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ORIGINAL RESEARCH

Microbiological Assessment of Selected Vegetables from Kathmandu, Nepal

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ABSTRACT

Objectives: To determine the microbial load and antibiotic susceptibility of bacterial isolates from selected vegetables in Kathmandu.

Method: This study was conducted in the department of microbiology, Tri-Chandra multiple campus from March to July 2025. A total of 30 samples including, moringa, cucumber, spinach, chili, peas, cauliflower, okra, carrot, cabbage, and brinjal, were collected in sterile containers from markets, hotels, and farms and were transported to the laboratory under cold chain. The samples were cultured on Plate Count Agar (PCA), Potato Dextrose Agar (PDA), and Violet Red Bile Agar (VRBA). The PCA and VRBA plates were incubated at 37°C for 24 hours and PDA plates at 28°C for 24-48 hours. Bacterial isolates were identified using Gram staining and various biochemical tests, and antibiotic susceptibility was performed. Fungal presence was detected by using Lactophenol Cotton Blue stain and parasites were identified by Wet Mount method.

Results: Spinach from vendor had the highest mesophilic bacterial load (1.54×10^6CFU/g) and lowest on peas from hotels (1.7×10^3CFU/g). Coliform were highest in vendor cabbage (2.89×10^5CFU/g), while peas consistently showed lowest counts (5×10^2CFU/g) from all sources. Bacteria such as *Pseudomonas* spp, *E. coli*, *Proteus* spp, *Enterobacter* spp, *S. aureus*, *Klebsiella* spp, and fungi like *Aspergillus* spp, *Fusarium* spp, *Alternaria* spp, *Cladosporium* spp, *Candida* spp, and parasites *G. lamblia*, *E. histolytica*, *Ascaris* were identified. Most of the bacterial isolates were resistant to the antibiotics used, including Ceftriaxone, Cotrimoxazole, Ceftazidime, Amikacin, Erythromycin, and Ciprofloxacin with only few exhibiting susceptibility.

Conclusion: The study revealed a significant presence of pathogens and antibiotic resistant strains highlighting the importance of improved food safety practices, regular microbial monitoring, and public awareness to reduce the risk of foodborne diseases in urban Nepal.

Keywords: Vegetable samples, bacterial count, fungi, parasites, antibiotic susceptibility

INTRODUCTION

Vegetables have a number of benefits to humans and their daily intake has been endorsed as an important aspect of maintaining a healthy life. The World Health Organization (WHO), and the Food and Agriculture Organization (FAO) recommend a daily intake of at least 400 grams of fruits and vegetables to reduce the risk of non-communicable diseases and ensure nutritional wellbeing (WHO and FAO, 2003). Vegetables can serve as vehicles for foodborne pathogens when exposed to unhygienic conditions. Contamination can occur at any stage from farm to the table, including use of untreated irrigation water, manure, processing, distribution, sale and during consumption (Eni et al., 2010), which results

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in foodborne human diseases (Shafa-ul-Haq et al., 2014; Bekele et al., 2017). Pathogenic microbes such as *E. coli*, *Salmonella* spp, *Pseudomonas* spp, *Aspergillus*, *Ascaris* and many others have been frequently associated with fresh production of vegetables. Since vegetables are often consumed raw or sometimes undercooked to preserve vitamin and mineral contents, which may result in various food-borne infections and disease outbreaks like typhoid fever, dysentery, diarrhoea, and cholera (Balali et al., 2020).

Microbial contamination of vegetables has been reported by several researchers inside and outside Nepal. In Brazil, Oliveira et al., (2011) reported that 43% of sampled vegetables were contaminated with fecal coliforms (Oliveira et al., 2011), whereas in Nepal, Sapkota et al., (2019) detected *E. coli* in 13.4% and *Salmonella* in 35.2% of vegetable samples from hotels and restaurants of Bharatpur, Chitwan (Sapkota et al., 2019). The studies conducted in Kathmandu have reported significant microbial contamination in vegetables like spinach, cabbage, tomato, cucumber, and carrots (Ghimire et al., 2020). Vegetables can serve as a major route for the transmission of infections (Rahman et al., 2022). In Saudi Arabia in 2018, consumption of food contaminated with foodborne bacteria resulted in 2,191 cases of illness and 11 deaths (Ministry of Health, 2019). Despite increasing reports of foodborne illness in Nepal, there is inadequate research on fresh produce for microbial contamination. The studies have been focused mostly on total bacterial load or the presence of indicator organisms such as coliforms, rather than identifying specific, disease-causing organisms. The growing global concern over antimicrobial resistance (AMR) has also further complicated the issue as many pathogens have developed resistance to commonly used antibiotics, making treatment and disease management more challenging (Razzaq et al., 2014). The situation is particularly alarming in nations where healthcare infrastructure is strained and advanced diagnostic and treatment options are limited. The prevalence of antibiotic resistance bacteria linked to fresh produce has been well documented and is increasing worldwide (Threlfall et al., 2000).

