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Nature's Pharmacy under Siege: Investigating Antibiotic Resistance Pattern in Endophytic Bacteria of Medicinal Plants

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Abstract

Antibiotic resistance poses a significant global health threat, necessitating a thorough understanding of its prevalence in various ecological contexts. Medicinal plants, renowned for their therapeutic properties, host endophytic bacteria that produce bioactive compounds. Understanding antibiotic resistance dynamics in these bacteria is vital for human health and antibiotic efficacy preservation. In this study, we investigated antibiotic resistance profiles in endophytic bacteria from five medicinal plants: Thankuni, Neem, Aparajita, Joba, and Snake plant. We isolated and characterized 113 endophytic bacteria, with varying resistance patterns observed against multiple antibiotics. Notably, 53 strains were multidrug-resistant (MDR), with 14 exhibiting extensive drug resistance (XDR). Thankuni-associated bacteria displayed 44% MDR and 11% XDR, while Neem-associated bacteria showed higher resistance (60% MDR, 13% XDR). Aparajita-associated bacteria had lower resistance (22% MDR, 6% XDR), whereas Joba-associated bacteria exhibited substantial resistance (54% MDR, 14% XDR). Snake plant-associated bacteria showed 7% MDR and 4% XDR. Genus-specific distribution revealed *Bacillus* (47%), *Staphylococcus* (21%), and *Klebsiella* (11%) as major contributors to MDR. Our findings highlight diverse drug resistance patterns among plant-associated bacteria and underscore the complexity of antibiotic resistance dynamics in diverse plant environments. Identification of XDR strains emphasizes the severity of the antibiotic resistance problem, warranting further investigation into contributing factors.

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Keywords

Antibiotic Resistance, Endophytic Bacteria, Medicinal Plants, Drug Resistance

1. Introduction

The emergence of antimicrobial resistance (AMR), whereby bacteria, viruses, fungi, parasites, or other microbes evade the effects of antibiotics, poses a significant global health threat. Despite varied projections, the World Health Organization (WHO) underscores the urgent need to address AMR (10 global health issues to track in 2021), which was responsible for an estimated 1.27 million deaths globally in 2019 [1] and there is a projection that AMR could lead to approximately 10 million deaths annually by 2050 [2]. While debates persist [3] [4], there is consensus among WHO and other stakeholders on the necessity for comprehensive action to combat AMR, as outlined in a number of reports [5] [6] [7].

Antibiotic resistance represents a pressing global health crisis, challenging the efficacy of conventional treatment methods and necessitating novel strategies for containment and management. With the rise of resistance across various ecological niches, understanding its prevalence in diverse environments is paramount for effective mitigation. Medicinal plants have been integral to therapeutic practices since ancient times [8], known to harbor a diverse array of endophytic bacteria [9]. The intricate interplay between medicinal plants and their associated endophytic bacteria underscores the importance of comprehensively understanding antibiotic resistance within this symbiotic relationship. Investigating the antibiotic resistance profiles of endophytic bacteria isolated from medicinal plants offers insights into the resilience and adaptability of microbial communities in response to antibiotic pressure. Such insights are crucial for safeguarding human health and preserving the effectiveness of antibiotics in both medical and agricultural contexts.

Human acquisition of antimicrobial-resistant bacteria (ARB) stems from various origins, encompassing human-to-human transmission, animal interaction, food consumption, and environmental exposure. Despite growing acknowledgment of the environment's role in AMR, uncertainties persist regarding its precise contribution to the emergence and dissemination of ARB [10]. In the years since the discovery of antibiotics, it has become evident that the utilization of these drugs in human medicine, veterinary medicine, and agriculture is associated with the pollution of various environmental components (such as surface water, groundwater, drinking water, municipal sewage, soil, vegetables, and sludge). This contamination has led to an increase in antibiotic resistance and has resulted in adverse ecological impacts [11]. The release of antibiotics into the environment through wastewater discharge, and improper disposal of pharmaceuticals can lead

to the selection pressure for resistant bacteria. Once present in the environment, antibiotic residues can adversely affect organisms across various trophic levels and pose risks to human health through the consumption of contaminated food and water. Additionally, they contribute to the proliferation of resistant bacterial populations and sustain selective pressures that promote the emergence and spread of resistance in different environmental compartments [12]. While numerous studies have explored AMR in diverse environmental samples like poultry litter [13] [14] [15] [16], municipal wastewater [17] [18] [19], and heavy metal-polluted soils [20], limited research has been conducted on the AMR of endophytic bacteria from medicinal plants.

