ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 03 | March - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

OCCURRENCE AND CHARACTERIZATION OF *PSEUDOMONAS* SPP. IN RAW VEGETABLES: A STUDY ON ANTIMICROBIAL RESISTANCE AND VIRULENCE TRAITS

Health Science

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ABSTRACT

Background & Objective: Raw vegetable consumption has gained significant popularity due to its nutritional benefits. However, these vegetables can serve as potential carriers of pathogenic bacteria, posing a risk to public health. This study investigates the occurrence of *Pseudomonas* spp. in fresh salad vegetables, focusing on their antimicrobial resistance and virulence traits. **Method:** A total of 40 samples, including tomato, beetroot, green chili, cucumber, and coriander, were collected from fresh vegetable markets in Haryana, India. The bacterial isolates were identified through cultural, morphological, and biochemical characterization using selective media and standard biochemical tests. **Result:** The findings revealed the presence of *Pseudomonas* spp. in multiple samples, with isolates testing positive for catalase, oxidase, and gelatin hydrolysis, confirming their identity. The study highlights the potential pathogenicity of *Pseudomonas aeruginosa*, an opportunistic pathogen known for its ability to produce virulence factors such as elastases, proteases, and exotoxins. The presence of these bacteria in fresh vegetables underscores the need for stringent food safety measures throughout the farm-to-fork supply chain. **Conclusion:** This research emphasizes the importance of effective washing, proper handling, and hygienic agricultural practices to minimize contamination risks. Continuous monitoring and awareness programs for farmers and consumers are crucial to mitigating the public health threats associated with *Pseudomonas* spp. in raw vegetables. Further studies on antimicrobial resistance patterns and genetic characterization of these isolates are recommended to develop targeted intervention strategies.

KEYWORDS

INTRODUCTION

Raw vegetable salads have gained widespread popularity as healthy and refreshing dietary options. They offer a convenient means to incorporate a diverse range of vegetables into our daily meals. Fresh fruit and vegetables are crucial components of a healthy diet.1 The desire for a healthy lifestyle has resulted in an increase in the consumption of fresh goods in recent years. 400g of fruits and vegetables should be consumed daily, according to a 2003 WHO and FAO initiative to promote fruit and vegetable consumption for health worldwide [http://surl.li/hojlz].

This is primarily due to their consumption without undergoing substantial cooking or processing, which may allow pathogenic microorganisms to survive and potentially cause harm to human health. Nevertheless, due to a variety of contamination sources, including dust, soil, manure, irrigation water, and wild animal feces, vegetables are now more widely recognized as potential carriers of food borne diseases.2

Some vegetables, including tomato, cucumber, carrot, green chili, lemon, coriander leaf, pepper mint, and beetroot rot, are typically consumed raw to obtain their valuable nutrients in their best form. Their traditional use in salad preparation is also well known around the world. It is also widely known that they are traditionally used to make salads.3The gram-negative organisms tend to predominate the bacterial population and are most frequently associated with raw vegetables. The gram-negative, non-fermenting Pseudomonas bacteria can adapt to a wide range of environmental factors, which allows them to colonize various niches. There are many different species in this genus, with Pseudomonas aeruginosa being the most significant one. This species is a significant human opportunistic pathogen of growing medical and veterinary significane.4

P. aeruginosa disrupts host defenses by using a diverse array of pathogenicity factors, such as adhesions and secretion toxins, effectors proteins, proteases, elastases, and pigments. Due to its syringe-like structure, the type 3 secretion system (T3SS) is a key tool for virulence that aids in cytotoxicity and acute infections by injecting powerful exotoxins known as effectors (ExoU, ExoS, ExoY, and ExoT) into the cytoplasm of the host cell.5

Other gram-negative bacteria include E. coli, Salmonella spp., and Vibrio spp. Apart from gram-negative bacteria, gram-positive bacteria like Lactobacillus are also involved in raw vegetables.6 Lactobacillus is known for its probiotic characteristics, along with Bifidobacterium.7

To reduce the risk of foodborne illnesses linked to raw vegetable salads, it is necessary to delve into insightful information that can be used to develop preventive measures, guidelines, and interventions. It is possible to improve food safety practices along the entire farm-to-fork supply chain by improving understanding of the factors causing contamination and the traits of pathogenic isolates.8

In conclusion, it is crucial to look into pathogenic isolates found in raw vegetable salads in order to protect consumer safety. It is also necessary to keep a check on the soil profile, manure, and water used for irrigation, which are potentially responsible for contamination, along with handling processes post-harvest.

