

Research Article

Multidrug-Resistant Bacteria In Fresh Produce And Community Samples: Phenotypic Profiling And Probiotic Consortium Screening.

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Running title: Multidrug-resistant pathogen in fresh produce.

Abstract

Bacteria resistant to antibiotics, prevalent in fresh produce and urban microbiomes, constitute an emerging public health exposure hazard. Phenotypic antimicrobial resistance profiles were characterized for culturable isolates derived from organic produce, conventionally farmed produce, and environmental community samples via a disk diffusion assay encompassing multiple antibiotics and a composite resistance index. Evaluation of 34 isolates across nine antibiotics revealed pronounced source-specific variations in resistance prevalence: organic produce isolates exhibited minimal resistance, conventional produce isolates were chiefly multidrug-resistant, and community isolates displayed near-ubiquitous resistance. Four probiotic consortia were subsequently assessed for antagonistic efficacy against these resistant strains using a categorical inhibition metric. A multi-species *Lactobacillus* and *Bifidobacterium* consortium exhibited the broadest inhibitory spectrum, whereas consortia with reduced *Lactobacillus/Bifidobacterium* diversity or a *Bacillus*-dominant formulation demonstrated constrained or substrate-dependent activity. These observations emphasize source-attributable enrichment of MDR traits and endorse multi-strain probiotic formulations as viable candidates for advanced validation in quantitative bioassays and produce-surface simulations.

Keywords: Microbes, Microflora, Pathogens, Antibiotics, Multi-Drug Resistance (MDR), Extended-Spectrum Beta-Lactamase (ESBL), Pesticide-Treated, Organic Farming, Probiotics, Phage Therapy, Antibiotic Stewardship, Predatory Bacteria.

INTRODUCTION

Microorganisms, encompassing bacteria, fungi, viruses, protozoa, and algae, are prevalent globally. These microflora communities are generally avirulent. However, pathogenic microbes have contaminated food systems, particularly fresh produce, leading to significant outbreaks of foodborne illnesses. Key pathogens of concern include *Salmonella*, associated with contamination in tomatoes, seed sprouts, and spices, and *Escherichia coli*, frequently found in leafy greens like spinach and lettuce. (Carstens et al., 2019) These pathogens can infect produce at any point in the supply chain, from cultivation and harvesting to processing, storage, and distribution. The risk is amplified when produce is consumed raw, such as in salads or as uncooked fruits and vegetables.

The increasing prevalence of multidrug-resistant strains among these foodborne pathogens poses a critical public health challenge, complicating treatment and elevating the risk of severe disease outcomes (Helmy et al., 2023). The widespread, persistent presence of antibiotic-resistant bacteria and their associated genes in the environment, largely due to the excessive and inappropriate use of human and veterinary antibiotics, exacerbates this global concern (Sarailoo et al., 2024; Sharma et al., 2024; Zhang et al., 2022). Gracie's Kitchens has initiated a recall of 150 fruit and vegetable products due to potential contamination with *Listeria monocytogenes*. This bacterium can cause severe illness, particularly in vulnerable populations such as the elderly, young children, and immunocompromised individuals (Yan et al., 2025). Highlighting the severity of this

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pathogen, *Listeria* is responsible for an estimated 1,600 infections and 260 deaths annually in the United States (Self et al., 2019; Sharma et al., 2025). Beyond *Listeria*, a notable incident involved a multi-state outbreak of *E. coli* O157:H7 infection linked to recalled yellow onions from Taylor Farms, which were distributed to McDonald's restaurants in several states. This outbreak affected 104 individuals across 14 states, leading to 34 hospitalizations and one fatality. While the specific *E. coli* strain was not isolated from onion or environmental samples, the recalled onions were identified as the probable source (Mitchell et al., 2024; Racine et al., 2024). Similarly, a multi-state outbreak of *Salmonella* *Africana* and *Salmonella* *Braenderup* was associated with Bedner Growers, Inc. and Thomas Produce Company in Florida. (Marshall et al., 2020; Mitchell et al., 2024) Although both farms had concluded their growing seasons and no product was actively on the market, *Salmonella* strains matching those in the outbreak were detected in water samples from both locations. This outbreak resulted in 155 hospitalizations without any reported deaths. Major outbreak events are further detailed in **Table 1**. These instances underscore the persistent threat posed by foodborne pathogens, particularly those exhibiting antimicrobial resistance, which can lead to widespread public health crises and economic burdens (Iwu & Okoh, 2019) (Rahman et al., 2021).

Table 1. Major outbreak events in food products. Numerous studies highlight a significant global burden of foodborne diseases linked to fresh produce.

