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Original Article

Comparison of Antimicrobial Resistance in Pseudomonas Isolated from Raw Spinach Grown with and Without Chemical Fertilizers

Usama Ali[1], Eman Fatima[1], Rehmat Ullah[1], Ijaz Ahmad[1], Azka Mubeen[1], Sidra Iqbal[1], Faizan Hameed[1]

[1] Department of Medical Lab Technology, Faculty of Allied Health Sciences, The Superior University Lahore, Lahore, Pakistan

***Corresponding author: Azka Mubeen, azkamubeen786@gmail.com**

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ABSTRACT

Background: Antimicrobial resistance is an increasing public health concern that extends into agricultural and food-production environments. Fresh leafy vegetables such as spinach may serve as reservoirs for resistant bacteria, particularly when consumed raw or minimally processed. **Objective:** This study aimed to compare the antimicrobial resistance patterns of Pseudomonas spp. isolated from raw spinach cultivated with chemical fertilizers and spinach cultivated without chemical fertilizers. **Methods:** An observational cross-sectional laboratory-based comparative study was conducted on 60 raw spinach samples, including 30 samples from chemically fertilized fields and 30 from non-fertilized fields. Samples were collected using the zigzag sampling technique and transported under cold-chain conditions. Presumptive Pseudomonas spp. were isolated using standard culture methods and identified through colony characteristics, Gram staining, oxidase testing, motility, and non-lactose-fermenting behavior. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, and results were interpreted according to Clinical and Laboratory Standards Institute criteria. Data were analyzed using frequencies, percentages, chi-square test, and Fisher's exact test where applicable. **Results:** Pseudomonas spp. were isolated from 24 of 60 samples (40.0%). Isolation was higher in fertilized spinach than in non-fertilized spinach, but the difference was not statistically significant (14/30, 46.7% vs. 10/30, 33.3%; $p = 0.292$). All isolates were susceptible to imipenem and meropenem. Cefotaxime resistance was significantly higher in fertilized isolates than non-fertilized isolates (10/14, 71.4% vs. 2/10, 20.0%; $p = 0.036$), as was ceftazidime resistance (6/14, 42.9% vs. 0/10, 0.0%; $p = 0.024$). Moxifloxacin resistance was detected only in fertilized isolates, but the difference was not statistically significant. **Conclusion:** Raw spinach cultivated with chemical fertilizers showed significantly higher cephalosporin resistance among Pseudomonas spp. isolates, while carbapenem susceptibility remained preserved. These findings support the need for antimicrobial resistance surveillance in fresh produce and improved monitoring of agricultural practices. **Keywords:** Pseudomonas spp.; antimicrobial resistance; spinach; chemical fertilizers; cephalosporin resistance; food safety; fresh produce.

INTRODUCTION

Antimicrobial resistance is a growing public health concern that extends beyond clinical settings into environmental, agricultural, and food-production systems. Fresh vegetables are increasingly recognized as potential vehicles for the transmission of resistant bacteria because they are frequently exposed to soil, irrigation water, fertilizers, handling surfaces, and post-harvest contamination (1). Leafy vegetables such as spinach are of particular concern because they are often consumed raw or minimally processed, allowing viable bacteria to reach consumers if hygiene and washing practices are inadequate (2).

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Among food-associated Gram-negative bacteria, Pseudomonas spp. are important because of their environmental adaptability, ability to survive under diverse conditions, and capacity to develop resistance through intrinsic and acquired mechanisms. These organisms can persist in soil, water, and fresh produce environments, and their presence on raw vegetables may indicate broader microbial contamination within the farm-to-consumer chain (3). Although Pseudomonas aeruginosa is widely known as an opportunistic pathogen, genus-level Pseudomonas contamination in fresh produce is also relevant from a food safety perspective, particularly when isolates demonstrate reduced susceptibility to clinically important antibiotics (4).

Agricultural practices may influence the microbial ecology of crop environments and contribute to the persistence of resistant organisms. Chemical fertilizers can alter soil microbial communities and may introduce or concentrate selective pressures, including changes in nutrient composition, metal content, and other environmental factors that favor resistant bacterial populations. In agricultural ecosystems, co-selection of antibiotic resistance may occur when resistance determinants are linked to tolerance mechanisms for environmental stressors such as heavy metals. However, the extent to which fertilizer-associated cultivation conditions influence antimicrobial resistance patterns in bacteria recovered from fresh produce remains insufficiently characterized (5,6).