MATERIAL & METHOD

The present study was conducted in the department of microbiology, SGT University, Gurugram.

Sample Collection

The fresh raw salad vegetables were collected separately in sterile plastic bags from two different zones of Haryana which includes Gurugram and Rewari. The samples were collected from fresh vegetables markets. A total number of 40 samples were collected which included Tomato(n=8), Beetroot(n=8), Green Chili(n=8), Cucumber(n=8) and Coriander(n=8). They were transferred to laboratory for analysis.

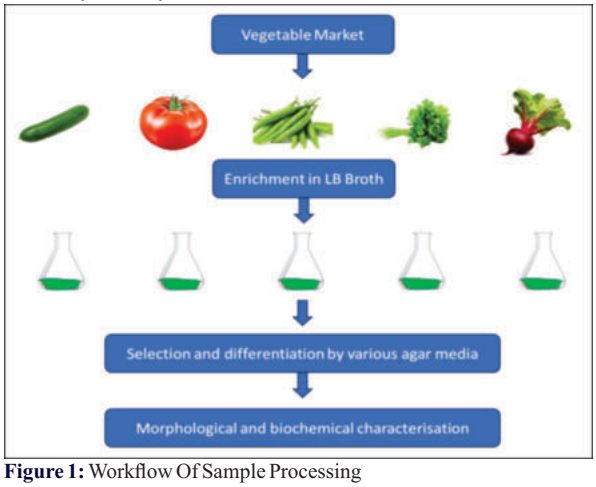

Figure 1: Workflow Of Sample Processing

Isolation Of Suspected Bacteria

The fresh vegetables were sterilized with peptone water and standard serial dilution was used to isolated bacteria from the collected peptone water. A dilution range of 10^{-1} - 10^{-10} was then made from collected peptone water. The final serial dilution of concentration 10^{-10} (10ml) was then mixed with 90 ml of LB Broth making up the final volume to 100ml.

The broth was incubated for 24hr correspondingly at 37°C. The assured cultures were streaked on Cetrimide Agar for suspected Pseudomonas spp., Mannitol salt phenol-red Agar for suspected Staphylococcus spp., CLED Agar for suspected E. coli and Lactobacillus MRS Agar for suspected Lactobacillus spp. The plates were incubated for 24hr at 37°C.

The plates were inspected for morphological characters, showing suspected growth of Lactobacillus spp. And Pseudomonas spp.Suspected bacteria were then subjected to biochemical tests for further identification.

Morphological Characterization

The bacterial isolates were then preceded to morphological characterization by Gram staining and motility test.

Gram's Staining

The first step in phenotypically identifying an unidentified bacterial isolate is gram staining. Based on the composition of their cell walls, Gram's Method divides a wide range of bacterial organisms into two major groups. Gram-negative cells are pink and have a thin peptidoglycan layer, whereas Gram-positive cells are purple and have a thick peptidoglycan layer. the variations in color that occur in bacterial cells when Gram staining is done.

Motility Test

Using SIM agar media, this test measures how easily bacteria can move across a liquid or semi-solid medium using their cilia, flagella, pseudopodia, or unique fibrils that give gliding motility.

Biochemical Characterization

According to the procedures outlined by Aneja (2008), the biochemical properties of bacterial isolates were identified. Several tests were carried out, including those for the formation of indole test, methyl red test, catalase test, oxidase test and gelatin test. A positive test indicated that the isolate had grown in the appropriate medium, but a negative test indicated that the isolate had not grown.

Methyl Red Test

This test distinguishes between two types of bacteria: one that creates a lot of organic acid (a stable end product like formic, acetic, lactic, or succinic acid), and the other that produces ethanol or acetoin (a non-acidic end product like acetoin or acetoin). When methyl red indicator is added, an organism that produces acid in the medium retains its red colour (a positive test), whilst other organisms display yellow colour (a negative test) when the pH of the medium rises. The KOH and naphthol solution in the VP reagent react with the acetoin to give a red colour (a positive test), while a lack of change in colour denotes a negative test.