S.No.	Outbreak Event / Produce / Place	Causal Agent (MDR Bacteria)	Reference
1	Bagged spinach outbreak, USA (2006)	<i>Escherichia coli</i> O157:H7 (antibiotic-resistant strain)	CDC, 2006; FDA Foodborne Outbreak Reports
2	Raw sprouts contamination, Europe (2011)	<i>E. coli</i> O104:H4 (extended-spectrum β -lactamase producer, ESBL)	European Food Safety Authority (EFSA), 2011
3	Leafy greens, California farms (2018–2020)	<i>E. coli</i> O157 and <i>Salmonella enterica</i> (multi-drug resistant isolates)	CDC FoodNet Surveillance Reports
4	Poultry supply chain, South Asia (2019)	<i>Campylobacter jejuni</i> (fluoroquinolone-resistant)	WHO Global Antimicrobial Resistance Surveillance System (GLASS), 2019
5	Street food samples, India (2020)	<i>Staphylococcus aureus</i> (methicillin-resistant, MRSA)	Journal of Food Protection, 2020
6	Urban wastewater and market vegetables, Africa (2021)	<i>Klebsiella pneumoniae</i> (carbapenem-resistant, CRE)	Frontiers in Microbiology, 2021
7	Ready-to-eat salads, UK supermarkets (2022)	<i>Listeria monocytogenes</i> (multidrug-resistant strain)	Food Standards Agency (FSA), 2022
8	Community gyms and schools, USA (2023)	<i>Staphylococcus aureus</i> (community-acquired MRSA, CA-MRSA)	CDC Emerging Infectious Diseases Journal, 2023
9	Organic vs. conventional produce comparison, global meta-analysis (2024)	Multiple MDR bacteria (<i>E. coli</i> , <i>Enterobacter</i> , <i>Salmonella</i>)	International Journal of Food Microbiology, 2024

When considering food safety, pesticide-treated fruits and vegetables present several harmful health effects on the human body (Benbrook et al., 2021). Organic farming avoids synthetic pesticides and relies on more natural methods for fruit and vegetable treatment, reducing chemical residues in pesticide-treated produce (Benbrook et al., 2021; Mie et al., 2016). However, while organic farming reduces chemical residues, it is not immune to contamination by harmful microorganisms such as multidrug-resistant bacteria, even with the use of natural fertilizers (Kiplimo et al., 2025). The presence of MDR bacteria in produce, including organic varieties, poses a significant public health risk as these resistant strains can cause infections that are difficult to treat with conventional antibiotics (Iwu & Okoh, 2019; Rahman et al., 2021). For instance, a 2023 study demonstrated MDR bacteria in fresh fruits in low-cost Ecuadorian markets, highlighting the potential for these pathogens to enter the food chain regardless of farming practices. It is therefore necessary to develop strategies to ensure food security and safety by reducing bacterial contamination in both farm and retail markets and to prevent or reduce the spread of antibiotic-resistant infections within the population. The presence of antibiotic-resistant bacteria and antibiotic resistance genes in fresh produce is increasingly reported as a cause of foodborne disease outbreaks, necessitating comprehensive intervention strategies (Rahman et al., 2021). (Kiplimo et al., 2025; Wu-Wu et al., 2023) Studies indicating a significant risk of outbreaks further amplify this heightened concern a significant risk of outbreaks stemming from antimicrobial resistance and multidrug-resistant pathogens in contaminated fresh produce, underscoring the urgent need to elucidate the mechanisms of farm-level contamination (Glaize et al., 2019).

Foodborne illnesses represent a significant public health challenge, with an estimated 48 million individuals experiencing such diseases annually (Mather et al., 2024). These illnesses are also widely documented as transmissible (Vourc'h et al., 2022).

Compounding this issue, several of these bacteria exhibit resistance to common medications (Farrukh et al., 2025). Future pandemics may not solely be caused by viruses like Covid, but rather by dangerous pathogens such as methicillin-resistant *Staphylococcus aureus* (Carrel et al., 2024). Treating antibiotic-resistant bacteria is significantly more difficult and would likely lead to elevated mortality rates, expensive medical treatments, and increased hospitalization (Baltazar & Campos, 2025). Therefore, understanding which bacteria are commonly resistant to antibiotics and their origins is crucial.

Antibiotics are vital medications that inhibit the growth of microorganisms, effectively preventing bacterial infections. The mechanisms of commonly used antibiotics are detailed in **Table 2**. However, bacterial pathogens, such as *Escherichia coli*, can develop resistance to antibiotics and transfer this resistance to other bacteria, thereby diminishing the effectiveness of standard treatments for common bacterial infections [2]. Of particular concern are certain strains of *E. coli* that have become multidrug-resistant (MDR), exhibiting resistance to multiple classes of antibiotics. The global rise of MDR microorganisms poses a significant threat to public health, rendering many infections untreatable with conventional therapies (Ahmed et al., 2024). These pathogens, including MDR *E. coli*, have developed resistance to critical antibiotics, such as cephalosporins and carbapenems, often through the production of extended-spectrum beta-lactamases (ESBL).

Table 2. Detailed Mechanisms of Commonly Used Antibiotics.

