjal (B)	6.6×10^6	6.82×10^6	6.52×10^6	5.625×10^6	6.585×10^6	2.985×10^6
2	Cabbage (C)	6.16×10^6	3.42×10^6	9.12×10^6	2.605×10^6	3.415×10^6	6.8×10^6
3	Carrot (Cr)	3.36×10^6	5.705×10^6	3.39×10^6	5.56×10^6	7.185×10^6	4.4×10^6
4	Capsicum (Cs)	4.105×10^6	4.64×10^6	2.06×10^6	7.09×10^6	5.56×10^6	3.11×10^6
5	Cauliflower (Ca)	5.35×10^6	4.95×10^6	2.475×10^6	5.55×10^6	2.51×10^6	3.255×10^6
6	Ladyfingers (L)	4.36×10^6	7.22×10^6	6.275×10^6	5.73×10^6	4.495×10^6	4.64×10^6
7	Pumpkin (P)	2.065×10^6	4.26×10^6	2.99×10^6	3.29×10^6	2.47×10^6	4.725×10^6
8	Onion (O)	4.97×10^6	3.46×10^6	4.28×10^6	1.8×10^6	5.585×10^6	2.135×10^6
9	Tomato (T)	3.215×10^6	2.7×10^6	5.585×10^6	5.58×10^6	4.675×10^6	6.325×10^6
10	Spinach (S)	6.3×10^6	9.3×10^6	3.425×10^6	4.325×10^6	6.52×10^6	7.355×10^6

Table 2: Comparison of total coliform count (Mean) found between Farm and Market vegetables.

S.N	Sample	Kathmandu		Bhaktapur		Lalitpur	
		Farm (1)	Market (2)	Farm (3)	Market (4)	Farm (5)	Market (6)
1	Brinjal (B)	0.01×10^6	0.42×10^6	1.07×10^6	0.98×10^6	0	1.195×10^6
2	Cabbage (C)	0.09×10^6	1.26×10^6	0.05×10^6	4.225×10^6	0.03×10^6	4.9×10^6
3	Carrot (Cr)	1.43×10^6	2.8×10^6	0.04×10^6	1.78×10^6	0.0537×10^6	2.205×10^6
4	Capsicum (Cs)	0.03×10^6	0	0.02×10^6	0.1×10^6	0	0.46×10^6
5	Cauliflower (Ca)	0.36×10^6	1.07×10^6	0.07×10^6	0.06×10^6	0	0.43×10^6
6	Ladyfingers (L)	0.24×10^6	1.725×10^6	3.105×10^6	4.87×10^6	0.02×10^6	1.68×10^6
7	Pumpkin (P)	0	0.01×10^6	0.06×10^6	0.61×10^6	0	0.24×10^6
8	Onion (O)	0.13×10^6	0	0	3.475×10^6	0	0.35×10^6
9	Tomato (T)	0	0	0.01×10^6	2.035×10^6	0	1.08×10^6
10	Spinach (S)	0.2×10^6	0	2.85×10^6	2.55×10^6	0	1.635×10^6

Table 3: Total number of isolates identified from local farms

SN.	Sample	No. of sample	*E.coli*	*Klebseilla* spp.	*Staphylococcus aureus*	*Pseudomonas* spp.
1	Brinjal	3	-	1	-	-
2	Cabbage	3	1	-	1	-
3	Carrot	3	-	1	1	-
4	Capsicum	3	-	-	1	1
5	Cauliflower	3	-	-	1	-
6	Ladyfingers	3	-	1	1	-
7	Pumpkin	3	-	-	1	-
8	Onion	3	-	-	-	-
9	Tomato	3	1	-	-	-
10	Spinach	3	-	2	2	-
Total isolates		**30**	**2**	**5**	**8**	**1**