The escalating use of antibiotics has led to soil pollution, with antibiotic residues infiltrating soil through human activities, particularly for soil fertilization [21]. Consequently, soil contamination with antibiotics, along with antibiotic-resistant bacteria (ARB) and genes (ARGs), has become a global challenge. The primary sources of this contamination are the application of manure as fertilizer and the irrigation of soil with treated wastewater [22]. Soil, being the primary reservoir of bacterial endophytes [23], can play a crucial role in the transmission of these bacteria to plants. Bacteria from the soil can enter and colonize the interior of plants as endophytes either early on, through the germination environment known as the spermosphere or later on through the rhizosphere and into the roots of both seedlings and mature plants [24]. Due to the widespread presence of antibiotic-resistant bacteria in soil, endophytic bacteria are also affected, leading to the development of antibiotic resistance.

Antibiotic resistance can arise through either mutations or the acquisition of resistance-conferring genes via horizontal gene transfer (HGT), with the latter recognized as the predominant factor driving the current pandemic of antimicrobial resistance (AMR). Evidence suggests HGT between soil bacteria and pathogens, with the direction of transfer remaining uncertain [25]. Antibiotic resistance genes (ARGs) have the capability to spread horizontally among bacteria through mobile genetic elements (MGEs) [26]. Mechanisms facilitating the horizontal transfer of ARGs encompass transformation, conjugation transfer, transduction, membrane vesicles (MVs), and DNA encapsulated within virus-like particles [27]. Several research investigations have indicated that antibiotic resistance genes (ARGs) present in soil can migrate to both the roots and leaves of plants [28]. This highlights the possibility that the consumption of medicinal plants containing endophytic bacteria could contribute to antimicrobial resistance via horizontal gene transfer. Hence, understanding the antibiotic resistance profile of endophytic bacteria becomes crucial in addressing this complex health challenge.

In this study, we explore the antibiotic resistance profiles of endophytic bacteria sourced from five distinct medicinal plants: Thankuni (*Centella asiatica*), Neem (*Azadirachta indica*), Aparajita (*Clitoria ternatea*), Joba (*Hibiscus rosa-sinensis*), and Snake plant (*Dracaena trifasciata*). By isolating and characte-

rizing 113 endophytic bacteria across these plant species, we aimed to elucidate the prevalence and patterns of multidrug resistance (MDR) and extensively drug-resistant (XDR) strains within the plant-associated microbial communities.

Through a systematic assessment of resistance against various antibiotics, we unveil the diversity and distribution of MDR and XDR strains among the investigated plant-associated bacteria. Furthermore, genus-specific analysis sheds light on the predominant contributors to antibiotic resistance, highlighting the differential susceptibility of bacterial genera to antimicrobial agents.

This research not only provides valuable insights into the complex dynamics of antibiotic resistance in diverse plant environments, but also underscores the urgency of addressing this global health challenge. The identification of XDR strains emphasizes the severity of the antibiotic resistance problem, underscoring the need for further investigation into the underlying factors shaping resistance within plant-associated bacterial communities.

2. Material and Method

A sequential work flow of this study is summarized in **Figure 1**.

