GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Assessment of Bacterial Contamination in Lettuce (*Lactuca sativa*) Sold in Al-Rass City, Saudi Arabia

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GSC Biological and Pharmaceutical Sciences, 2025, 31(01), 082-088

Publication history: Received on 22 February 2025; revised on 05 April 2025; accepted on 07 April 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.31.1.0136

Abstract

Lettuce (*Lactuca sativa*), a widely consumed leafy vegetable, is susceptible to bacterial contamination, posing potential health risks to consumers. This study aimed to assess the microbiological quality of lettuce sold in Al-Rass City, Saudi Arabia. A total of 10 samples were randomly collected from local markets and analyzed for bacterial contamination. Coliform bacteria were detected in only three samples using the Petrifilm™ method. The total bacterial count, determined through plate count analysis, ranged from 190 CFU/g to an uncountable level. Further identification using the MicroScan system confirmed the presence of *Providencia rettgeri* and *Citrobacter gillenii* in two samples. Antibiotic susceptibility testing revealed that *P. rettgeri* exhibited resistance to 8 out of 27 tested antibiotics, whereas *C. gillenii* was sensitive to all 27 antibiotics. These findings highlight the potential microbial risks associated with fresh lettuce and emphasize the importance of proper washing before consumption to minimize health hazards.

Keywords: *Lactuca sativa*; Vegetables; Food contamination; Bacteria; Health

1. Introduction

Plants have been indispensable to human survival since the dawn of civilization, serving as a critical source of sustenance and medicinal resources, as documented by fossil evidence tracing back to the earliest emergence of humankind (1, 2). Including fresh fruits and vegetables in the daily diet is crucial for maintaining good health. Furthermore, plant-derived natural products have long formed the fundamental basis for primary healthcare systems and therapeutic practices, many of which have become integral to the modern pharmaceutical drug development (3). Vegetables play a crucial role in maintaining and promoting good health, as they are essential for sustaining a balanced and nutritious diet. Both the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend a daily intake of a minimum of 400 grams of fruits and vegetables to support overall well-being (4). In recent years, there has been a notable rise in the incidence of foodborne illnesses, resulting in increasing rates of disease, fatalities, and significant economic losses worldwide (5). According to the World Health Organization, infectious diseases remain the second leading cause of death globally (6). Therefore, food products and vegetables should be handled carefully and following proper hygiene practices.

Lettuce (*Lactuca sativa* L.) is one of the most famous leafy vegetable classified under the Cicoreae tribe of the Compositae family. Archaeological evidence, such as depictions in Egyptian tomb paintings, indicates that its cultivation dates back to at least 4500 BC (7). The genus *Lactuca* is documented to comprise between 100 and 111 species, according to taxonomic research. These species are mainly distributed across warm and temperate zones, particularly in the Northern Hemisphere. Within the category of leafy vegetables, *Lactuca sativa* is regarded as the most prominent

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and economically important. It is widely cultivated on a commercial scale in many parts of the world and is primarily grown for consumption as a vegetable (8). Lettuce is among the most globally consumed vegetables, yet its nutritional significance has often been overlooked. It is low in calories, fat, and sodium, while serving as a valuable source of dietary fiber, iron, folate, and vitamin C. Additionally, lettuce contains a variety of bioactive compounds that contribute to its health-promoting properties (9). The nutritional and antioxidant profiles of lettuce vary significantly across different varieties, with distinct variations observed between green and red types. The health advantages of consuming lettuce are largely influenced by its composition, particularly its antioxidant content, which serves as a key bioactive nutrient. These benefits are contingent on the biochemical activity of these compounds once absorbed into the bloodstream. Importantly, some components must first be liberated from the food matrix and may be transformed during digestion before they can deliver their positive effects (10).

The Arabian Peninsula is marked by its arid climate and relatively low biodiversity. Despite these challenging conditions, it sustains a variety of plant life, including numerous species with culinary and medicinal uses that have adapted to thrive in such extreme environments (11). Al-Rass province is situated in the southwestern part of the Qassim region, in the central area of Saudi Arabia. It lies to the east of the Arabian Shield and is approximately 400 kilometres away from Riyadh, the capital city (12). Vegetables are widely cultivated in the Qassim region and Al-Rass province for local consumption. However, to the best of our knowledge, no research study has evaluated the microbiological quality of vegetables sold in the markets of Al-Rass province. Therefore, the current study aims to assess the microbiological quality of lettuce, which is widely sold in vegetable markets in Al-Rass town, Saudi Arabia.