Antibiotic	Mechanism of Action	Commonly Treated Infections / Medical Conditions
Ampicillin	Attaches to penicillin-binding proteins (PBPs) and inhibits bacterial cell wall synthesis; also triggers autolysis leading to bacterial self-destruction.	Respiratory tract infections, urinary tract infections (UTIs), meningitis, and gastrointestinal infections.
Gentamicin	Binds irreversibly to the 30S ribosomal subunit, inhibiting protein synthesis and causing misreading of mRNA in Gram-negative bacteria.	Severe systemic infections, sepsis, pneumonia, and infections caused by <i>Pseudomonas</i> and <i>Enterobacteriaceae</i> .
Ciprofloxacin	Inhibits bacterial DNA gyrase and topoisomerase IV, blocking DNA replication and transcription.	Urinary tract infections, gastrointestinal infections, anthrax exposure, and respiratory tract infections.
Tetracycline	Binds to the 30S ribosomal subunit, blocking attachment of aminoacyl-tRNA to the ribosome, halting protein synthesis.	Acne, respiratory infections, tick-borne diseases (e.g., Lyme disease), and some sexually transmitted infections (STIs).
Erythromycin	Binds to the 50S ribosomal subunit, blocking translocation during protein synthesis.	Respiratory tract infections, skin infections, and alternative therapy for penicillin-allergic patients.
Methicillin	A β -lactam antibiotic that inhibits cell wall synthesis by binding to PBPs, preventing cross-linking of peptidoglycan.	Historically used for <i>Staphylococcus aureus</i> infections; now replaced by other β -lactams due to MRSA resistance.
Imipenem	A carbapenem that binds to PBPs and inhibits cell wall synthesis, resistant to most and respiratory infections; hospital- β -lactamases.	Complicated intra-abdominal, urinary, acquired infections.
Vancomycin	Binds to D-Ala-D-Ala termini of peptidoglycan precursors, blocking cross-linking and weakening the cell wall.	MRSA infections, endocarditis, bloodstream infections, and <i>Clostridioides difficile</i> colitis.
Chloramphenicol	Binds to the 50S ribosomal subunit and inhibits peptidyl transferase activity, blocking peptide bond formation.	Typhoid fever, meningitis, and certain eye infections (topical use).

The development of antibiotic resistance arises from multiple mechanisms, including natural selection, genetic mutations, and the acquisition of resistance-conferring genes. Bacteria can acquire resistance through horizontal gene transfer, which involves the direct exchange of genetic material between cells (Elshobary et al., 2025; Schindel, 2013; Urban-Chmiel et al., 2022). Due to their prokaryotic nature, bacteria have a remarkable ability to share resistance genes, enabling rapid dissemination across populations. These horizontally acquired genes often confer resistance to specific antimicrobial agents. A key example is efflux pumps, which actively expel antibiotics from the bacterial cell, preventing the buildup of therapeutic concentrations (Lomovskaya & Watkins, 2001; Nishino et al., 2021). This mechanism typically targets particular antibiotics, allowing resistant strains to survive and multiply during treatment. Highly resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Mycobacterium tuberculosis*, exemplify the severe challenges of antibiotic resistance (Halawa et al., 2024). MRSA is a leading cause of healthcare-associated infections, while MDR-TB represents a major global health threat due to resistance to multiple first-line antituberculosis drugs (Karnan et al., 2024). Bacteria producing extended-spectrum beta-lactamases further complicate treatment, enzymes that degrade key antibiotics like cephalosporins and penicillins, rendering

them ineffective (Alen et al., 2023; Shaikh et al., 2014). As a result, ESBL-producing infections are difficult to treat and often lead to poor outcomes. **Table 3** outlines additional key mechanisms of antibiotic resistance.

Table 3. Detailed Summary of Major Antibiotics, Their Mechanisms of Action, and Commonly Treated Infections.

S. No.	Resistance Mechanism	Affected Antibiotics	References
1	Enzymatic degradation of antibiotics — bacteria produce enzymes that inactivate or hydrolyze Aminoglycosides (e.g., Gentamicin). antibiotics.	β -lactam antibiotics (e.g., Penicillin, Ampicillin, Cephalosporins);	β -lactam antibiotics (e.g., Penicillin, Ampicillin, Cephalosporins); Rev., 2010
2	Target site modification — alteration of antibiotic binding sites, reducing drug affinity.	Macrolides (Erythromycin), Fluoroquinolones (Ciprofloxacin), β -lactams (Methicillin).	Blair et al., Nat. Rev. Microbiol., 2015
3	Efflux pump activation — increased expression of membrane transporters that expel antibiotics.	Tetracyclines, Fluoroquinolones, Macrolides, Chloramphenicol.	Li & Nikaido, Drugs, 2009
4	Reduced membrane permeability — changes in porin proteins or membrane structure limiting antibiotic entry.	β -lactams (Carbapenems), Aminoglycosides.	Delcour, Biochim. Biophys. Acta, 2009
5	Bypass of metabolic pathways — bacteria develop alternative pathways to avoid the antibiotic target.	Sulfonamides, Trimethoprim.	Sköld, Int. J. Antimicrob. Agents, 2000
6	Biofilm formation — bacterial communities encased in protective matrices that block antibiotic penetration.	Broad range: β -lactams, Fluoroquinolones, Aminoglycosides.	Hall & Mah, Nat. Rev. Microbiol., 2017
7	Horizontal gene transfer (HGT) — Multiple classes acquisition of resistance genes via plasmids, transposons, or bacteriophages.	Carbapenems, Macrolides, Tetracyclines, Aminoglycosides.	Von Wintersdorff et al., Front. Microbiol., 2016

Resistant bacteria spread via various routes, including person-to-person contact, airborne transmission, and environmental contamination. In food safety contexts, these bacteria can contaminate products throughout production and handling, posing substantial public health risks (Bava et al., 2024; Samtiya et al., 2022).