2.1. Sample Collection

Five medicinal plant samples, Thankuni (*Centella asiatica*), Neem (*Azadirachta indica*), Joba (*Hibiscus rosa-sinensis*), Aparajita (*Clitoria ternatea*), and Snake plant (*Dracaena trifasciata*) were selected for this experiment. We have chosen these plants to represent a variety of plants, including herbs such as Thankuni, Aparajita, and Snake plant, shrubs like Joba, and trees such as Neem. This selection allows us to assess the antibiotic resistance profile of endophytic bacteria across diverse medicinal plants. Additionally, these medicinal plants are occasionally ingested as herbal remedies, presenting a potential pathway for the transmission of resistant bacteria from plants to humans. These samples were collected from various field environments, including the Botanical Garden of the University of Dhaka, as well as field areas, residential zones, and academic premises at the university. These areas were chosen based on the fact that they provide a controlled and well-maintained environment with a diverse collection of plant species from various regions and ecosystems. This diversity offers a unique opportunity to access a wide range of plant species with potential medicinal properties. The majority of these areas utilized both organic and inorganic fertilizers, including herbicides, insecticides, and fungicides. For the isolation of endophytic bacteria, different parts (leaves, stems, roots, and flowers) of healthy plants were collected. Using a sterile knife and forceps, plant parts were collected and placed in clean plastic zipper bags. They were then brought to the laboratory, where they were immediately used for the isolation of bacterial endophytes. Fresh plant materials were used for the isolation of the bacterial endophytes to minimize the chances of contamination.

