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RESEARCH PAPER

TITLE

ISOLATION AND IDENTIFICATION OF BACTERIAL PATHOGENS IN FRESH VEGETABLES AVAILABLE TO LOCAL CONSUMERS IN NAROWAL CITY, PAKISTAN

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ISOLATION AND IDENTIFICATION OF BACTERIAL PATHOGENS IN FRESH VEGETABLES AVAILABLE TO LOCAL CONSUMERS IN NAROWAL CITY, PAKISTAN

ABSTRACT

Vegetables are a wonderful source of proteins, nutrients, dietary fibers, carbohydrates, and vitamins. They can help with digestive system inflammation, and maintained of blood pressure, skin glowing, and eye protection. Vegetables are full of cancer-fighting antioxidants and nutrients. They are abundant in phytochemicals and antioxidants, which may help the immune system in reducing chronic inflammation. In the present study, five different type of vegetables were taken as samples i.e., Potato, green chili, carrot, ladyfinger and Tomato were collected from main market of Narowal. Five samples of each vegetable product was collected. Ten grams of each vegetable sample was weighed and then homogenized in 90ml saline solution. Serial dilutions of samples were made up to 10^{-5} with sterile normal saline Gram positive and gram-negative bacteria were isolated by the using gram staining procedure. Bacterial identification was taken place by using different biochemical tests. Antibiotic resistance was checked by disk diffusion method on Muller-Hinton agar medium by using antibiotic disks. Different types of bacteria were identified in collected samples, Escherichia coli, Klebsiella, Coagulase negative staphylococcus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Serratia marcesscens. The gram-negative bacteria were Klebsiellaa and Escherichia coli while gram-positive in nature were Serratia marcesscens, Staphylococcus and Pseudomonas aeruginosa, epridermidis, Coagulase negative staphylococcus. In this study, all samples of vegetables were contaminated with bacteria. The highest microbial load (9.0 X 10^5CFU/ml) was shown in tomato while the lowest microbial load (1.0 X 10^6 CFU/ml) was shown in Carrot. Ladyfinger showed the lowest significance in the viable bacterial count and green chili showed a highly significant viable bacterial count. Applied different types of disks and checked the resistivity and sustainability of isolated organisms. It was concluded that the bacterial load was highest in local market vegetable samples. It indicates that the hygienic conditions in these markets are not sufficient. Therefore, proper vegetable handling is needed the foodborne diseases and other infections in humans.

KEYWORDS: vegetables, total viable count, biochemical testing, gastrointestinal diseases

1. INTRODUCTION

Vegetables are a great source of fiber, vitamins, and minerals, however, servings of mixed green portions may include germs that trigger sickness flare-ups. Vegetables are frequently ingested raw, improperly cleaned, and infrequently subjected to processes that reduce their microbiological contamination. These commodities turn into a source of

infection and propagate bacteria that cause food poisoning in humans **(Chandra et al., 2022).** Vegetables, have high nutritional content and protect against serious diseases including diabetes, cancer, osteoporosis, and heart disease. Vegetables are also low in fat and include elements like minerals, phytochemicals, and vitamins. Carotenoids and antioxidants are also among them. They guard against cancer, heart disease, and stroke. **(Okwori & Bishop, 2017).** The microbial growth of vegetables is influenced by various abiotic factors, including pH, temperature, moisture, corrosivity, water action, oxygen concentration, and redox potential. When human consumption was a global problem, microbial contamination rendered the ready-to-eat food and beverages offered by roadside vendors unfit and dangerous to health **(Mamum et al., 2021).** Leafy meals carry a variety of microbial flora from the farm to the table, which may develop and spread from many sources. At every stage, including development, harvesting, transporting, storage, packing, and sale to final consumers, products are exposed to possible microbial contamination. The quantity and types of harmful foreign invaders that are present in vegetables are consumed by humans can also increase or decrease. These can cause fatal diseases and illnesses **(Rida & Deeba, 2018).** Raw vegetables can harbor a variety of microorganisms that can spread throughout the plant, from the root to the tip of the shoot, or they can appear as tiny colonial patches impregnated with plant sap or supplemental cells from which they can flourish and spread. Vegetables degrade slowly and are directly exposed to microorganisms. As a result, vegetables get rapidly contaminated by soil, residue, water, and careless or unclean handling when being harvested or handled. Product degradation caused by microbial movement involves several complex mechanisms that reduce organoleptic quality and trigger customer rejection of the product **(Ali & Basharat, 2019).** The use of bacterially contaminated food causes a variety of illnesses, including kidney stones, decreased risk of heart attack blockage, etc. Considering the significance of vegetables in our lives, this study was created to identify bacterial isolates found in vegetables purchased from local market sellers in Narowal City, Pakistan.