The rising prevalence of antibiotic resistance highlights the need to understand its mechanisms, curb its spread, and develop effective interventions. Essential strategies to combat this crisis and preserve antibiotic efficacy include enhanced surveillance, rigorous hygiene, and innovative approaches (Elshobary et al., 2025; Mmbando et al., 2025; Singha et al., 2024). These include antibiotic stewardship programs (Elshobary et al., 2025), phage therapy (Singha et al., 2024), probiotics to reshape microbial communities, predatory bacteria, combination antibiotic therapies to boost effectiveness and hinder resistance, and real-time monitoring of multidrug resistance. This multifaceted approach is critical to address the complex challenge of antibiotic resistance, which stems from various bacterial mechanisms including enzymatic modification, reduced binding affinity, changes in cell permeability, and the overexpression of efflux pumps (Elshobary et al., 2025). (Darby et al., 2022; Elshobary et al., 2025) This study comprehensively evaluated the prevalence of multidrug-resistant bacteria in local community environments and agricultural products, confirming substantial contamination risks. The study collected bacterial samples from diverse sources, including public schools,

organic produce, and conventionally grown produce, followed by rigorous testing and analysis that definitively characterized their antimicrobial and MDR profiles. The study successfully identified agricultural products with high levels of resistant bacteria, demonstrated the superior efficacy of probiotics over specific antibiotics against these strains, and validated practical strategies using readily available tools to effectively curb resistance dissemination. Moreover, artificial intelligence-based methodologies robustly monitor resistance patterns, enhance clinical interventions, and optimize preventive measures (Lastra et al., 2024; Saeed et al., 2023). These findings directly inform and enable targeted interventions to manage MDR bacteria in community and agricultural settings.

MATERIALS AND METHODS

All reagents were purchased from Amazon unless otherwise specified.

Sample Collection and pathogen isolation

Samples for this research were collected from a community garden, various grocery stores, and two different high schools. A total of 41 samples were tested and analyzed, specifically from strawberries, apples, bell peppers, spinach, coriander, lettuce, and commonly used door handles in the two schools. These samples encompassed bacteria found in some of the most contaminated leafy vegetables and fruits. Additionally,

the samples from regularly used high school door handles represented the general bacterial community of St. Louis, MO, U.S.

To collect these samples, sterile cotton swabs were used to thoroughly collect bacteria from the sources. The swabs were then placed into individual tubes, each containing 2 ml of nutrient broth. Subsequently, the samples were stored at room temperature for 72 hours to allow for bacterial growth. Following incubation, to determine which bacterial samples were antibiotic-resistant, petri dishes containing antibiotics and control petri dishes were prepared. The agar mixture was created by mixing 12.5 grams of Luria-Bertani Broth Miller, 6.25 grams of agar, and 500 ml of water, then heated to 121°C. After the agar cooled, it was poured into sterile petri dishes.

Antibiotic resistance test

Antibiotics (gentamicin and ampicillin) were incorporated into the samples. These antibiotics serve to inhibit bacterial growth. Bacterial cultures were grown in Luria-Bertani broth, LB agar, and plain LB medium. Samples were collected from a diverse range of commonly consumed fruits and vegetables, as well as environmental surfaces. Variations in bacterial growth patterns were observed, with some isolates exhibiting robust growth and others demonstrating reduced or delayed proliferation. Bacterial isolation was performed using antibiotic-supplemented agar plates (e.g., those containing ampicillin) to selectively culture and isolate resistant bacterial strains from the samples. This process aimed to identify the specific antibiotics against which the bacteria exhibited growth or attenuated proliferation.

The Antibiotic Resistance Diffusion Disc Method was employed; wherein antibiotic discs assess antimicrobial susceptibility. The presence of a clear "zone of inhibition" around a disc indicates that the bacteria are susceptible to that antibiotic, preventing their growth. Conversely, the absence of such a clear zone signifies bacterial resistance to the particular antibiotic. Each zone of inhibition was measured meticulously to quantify the degree of bacterial susceptibility or resistance, thereby providing a comprehensive antibiotic profile for each isolate (Schindel, 2013) (Angamarca et al., 2023) (Kumar et al., 2020).

Probiotic Culture and screening for inhibition of antibiotic-resistant bacteria

Commercially available probiotic consortia, including various *Lactobacillus* strains procured from commercial pharmacies, were cultured on MRS agar and screened for antagonistic activity against MDR bacterial isolates recovered from organic and conventional produce samples. Resistance was first confirmed via antibiotic disc diffusion assays measuring zones of inhibition (Angamarca et al., 2023; Kumar et al., 2020; Schindel, 2013), with resistant targets then challenged by co-culturing probiotics and pathogens via streaking on agar plates, followed by incubation to assess inhibition zones. Growth suppression was categorized using a rubric (I, II, or NI) and converted to quantitative scores (I = 1, II = 0.5, NI = 0) for comparative analysis across produce types and consortia, and summarized as inhibition events per consortium.