The importance of this research stems from the real and present threat of foodborne illness. This study aimed to provide an assessment of vegetables from farms, hotels and markets of Kathmandu to detect the presence of foodborne organisms. The study also seeks to address the existing research gaps by isolating and identifying bacteria from selected vegetables in Kathmandu, evaluating their antibiotic susceptibility patterns, and comparing contamination levels between different sources. The results provide deep insight into microbial risks in vegetable consumption and help inform strategies for improved food safety, public health interventions, and policy development in Nepal.

METHODS

Sample collection

A total of 30 fresh vegetable samples were randomly collected from various retail outlets, including local markets, hotels, and vendors across Kathmandu. Each sample was placed in sterile, labeled polyethylene bags and were transported immediately to the microbiology laboratory in the department of microbiology, Tri-Chandra Multiple campus maintaining a cold chain and analyzed.

Sample preparation, enumeration and identification of microorganisms

Twenty-five grams of each sample was homogenized in 225 ml sterile saline solution in a sterile conical flask (Odumeru et al., 1997). Six fold serial dilutions were prepared in different test tubes and the prepared samples of respective dilution were poured on PCA, VRBA plates and incubated for 37°C for 24 hours for bacterial count and on PDA plates at 28°C for 24 to 48 hours for yeast and mold, (Acharya, 2021).

The number of visible colonies were counted, subcultured on NA and calculated by using a standard formula (Ema et al., 2022).

CFU/g = (No. of colonies/ inoculum size) × Dilution factor

Identification of bacteria was done using Gram staining and biochemical tests such as Catalase, Oxidase, IMViC series, Triple Sugar Iron (TSI) test, and motility test (Shoaib et al., 2020). The fungal growth on the PDA plate was subcultured onto fresh PDA plates and incubated at room temperature for 5 days. A piece of cellophane tape was used to slightly lift the fungal colonies, sticky side was placed on a slide containing a drop of Lactophenol Cotton Blue (LCB) and examined under microscope at 10x and 40x (Mohammed, 2024). Antibiotic susceptibility test of bacterial isolates was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) plate. The results were interpreted as sensitive, intermediate, or resistant (Bayot & Bragg, 2024). Parasites were identified using the Wet Mount method, with careful observation for motile trophozoites, cysts, eggs, or larvae, and their morphological characteristics were recorded (Demeke et al., 2021).

RESULTS

Table 1: Total mesophilic count (PCA) and coliform count (VRBA) of different vegetables

Sample	Source	Total mesophilic count Average (CFU/g)	Total coliform count Average (CFU/g)
Moringa	Farm	1.07×10^6	2.52×10^5
	Vendor	1.21×10^6	3.15×10^5
	Hotel	9.22×10^5	2.38×10^5
Cucumber	Farm	1.63×10^5	1.57×10^5
	Vendor	2.24×10^5	2.19×10^5
	Hotel	5.77×10^4	2.7×10^4
Spinach	Farm	1.32×10^6	2.79×10^5
	Vendor	1.54×10^6	2.98×10^5
	Hotel	7.2×10^5	1.57×10^5
Green chilli	Farm	3.83×10^4	3.35×10^4
	Vendor	5.15×10^5	9.65×10^4
	Hotel	3.51×10^4	1.4×10^4
Peas	Farm	1.1×10^4	2.5×10^3
	Vendor	1.35×10^4	3.75×10^3
	Hotel	1.7×10^3	5×10^2
Cauliflower	Farm	4.1×10^5	2.37×10^5
	Vendor	4.69×10^5	2.98×10^5
	Hotel	2.52×10^5	9.6×10^4
Lady's finger	Farm	4.7×10^4	4.2×10^4
	Vendor	1.03×10^5	6.83×10^4
	Hotel	3.9×10^4	2×10^4
Carrot	Farm	1.04×10^5	3.4×10^4
	Vendor	2.67×10^5	4.15×10^4
	Hotel	6.89×10^4	1.75×10^4
Cabbage	Farm	3.17×10^5	9.14×10^4
	Vendor	3.93×10^5	2.89×10^5
	Hotel	1.62×10^5	4.15×10^3
Brinjal	Farm	1.65×10^5	6.17×10^4
	Vendor	1.71×10^5	6.91×10^4
	Hotel	3.33×10^4	3.25×10^4