2. MATERIALS AND METHOD

Sample Collection

Five different types of vegetables were purchased from the local markets in Narowal. The vegetable samples were Tomato *(Solanum lycopersicum)*, Potato (*Solanum tuberosum*), Green Chili *(Capsicum frutescens)*, Lady finger *(Abelmoschus esculentus)*, and Carrot (*Daucus corata*). These vegetable samples were put into a plastic polythene bag and brought into the Zoology lab of Minhaj University Lahore.

Preparation of Stock Solution

One gram of each vegetable sample was weighed, crushed, and added to a mortar and pestle together with 10 ml of distilled water to create a suspension. The serial dilution of this sample ranged from 10^{-1} to 10^{-5}. For each serial dilution concentration, 1 ml from each serially diluted test tube was dispensed into a Petri plate. All the plates were solidifying. Then Petri plates were incubated for 24 hours at 37°C. On petri plates, bacterial colonies were examined and noted.

Bacterial isolation and identification

On nutrient agar plates, 100ul of vegetable suspension from each dilution was dispersed. Enumerated bacteria were isolated and grown on nutrient agar. Petri plates were incubated for bacterial growth at 37^{o}C for 24 hours. Following incubation, the morphologically distinct bacterial colonies were isolated and examined. The morphology of different bacterial isolate colonies was examined in terms of their forms, sizes, surfaces, colors, opacities, and elevations. Bacteria were identified on the basis of morphological characteristics and different biochemical tests by using a standard protocol as described elsewhere **(Jolt *et al.*, 1994).**

Bacterial Enumeration

The Spread plate method was used to count the bacteria to calculate colony colony-forming unit (CFU/ml). Then streaking method was used to purify the culture of each bacterial strain.

Antibiotic sensitivity test:

The antibiotic sensitivity of S*erratia marcesscens, Kleblsiella species, Pseudomonas aeruginosa, Coagulase-negative staphylococcus epidermis*, and *Escherichia coli was* checked by disk diffusion method on Muller-Hinton agar medium by using antibiotic disks. A swab of the cell suspension was subsequently spread in three directions on the entire surface of a Mueller Hinton agar plate (MHA) and left for 15 minutes to air dry at room temperature before antibiotic disks were applied to the agar. Then antibiotic discs will be placed on the plate with the help of forceps. Then plates were incubated at 37°C for 24 hours. After 24 hours, antibiotic resistance will be checked by measuring the zone of inhibition around the disks. The antibiotic resistance of bacterial isolates was assessed against the following antibiotics, Amoxicillin, Pencillium, Chloramphenicol, and Amikacin.

Statistical Analysis:

For statistical analysis, ANOVA was performed to determine the significance of the difference of contamination between vegetable samples. Significance was assessed at 95% conviction ($p<0.05$) (Inc, SPSS, 1999).

3. RESULTS

A total of six bacterial species were identified from different samples of vegetables. Identified bacteria were *Pseudomonas aeruginosa, Serratia marcessens, Klebsiella* species, *Staphylococcus epidermidis, Coagulase-negative staphylococcus* species, *Escherichia coli*, and, from various vegetables at the local market in Narowal City. (Table 1)

TOTAL VIABLE BACTERIAL COUNT:

In this study, all vegetable samples were contaminated. The bacterial count of sampled vegetables varied with the types. The range of microbial count of Tomato was 9.0 X 10^5 CFU/ml, Carrot was 1.0X10^5 CFU/ml, Potato was 7.0 X 10^5 CFU/ml, Ladyfinger was 6.0X10^5 CFU/ml and Green chili was 1.7 X10^5 CFU/ml (Table 2)

Table 1: Colony forming unit/ml of vegetable samples collected from local market of Narowal City

Vegetables	Bacterial samples
Tomato	9.0×10^5
Carrot	1.0×10^5
Ladyfinger	6.0×10^5
Green Chili	1.7×10^5
Potato	7.0×10^5

Table 2: Mean viable count of isolated Bacteria species from vegetable samples collected from Narowal.