Spinach represents a useful model for investigating this issue because its broad leaf surface, close contact with soil, and common raw consumption increase the relevance of microbial contamination. While previous studies have documented antimicrobial-resistant bacteria in food and environmental reservoirs, limited local data are available comparing resistance patterns of Pseudomonas spp. isolated from spinach cultivated under different fertilizer practices. Such data are important for understanding whether routine agricultural inputs may be associated with differences in resistance profiles among produce-associated bacteria (7,8).

Therefore, the present study aimed to compare the antimicrobial resistance patterns of Pseudomonas spp. isolated from raw spinach grown with chemical fertilizers and spinach grown without chemical fertilizers. The study specifically assessed the prevalence of Pseudomonas spp. in both cultivation groups and compared in vitro susceptibility patterns to selected antimicrobial agents using the Kirby-Bauer disk diffusion method.

MATERIALS AND METHODS

This observational cross-sectional laboratory-based comparative study was conducted at Concept Laboratory, Shalimar Link Road, Lahore, over a four-month period after approval of the research synopsis. The study was designed to compare the occurrence and antimicrobial susceptibility patterns of Pseudomonas spp. isolated from raw spinach cultivated with chemical fertilizers and spinach cultivated without chemical fertilizers.

A total of 60 fresh spinach samples were included in the study. The samples were divided equally into two groups: 30 samples from spinach cultivated with chemical fertilizers and 30 samples from spinach cultivated without chemical fertilizers. Sampling sites were selected on the basis of availability and confirmation of fertilizer-use history. The fertilizer status of each field was documented through information provided by the grower or farm manager. Fresh, healthy spinach leaves collected at harvest were included. Decayed, rotten, physically damaged, washed, processed, disinfectant-treated, or chemically preserved leaves were excluded (9,10). Samples with unclear fertilizer history or those collected without permission from the farm owner were also excluded.

Samples were collected using the zigzag sampling technique to improve field-level representation. During collection, each field was traversed in a zigzag pattern, and spinach leaves were obtained at regular intervals from different points across the field. Immediately after collection, samples were placed in sterile sealed sampling bags, labelled according to cultivation group, and transported to the

laboratory in a portable cooler containing ice packs. A cold-chain temperature of approximately 4°C was maintained during transport. All samples were delivered to the laboratory within four hours of collection and processed within 24 hours to preserve microbiological integrity.

For microbiological processing, spinach samples were handled under aseptic conditions. Each sample was examined for bacterial contamination using standard culture-based methods. Isolation of presumptive Pseudomonas spp. was performed on appropriate selective culture media, followed by colony observation and preliminary biochemical identification. Presumptive isolates were identified on the basis of characteristic colony morphology, Gram staining, oxidase reaction, motility, and non-lactose-fermenting behavior. Isolates showing features consistent with Pseudomonas spp. were selected for antimicrobial susceptibility testing.