The average total mesophilic and coliform counts indicate that spinach obtained from vendors had the highest mesophilic load (1.54×10^6CFU/g), while cabbage from vendors showed the highest coliform load (2.89×10^5CFU/g). In contrast, peas consistently exhibited the lowest microbial counts (5×10^2CFU/g) across all sources.

Table 2: Identification of the isolates

Isolates	Gram staining	Catalase	Oxidase	O/F	Indole	Motility	MR	VP	Citrate	TSIA	H_2S	Organism
Isolate 1	-	+	-	Fermentative	-	+	+	-	+	A/A	-	*Enterobacter* spp
Isolate 2	-	+	-	Fermentative	+	+	+	-	-	A/A	-	*E. coli*
Isolate 3	-	+	+	Oxidative	-	+	-	-	+	K/K	-	*P. aeruginosa*
Isolate 4	-	+	-	Fermentative	-	-	-	+	+	A/A	-	*Klebsiella* spp
Isolate 5	-	+	-	Fermentative	-	+	+	-	+	K/A	+	*Proteus* spp
Isolate 6	+	+	-	Fermentative	-	-	+	+	+	A/A	-	*S. aureus*

The isolates were identified as *Enterobacter* spp, *E. coli*, *P. aeruginosa*, *Klebsiella* spp, *Proteus* spp, and *S. aureus*, based on their biochemical characteristics.

Table 3: Fungi identified from vegetables

Vegetable	Source	Colony morphology on PDA	Colony character on bottom side of PDA plate	Microscopic observation	Fungi
Cabbage	vendor	grayish green or black	dark brown	transversely and longitudinally septate, beaked conidia	*Alternaria* spp
Spinach	farm	greenish black and powdery	black	conidia head in mass at the apex of conidiophore	*Cladosporium* spp
Cucumber	farm	white to pink, wooly	dark purple	sickle shaped transversely septate macroconidia produced in sporodochia	*Fusarium* spp
Brinjal	farm	greenish blue, black or green	light yellow	conidiophore arising from foot cell, conidia on phialides	*Aspergillus* spp
Carrot	farm	large round, smooth creamy white	white	small, oval to spherical budding yeast cells, no hyphae	*Candida* spp

The genera of fungal isolates were detected as *Alternaria*, *Cladosporium*, *Fusarium*, *Aspergillus*, and *Candida*, based on their macroscopic and microscopic characters.

Table 4: Identification of parasites on vegetables

Sample	Source	Microscopic observation	Parasite
Cauliflower	Vendor	ovoid, visible nuclei and fibrils	*G. lamblia*
Spinach	Hotel	spherical, nuclei present + ovoid, visible nuclei and fibrils	*E. histolytica* + *G. lamblia*
Soijan	Farm	round, thick shell with an external mammillated layer	*Ascaris*

Parasitological examination revealed the presence of *Giardia lamblia, Entamoeba histolytica, Giardia lamblia* and *Ascaris,* based on their microscopic observation.