Catalase Test

The enzyme catalase, which protects cells from harmful byproducts of oxygen metabolism like hydrogen peroxide (H2O2), a potent oxidizing agent, was discovered using this test in bacteria. Hydrogen peroxide (H2O2) is converted into water and oxygen by the catalase enzyme (O2). Catalase was discovered to be present when bubbles formed as a result of O2 evolution during this test, which involved saturating the culture with a 3% H2O2 solution.

Indole Test

A 48-hour growth period was allowed for all isolates in peptone broth while they were kept at 37 °C. After incubation, 0.5 ml of Kovac's reagent was added to each tube and thoroughly mixed. After mixing, the tubes were left alone for five minutes, and the positive development of cherry red color in the medium's top layer was noted. The bacteria's phenotype and color development are related.

Oxidase Test

An isolated colony was placed on the oxidase disc to conduct the oxidase test. At 25–30°C, the outcome was visible in 5–10 seconds. A color change that occurs within 10 seconds is regarded as a successful oxidase reaction.

Gelatin Test

This test's primary objective is to identify an organism's capacity to produce the gelatinase enzyme. Collagen is hydrolyzed to create gelatin, a globular protein. The main building block of connective tissues and tendons in both humans and animals is collagen. Gelatin dissolves in water at a temperature of 50°C, and it is a liquid above 25°C and a solid below 25°C. Even 4°C temperature cannot restore the gel-like characteristics of gelatin after it has been hydrolyzed. Gelatin is an amino acid polymer held together by peptide bonds. By adding acidic HgCl2, gelatin protein can be found (mercuric chloride). With gelatin protein, HgCl2 precipitates white. When an extracellular gelatinase is produced, it hydrolyzes gelatin and releases its constituent amino acids and short peptides. The microorganisms use these amino acids after that. The microorganism that hydrolyzes gelatin does not produce white precipitate when HgCl2 is used in conjunction with amino acids.

RESULTS AND DISCUSSION

Isolation And Identification

Out of 40 samples from two different zones, 8 bacteria were isolated from 4 samples and identified on the basis of their cultural (growth), morphological, and biochemical characterization.

Biochemical Characteristics Of Lactobacillus And Pseudomonas

The biochemical tests were performed to give confirmatory results for Lactobacillus and Pseudomonas. Four isolates were positive for catalase, oxidase, and gelatin test while negative for indole acetic test and methyl red test, confirming the presence of Pseudomonas spp. The other four isolates were negative for the methyl red test, catalase, oxidase, gelatin test, and indole acetic test, confirming the presence of Lactobacillus spp.

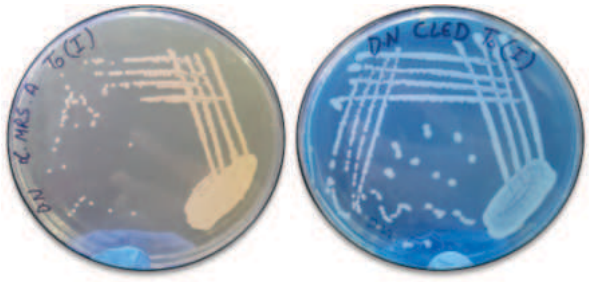

Figure 2: Lactobacillus spp. growth on LMRS Agar (left) and Pseudomonas spp. Growth on CLED Agar (Right)

Figure 3: Pseudomonas spp. Growth on Cetrimide Agar

Table 1: Morphological Characterization of Isolated Pseudomonas spp. And Lactobacillus spp.

S. no	Sample Name	Isolates	1	2	3
1	Tomato	PD1	G-	Single Rods	M+
2	Tomato	PD3	G-	SingleRods	M+
3	Tomato	LB1	G+	SingleRods	M-
4	Tomato	LB3	G+	SingleRods	M-
5	Cucumber	PD2	G-	SingleRods	M+
6	Cucumber	PD4	G-	SingleRods	M+
7	Cucumber	LB2	G+	SingleRods	M-
8	Cucumber	LB4	G+	SingleRods	M-

PD1-4= Pseudomonas spp. Isolates, LB1-4= Lactobacillus spp. Isolates, 1= Gram Staining, 2= Morphology, 3= Motility

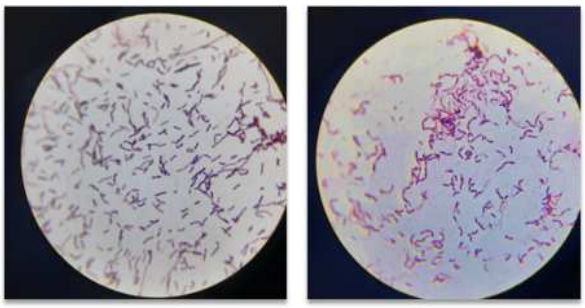

Figure 4: Gram positive, single rods (Lactobacillus spp.) on left and Gram negative, single rods (Pseudomonas spp.) on right.