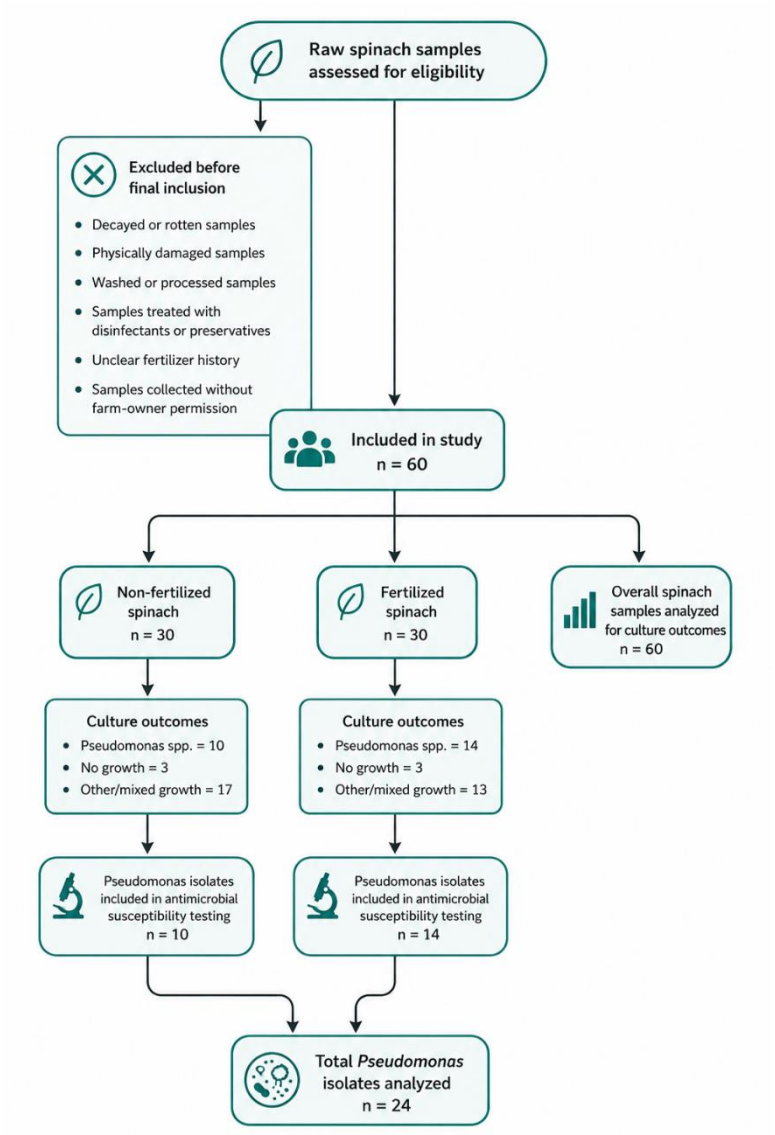

Figure 1. Flow Diagram of Spinach Sample Selection, Group Allocation, Culture Outcomes, and Pseudomonas Isolates Included in Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. A bacterial suspension was prepared from each confirmed isolate in sterile normal saline and adjusted to match the 0.5 McFarland turbidity standard. The standardized inoculum was evenly swabbed over the surface of Mueller-Hinton agar plates using a sterile swab. Antibiotic discs were then placed aseptically on the inoculated agar surface at appropriate distances to prevent overlapping zones of inhibition. The plates were incubated aerobically at 37°C for 18–24 hours.

The antimicrobial agents assessed included imipenem, meropenem, amikacin, cefotaxime, ceftazidime, moxifloxacin, and piperacillin-tazobactam. After incubation, inhibition zones were measured in millimeters using a calibrated ruler. Susceptibility patterns were interpreted as susceptible, intermediate, or resistant according to Clinical and Laboratory Standards Institute criteria. The primary outcome variable was antimicrobial resistance among Pseudomonas spp. isolates. Secondary variables included cultivation group, culture outcome, isolation frequency of Pseudomonas spp., and distribution of other bacterial growth patterns.

Data were entered and analyzed using SPSS. Descriptive statistics were calculated as frequencies and percentages for categorical variables, including culture outcome, isolate distribution, and antimicrobial susceptibility category. The prevalence of Pseudomonas spp. was calculated overall and separately for fertilized and non-fertilized spinach samples. Antimicrobial susceptibility patterns were compared between isolates obtained from fertilized and non-fertilized spinach. Associations between categorical variables were assessed using the chi-square test, while Fisher's exact test was applied where cell counts were small. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 60 raw spinach samples were analyzed, including 30 (50.0%) samples from spinach cultivated without chemical fertilizers and 30 (50.0%) samples from spinach cultivated with chemical fertilizers. Overall, Pseudomonas spp. were isolated from 24 samples (40.0%). Six samples (10.0%) showed no bacterial growth, while 30 samples (50.0%) yielded other or mixed bacterial growth.

In the non-fertilized group, Pseudomonas spp. were isolated from 10 of 30 samples (33.3%), while 3 samples (10.0%) showed no growth and 17 samples (56.7%) showed other or mixed growth. In the fertilized group, Pseudomonas spp. were isolated from 14 of 30 samples (46.7%), while 3 samples (10.0%) showed no growth and 13 samples (43.3%) showed other or mixed growth. The difference in Pseudomonas isolation between the two cultivation groups was not statistically significant ($p = 0.292$).