2. Material and methods

2.1. Sample collection and bacterial culturing

In February 2025, 10 lettuce leaf samples were collected in the early morning from various vegetables stores in Al-Rass city. The stores were carefully selected to ensure sample diversity from multiple areas of the city, aiming to represent different sources of lettuce. After collection, the samples were transported to the laboratory, ensuring they were placed in sterile bags to maintain cleanliness and prevent contamination during transit.

Upon arrival at the laboratory, initial microbial analysis of the samples was initiated using sterile cotton swaps to collect from the inner leaves of lettuce (Figure 1). Laboratory cultures were prepared using selective growth media (nutrient agar) to identify the presence of any microorganisms on the lettuce leaves. The samples were examined to detect microbial growth, with a focus on identifying the types of microorganisms. Pure cultures were then prepared from each colony to get pure bacterial cultures. To avoid fungal growth, drops of clotrimazole (antifungal agent) was added to the molten nutrient agar (about 20 microliters to 500 ml nutrient agar). All observations regarding the condition of the samples and the results of the initial analysis were recorded to ensure the accuracy of the findings in subsequent stages of the research.

Figure 1 Collection of bacterial samples from the inner surface of lettuce leaves

2.2. Gram- staining

The Gram-staining procedure was performed according to standard microbiological protocols (13), to differentiate bacterial isolates based on their cell wall characteristics. Initially, a thin smear of each bacterial isolate was prepared on a clean glass slide and heat-fixed to ensure adherence. The slides were then flooded with crystal violet solution (primary stain) and allowed to stand for 60 seconds. Following this, the slides were gently rinsed with distilled water to remove excess stain. Subsequently, Gram's iodine solution (mordant) was applied to the smears and left for 60 seconds to form a crystal violet-iodine complex. The slides were rinsed again with distilled water to remove unbound iodine. Decolorization was carried out using acetone, applied dropwise for 10–15 seconds, until the solvent flowed colorlessly from the slides. The slides were immediately rinsed with distilled water to halt the decolorization process. Finally, the smears were counterstained with safranin solution (secondary stain) for 60 seconds, followed by a final rinse with distilled water. The slides were air-dried and examined under a light microscope using the oil immersion objective (100x). Bacterial isolates were classified as Gram-positive or Gram-negative based on their retention of the primary stain (purple) or uptake of the counterstain (pink/red), respectively. All observations were documented, and representative images were captured for further analysis and reference.

2.3. Total bacterial count

The total bacterial count was determined using the pure-plate method to quantify viable microorganisms present in the samples as reported in literature (14), with some modifications.. Serial dilutions of the sample were prepared in sterile phosphate-buffered saline (PBS) to achieve a concentration range suitable for counting. From each dilution, 1 mL was aseptically transferred into sterile Petri dishes. Approximately 15–20 mL of molten nutrient agar, cooled to 45–50°C, was poured into each Petri dish and gently swirled to ensure uniform mixing. The agar was allowed to solidify at room temperature.

The plates were then inverted and incubated at 37°C for 24–48 hours under aerobic conditions. After incubation, colonies were counted using a colony counter, and only plates containing 30–300 colonies were considered for accurate enumeration. The total bacterial count was expressed as colony-forming units per gram (CFU/g) of the sample, calculated using the formula:

$$Total\ bacterial\ count = \frac{Number\ of\ colonies \times Dilution\ factor}{Volum\ of\ inculum}$$

Appropriate controls, including sterile media and uninoculated plates, were maintained to validate the results.

2.4. Identification of Fecal Bacteria Using Petrifilm™ Test

The presence of fecal bacteria from animal or human sources in the collected samples was assessed using 3M™ Petrifilm™ plates, following the manufacturer's instructions. Briefly, 1 mL of each sample suspension was aseptically applied to the center of the Petrifilm™ plate. The inoculum was spread evenly using a sterile spreader, ensuring uniform distribution. The plates were then incubated at 35 ± 1°C for 24 ± 2 hours. After incubation, the plates were examined for the presence of coliforms. Coliform colonies were identified by the formation of red spots. Colonies were enumerated using a colony counter, and results were expressed as colony-forming units per gram (CFU/g) of the sample (15).