PD1-4 = Pseudomonas spp. Isolates, LB1-4 = Lactobacillus spp. Isolates, 1 = Indole Acetic Acid test, 2 = Methyl Red test, 3 = Catalase test, 4 = Oxidase test, 5 = Gelatin test

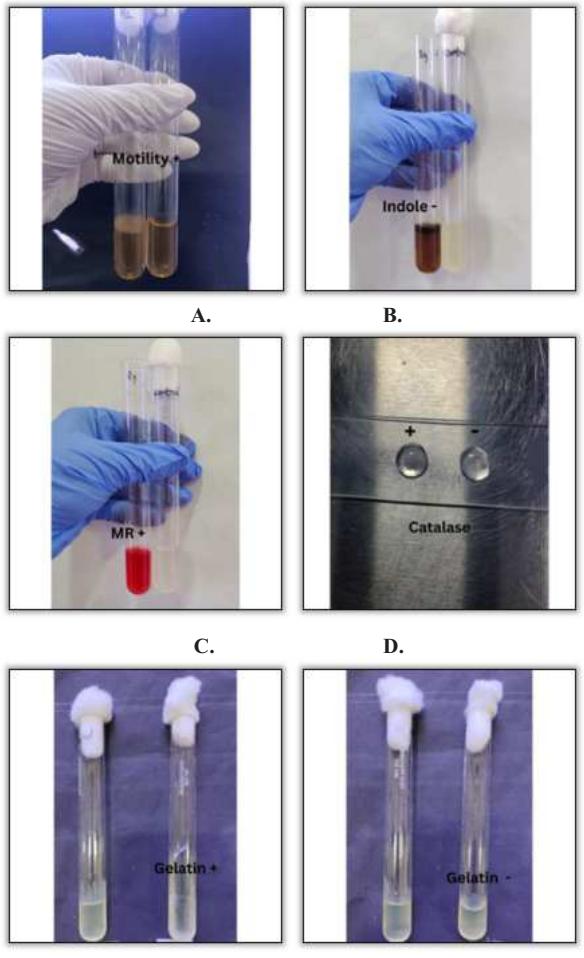

Figure 5: A = Motility test, B = Indole Acetic Acid test, C = Methyl Red test, D = Catalase test, E = Gelatin positive test, F = Gelatin negative test

DISCUSSION

Fresh vegetable samples frequently contain samples of the bacterial genera Lactobacillus spp. and Pseudomonas spp.9 While Pseudomonas spp. can pose a serious risk to human health due to their potential pathogenicity**;10**, Lactobacillus spp. are generally regarded as beneficial and are frequently connected with fermented foods **.11** The pathogenicity of Pseudomonas spp. in fresh vegetables and its implications for food safety will be the main topics of this discussion.

Pseudomonas spp., and Pseudomonas aeruginosa in particular, are well-known for their opportunistic pathogenicity and capacity to infect humans. Pseudomonas spp. have been discovered to contaminate a variety of foods, including fresh vegetables, even though they are primarily linked to nosocomial infections. This contamination can happen at different points in the production and processing of vegetables, from irrigation water handling to post-harvest handling.12 Contrarily, Lactobacillus spp., which are present in fresh vegetables, are generally thought to be advantageous and have had their probiotic properties thoroughly investigated. 13 In addition to assisting in the fermentation process, Lactobacillus spp. can improve the nutritional value and shelf life of fermented vegetables.