Table 4: Total number of isolated identified from local markets

S.N	Sample	No. of sample	*E. coli*	*Klebsiella* spp.	*Staphylococcus aureus*	*Serratia marcescens*	*Pseudomonas* spp.
1	Brinjal	3	-	1	-	1	-
2	Cabbage	3	1	-	-	-	-
3	Carrot	3	-	1	2	-	-
4	Capsicum	3	-	1	-	-	-
5	Cauliflower	3	1	-	-	-	1
6	Ladyfingers	3	-	1	1	-	-
7	Pumpkin	3	-	2	2	-	-
8	Onion	3	-	-	-	-	-
9	Tomato	3	-	-	2	1	-
10	Spinach	3	1	-	-	-	-
Total isolates	**30**	**3**	**6**	**7**	**2**	**1**	

Antibiotic Susceptibility Profile of *Klebsiella* spp. [N=11] and *E. coli* [N=5]

The overall antimicrobial susceptibility revealed, all 11 isolates of *Klebsiella* spp. was sensitive to Chloramphenicol, Cotrimoxazole, Amikacin and Gentamicin (100%) each as shown in Table 5. Out of 5 *E. coli* isolated, all isolates were sensitive to Chloramphenicol, Tetracycline and Gentamicin (100%) each as shown in Table 6.

Antibiotic Susceptibility Profile of *Serratia marcescens* [N=2]

Out of 2 *Serratia marcescens* isolated, all isolates were sensitive to 4 group of antimicrobial drugs as Ciprofloxacin, Chloramphenicol, Amikacin and Gentamicin (100%) each. All isolates was resistant to 3 group of antimicrobial drug as Ampicillin, Tetracycline and Ceftazidime (100%) each and intermediate to only one group i.e. Cefotaxime (100%).

Antibiotic Susceptibility Profile of *Pseudomonas* spp. [N=2]

Out of 2 *Pseudomonas* spp. isolated, all isolates were sensitive to 3 group of antimicrobial drug such as Tetracycline, Cotrimoxazole, Amikacin, Gentamicin (100%) each Similarly, all isolates were resistant to 2 group as Cefotaxime and Ciprofloxacin (100%) each.

Antibiotic Susceptibility Profile of *Staphylococcus aureus* [N=15]

The antimicrobial susceptibility pattern of *S. aureus* are summarized in Table 7. Among the antibiotics evaluated, *S. aureus* was found to be highly susceptible towards Linezolid and Cotrimoxazole (100%) each, followed by Gentamicin and Tetracycline (93.33%) each, Clindamycin (80%), Erythromycin (46.67%), Penicillin, Cefoxitin and Ciprofloxacin (20%) each. Among 15 isolates, 12 were resistant to 2 group of antimicrobial drug as Penicillin and Cefoxitin (80%) each.

Determination of Methicillin-resistant Staphylococcus aureus (MRSA)

80% (12/15) isolates were resistant to Cefoxitin confirming Methicillin-resistant *Staphylococcus aureus* (MRSA). All the 12 MRSA isolates were sensitive to Vancomycin (100%) indicating that vancomycin would be an effective treatment option for these strains.

Distribution of Multi Drug Resistance (MDR)

Out of 20 *Enterobacteriaceace* isolates, 7(35%) were identified as multidrug-resistant. All the isolates were resistant to Ampicillin (100%), 4 were resistant to Ciprofloxacin (57.14%), 3 were resistant to Ceftazidime and Cefotaxime (46.86%) each. Similarly, 7 out of 15 *Staphylococcus aureus* isolates tested were found to be multidrug-resistant. Among 7 MDR isolates, all the isolates were resistant to Penicillin (100%) and Cefoxitin (100%) and 6 were resistant to Ciprofloxacin (85.71%), 1 isolate was resistant to Gentamicin (14.29%) respectively. Among 7 MDR isolates, 3 were screened as suspected ESBL producers. The isolates were further tested for phenotypic confirmation test and none were conformed ESBL.

Table 5: Antibiotic Susceptibility Profile of Klebsiella spp.