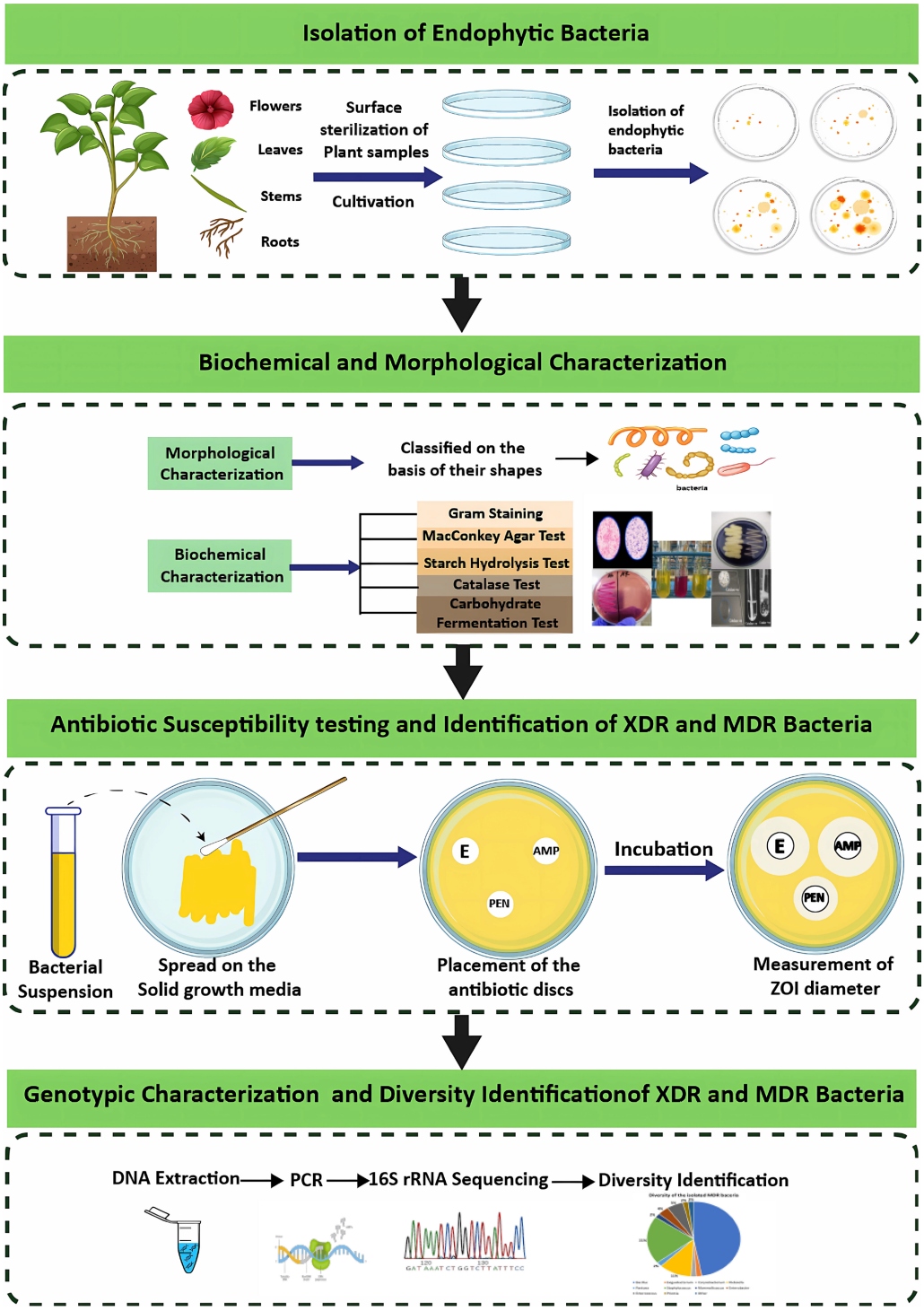

Figure 1. A sequential work-flowchart of the study.

2.2. Isolation of Endophytic Bacteria

This study employed a modified version of the isolation procedure [29] to effectively remove potential epiphytic bacteria from the surface of collected plant samples. Initially, the plant samples underwent a series of washes to eliminate adhering soil particles and surface microbial epiphytes. This process involved rinsing

the samples under slow-running tap water for 15 minutes, followed by consecutive washes with 0.5% Tween20, 2% sodium hypochlorite, 70% ethanol, and 2% mercuric chloride. After each chemical wash, the samples were thoroughly rinsed with sterilized deionized water (dH2O) three times. Finally, the samples were washed with autoclaved nano-pure (Milli-Q) water and dried on sterile tissue paper. The final rinse water was collected as a negative control. All materials and solutions used in the process were autoclaved and exposed to UV light to prevent contamination.

Following surface sterilization, the plant samples were ground using an autoclaved mortar and pestle. The resulting samples were suspended in autoclaved 0.9% saline solution and diluted serially to obtain dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Subsequently, 100 µL of each dilution was spread onto tryptic soy agar (TSA) growth media in Petri dishes. Control plates were prepared by spreading the water collected from the final rinse of the sample to ensure the absence of epiphytes post-washing. The plates were then incubated at 37˚C for 24 - 48 hours. Morphologically distinct bacterial colonies were selected as individual endophytic bacterial colonies. Each colony was preserved in a 50% glycerol solution for further use and stored at −20˚C.

2.3. Morphological and Biochemical Characterization of the Endophytic Bacteria

For morphological characterization, the endophytic bacteria from Thankuni, Neem, Aparijita, Joba, and Snake plant bacterial isolates were evaluated based on their surface structure, color intensity, size, form, and appearance using a microscope.

Five different tests—Gram Staining, MacConkey Agar Test, Starch Hydrolysis Test, Carbohydrate Fermentation Test, and Catalase Test were conducted for the biochemical characterization of the endophytes. All the tests were done following the protocols described in [30] and ASM MicrobeLibrary (http://www.microbelibrary.org/).

2.4. Antibiotic Susceptibility Assay

The disk diffusion susceptibility assay was employed to assess the susceptibility or resistance of endophytic bacteria to a range of antibiotics. Bacterial cultures isolated from Thankuni, Aparajita, Joba, and Snake plant were grown in tryptic soy broth (TSB) media, while Neem-associated bacteria were cultured in brain heart infusion (BHI) broth, followed by overnight incubation at 37˚C on a shaker operating at 180 rpm. Subsequently, the optical density (OD) of the overnight cultures was measured at a wavelength of 600 nm, with an absorbance of 0.125 corresponding to a cell density of 2×10^8 cells/mL.