Table 1. Culture outcomes of raw spinach samples by cultivation group

Culture outcome	Non-fertilized spinach, n (%)	Fertilized spinach, n (%)	Total, n (%)
Pseudomonas spp.	10 (33.3)	14 (46.7)	24 (40.0)
No growth	3 (10.0)	3 (10.0)	6 (10.0)
Other or mixed growth	17 (56.7)	13 (43.3)	30 (50.0)
Total	30 (100.0)	30 (100.0)	60 (100.0)

Distribution of Bacterial Isolates

Among all spinach samples, Pseudomonas spp. were the most frequently recovered isolates, accounting for 24 of 60 samples (40.0%). Other bacterial growth included mixed growth in 5 samples (8.3%), Klebsiella spp. in 5 samples (8.3%), Escherichia coli in 4 samples (6.7%), Enterobacter spp. in 4 samples (6.7%), Citrobacter spp. in 4 samples (6.7%), Staphylococcus spp. in 4 samples (6.7%), and normal flora in 4 samples (6.7%). No growth was observed in 6 samples (10.0%).

In the non-fertilized group, Pseudomonas spp. were detected in 10 samples (33.3%), followed by mixed growth, Klebsiella spp., and E. coli in 3 samples each (10.0%). In the fertilized group, Pseudomonas spp. were detected in 14 samples (46.7%), followed by no growth in 3 samples (10.0%) and several other bacterial categories at lower frequencies. Antimicrobial susceptibility testing was performed on 24 Pseudomonas spp. isolates, including 10 isolates from non-fertilized spinach and 14 isolates from fertilized spinach. All isolates were susceptible to imipenem and meropenem, with 24 of 24 isolates (100.0%) showing susceptibility to both carbapenems.

Amikacin showed mixed activity, with 12 isolates (50.0%) susceptible and 12 isolates (50.0%) intermediate; no isolate was resistant. In the non-fertilized group, 4 of 10 isolates (40.0%) were susceptible and 6 (60.0%) were intermediate. In the fertilized group, 8 of 14 isolates (57.1%) were susceptible and 6 (42.9%) were intermediate.

Cefotaxime resistance was observed in 12 of 24 isolates (50.0%) overall. Resistance was higher among isolates from fertilized spinach, where 10 of 14 isolates (71.4%) were resistant, compared with 2 of 10 isolates (20.0%) from non-fertilized spinach. The difference in cefotaxime resistance between cultivation groups was statistically significant (p = 0.036).

Table 2. Distribution of bacterial isolates recovered from spinach samples

Isolate or culture category	Non-fertilized spinach, n (%)	Fertilized spinach, n (%)	Total, n (%)
Pseudomonas spp.	10 (33.3)	14 (46.7)	24 (40.0)
No growth	3 (10.0)	3 (10.0)	6 (10.0)
Mixed growth	3 (10.0)	2 (6.7)	5 (8.3)
Klebsiella spp.	3 (10.0)	2 (6.7)	5 (8.3)
Escherichia coli	3 (10.0)	1 (3.3)	4 (6.7)
Enterobacter spp.	2 (6.7)	2 (6.7)	4 (6.7)
Citrobacter spp.	2 (6.7)	2 (6.7)	4 (6.7)
Staphylococcus spp.	2 (6.7)	2 (6.7)	4 (6.7)
Normal flora	2 (6.7)	2 (6.7)	4 (6.7)
Total	30 (100.0)	30 (100.0)	60 (100.0)

Ceftazidime resistance was observed in 6 of 24 isolates (25.0%) overall. All resistant isolates were from fertilized spinach, where 6 of 14 isolates (42.9%) were resistant. No ceftazidime resistance was detected among isolates from non-fertilized spinach. The difference in ceftazidime resistance between cultivation groups was statistically significant (p = 0.024).