Antibiotic Groups	Antibiotics	Antibiotic Susceptibility Pattern					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Cephalosporin	Cefotaxime (CTX 30)	5	45.46	2	18.18	4	36.36
Fluoroquinolones	Ciprofloxacin (CIP 5)	4	36.36	1	9.09	6	54.55
Phenicols	Chloramphenicol (C 30)	11	100	0	0	0	0
β-Lactam	Ampicillin (AMP 10)	10	90.91	1	9.09	0	0
Tetracycline	Tetracycline (TE 30)	9	81.82	2	18.18	0	0
Trimethoprim	Cotrimoxazole (COT 25)	11	100	0	0	0	0

Cephalosporin	Ceftazidime (CAZ 30)	5	45.46	4	36.36	2	18.18
Aminoglycosides	Amikacin (AK 30)	11	100	0	0	0	0
	Gentamicin (GEN 10)	11	100	0	0	0	0

Table 6: Antibiotic Susceptibility Profile of *E. coli*

Antibiotic Groups	**Antibiotics**	**Antibiotic Susceptibility Pattern**					
		Sensitive		**Intermediate**		**Resistant**	
		No.	**%**	**No.**	**%**	**No.**	**%**
Cephalosporin	Cefotaxime (CTX 30)	4	80	0	0	1	20
Fluoroquinolones	Ciprofloxacin (CIP 5)	3	60	1	20	1	20
Phenicols	Chloramphenicol (C 30)	5	100	0	0	0	0
β-Lactam	Ampicillin (AMP 10)	1	20	0	0	4	80
Tetracycline	Tetracycline (TE 30)	5	100	0	0	0	0
Trimethoprim	Cotrimoxazole (COT 25)	4	80	0	0	1	20
Cephalosporin	Ceftazidime (CAZ 30)	4	80	1	20	0	0
Aminoglycosides	Amikacin (AK 30)	4	80	0	0	1	20
	Gentamicin (GEN 10)	5	100	0	0	0	0

Table 7: **Antibiotic Susceptibility Profile of *S. aureus***

Antibiotic Groups	**Antibiotics**	**Antibiotic Susceptibility Pattern**					
		Sensitive		**Intermediate**		**Resistance**	
		No.	**%**	**No.**	**%**	**No.**	**%**
Oxazolidinone	Linezolid (LZ 30)	15	100	0	0	0	0
β-Lactam	Penicillin (P 10)	3	20	0	0	12	80
Aminoglycosides	Gentamicin (GEN 10)	14	93.33	0	0	1	6.67
Macrolides	Erythromycin (E 15)	7	46.67	8	53.33	0	0
Lincosamides	Clindamycin (CD 2)	12	80	3	20	0	0
Tetracycline	Tetracycline (TE 30)	14	93.33	1	6.67	0	0
Cephamycin	Cefoxitin (CX 30)	3	20	0	0	12	80
Fluoroquinolones	Ciprofloxacin (CIP 5)	3	20	5	33.33	7	46.67
Trimethoprim	Cotrimoxazole (COT 25)	15	100	0	0	0	0

DISCUSSION

In this study, a total of 60 vegetable samples were analyzed for microbial contamination. All samples demonstrated positive growth (100%) in the total plate count, indicating the presence of microorganisms in all samples. For the total coliform count, 46 samples (76.67%) were positive for coliform bacteria, whereas 14 samples (23.33%) showed no coliform growth.

For total plate count, high contamination of bacteria was observed in spinach (9.3×10^6) from local market as compared to spinach sourced from local farm (3.425×10^6). Comparing both market and farm sources, the highest level of contamination was observed in market-sourced spinach. Similarly, total coliform count was found highest in cabbage (4.9×10^6) from local market as compared to local farm (0.03×10^6). Leafy vegetables like cabbage and spinach are more prone to bacterial growth primarily due to their

high water content, which facilitates microbial proliferation (Mogren et al., 2018). The high moisture content of these vegetables, combined with their surface area, creates an environment where bacteria can thrive, especially if the vegetables are not properly washed or stored (Olaimat et al., 2012). This increased contamination is attributed to factors such as improper handling, inadequate storage conditions, extended transportation, and the practice of sprinkling water to maintain a fresh appearance (Alum, 2016). Due to this, spoilage of vegetable occurred more in market as compared to the farm.