Table 5: Antibiotic susceptibility pattern of *E. coli, Klebsiella* spp, *Proteus* spp and *Enterobacter* spp

Antibiotics	Antibiotic Susceptibility Pattern							
	E. coli		*Klebsiella* spp		*Proteus* spp		*Enterobacter* spp	
	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)
Ceftriaxone (CTR 30)	2 (33.3%)	4 (66.7%)	3 (50%)	3 (50%)	3 (50%)	3 (50%)	1 (16.6%)	5 (83.4%)
Ceftazidime (CAZ 30)	0	6 (100%)	3 (50%)	3 (50%)	4 (66.7%)	2 (33.3%)	2 (33.3%)	4 (66.7%)
Cotrimoxazole (COT 30)	4 (66.7%)	2 (33.3%)	6 (100%)	0	2 (33.3%)	4 (66.7%)	2 (33.3%)	4 (66.7%)
Amikacin (AK 30)	5 (83.4%)	1 (16.6%)	4 (66.7%)	2 (33.3%)	6 (100%)	0	1 (16.6%)	5 (83.4%)

The antibiotic pattern of isolates showed that amikacin was the most effective against all isolates, particularly *Proteus* (100% sensitive) and *E. coli* (83.4% sensitive), while ceftazidime exhibited the poorest activity, with *E. coli* showing 100% resistance and high resistance also observed in *Enterobacter* (66.7%), *Klebsiella* (50%), and *Proteus* (33.3%).

Table 6: Antibiotic susceptibility pattern of *Pseudomonas* spp

Antibiotics group	Antibiotics	Susceptibility Pattern	
		Sensitive No. (%)	Resistant No. (%)
Cephalosporin	Ceftriaxone (CTR 30)	3 (50%)	3 (50%)
Cephalosporin	Ceftazidime (CAZ 30)	2 (33.3%)	4 (66.7%)
Trimethoprim	Cotrimoxazole (COT 30)	3 (50%)	3 (50%)
Aminoglycosides	Amikacin (AK 30)	1 (16.6%)	5 (83.4%)
Fluoroquinolone	Ciprofloxacin (CIP 5)	0	6 (100%)

Among the tested antibiotic groups, cotrimoxazole and ceftriaxone showed moderate effectiveness (50% sensitivity), *Pseudomonas* spp was found to be 100% resistant against ciprofloxacin while lowest sensitivity against amikacin.

Table 7: Antibiotic susceptibility pattern of *S. aureus*

Antibiotics group	Antibiotics	Susceptibility Pattern	
		Sensitive No. (%)	Resistant No. (%)
Macrolides	Erythromycin (E 15)	2 (33.3%)	4 (66.7%)
Cephalosporin	Ceftazidime (CAZ 30)	3 (50%)	3 (50%)
Trimethoprim	Cotrimoxazole (COT 30)	1 (16.6%)	5 (83.4%)
Aminoglycosides	Amikacin (AK 30)	2 (33.3%)	4 (66.7%)
Fluoroquinolone	Ciprofloxacin (CIP 5)	3 (50%)	3 (50%)

Across the antibiotic groups, ciprofloxacin and ceftazidime showed the highest effective (50%), erythromycin and amikacin displayed moderate effective (66.7%), and cotrimoxazole was the least effective towards *S.aureus*.

DISCUSSION

Among all the vegetables examined, the highest isolation of bacteria was from vendor samples with a total number of 5 types (*E. coli, Enterobacter, P. aeruginosa, Proteus* spp *and S. aureus*) species. Farm samples yielded 4 types of bacteria (*E. coli, P. aeruginosa, Enterobacter* spp, and *Klebsiella* spp) and hotel samples showed 3 (*E. coli*, *Pseudomonas* spp, and *S. aureus*). These results are consistent with previous study conducted by Falomir, in which *Klebsiella* spp, *Enterobacter* spp, *E. coli* along with other species were present on vegetables (Falomir et al., 2010). Among the total samples, vendor spinach had the highest mesophilic count (1.54×10^6CFU/g) and vendor cabbage showed the highest coliform count (2.89×10^5CFU/g), while peas had the lowest counts for both bacteria (5×10^2CFU/g) from all sources. A study on microbiological safety of raw, freshly consumed vegetables from open markets in Lusaka District found that these vegetables harbor foodborne pathogens, posing potential health risks to consumers (Chakopo, 2017).