Vegetables	Isolated bacterial stain
Tomato	*Staphylococcus epidermis* *Pseudomonas aeruginosa* *Escherichia coli* *Klebsiella species 3*
Carrot	*Serratia marcessens* *Coagulase negative* *Staphylococcus epidermis*
Green Chili	*Coagulase negative staphylococcus species 3* *Staphylococcus epidermis* *Escherichia coli* *Serratia marcessens*
Potato	*Staphylococcus epidermis* *Serratia marcessens* *Pseudomonas aeruginosa* *Klebsiella species 1*
Ladyfinger	*Pseudomonas aeruginosa* *Staphylococcus epidermis* *Coagulase negative staphylococcus species 2* *Escherichia coli* *Serratia marcessens* *Klebsiella species 2*

Gram staining and endospore staining were used to separate bacterial species into Gram-Negative and Gram-Positive bacteria. Positive results of Gram staining were showed by *Serratia marcessens, Staphylococus Epidermis, Psedomonas aeruginosa, Coagulase negative staphylococcus*. While *Klebeblsilla species* and *E. coli* showed Negative results. *Serratia marcessens, Staphylococus* and *Psedomonas aeruginosa* showed positive results for endospore staining, While *Coagulase negative staphylococcus, Staphylococus* and *Psedomonas aeruginosa* showed negative results.

Biochemical characterization of

isolated bacteria was given in (Table 3).

Table 3: Biochemical testing of isolated bacterial strains isolated from Vegetable samples of Narowal City

Bacterial species	Gram Staining	Endospore staining	Citrate test	Motility Test	Catalase test	Oxidase test	D-NASE test	Coagulase test	H_2S
Serratia marcessens	+	+	+	+	+	-	+	+	-
Staphylococcus Epidermidis	+	+	-	-	+	-	-	-	+
Klebsilla species	-	-	+	-	+	-	-	-	
Pseudomonas aeruginosa	+	+	+	-	+	+	-	-	-
Escherichia coli	-	-	-	-	+	-	-	-	-
Coagulase negative staphylococcus	+	-	-	-	+	-	-	-	+

(-) Negative, (+) Positive.

There was a significant increase in viable bacterial count in all the vegetables (Table 4) which indicates that the bacterial contamination in all vegetables was very high.

Table 4: Mean bacterial count comparison among all vegetable samples collected from local market of Narowal City.

Variation Source	Mean squares	Prob.	F-value	Sum of squares	Degrees of freedom
Samples	258.667	0.0019	4.97**	1034.7	4
Vegetable (V)	612.000	0.0000	11.77**	2448.0	4
Sample x V	422.833	0.0000	8.13**	6765.3	16
Error	52.000			2600.0	50
Total				12848.0	74

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = highly significant (P<0.01)

Significant increases in bacterial count were shown in all five samples of Tomato and potato (Table 5). A highest peak of bacterial load were shown by Green chilli vegetable sample 3 then, it was followed by sample 2 of tomato and then vegetable sample 4 of potato vegetable samples. All the vegetable samples of ladyfinger showed the least bacterial load (Fig 3)

Table 5: Mean±SE of the bacterial count of vegetable samples of Narowal City, Pakistan