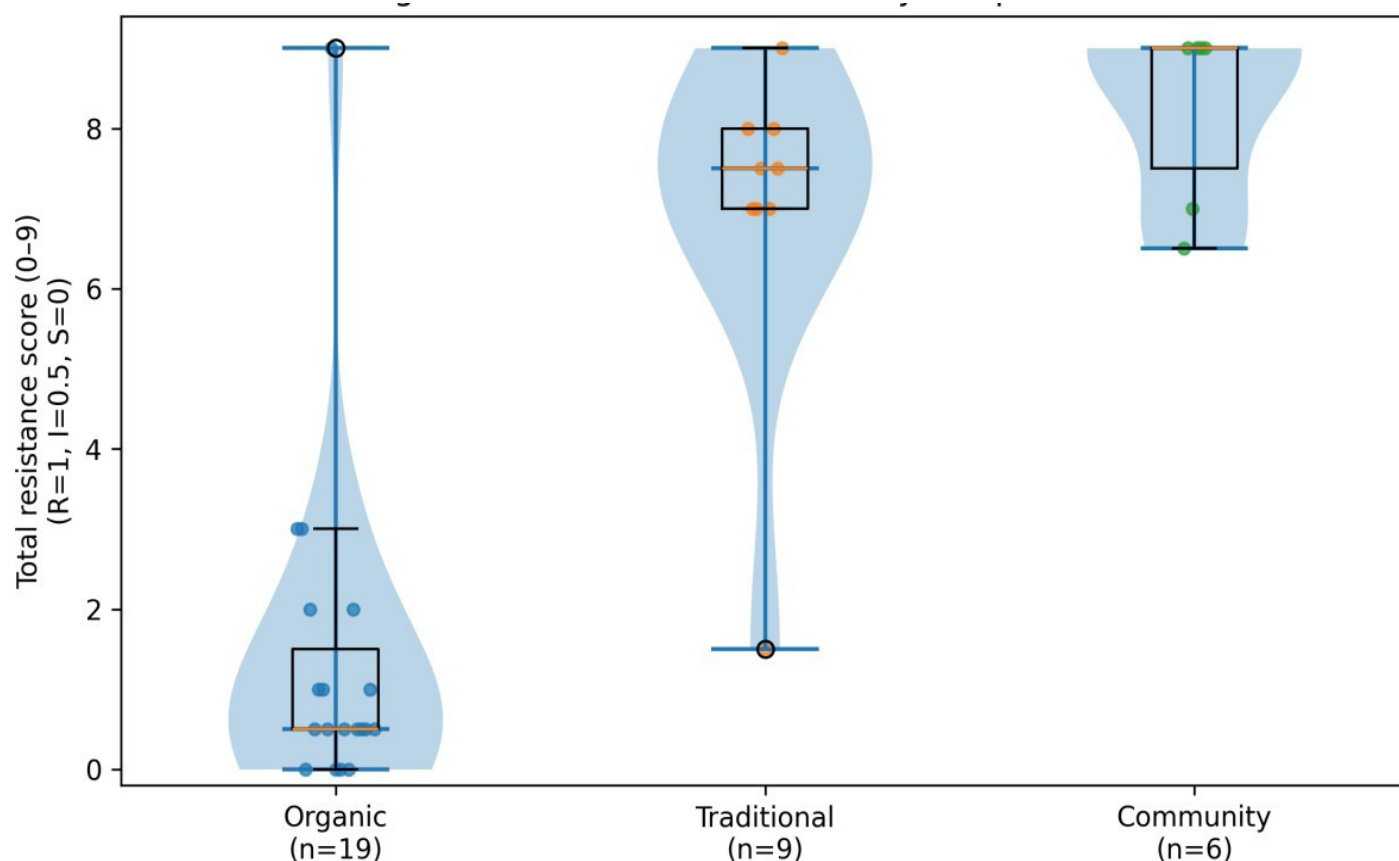
RESULT

Across 34 isolate-level susceptibility profiles spanning nine antibiotics (chloramphenicol, neomycin, penicillin, kanamycin, novobiocin, streptomycin, erythromycin, tetracycline, and ampicillin), resistance patterns were strongly dependent on sample source (organic produce, traditional/conventional produce, and "community site"). Employing the scoring scheme depicted in the table ($R = 1$, $I = 0.5$, $S = 0$) to derive a composite "total resistance" score per isolate, isolates from organic produce demonstrated low overall resistance (mean = 1.34, median = 0.5, $n = 19$), while those from traditional produce exhibited predominantly multidrug resistance (mean = 6.94, median = 7.5, $n = 9$). Community site isolates displayed near-universal resistance across the antibiotic panel (mean = 8.42, median = 9, $n = 6$), with most profiles showing "R" in nearly every category. Applying an operational MDR threshold of resistance to ≥ 3 antibiotic classes yielded prevalences of 15.8% in organic isolates, 88.9% in traditional isolates, and 100% in community isolates. Notably, the organic dataset encompassed both fully susceptible isolates and sporadic instances of elevated resistance, highlighting intra-group heterogeneity (**Table 4 and Figure 1**).

Table 4. Sample details from various sources and the antibiotic disc diffusion test results.