A total of 35 isolates were identified from samples collected within the Kathmandu Valley. The isolates from both local farms and markets include *Staphylococcus aureus*, *Klebsiella* spp., *Pseudomonas spp*., and *Escherichia coli*. Additionally, *Serratia marcescens* was also found in the market. The detection of *Serratia marcescens* on brinjal and tomato in this study is significant because *S. marcescens* are opportunistic pathogens that can be widespread on vegetables (Michael et al., 2005). Their presence poses serious food safety risks, highlighting the need for enhanced monitoring and preventive measures. However, some studies have also reported the presence of *S. marcescens* on some vegetables (Falomir et al., 2010; Akinyele et al., 2013; Akoachere et al., 2018). The highest number of isolates was found in spinach and pumpkin i.e., *Klebsiella* spp. and *Staphylococcus aureus* in local farm and market. The elevated incidence of *Klebsiella* spp. may be linked to fecal contamination from animal manure and contamination from irrigated water during the pre-harvest period (Iwu et al., 2019). *Staphylococcus aureus* was the predominant among all isolates likely due to its presence in normal microbial flora of the mucus membrane and on the human skin of food handlers and sellers. This prevalence is primarily attributed to cross-contamination resulting from inadequate hand washing or improper food handling practices (Castro et al., 2016). This is a significant public health concern because the pathogen can lead to both food-borne infections and food-borne intoxication. Overall, this study revealed the presence of a high load of microorganisms in the commonly consumed vegetables items in both local farm and market in Kathmandu Valley.

In this study, antimicrobial resistant bacteria contamination in the vegetables was 58.33%. Tetracycline was the most effective antibiotic as 80% isolates were sensitive but contradicted to *S. marcescens.* The Enterobacteriaceace isolates demonstrated sensitivity to Amikacin and Gentamicin, a finding that aligns with the results reported in Österblad's et al. (1999). Most *S. aureus* isolates was susceptible towards linezolid and resistance towards Penicillin similar to the study conducted in China (Wu et al., 2018). The increased resistance towards penicillin (80%) in this study is probably due to acquisition of resistance gene in *S. aureus* (Lyon and Skurray, 1987). *S. aureus* showed susceptibility to most of the other antimicrobial test. Antibiotic susceptibility pattern shown by the Enterobacteriaceae and *S. aureus* isolates were variable.

In this study, 80% (12/15) of the isolates were found to be MRSA by cefoxitin disc diffusion method. This prevalence is notably higher compared to other research findings. For instance, a study conducted in Korea (Hong, 2015) reported a much lower MRSA prevalence rate of 20.75%, while a study from China identified 60% of the strains as MRSA (Wu et at., 2018). The rate observed in this study suggests a more significant issue with MRSA in the studied population or region, indicating a potentially higher level of antibiotic resistance compared to these other settings. The variation in MRSA isolation rates across different studies could be attributed to differences in study locations, time periods, and hygienic conditions (Hardy et al., 2006). None of the MRSA isolates was detected resistance to vancomycin. Thus, the monitoring of MRSA isolates and studying their antimicrobial resistance could be an important tool to prevent the transmission of infectious pathogens (Jia et al., 2020).

Out of the 35 isolates, 14 exhibited multi-drug resistance. Specifically, 46.67% of *S. aureus* isolates displayed resistance to multiple antimicrobial agents, with resistance spanning between two - five different classes of antibiotics. On the other hand, 35% of the *Enterobacteriaceace* isolates showed MDR against two to three antimicrobial classes. The application of organic manure to agricultural fields and vegetables has emerged as a significant contributor to the dissemination of multidrug-resistant bacteria (Zalewska et al., 2021). The prevalence of pathogenic bacteria in raw, unwashed vegetables could be a source of multidrug-resis-

tant bacteria because these vegetables may harbor bacteria that have developed resistance to multiple antibiotics (Tenover, 2001). The findings of this study have important implications underscoring potential health risks for consumers, particularly those with compromised immune systems. It's crucial to address these risks to protect vulnerable populations and improve overall food safety.