Sample	Vegetable					Mean
	Green chili	Potato	Lady finger	Tomato	Carrot	
Sample 1	20.00±0.00efgh	30.00±5.77de	20.00±0.00e-h	43.33±6.67bc	16.67±3.33fgh	26.00±3.06AB
Sample 2	20.00±5.77efgh	30.00±0.00de	23.33±3.33d-g	46.67±3.33ab	33.33±3.33cd	30.67±2.84AB
Sample 3	56.67±6.67a	16.67±3.33fgh	16.67±3.33fgh	26.67±6.67def	20.00±0.00e-h	27.33±4.41AB
Sample 4	13.33±3.33gh	43.33±3.33bc	10.00±0.00h	23.33±6.67d-g	33.33±3.33cd	24.67±3.63AB
Sample 5	13.33±1.67gh	30.00±5.77de	10.00±2.89h	23.33±3.33d-g	20.00±5.77e-h	19.33±2.48AB
Mean	24.67±4.64B	30.00±2.76A	16.00±1.7AB	32.67±3.45C	24.67±2.36B	

Comparison among interaction means is represented by small letters and capital letters represents overall mean. Similar letters in rows or columns give indication of statistically **non-significant (P>0.05)**.

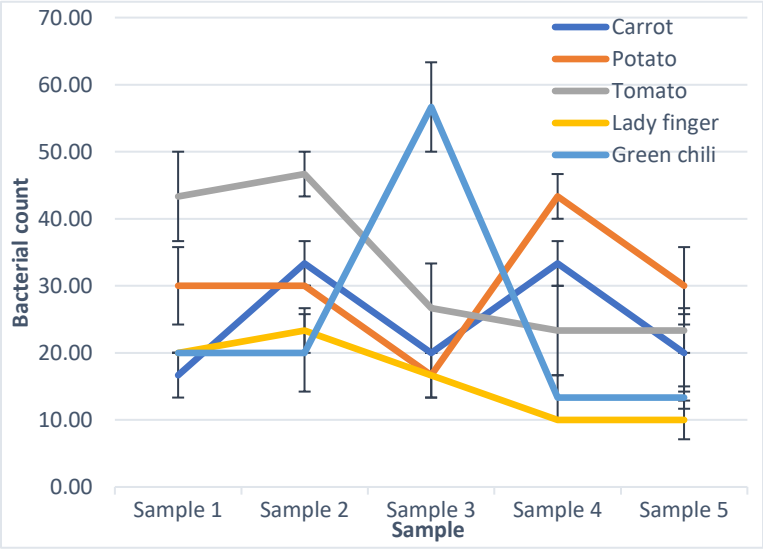

Fig 3 : Bacterial load

CFU/ml of all vegetable samples

Antibiotic sensitivity of isolated bacteria of vegetable samples collected from the local market of Narowal City

Different antibiotics were used to check the antibiotic resistivity and sensitivity against vegetable bacteria isolates. The results showed that *Serratia marcesscens* and *Escherichia coli* exhibited significant levels of resistance to Pencilium, Ampicillin, and Amikacin as well as sensitivity to chloramphenicol. Amoxicillin Ampicillin, and Amikacin were all highly resistant to *Klebsiella* species while chloramphenicol showed sensitivity against *Klebsiella* species. Coagulase-negative Staphylococcus species exhibited chloramphenicol sensitivity and resistance to ampicillin and penicillin. Amikacin showed intermediate pattern against Coagulase-negative *Staphylococcus species*. *Escherichia coli* exhibited resistance to Gentamicin, Chloramphenicol and Amikacin but susceptibility to Ampicillin. While *Pseudomonas aeruginosa* shown sensitivity to amikacin and Penicillium, it demonstrated resistance to ampicillin and chloramphenicol.

(Table 6)

Table 6: The patterns of Antibiotic Susceptibility showed by different isolated bacteria collected from vegetable samples of Narowal City.

Bacterial strains	**Antibiotic resistivity and sensitivity**	**Antibiotic disk**
Serratia marcesscens	Amikacin,	R
	Penicillium	R
	Amoxicillin	R
	Chloramphenicol	S
Klebsiella species	Ampicillin	R
	Amoxicillin	R
	Amikacin,	R
	Chloramphenicol	S
Pseudomonas aeruginosa	Amikacin	S
	Chloramphenicol	R
	Ampicillin	R
	Penicillium	S
Coagulase negative staphylococcus	Penicillium	R
	Amoxicillin	R
	Amikacin	I
	Chloramphenicol	S
Staphylococcus epidermidis	Chloramphenicol	S