Moxifloxacin showed full susceptibility among isolates from non-fertilized spinach, with 10 of 10 isolates (100.0%) susceptible. Among isolates from fertilized spinach, 12 of 14 isolates (85.7%) were intermediate and 2 of 14 isolates (14.3%) were resistant. Overall, 10 isolates (41.7%) were susceptible, 12 (50.0%) were intermediate, and 2 (8.3%) were resistant. The difference in moxifloxacin resistance between groups was not statistically significant (p = 0.493). Piperacillin-tazobactam showed high susceptibility overall, with 20 of 24 isolates (83.3%) susceptible and 4 isolates (16.7%) intermediate. All 10 isolates (100.0%) from non-fertilized spinach were susceptible. Among isolates from fertilized spinach, 10 of 14 isolates (71.4%) were susceptible and 4 of 14 isolates (28.6%) were intermediate. No isolate was resistant to piperacillin-tazobactam.

Table 3. Antimicrobial susceptibility profile of Pseudomonas spp. isolates by cultivation group

Antibiotic	Non-fertilized spinach S/I/R, n	Fertilized spinach S/I/R, n	Total S/I/R, n
Imipenem	10/0/0	14/0/0	24/0/0
Meropenem	10/0/0	14/0/0	24/0/0
Amikacin	4/6/0	8/6/0	12/12/0
Cefotaxime	0/8/2	0/4/10	0/12/12
Ceftazidime	2/8/0	0/8/6	2/16/6
Moxifloxacin	10/0/0	0/12/2	10/12/2
Piperacillin-tazobactam	10/0/0	10/4/0	20/4/0

Abbreviations: S, susceptible; I, intermediate; R, resistant.

Table 4. Resistance frequency among Pseudomonas spp. isolates by cultivation group

Antibiotic	Non-fertilized resistant isolates, n/N (%)	Fertilized resistant isolates, n/N (%)	Total resistant isolates, n/N (%)	p-value
Imipenem	0/10 (0.0)	0/14 (0.0)	0/24 (0.0)	—
Meropenem	0/10 (0.0)	0/14 (0.0)	0/24 (0.0)	—
Amikacin	0/10 (0.0)	0/14 (0.0)	0/24 (0.0)	—
Cefotaxime	2/10 (20.0)	10/14 (71.4)	12/24 (50.0)	0.036
Ceftazidime	0/10 (0.0)	6/14 (42.9)	6/24 (25.0)	0.024
Moxifloxacin	0/10 (0.0)	2/14 (14.3)	2/24 (8.3)	0.493
Piperacillin-tazobactam	0/10 (0.0)	0/14 (0.0)	0/24 (0.0)	—

Resistance was most frequently observed against cefotaxime, followed by ceftazidime and moxifloxacin. Overall, cefotaxime resistance was detected in 12 of 24 isolates (50.0%), ceftazidime resistance in 6 of 24 isolates (25.0%), and moxifloxacin resistance in 2 of 24 isolates (8.3%). No resistance was detected against imipenem, meropenem, amikacin, or piperacillin-tazobactam. Among isolates from non-fertilized spinach, resistance was observed only against cefotaxime, with 2 of 10 isolates (20.0%) resistant. Among

isolates from fertilized spinach, resistance was observed against cefotaxime in 10 of 14 isolates (71.4%), ceftazidime in 6 of 14 isolates (42.9%), and moxifloxacin in 2 of 14 isolates (14.3%).

The overall prevalence of Pseudomonas spp. in raw spinach samples was 40.0%. Isolation was higher in fertilized spinach than in non-fertilized spinach, but the difference was not statistically significant. All Pseudomonas spp. isolates were susceptible to imipenem and meropenem. Cefotaxime and ceftazidime showed the highest resistance frequencies, with significantly higher resistance among isolates from fertilized spinach. Moxifloxacin resistance was detected only in the fertilized group, but the group difference was not statistically significant.