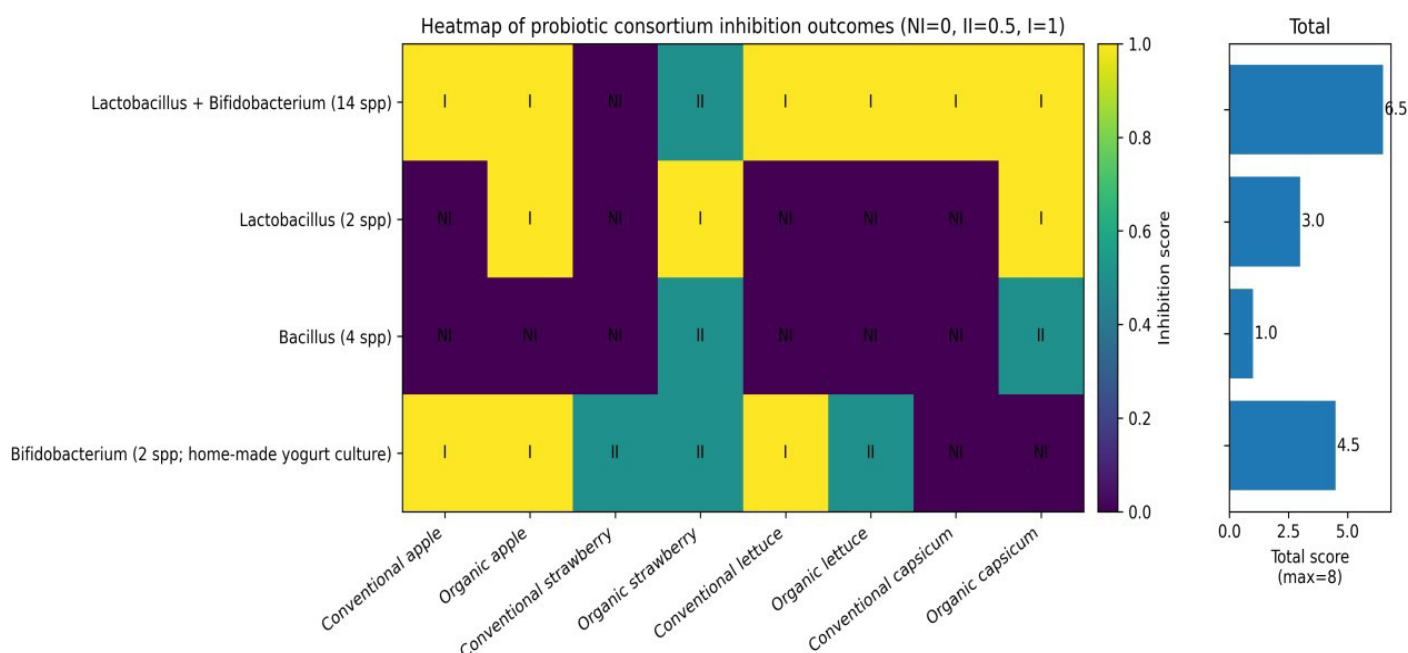
Sample	Chloromphenicol	Neomycin	Penicillin	Kanamycin	Novobiocin	Streptomycin	Erythromycin	Tetracycline	Ampicillin	Total Resistance
organic Capsicum	R	S	S	S	S	S	R	R	S	3
organic Capsicum	S	R	S	R	S	S	S	R	S	3
organic Capsicum	S	S	S	S	S	S	S	S	S	0
organic strawberry	S	R	R	S	S	S	S	S	S	2
organic Lettuce	S	S	S	I	S	S	S	S	S	0.5
organic Apple	S	S	I	I	S	S	S	S	S	1
organic Apple	S	S	S	S	S	R	S	S	S	1
Community place	R	S	R	R	R	S	R	I	R	6.5
Community place	R	R	R	R	R	R	R	R	R	9
organic Capsicum	S	S	S	S	S	S	S	I	S	0.5
organic strawberry	R	R	R	R	R	R	R	R	R	9
Traditional apple	R	R	R	I	R	R	R	I	R	8
Traditional apple	R	S	R	R	R	R	R	S	R	7
organic Apple	S	S	S	S	S	S	S	S	S	0
Traditional Capsicum	I	R	R	R	I	R	R	S	R	7
Traditional Lettuce	I	R	I	R	R	R	R	I	R	7.5
organic Lettuce	S	S	S	S	S	S	S	S	S	0
organic Lettuce	S	S	S	S	S	I	S	S	S	0.5
organic Capsicum	S	S	S	S	S	S	S	S	S	0
organic strawberry	I	S	S	R	S	S	I	S	S	2
organic spinach	S	S	S	S	S	I	S	S	S	0.5
Community place	I	R	R	R	R	R	R	I	R	7
Community place	R	R	R	R	R	R	R	R	R	9
Traditional Spinach	R	S	I	S	S	S	S	S	S	1.5
Traditional apple	I	I	I	R	R	R	R	I	R	7
organic Apple	S	S	S	S	I	S	S	S	S	0.5
organic strawberry	S	S	I	S	S	S	S	S	S	0.5
organic Capsicum	S	S	I	S	S	S	I	S	S	1
organic Lettuce	S	S	S	S	S	S	I	S	S	0.5
Traditional apple	S	R	R	R	R	R	R	R	R	8
Traditional Spinach	R	R	R	R	R	I	R	S	R	7.5
Traditional stawberry	R	R	R	R	R	R	R	R	R	9
Community place	R	R	R	R	R	R	R	R	R	9
Community place	R	R	R	R	R	R	R	R	R	9

Figure 1. (violin + box plot with individual data points) for total resistance from nine different antibiotics by source and data derived from Table 1



When aggregating data across all samples, kanamycin and erythromycin exhibited the highest weighted resistance burdens (weighted resistance fraction ≈ 0.515 each), followed by penicillin (≈ 0.50) and streptomycin (≈ 0.456). Tetracycline displayed the lowest overall resistance (weighted fraction ≈ 0.353) among the antibiotics evaluated (**Figure 1**). Stratification by sample source indicated that this global resistance profile was primarily propelled by isolates from traditional produce and community sites, whereas organic isolates were largely susceptible across the panel, with only infrequent resistant or intermediate outcomes. A minor discrepancy was noted in one "community site" entry: comprising two intermediates and seven resistant isolates, it should sum to 8.0 per the scoring scheme ($R=1, I=0.5, S=0$), yet the table lists 7.0. This likely reflects a transcription, rounding, or scoring anomaly that warrants correction during manuscript revision to uphold reproducibility. Further rigorous data validation and re-evaluation of the scoring rubric will be undertaken to ensure the utmost accuracy and consistency in the reported resistance profiles. A semi-quantitative antagonism screen was performed using four probiotic consortia against bacterial isolates recovered from eight produce categories. Inhibition outcomes were categorically scored as strong inhibition (I), intermediate inhibition, or no inhibition.