Tryptic soy agar (TSA) and king's B (KB) medium agar plates were prepared for the disk diffusion assay. Diluted cultures of endophytic bacteria were spread onto TSA agar plates for Thankuni, Aparajita, Joba, and Snake plant-associated bacteria, while Neem-associated bacteria were spread on KB medium plates. An-

tibiotic filter paper discs, each containing one of 14 different antibiotics, namely penicillin (PEN) 10 µg, cephradine (RAD) 30 µg, clindamycin (CLI) 2 µg, erythromycin (ERY) 15 µg, kanamycin (KAN) 30 µg, ampicillin (AMP) 30 µg, Ciprofloxacin (CIP) 5 µg, gentamicin (GEN) 10 µg, doxycycline (DOX) 30 µg, Amoxicillin (AMX) 30 µg, vancomycin (VAN) 30 µg, ceftriaxone (CTR) 30 µg, chloramphenicol (CHL) 30 µg, and cotrimoxazole (COT) 30 µg, were then placed onto the agar surface. We chose these 14 antibiotics from 10 distinct categories to serve as references for the 1st, 2nd, and 3rd generation antibiotics, enabling us to evaluate the susceptibility and resistance of the bacteria.

The plates were subsequently incubated at 37˚C for 16 - 18 hours. Following incubation, the diameter of the zones of inhibition (ZOI) surrounding each antibiotic disc was measured, and the sensitivity or resistance of the endophytic bacteria to each antibiotic was determined based on the observed ZOI.

2.5. Genotypic Characterization of Antibiotic-Resistant Endophytes

The genotypic characterization of antibiotic-resistant endophytic bacteria was conducted through the extraction of genomic DNA, followed by polymerase chain reaction (PCR) amplification of the 16S rRNA gene, and subsequent sequencing of the amplified fragment. This methodological approach allowed for the identification and analysis of genetic markers within the bacterial isolates, providing valuable insights into their taxonomic classification and antibiotic resistance profiles.

2.5.1. DNA Extraction

For the extraction of total genomic DNA, the Thermo Scientific GeneJET Genomic DNA Purification Kit was employed following the manufacturer's recommended protocol. This kit is designed to efficiently isolate high-quality genomic DNA from various sample types. The protocol involves several steps, including cell lysis, protein precipitation, DNA binding to a silica membrane, washing to remove contaminants, and elution of purified DNA.

2.5.2. PCR Amplification

To amplify the 16S rRNA gene from bacterial genomic DNA, PCR was conducted using the universal primers 27-F (AGAGTTTGATCCTGGCTCAG) and 1492-R (GGTTACCTTGTTACGACTT). Each PCR reaction contained 25 µL of reaction mixture, comprising 12.5 µL of PCR Master Mix, 2 µL of 100ng/µL DNA template, 2.5 µL of each 10 mM primer, and nuclease-free water to achieve a final volume of 25 µL. A negative control was included in each PCR run.

PCR amplification was performed through a 35-cycle program, beginning with an initial denaturation step at 95˚C for 5 minutes, followed by 35 cycles of denaturation at 95˚C for 30 seconds, annealing at 58˚C for 40 seconds, and elongation at 72˚C for 1 minute and 40 seconds. A final elongation step was conducted at 72˚C for 7 minutes, after which the reaction was held at 4˚C indefinitely.

2.5.3. Gel Electrophoresis

To confirm the amplification of the target gene, the PCR products were run through agarose gel electrophoresis. The PCR product's size was approximately 1500 base pairs. 2% agarose gel was prepared in Tris-acetate-ethylenediaminetetraacetic acid (EDTA) (TAE) buffer and used to perform the electrophoresis. The same buffer was used to run the gel. The gel was submerged with the 1x TAE (40 mM Tris-acetate, 1 mM EDTA) running buffer. In this electrophoresis, a 1-kilo base-pair DNA ladder was used. The electrophoresis was run at a constant voltage of 120 V for 40 minutes. After completing the electrophoresis, the gel was placed under a UV-illuminator (Alphalmager Mini System, Proteinsimple) and imaged using special software (Alpha view software), which was included in this system.

2.5.4. DNA Sequencing and Taxonomic Identification of the Endophytes

The PCR-amplified products of 16S rRNA genes of the endophytes underwent sequencing using Sanger's method. Subsequent sequence analysis was conducted using Genious Prime software, aligning the sequences with reference sequences of the 16S rRNA gene obtained from the National Center for Biotechnology Information (NCBI) database via the basic local alignment search tool (BLAST). Species identification was accomplished by comparing the acquired sequences with those in the GenBank database, selecting the sequence with the highest maximum identity score. In instances where the identity of the best match was below 99% and the query cover was less than 96%, only genus-level assignment was made.