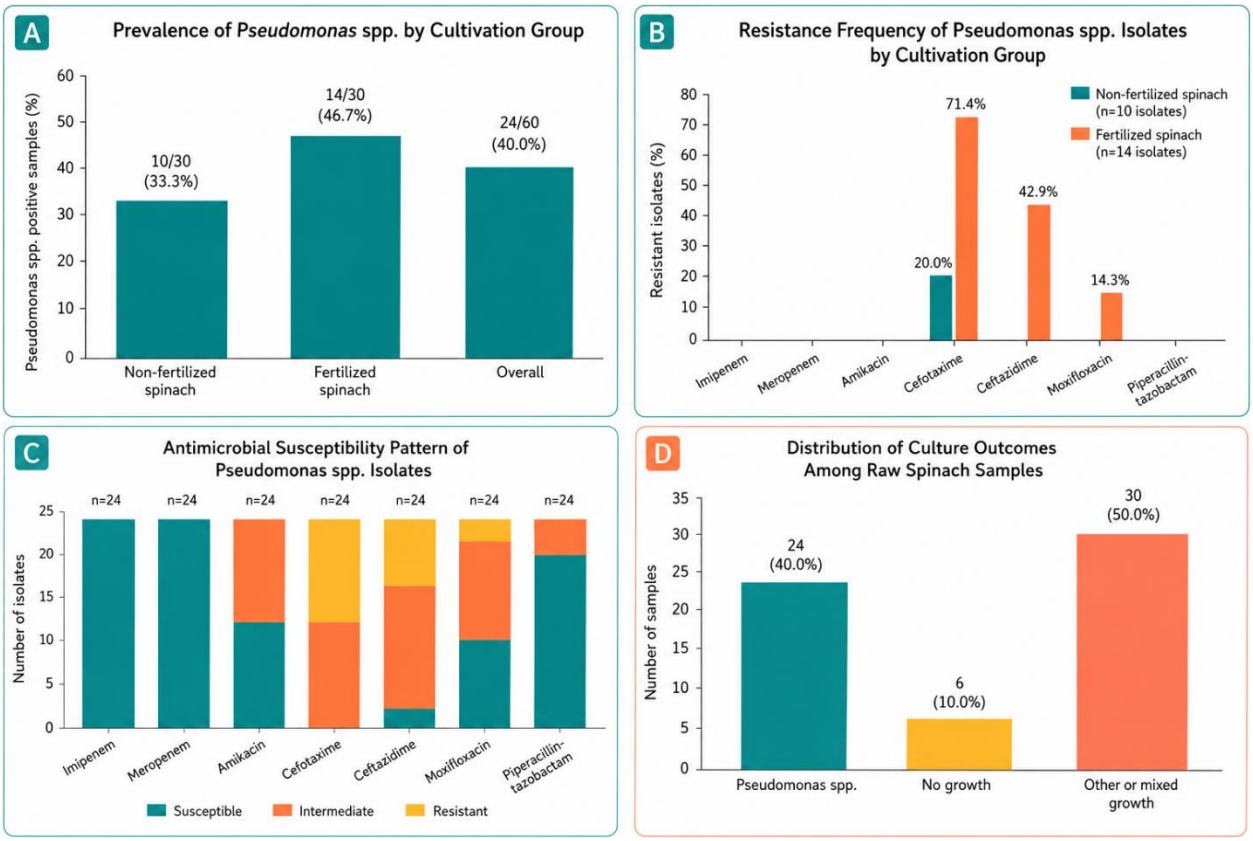

Figure 2. Prevalence, antimicrobial resistance, susceptibility patterns, and culture outcomes of Pseudomonas spp. isolated from raw spinach samples.

Panel A illustrates the prevalence of Pseudomonas spp. among cultivation groups, showing a higher proportion of positive samples in fertilized spinach (46.7%; 14/30) compared with non-fertilized spinach (33.3%; 10/30), with an overall prevalence of 40.0% (24/60). Panel B demonstrates the resistance frequency of Pseudomonas isolates according to cultivation group, where isolates from fertilized spinach exhibited markedly higher resistance to cefotaxime (71.4%), ceftazidime (42.9%), and moxifloxacin (14.3%), whereas isolates from non-fertilized spinach showed lower resistance, limited mainly to cefotaxime (20.0%). Panel C presents the antimicrobial susceptibility profile of the 24 Pseudomonas isolates, highlighting high susceptibility to imipenem, meropenem, and piperacillin–tazobactam, while variable intermediate and resistant responses were observed against cephalosporins and fluoroquinolones. Panel D summarizes the overall culture outcomes among raw spinach samples, indicating that 40.0% yielded Pseudomonas spp., 10.0% showed no microbial growth, and 50.0% demonstrated other or mixed bacterial growth. Together, the findings suggest a greater prevalence and antimicrobial resistance burden of Pseudomonas spp. in fertilized spinach samples compared with non-fertilized counterparts.