The *Lactobacillus* + *Bifidobacterium* consortium showed the broadest activity, producing I in 7/8 conditions and only failing to inhibit isolates from conventional strawberry (with intermediate inhibition against organic strawberry, suggesting partial but incomplete suppression in that matrix). By contrast, the *Lactobacillus* consortium displayed more selective activity: it inhibited isolates from organic apple, organic strawberry, and organic capsicum, but failed against those from conventional apple, conventional strawberry, conventional lettuce, organic lettuce, and conventional capsicum indicating a narrower spectrum or reduced competitiveness under several isolate backgrounds. (**Figure 2**)

Figure 2. Clustered heatmap of inhibition scores by different commercially available probiotic consortium.

The *Bacillus* consortium exhibited the weakest overall performance, showing NI across 6/8 produce categories and only intermediate inhibition against isolates from organic strawberry and organic capsicum, which highlights its limited antagonistic potential in this screen. Meanwhile, the *Bifidobacterium* consortium demonstrated strong inhibition against both apple categories and conventional lettuce, intermediate inhibition against both strawberry categories and organic lettuce, but no inhibition against both capsicum categories revealing a produce-type-dependent profile distinct from the *Lactobacillus*-dominant formulations.

Overall, these data suggest that probiotic consortia efficacy in inhibiting bacterial growth depends heavily on both the specific strains employed and the environmental context of the bacterial isolate, underscoring the challenges in developing universal probiotic interventions.

DISCUSSION

The present study investigated antibiotic and multidrug resistance phenotypes in bacterial isolates from organic, traditionally grown produce, and community-sourced environmental samples. These data reveal marked enrichment of MDR in isolates from traditional produce and community sites compared to organic produce. Near-pan-resistant community profiles suggest environments harboring broad intrinsic resistance, repeated antimicrobial selection, or cross-contamination amplifying resistant strains. In contrast, organic isolates predominantly showed full susceptibility or minimal resistance, though occasional high-resistance cases indicate that organic status alone does not preclude exposure risks from point-source events.

Mechanistically, elevated β -lactam and aminoglycoside resistance in high-resistance groups aligns with common environmental bacterial mechanisms like enzyme inactivation, efflux, and permeability changes, while strong erythromycin resistance reflects widespread macrolide determinants across Gram-positive and -negative taxa. Lower tetracycline resistance may stem from underrepresentation of resistant taxa or phenotypic assay limits for borderline cases in mixed communities. These phenotypic data capture only culturable fractions under specific conditions—not the full microbiome—potentially underestimating the resistance reservoir, as unculturable components harbor diverse mechanisms (Rodríguez et al., 2006).

These findings justify probiotic screening strain selection: traditional and community isolates form a stringent panel, while organic isolates serve as low-resistance comparators to discern broad- from resistance-specific inhibition. This manuscript specifies unique isolates advanced from each category and any enrichment of resistant profiles. Absent standardized breakpoints for environmental bacteria, interpretations relied on clinical thresholds for *E. coli* and *S. aureus* as proxies (Mafiz et al., 2021; VOİDAROU et al., 2009). Finally, several limitations should be acknowledged: (1) the dataset appears to represent a modest number of isolates per produce type and category (with some categories having $n=1$ entry), limiting statistical power; (2) intermediate calls were incorporated as 0.5 by convention, but interpretation thresholds may vary by organism and guideline, and (3) “community place” is operationally broad—defining the sampling context (surface type, handling, and environmental conditions) will strengthen causal interpretation. Addressing these points will make the resistance dataset a strong

foundation for the subsequent probiotic efficacy results and the overall argument that targeted biocontrol strategies may be necessary to reduce the MDR load associated with certain produce-handling environments.

Furthermore, future research could benefit from integrating metagenomic approaches to provide a more holistic understanding of the resistome, including non-culturable bacteria and mobile genetic elements, which are crucial for the dissemination of antibiotic resistance genes within urban agricultural systems (Harrelson et al., 2025). Such molecular characterization would allow the identification of specific genetic mechanisms underlying resistance and elucidate potential horizontal gene transfer events (Abraham et al., 2025). Probiotics are live microorganisms that have beneficial effects on the body and improve or restore the gut microbiota. Probiotic bacteria have the ability to influence particular immune functions and immunological homeostasis by interacting with and stimulating commensal microflora and intestinal immune cells (Mazziotta et al., 2023). Probiotics have the potential to improve gut microflora by encouraging the growth of beneficial bacteria and suppressing the growth of harmful pathogens, such as MDR bacteria (Wieërs et al., 2021). Wieërs et al., 2021 suggested that patients treated with probiotics upon admission to the hospital had fewer MDR pathogens like *Pseudomonas aeruginosa* in the digestive tract.