	Amoxicillin	R
	Penicillium	R
	Amikacin	R
Escherichia coli	Gentamicin	R
	Ampicillin	S
	Amikacin	R
	Chloramphenicol	R

S=Sensitive, R=Resistant

4. DISCUSSION

The present study showed that pathogenic bacteria can infect vegetables while they are growing in fields or farms, as well as after being harvested, refined, and distributed. Numerous fresh food types are regularly linked to microorganisms in a number of ways that affect the general quality and cleanliness needs **(Biswas et al, 2020)**

Furthermore, it is possible that the vegetables sold in supermarkets were kept for an extended period of time, which would have allowed dangerous germs to gather. The storage conditions significantly affect the multiplication of microbial pathogens over time. Bacteria present on stored vegetables may transfer and cross-contaminate when vegetables are pre-washed with the same wash water by a vendor or processor **(Ohiduzzaman *et al.*, 2022).** Water used to wash and spray vegetables is a common source of illnesses. Water from contaminated fields, unclean reservoirs, leaks from reservoirs, and areas where manure is stored are the most frequent sources of water pollution. The same rules that apply to drinking water should also apply to water used for spraying and washing vegetables **(Osafa *et al*, 2022).**

Food preparation and serving spaces are great evidence of the cleanliness of the space where food is prepared or served. In addition, the open presentation of the foods, the absence of facilities for disposing of sewage and running water, as well as the inadequate storage conditions, all contributed to various forms of contamination **(Lidia *et.al*., 2021).**

The current analysis revealed significant levels of bacterial contamination in various vegetable samples, all of which clearly demonstrated the unsanitary state of open markets. In contrast to Ladyfinger, green chili has a high microbiological load of bacteria owing to environmental contamination.

Gram-negative bacteria included *Escherichia coli and Klebsiella species*, whereas Gram-positive bacteria included *Staphylococcus epridermidis, Serratia marcescens, Coagulase-negative staphylococcus,* and *Pseudomonas aeruginosa.* The presence of *Serratia marcessens, Klebsilla species, Pseudomonas aeruginosa, and Staphylococcus epidermidis* indicated that the local population is under serious health problems which can cause a variety of illnesses, including abdominal infections, pneumonia, urinary tract infections, bacteremia, pelvic infections, and meningitis. Due to isolated *Escherichia coli*, children and adults who consume contaminated food commonly experience diarrhea, nausea,

fever, and cramping.

All of the isolated bacteria were found to be rod-shaped. Similar results have been reported by **(Rahman *et al.*, 2016)** and **(Ahmed *et al.*,2019).** These results suggest that these microorganisms are to blame for a range of food-borne ailments, such as food poisoning, diarrhea due to digestive problems, cholera, typhoid, and pneumonia. Since raw vegetables were unfit for consumption, it was suggested that vegetables should be cleansed with clean water or another washing technique before being consumed as salad or meal **(Mir *et al,* 2018).**

Vegetables sold in local markets were dusty and crowded. Air, water, and the environment could easily pollute them. On the other hand, supermarket salespeople should follow hygienic instructions before handling the vegetables, in order to prevent microbial contamination. Supermarkets were likewise found in enclosed, tidy buildings. The government should thus concentrate more on checking the local markets and set up some seminars to teach vegetable handlers about sanitary handling techniques. These typical salad ingredients must be easily accessible in order for city dwellers to live their everyday lives. Food may spread a range of viruses when improperly handled, especially with samples, and cause a number of food-borne illnesses.

CONCLUSION:

The existence of bacteria in raw vegetables is harmful to human health. Since it can result in food-borne sickness and other illnesses that can affect people in any situation. It was determined that Narowal's vegetable purchasing and selling area is now in an unsanitary state. These vegetables are contaminated with pathogenic bacteria that may cause harmful diseases. *Serratia marcesscens, Staphylococcus epidermidis, Klebsiella species* and Coagulase-*negative staphylococcus* showed sensitivity to Chloramphenicol while *Pseudomonas aeruginosa* showed sensitivity to Amikacin and Penicillium. *Escherichia coli* showed sensitivity to Ampicillin. All the bacterial isolates showed resistance patterns among other tested drugs. Awareness about guidelines regarding food safety while selling and buying vegetables should be developed among vegetable sellers in the local market of Narowal. Vegetables should be prevented from rotting so that bacterial infections and food-borne illnesses don't occur. Cleaning surfaces, tools, and hands thoroughly can help prevent the transmission of germs into food. A preventative maintenance program and structured cleaning programs should be conducted. The use of controlled temperature, lighting, and ventilation as well as waste storage, and control of food hazards were suggested as ways to maintain the hygiene standards in these markets. When handling the vegetables, handlers should use gloves.