3. Results

3.1. Isolation of Endophytic Bacteria

A comprehensive investigation into the antibiotic resistance profiles of endophytic bacteria was conducted, utilizing specimens sourced from five distinct plant species. A total of 113 endophytic bacterial isolates were successfully isolated, cultured, and characterized from these plant sources. Specifically, 18 isolates were associated with Thankuni (labeled as T1 to T18), 15 with Neem (labeled as N1 to N15), 18 with Aparajita (labeled as A1 to A18), 35 with Joba (labeled as J1 to J35), and 28 with Snake plant (labeled as S1 to S28). This extensive sampling strategy allowed for a comprehensive assessment of antibiotic resistance profiles across a diverse range of endophytic bacterial communities associated with different plant hosts.

3.2. Morphological and Biochemical Characterization

The initial morphological characterization of the bacteria involved the observation and differentiation of colony characteristics, including size, color, and texture. Subsequently, a series of biochemical tests were conducted to further delineate their characteristics. Gram staining was employed to classify the bacteria based on cell wall structure, while the MacConkey Agar Test provided insight

into their ability to ferment lactose. Additional tests, such as the Starch Hydrolysis Test and Carbohydrate Fermentation Test, were performed to assess the bacteria's capacity to utilize specific substrates. Furthermore, the Catalase Test was conducted to determine the presence of the enzyme catalase, which aids in the breakdown of hydrogen peroxide. These biochemical assays collectively contributed to the comprehensive biochemical characterization of the bacterial isolates, and the summarized results are depicted in Figure 2.