DISCUSSION

The present study compared the occurrence and antimicrobial susceptibility patterns of Pseudomonas spp. isolated from raw spinach cultivated with and without chemical fertilizers. The overall isolation rate of Pseudomonas spp. was 40.0%, with a higher proportion recovered from fertilized spinach than from non-fertilized spinach, although this difference was not statistically significant. The most important finding was the significantly higher resistance to cefotaxime and ceftazidime among isolates from fertilized spinach, whereas all isolates remained susceptible to imipenem and meropenem. These findings suggest that fertilizer-associated cultivation conditions may be linked with differences in cephalosporin resistance patterns among spinach-associated Pseudomonas spp.

The recovery of Pseudomonas spp. from a substantial proportion of raw spinach samples highlights the relevance of leafy vegetables as potential reservoirs of environmental and opportunistic bacteria. Spinach is commonly exposed to soil, irrigation water, agricultural inputs, and handling during harvesting and transport, all of which may contribute to microbial contamination (11). Because spinach is frequently consumed raw or minimally processed, the presence of antimicrobial-resistant bacteria on its surface has food safety significance. Although Pseudomonas spp. are widely distributed in natural environments, their detection in fresh produce becomes more concerning when isolates show reduced susceptibility to clinically important antimicrobial agents (12).

The higher isolation frequency of Pseudomonas spp. in fertilized spinach may reflect the influence of agricultural practices on soil and plant-associated microbial communities. Chemical fertilizers can alter nutrient availability, soil physicochemical conditions, and microbial competition in the rhizosphere. These changes may favor the persistence or proliferation of certain bacterial groups, including environmentally adaptable organisms such as Pseudomonas spp. However, the non-significant difference in isolation rate indicates that fertilizer use alone cannot explain the distribution of isolates in this study. Other factors, including irrigation water quality, soil contamination, field hygiene, harvesting practices, storage conditions, and local environmental variation, may also have contributed to the observed pattern (13).

The resistance findings are more notable than the difference in isolation frequency. Cefotaxime resistance was detected in 71.4% of Pseudomonas isolates from fertilized spinach compared with 20.0% from non-fertilized spinach, while ceftazidime resistance was found only among isolates from fertilized spinach. This pattern indicates that resistance was not evenly distributed across the two cultivation groups, but was concentrated particularly in relation to third-generation cephalosporins (14,15). Such findings are consistent with the concept that agricultural environments may act as reservoirs where resistant bacteria are maintained and disseminated through soil, water, and crop surfaces (16).

Several mechanisms may explain the higher cephalosporin resistance observed among isolates from fertilized spinach. Fertilized agricultural soils may be exposed to selective pressures from fertilizer components, environmental contaminants, heavy metals, or residues introduced through agricultural inputs. Co-selection may occur when genes associated with resistance to antibiotics and tolerance to environmental stressors are located on shared mobile genetic elements. Under such conditions, exposure to non-antibiotic selective agents may indirectly favor bacteria carrying antimicrobial resistance determinants (17,18). In addition, Pseudomonas spp. possess intrinsic resistance mechanisms, including low outer-membrane permeability, efflux pumps, and inducible beta-lactamase activity, which may contribute to reduced susceptibility to beta-lactam antibiotics.

The complete susceptibility of all isolates to imipenem and meropenem is an important finding. Carbapenems are generally reserved for severe infections caused by multidrug-resistant Gram-negative bacteria, and carbapenem-resistant Pseudomonas is a major public health concern. In this study, no carbapenem resistance was detected among spinach-associated isolates, suggesting preserved in vitro activity of these agents in the sampled isolates. However, this finding should be interpreted carefully

because the sample size was limited and the isolates were obtained from a defined local sampling frame. Continued surveillance remains important because carbapenem resistance can emerge through acquired carbapenemases, porin alterations, efflux mechanisms, and other adaptive pathways (19).