REFERENCES:

Ahmed, S., Siddique, Md. A., Rahman, M., Bari, Md. L., Ferdousi, S. 2019. A study on the prevalence of heavy metals, pesticides, and microbial contaminants and antibiotics resistance pathogens in raw salad vegetables sold in Dhaka, Bangladesh. *Heliyon.* 1-21.

Ali, B. 2019. "Functional and Genetic Diversity of Bacteria Associated with the Surfaces of Agronomic Plants" *Plants* 8, no. 4: 91. https://doi.org/10.3390/plants8040091

Bishop, H. G., & Okwori, G. O. 2017. *Escherichia coli* and *Staphylococcus aureus*

Contaminations of carrots sold within Zaria, Nigeria and their antibiotic Susceptibility Profiles. *Open Access Journal Science, 1*, 110-113.

Biswas, B., Azad, M. A. K., Absar, N., Islam, S., & Amin, S. 2020. Isolation and Identification of Pathogenic Bacteria from Fresh Fruits and Vegetables in Chittagong, Bangladesh. *Journal of Microbiology Research 10*(2), 55-58.

Chandra, A. K., Raj, H., Ranjan, H., Anand, K., & Kumari, P. 2022. Isolation and Characterization and sporulation of fungi from decaying vegetables and fruits of local Vegetables market in Hazaribag india, *Plant Archives, 22*(2), 426-430.

Inc, S., *SPSS base 9.0 applications guide*. 1999: Prentice Hall.

Jolt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST 1994. Bergey's manual of systematic bacteriology. 9th ed. Baltimore: Williams & Wilkins Co. p. 786.

Lidia, R. R., Rojo-Bezares, B., Lozano, C., López, M., Chichón, G., Torres, C and Sáenz, Y. 2021. "Occurrence of *Pseudomonas* spp. in Raw Vegetables: Molecular and Phenotypical Analysis of their Antimicrobial Resistance and Virulence-Related Traits" *International Journal of Molecular Sciences* 22, n o. 23: 12626. https://doi.org/10.3390/ijms222312626

Mamum, M. R., Das, K. K., Rana, M. S., Tanim, H. S., & Feroz, F. 2021. Comparative study on the microbiological quality of vegetables collected from local markets and super shops of Dhaka city. *Stamford Journal of Microbiology, 11*(1), 24- 27.

Mir, S., Shah, M., Mir, M ., Dar, B., Greiner, R & Roohinejad, S 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. 10.1016/j.foodcont.2017.10.006.

Ohiduzzaman, Islam, S.M, Arif, M., Saddam, Md., Uddain, J., Hossain, M. T., Kabir, S. M. L 2022. Microbial quality evaluation of fresh vegetables from distinct markets in urban areas of Bangladesh. 6 (1). 1-9. 10.3329/aajfss.v6i1.59694.

Osafo, R**.,** **Balali**, G. I ., Amissah-Reynolds, P. K., Gyapong,F" Addy, R., Nyarko, A. A., and Wiafe, P, 2022. Microbial and parasitic contamination of vegetables in developing countries. *Journal of Food Quality*,1-24 https://doi.org/10.1155/2022/4141914

Rehman ZU, Khan S, Qin K et al 2016. Quantification of inorganic arsenic exposure and cancer risk via consumption of vegetables in southern selected districts of Pakistan. Sci Total Environ 550:321–329. https://doi.org/10.1016/j.scitotenv.2016.01.094

Rida, B., & Deeba, F. 2018. Microbiological safety assessment of fresh fruits and Vegetables collected from main markets of Multan, Pakistan. *Journal of BioSource Management*, *5*(2),1-7.