2.5. Bacterial Identification and Antibiotic Susceptibility Profiling

The scientific identification and antibiotic susceptibility profile of fecal bacteria isolated from the tested samples were determined using the MicroScan WalkAway® system, following standardized protocols (16, 17). Pure bacterial colonies were suspended in sterile saline, and the turbidity was adjusted to a 0.5 McFarland standard. The suspension was then inoculated into MicroScan® panels, specifically designed for Gram-negative or Gram-positive bacteria, depending on the initial Gram-staining results. The panels were loaded into the MicroScan WalkAway® instrument, where biochemical substrates and antibiotic dilutions were automatically dispensed. The system incubated the panels at 35 ± 2°C for 18–24 hours. Biochemical reactions were analyzed to identify the bacterial species based on metabolic profiles, while antibiotic susceptibility was determined by measuring minimum inhibitory concentrations (MICs) for a range of antibiotics. Results were interpreted using the instrument's software, which provided the scientific name of the identified bacteria and categorized their susceptibility as susceptible, intermediate, or resistant based on Clinical and Laboratory Standards Institute (CLSI) guidelines.

3. Results

As shown in Table 1, the analysis of 10 lettuce samples revealed notable bacterial contamination profiles. Morphological and Gram stain characterization demonstrated microbial diversity across samples (Figure 2). Gram-positive (G+) bacteria dominated (80% of samples), with cocci and bacilli morphologies observed either singly or in combination. Mixed Gram-positive and Gram-negative (G+/G−) populations were identified in Samples 1 and 6, while Sample 9 uniquely exhibited Gram-negative (G−) bacilli.

Table 1 Microbial Contamination in Lettuce Samples: Analysis of Bacterial Load, Diversity, and Fecal Contamination Indicators

Sample ID	Morphology	Gram Stain Classification	Petrifilm™ Result	Bacterial Count (CFU/g)	Ambient Temperature (°C)	Ambient Humidity (%)
1	Cocci and Bacilli	Mixed (G+/G−)	No coliforms	–	6	56
2	Cocci and Bacilli	Gram-positive (G+)	No coliforms	–	7	53
3	Cocci	Gram-positive (G+)	No coliforms	–	6	54
4	Bacilli	Gram-positive (G+)	No coliforms	–	7	58
5	Bacilli	Gram-positive (G+)	Coliforms (+)	Over detection limit	8	55
6	Cocci	Mixed (G+/G−)	No coliforms	–	6	57
7	Cocci and Bacilli	Gram-positive (G+)	No coliforms	–	7	56
8	Bacilli	Gram-positive (G+)	Coliforms (+)	290	8	54
9	Bacilli	Gram-negative (G−)	No coliforms	–	7	59
10	Cocci and Bacilli	Gram-positive (G+)	Coliforms (+)	190	6	52

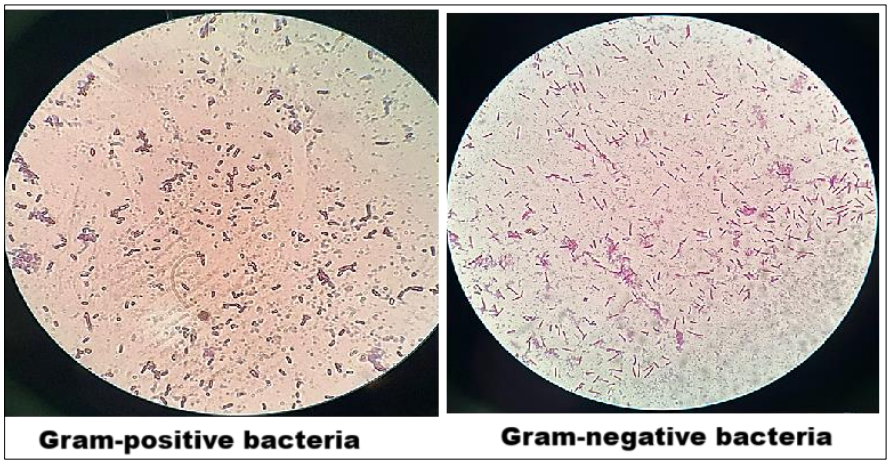

Figure 2 Gram-staining results of bacterial isolates obtained from lettuce leaves