Preventive actions need to be taken at every stage of the production and supply chain to guarantee the security of fresh vegetables. The risk of Pseudomonas spp. contamination can be greatly reduced by using sound agricultural practices, such as efficient irrigation water management, hygiene standards, and pest control.14

The ability of Pseudomonas spp. to cause foodborne illnesses and their pathogenic nature make them a potential risk to human health when they are present in fresh vegetables. For the creation and application of efficient preventive measures, it is essential to comprehend the factors influencing their pathogenicity, such as the production of virulence factors and antibiotic resistance.10

Furthermore, it's critical to spread knowledge about the dangers that could result from Pseudomonas spp. contamination of fresh vegetables among farmers, food handlers, and consumers. The prevalence of foodborne illnesses brought on by these pathogens can be decreased with proper education on hygiene practices, proper storage and handling, and the significance of eating properly cooked or washed vegetables.15

Although the focus of this discussion has been on the pathogenicity of Pseudomonas species in fresh vegetables, it is important to take into account the general microbiological safety and efficacy of these products. The presence of advantageous bacteria, like Lactobacillus spp., should also be taken into account since they can aid in fermentation and possibly have health advantages.16

CONCLUSION

Contamination from Pseudomonas spp. in fresh vegetables poses a serious threat to food safety. For putting effective preventive measures in place, it is essential to understand the pathogenicity and antibiotic resistance profiles of these bacteria. We can reduce the risk of Pseudomonas spp. contamination and guarantee the safety of fresh vegetable consumption by implementing comprehensive strategies that include good agricultural practices, strict post-harvest handling, and the investigation of novel interventions. To address the issues related to Pseudomonas spp. pathogenicity in fresh vegetables and to protect the public's health, ongoing research, surveillance, and education efforts are required.

REFERENCES

1. Adams MR, Moss MO. *Food Microbiology*. New Age International; 2007
2. Ruiz-Roldán L, Rojo-Bezares B, Lozano C, López M, Chichón G, Torres C, Sáenz Y. Occurrence of *Pseudomonas* spp. in raw vegetables: Molecular and phenotypical analysis of their antimicrobial resistance and virulence-related traits. *Int J Mol Sci.* 2021;22(23):12626. doi:10.3390/ijms222312626.
3. Nipa M, et al. Prevalence of multi-drug resistant bacteria on raw salad vegetables sold in major markets of Chittagong City, Bangladesh. *Middle-East J Sci Res.* 2011;10:70–77.
4. Ma Y, et al. Considerations and caveats in combating ESKAPE pathogens against nosocomial infections. *Adv Sci.* 2019;7(1):1901872.
5. Moradali MF, Ghods S, Rehm BHA. *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and persistence. *Front Cell Infect Microbiol.* 2017;7:Article 39.
6. Bjarnsholt T. The role of bacterial biofilms in chronic infections. *APMIS.* 2013;121(S136):1–58.
7. Matsumoto T, et al. Efficacies of alkaline protease, elastase and exotoxin A toxoid vaccines against gut-derived *Pseudomonas aeruginosa* sepsis in mice. *J Med Microbiol.* 1998;47(4):303–308.
8. Liao C, et al. Virulence factors of *Pseudomonas aeruginosa* and antivirulence strategies to combat its drug resistance. *Front Cell Infect Microbiol.* 2022;12:926758.
9. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients.* 2017;9(9):1021.
10. Pena RT, et al. Relationship between quorum sensing and secretion systems. *Front Microbiol.* 2019.
11. Michalska M, Wolf P. *Pseudomonas* exotoxin A: Optimized by evolution for effective killing. *Front Microbiol.* 2015;6:963.
12. Li S, et al. Anti-biofilm effect of novel thiazole acid analogs against *Pseudomonas aeruginosa* through IQS pathways. *Eur J Med Chem.* 2018;145:64–73.
13. Jyot J, et al. Type II secretion system of *Pseudomonas aeruginosa*: In vivo evidence of a significant role in death due to lung infection. *J Infect Dis.* 2011;203(10):1369–1377.
14. Morello E, et al. *Pseudomonas aeruginosa* lipoxygenase LoxA contributes to lung infection by altering the host immune lipid signaling. *Front Microbiol.* 2019;10:1826.
15. Garg, N., Churey, J.J. and Splittstoesser, D.F. (1990) 'Effect of Processing Conditions on the Microflora of Fresh-Cut Vegetables', Journal of Food Protection, 53(8), pp. 701–703.
16. Ellis, T.N. and Kuehn, M.J. (2010) 'Virulence and Immunomodulatory Roles of Bacterial Outer Membrane Vesicles', Microbiology and Molecular Biology Reviews : MMBR, 74(1), pp. 81–94.