Broad-spectrum vs selective inhibition

The most prominent finding is the superior breadth of Consortium 1 (*Lactobacillus* + *Bifidobacterium* multi-species), which inhibited isolates from nearly all produce categories. Broad-spectrum activity in multi-strain *Lactobacilli*/*bifidobacteria* blends is biologically plausible because such consortia can deploy multiple inhibitory mechanisms simultaneously, including organic acidification, competitive nutrient depletion, bacteriocin-like antimicrobials, and quorum-interference effects mechanisms that often vary by strain and collectively increase coverage against heterogeneous target isolates. In contrast, the narrower profile of Consortium 2 (two *Lactobacillus spp.*) suggests either (i) reduced mechanistic diversity, (ii) insufficient inhibitory metabolite production under the assay conditions, or (iii) mismatch between probiotic strains and the dominant isolate taxa recovered from certain produce types (notably lettuce and conventional samples).

Produce-type dependence and matrix effects

Across consortia, strawberry- and capsicum-derived isolates appeared more difficult to inhibit, reflected by frequent NI outcomes and shifts from I → II/NI depending on consortium. Strawberries can carry acid-tolerant and stress-adapted microbes (Lyu et al., 2025); isolates adapted to low pH and high sugar microenvironments may resist inhibition by lactic acid bacteria because acidification provides less incremental

stress. Capsicum surfaces (waxy cuticle, hydrophobic micro-niches (Zeisler-Diehl et al., 2018), and diverse epiphytic communities) may favor isolates with strong adhesion/biofilm potential and stress tolerance (Mamphogoro et al., 2020), reducing the apparent effectiveness of probiotics in agar-based antagonism formats. The consistent NI for capsicum with Consortium 4 specifically suggests that the home-made bifidobacterial blend may not generate inhibitory factors effective against capsicum-associated taxa or may not grow/express antagonistic traits optimally under the screening conditions used. Conversely, the heightened susceptibility of lettuce and coriander isolates to inhibition by Consortium 4 may indicate a specific efficacy of its bifidobacterial components against microbial profiles typical of these leafy greens (Fijan, 2016).

Organic vs conventional differences likely reflect isolate composition

Consortium 2 inhibited organic apple/strawberry/capsicum isolates but failed against most conventional counterparts, implying that the recovered isolates from conventional samples may include organisms with higher intrinsic tolerance to acids/bacteriocins, stronger competitive fitness, or different cell-envelope structures (e.g., Gram-negative dominance) that reduce susceptibility to probiotic metabolites. However, this pattern should be interpreted cautiously: “organic versus conventional” is a proxy for multiple upstream variables (handling, wash water, storage, supply chain surfaces), so the observed differences are most parsimoniously explained by differences in isolate identity and resistance traits, rather than farming practice per se. Similarly, antagonistic interactions of lactic acid bacteria isolates have been shown to be strain-dependent, with varying inhibitory effects against different pathogens, including *Enterococcus faecalis* and *Pediococcus pentosaceus* isolates (Ben-Miled et al., 2023; Szutowska & Gwiazdowska, 2020).

The *Bacillus* blend (Consortium 3) was largely inactive (NI in 6/8). This may indicate truly limited antagonism against the tested isolate panel, but it can also be an artifact of screening format: *Bacillus*-based inhibition can be highly dependent on sporulation state, medium composition, oxygenation, incubation time, and induction of lipopeptide/peptide antibiotics. If *Bacillus* strains were not in a metabolically active state (or if assay conditions did not promote antimicrobial production), their apparent inhibition would be underestimated. A targeted follow-up (e.g., longer pre-incubation, different media, cell-free supernatant testing) would clarify whether the weak performance reflects biology or methodology.

Practical implication: rational down-selection for confirmatory testing

From a translational standpoint, the screen supports Consortium 1 as the lead candidate for confirmatory studies because it delivers consistent inhibition across most produce categories, suggesting robustness to isolate heterogeneity. Consortium 4 may be a strong secondary candidate for applications targeting apple/lettuce-associated isolates but would require reformulation or pairing with complementary strains for capsicum coverage. Consortium 2 may be useful in specific contexts (organic-derived isolate panels) but lacks reliability across produce types. Consortium 3 is not favored based on these screening outcomes unless further optimization demonstrates stronger activity under conditions that better reflect *Bacillus* antimicrobial expression. Furthermore, certain combinations of *Lactobacillus* and *Bifidobacterium* strains have demonstrated synergistic inhibitory effects against pathogens like *Salmonella**, highlighting the potential for enhanced efficacy through careful consortia design rather than relying on single-strain applications (Zhang et al., 2021).

CONCLUSION

Overall, phenotypic susceptibility profiling showed a strong source-dependent resistance burden, with isolates from traditional produce and community sampling sites exhibiting markedly higher multidrug resistance than isolates recovered from organic produce. These findings justify prioritizing high-resistance isolates as stringent challenge strains for downstream probiotic-consortium screening and support the need for targeted interventions to reduce the MDR-associated microbial load in produce handling environments. Furthermore, the multi-species *Lactobacillus* + *Bifidobacterium* consortium showed the broadest inhibitory activity across produce-derived isolates, whereas the *Bacillus* consortium exhibited minimal inhibition and the smaller *Lactobacillus*/*Bifidobacterium* blends showed produce-dependent selectivity. These results support prioritizing the multi-species consortium for confirmatory, quantitative testing in produce-surface models to validate robustness and real-world applicability.

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