Amikacin and piperacillin-tazobactam also showed favorable activity, with no resistant isolates detected. Amikacin produced a mixed susceptible and intermediate pattern, while piperacillin-tazobactam showed high susceptibility with only intermediate results among some fertilized-sample isolates. These findings suggest that resistance was not broad across all tested antimicrobial classes (20). Instead, the main resistance signal was concentrated in cephalosporins, particularly cefotaxime and ceftazidime. This distinction is important because it indicates a selective resistance pattern rather than generalized multidrug resistance among all isolates.

Moxifloxacin showed reduced activity among isolates from fertilized spinach, with intermediate results in most fertilized-group isolates and resistance in a small proportion. Although the difference in resistance was not statistically significant, the presence of reduced susceptibility only in the fertilized group may still be epidemiologically relevant. Fluoroquinolone susceptibility can be influenced by chromosomal mutations, efflux pump activity, and environmental selection pressures. Larger studies would be needed to determine whether the observed moxifloxacin pattern represents a consistent association or a chance finding related to the small number of isolates (21,22).

The findings have practical implications for food safety and antimicrobial resistance surveillance. Fresh vegetables are not usually considered major drivers of clinical antimicrobial resistance compared with hospitals, livestock systems, or wastewater environments, but they may serve as points of human exposure to resistant environmental bacteria. The detection of cephalosporin-resistant Pseudomonas spp. in raw spinach suggests that produce-associated bacteria should be included in broader One Health surveillance frameworks. Monitoring should consider not only clinical isolates but also organisms recovered from soil, irrigation water, fertilizers, fresh produce, and food-handling environments.

This study has several limitations. First, the sample size was relatively small, with 60 spinach samples and 24 Pseudomonas isolates, which limits statistical power and generalizability. Second, the study used a cross-sectional design, so it can identify associations but cannot establish causality between chemical fertilizer use and antimicrobial resistance. Third, fertilizer exposure was based on cultivation history rather than direct measurement of fertilizer composition, application rate, heavy metals, antibiotic residues, or other contaminants. Fourth, molecular confirmation of isolates and detection of resistance genes were not performed, so the mechanisms underlying cephalosporin resistance could not be determined. Fifth, environmental variables such as irrigation water quality, soil characteristics, farm location, season, and post-harvest handling were not fully controlled. These limitations should be considered when interpreting the findings.

Despite these limitations, the study provides useful preliminary evidence that raw spinach may harbor Pseudomonas spp. with differing antimicrobial susceptibility patterns depending on cultivation conditions. The significantly higher cefotaxime and ceftazidime resistance among isolates from fertilized spinach supports the need for improved monitoring of agricultural inputs and fresh produce contamination. Future studies should include larger sample sizes, multiple geographic locations, detailed fertilizer characterization, testing for heavy metals and antibiotic residues, molecular identification of isolates, and resistance gene analysis (23). Such work would help clarify the pathways through which agricultural practices may influence antimicrobial resistance in produce-associated bacteria.

Overall, the findings emphasize the importance of integrating agricultural hygiene, fertilizer management, produce safety, and antimicrobial resistance surveillance. Reducing microbial contamination in leafy vegetables requires coordinated attention to field practices, irrigation water, harvesting methods, storage conditions, and consumer-level washing practices. The observed

cephalosporin resistance pattern among fertilized-spinach isolates suggests that cultivation practices may be one component of a broader environmental resistance landscape, warranting continued investigation within a One Health framework.

CONCLUSION

This study demonstrated that Pseudomonas spp. were isolated from a substantial proportion of raw spinach samples, with a higher isolation rate in spinach cultivated with chemical fertilizers than in spinach cultivated without chemical fertilizers, although this difference was not statistically significant. Isolates from fertilized spinach showed significantly higher resistance to cefotaxime and ceftazidime, while all isolates remained susceptible to imipenem and meropenem. These findings suggest an association between fertilizer-associated cultivation conditions and increased cephalosporin resistance among spinach-associated Pseudomonas spp., highlighting the need for improved agricultural hygiene, careful fertilizer management, and routine antimicrobial resistance surveillance in fresh produce.

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