To identify whether the bacteria were coliforms, we conducted the Petrifilm™ test (Figure 3). Coliform bacteria were detected in three samples (Samples 5, 8, and 10), while the remaining seven samples tested negative (no coliform bacteria, but other environmental bacteria). Total coliform bacterial counts, quantified via plate count analysis, exhibited significant variability. Sample 5 exceeded the detection limit, while Samples 8 and 10 yielded 290 CFU/g and 190 CFU/g, respectively. Ambient storage conditions during sampling ranged between 6–8°C and 52–59% humidity,

with no direct correlation between environmental parameters and microbial load or coliform presence. Further identification via the MicroScan system confirmed two clinically relevant species: *Providencia rettgeri* and *Citrobacter gillenii* in Samples 5 and 10, respectively. It is important to mention that *Citrobacter gillenii* and *Providencia rettgeri* are not exclusively fecal bacteria, but they can be associated with the intestinal environment and opportunistic infections. Antibiotic susceptibility testing (Table 2) revealed divergent resistance profiles. *P. rettgeri* demonstrated resistance to 8 of 27 antibiotics, including ampicillin, ceftazidime, and trimethoprim-sulfamethoxazole, indicating multidrug resistance (MDR). In contrast, *C. gillenii* remained susceptible to all tested antibiotics.

Table 2 Fecal bacteria detected in some lettuce samples and their antibiotic profiles

Bacteria	Sensitive to	Resistant to
Citrobacter gillenii	Amikacin,Amoxicillin/Clavulanic Acid,Ampicillin/Sulbactam,Ampicillin,Aztreonam,Ceftazolin,Cefepime,Cefotaxime,Cefotaxime/Clavulanic Acid,Cefoxitin,Ceftazidime,Ceftazidime/Clavulanic Acid,Cefuroxime,Ciprofloxacin,Colistin,Ertapenem,Gentamicin,Imipenem,Levofloxacin,Meropenem,Moxifloxacin,Nitrofurantoin,Norfloxacin,Piperacillin/Tazobactam,Tigecycline,Tobramycin,Trimethoprim/Sulfamethoxazole	None
Providencia rettgeri	Amikacin, Cefepime, Cefotaxime/Clavulanic Acid,Cefoxitin,Ceftazidime,Ceftazidime/Clavulanic Acid,Cefuroxime,Ciprofloxacin,Colistin,Ertapenem,Gentamicin,Imipenem,Levofloxacin,Meropenem,Moxifloxacin,Nitrofurantoin,Norfloxacin,Piperacillin/Tazobactam,Tigecycline,Tobramycin,Trimethoprim/Sulfamethoxazole	Amoxicillin/Clavulanic Acid, Ampicillin/Sulbactam, Ampicillin, Aztreonam, Ceftazolin, Cefotaxime,

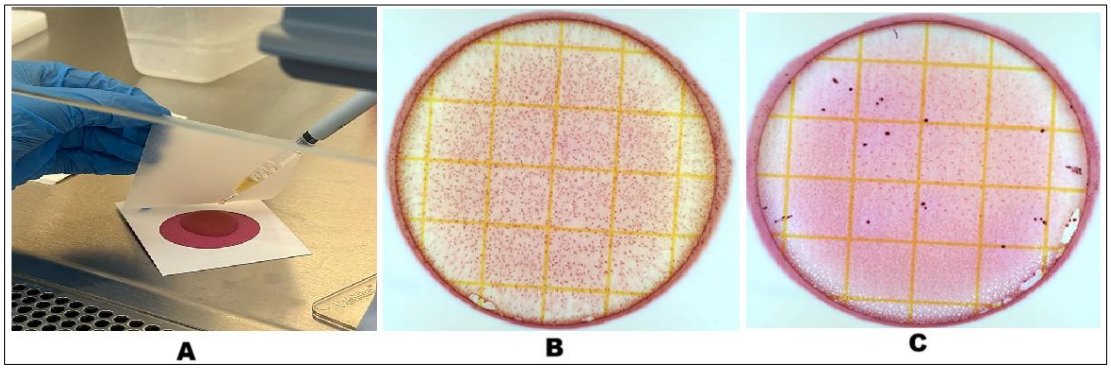

Figure 3 Identification and enumeration of fecal bacteria using the Petrifilm™ Test (A), and bacterial counting (B and C)