Fungi contribute to the spoilage of vegetables because of their pathogenicity to the harvested products as they produce harmful mycotoxins during pathogenesis (Abdulla et al., 2016). Farm samples were more prone to fungal contamination compared to vendor and hotel samples likely due to various factors such as direct exposure to soil, airborne spores, physical damage during harvesting, lack of sanitation, high humidity and moisture. The microbial quality of irrigation water is crucial, as contamination with animal or human waste can introduce pathogens into vegetables during both preharvesting and postharvesting (Suslow, 1997). In this study the identified fungi included *Alternaria* spp (cabbage), *Cladosporium* spp (spinach), *Fusarium* spp (cucumber), *Aspergillus* spp (brinjal), and *Candida* spp (carrot). A study in Nagpur, India showed frequent association of similar organisms in vegetables (Kakde et al., 2001). Parasites were detected only in cauliflower, spinach, and soijan, while the remaining vegetables tested negative. Parasitic examination revealed the presence of *G. lamblia*, *E. histolytica*, and *Ascaris* in the samples. Green leafy vegetables like lettuce have uneven surfaces that may allow parasitic eggs, cysts, and oocysts to adhere more easily, either during cultivation or when washed with contaminated water (El Said Said, 2012). Similarly, the presence of such parasitic species were reported by a study in Ethiopia (Alemu et al., 2020). Vendor samples are exposed to unhygienic handling during transportation, storage, and marketing, where vegetables are often kept in open environments and handled repeatedly without proper hygiene. These conditions allow microorganisms to multiply and persist, leading to higher bacterial counts. Hotel samples often have reduced bacterial diversity due to washing, peeling or proper storage but may still contain some species of bacteria. The variation in contamination level of the vegetables may be due to the differences in shape and surface of each vegetable.

Antibiotic susceptibility testing revealed high resistance among the isolates. All 6 isolates of *E. coli* i.e. 100% were resistant to Ceftazidime, followed by 66.7% to Ceftriaxone, 33.3% to Cotrimoxazole and 16.6% to Amikacin. *Klebsiella* spp showed sensitivity to Cotrimoxazole, and 66.7% susceptibility to Amikacin. *Proteus* spp were sensitive to Amikacin. In contrast, *Enterobacter* spp exhibited low sensitivity overall with only 16.6% susceptibility to Amikacin. A study conducted in Ethiopia has also reported a high burden of antimicrobial resistance among *Klebsiella* and *E. coli* (Kitaba et al., 2024). Several studies have found comparable interpretations on susceptibility patterns of *Proteus* and *Enterobacter* (Basnet et al., 2024). For *Pseudomonas* isolates, Ceftriaxone and Cotrimoxazole were highly effective with 50% sensitivity, followed by Ceftazidime 33.3%, Amikacin 16.6% and complete resistance to Ciprofloxacin. A similar resistance pattern was reported from a tertiary care hospital in Kathmandu (Shrestha et al., 2023). Research in South East Nigeria has pointed to the possible threat of *S. aureus* and its control measures (Nwankwo & Nasiru, 2011). These results indicate widespread bacterial contamination and variable antibiotic resistance patterns among vegetable associated isolates, suggesting that commonly used antibiotics may not reliably control infections. It also highlights Amikacin as a potentially useful treatment option.

CONCLUSION

The study depicted microbial variety and their burden in vegetable items sold in different markets, hotels and farms. Bacteria such as *S. aureus, E. coli, Proteus* spp, *Pseudomonas* spp, *Enterobacter* spp and *Klebsiella* spp were identified. Fungal species such as *Alternaria* spp, *Cladosporium* spp, *Aspergillus* spp, *Fusarium* spp and *Candida* spp were detected in these vegetables. Additionally, the presence of parasites such as *Ascaris, E. histolytica* and *G. lamblia* was confirmed in the samples during this study. The antibiotic susceptibility patterns showed a concerning level of resistance towards various antibiotics. Most of the isolates were resistant to all the antibiotics used such as ceftriaxone, ceftazidime, amikacin, erythromycin and ciprofloxacin. However, some susceptibility was observed, underscoring the importance of proper antibiotic use and continuous monitoring to combat antimicrobial resistance in foodborne pathogens. These results indicate a prevalence of drug resistance among vegetable associated pathogens.

Thus, the survival of bacteria, fungi and parasites within the fresh vegetables revealed the importance of maintaining proper sanitary conditions during harvesting, processing, storage and handling of the vegetables consumed. It also notified the importance of searching for ways to minimize the risk of getting different diseases. The findings of this research may also help form a policy guideline for safe consumption of raw vegetables based on the capacity of the particular vegetable to resist contaminating pathogens and also contribute to ensure food safety and security.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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