This study provides compelling evidence supporting the hypothesis that vegetables obtained from the market exhibit a higher count of microorganisms compared to those sourced from the farm. These findings highlight the need for enhanced attention to food safety measures in market environments, including improved hygiene practices, regular monitoring, and rigorous sanitation protocols. Future research should focus on identifying specific microbial species, evaluating their pathogenic potential, and exploring targeted interventions to mitigate microbial contamination in vegetables.

Conclusion

The study revealed that a wide range of bacteria was present in vegetables collected from different areas within the Kathmandu valley. Notably, high bacterial counts were found in vegetables such as cabbage and spinach highlighting that poor handling practices and inadequate washing can exacerbate contamination, leading to higher bacterial loads and an elevated risk of infection for consumers. It underscores the necessity for enhanced protocols and practices throughout every phase of vegetable production, distribution and consumption.

ACKNOWLEDGEMENTS

We are very grateful to all the farmers and seller for helping in sample collection and providing information. We would also like to thank all the teachers and staffs, Department of Microbiology, Tri-Chandra Multiple campus, Ghantaghar, Kathmandu for their support and guidance throughout the completion of this study.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFEREBCES

Acharya, M., Joshi, P. R., Thapa, K., Aryal, R., Kakshapati, T., & Sharma, S. (2017). Detection of metallo-β-lactamases-encoding genes among clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital, Kathmandu, Nepal. *BMC Research Notes*, *10*(1), 718. https://doi.org/10.1186/s13104-017-3068-9

Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J., … Zhong, N. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine*, *382*(18), 1708–1720.

Langford, B. J., So, M., Raybardhan, S., Leung, V., Westwood, D., MacFadden, D. R., Daneman, N. (2020). Bacterial co-infection and secondary infection in patients with COVID-19: A living rapid review and meta-analysis. *Clinical Microbiology and Infection*, *26*(12), 1622–1629. https://doi.org/10.1016/j.cmi.2020.07.016

Magleby, R., Westblade, L. F., Trzebucki, A., Simon, M. S., Rajan, M., Park, J., Satlin, M. J. (2021). Impact of Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load on Risk of Intubation and Mortality Among Hospitalized Patients With Coronavirus Disease 2019. *Clinical Infectious Diseases*, *73*(11), e4197–e4205. https://doi.org/10.1093/cid/ciaa851

Rawson, T. M., Moore, L. S. P., Zhu, N., Ranganathan, N., Skolimowska, K., Gilchrist, M., Holmes, A. (2020). Bacterial and Fungal Coinfection in Individuals with Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. *Clinical Infectious Diseases*, *71*(9), 2459–2468.

Shakya, G., Prasad Upadhyay, B., Rijal, N., Adhikari, S., Sharma, S., & Kansakar, P. (2019). Changing Trends of Antibiotic Resistance in Escherchia coli. *Journal of Health and Allied Sciences*, *2*(1), 42–45.

Sharma, S., Acharya, J., Caugant, D. A., Banjara, M. R., Ghimire, P., & Singh, A. (2021). Detection of *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* in Culture Negative Cerebrospinal Fluid Samples from Meningitis Patients Using a Multiplex Polymerase Chain Reaction in Nepal. *Infectious Disease Reports*, *13*(1), 173–180. https://doi.org/10.3390/idr13010019

Tille, P. M. (2020). *Bailey & Scott's diagnostic microbiology*. S.l.: churchill livingstone.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., … Cao, B. (2020). Clinical course and risk factors for mortality

of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *The Lancet, 395*(10229), 1054–1062.