4. Discussion

The detection of coliforms in 30% of lettuce samples highlights potential fecal contamination, implicating compromised hygiene during cultivation, handling, or distribution. While coliforms themselves are not universally pathogenic, their presence signals inadequate sanitation practices, aligning with prior studies linking fresh lettuce contamination to irrigation water and post-harvest handling (18-20). Notably, the uncountable bacterial load in Sample 5 suggests either excessive initial contamination or inadequate storage conditions, despite ambient temperatures (6–8°C) typically inhibiting microbial proliferation (21). This discrepancy underscores the possibility of temperature abuse or pre-existing high contamination levels prior to refrigeration. The identification of *P. rettgeri* and *C. gillenii* raises public health concerns. *P. rettgeri*, an opportunistic pathogen associated with urinary tract and bloodstream infections (22, 23), exhibited resistance to critical beta-lactams and sulfonamides. Its MDR profile likely stems from horizontal gene transfer in agricultural environments, potentially linked to antibiotic use in livestock or contaminated water sources (23, 24). Conversely, the susceptibility of *C. gillenii* to all antibiotics suggests a lack of recent antimicrobial exposure, though its presence in food matrices remains concerning due to its role in infections (25).

The predominance of Gram-positive bacteria (e.g., *Bacillus*, *Staphylococcus*) in most samples aligns with their resilience in dry, cool environments. The absence of coliforms in other samples, despite its G− classification, may reflect non-fecal Enterobacteriaceae or limitations in Petrifilm™ selectivity.While storage humidity (52–59%) falls within typical refrigeration ranges, the persistent survival of pathogens like *P. rettgeri* emphasizes the need for stringent cold chain management (26). Previous studies have documented biofilm formation in *Providencia* spp., enhancing resistance to environmental stress (27), which may explain its viability under suboptimal conditions. Large volumes of synthetic or semi-synthetic antibiotics, originating from hospitals and pharmaceutical industry waste, are discharged into wastewater treatment plants. These antibiotics often remain in their active form, accumulating in the environment. They are absorbed by plants and soil microorganisms, ultimately entering ecological cycles and impacting various life forms (28). Finally, this study's sample size (n=10) and geographic specificity limit broader generalizations. Additionally, the focus on two pathogens overlooks potential co-contaminants, such as *Listeria* or *Salmonella*. Future work should integrate metagenomic sequencing to elucidate full microbial profiles and trace resistance gene origins. Longitudinal monitoring of supply chains and antibiotic usage in agriculture is critical to mitigate MDR dissemination.

5. Conclusion

The findings of the current study highlight the critical need for stringent monitoring of the microbiological quality of fresh produce. The detection of multidrug-resistant *Providencia rettgeri* in retail lettuce presents a significant public health concern, particularly for immunocompromised individuals. To mitigate contamination risks, implementing strict hygiene protocols at both the production and post-harvest stages is essential. Additionally, consumers are strongly advised to thoroughly wash lettuce before consumption to minimize potential exposure to harmful bacteria. Future studies with larger sample sizes are recommended to provide a more comprehensive assessment of bacterial contamination patterns and resistance profiles in fresh produce.

Compliance with ethical standards

Acknowledgments

The authors would like to express their gratitude to the research supervisor, the college's deputy for student affairs in Al-Rass, and the supervisor of the university's headquarters in Al-Rass. Additionally, we would like to acknowledge the following individuals for their assistance in analyzing the samples: Mr. Ahmed Bashir from Al-Rass Food Safety Laboratory and Mr. Abdulmohsen Mohammed Althyab from King Saud Hospital.

Disclosure of conflict of interest

There is no conflict of interest.

Clarification

This article is a summarized from the BSc dissertation of the mentioned authors.

Availability of Data and Materials

The data results obtained in this study are available from the corresponding author upon request.

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