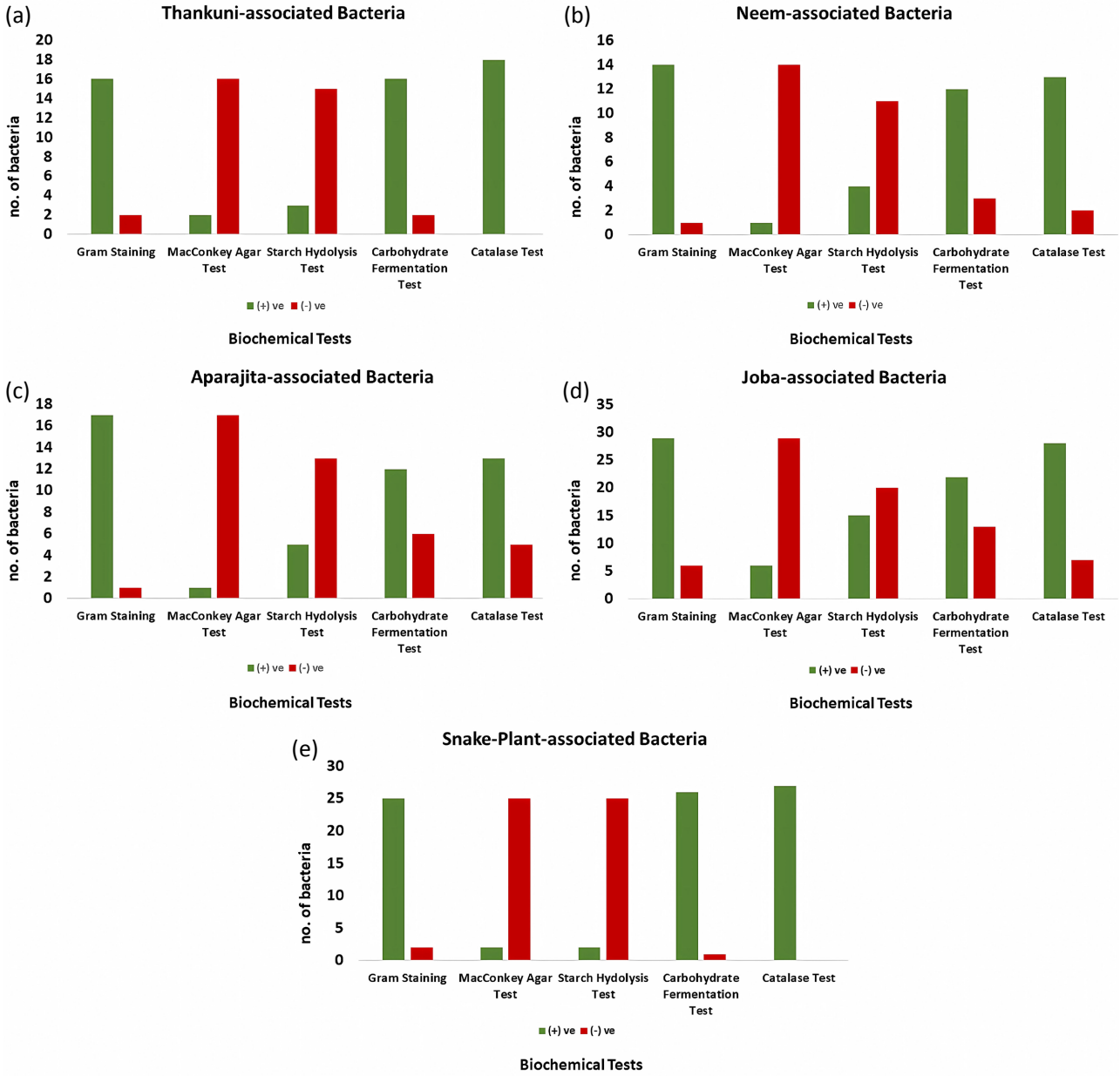

Figure 2. Biochemical characteristics of the isolated endophytic bacteria. Five biochemical tests were performed to assess the biochemical characteristics of the endophytic bacteria. The figure illustrates the number of bacteria demonstrating positive (depicted by green bars) and negative (depicted by red bars) outcomes in the Gram staining, MacConkey agar test, starch hydrolysis test, carbohydrate fermentation test, and catalase test, respectively. The data are categorized based on the plant sources from which the bacteria were isolated, namely (a) Thankuni, (b) Neem, (c) Aparajita, (d) Joba, and (e) Snake plant.

3.3. Antibiotic Susceptibility Test

The disk diffusion method, recognized as the gold standard for assessing antibiotic susceptibility, was utilized to evaluate the susceptibility of isolated bacteria to 14 different antibiotics spanning 10 distinct categories (Table 1). The zone of inhibition (ZOI) resulting from each antibiotic was measured, and the bacteria were categorized as resistant, intermediate, or sensitive following the Clinical and Laboratory Standards Institute (CLSI) guidelines outlined in the 27th edition (2017) of the "Performance Standards for Antimicrobial Susceptibility Testing". The distribution of resistant, intermediate, and sensitive bacteria against the antibiotics is depicted in Figure 3. Detailed information regarding the ZOI of each bacterium against the antibiotics utilized in the study can be found in Table S1.

All endophytic bacteria identified in this study exhibited varying degrees of antibiotic resistance. Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria are distinctively categorized in medical literature, delineating diverse patterns of antimicrobial resistance. MDR bacteria, as defined by the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC), encompass organisms exhibiting acquired resistance to at least one agent in three or more antimicrobial categories. Conversely, XDR bacteria manifest resistance to at least one agent in all but two or fewer antimicrobial categories, maintaining susceptibility to only one or two categories. Pan-drug-resistant (PDR) bacteria demonstrate resistance to all antimicrobial agents, rendering them impervious to treatment options [31].

Table 1. Antimicrobial categories and agents used to define MDR, XDR, and PDR.

Antimicrobial category	Antimicrobial agent
Penicillins	Penicillin
	Ampicillin
	Amoxicillin
Lincosamides	Clindamycin
Aminoglycosides	Kanamycin
	Gentamycin
Fluoroquinolones	Ciprofloxacin
Glycopeptides	Vancomycin
Macrolides	Erythromycin
Folate pathway inhibitors	Cotrimoxazole
Phenicols	Chloramphenicol
Tetracyclines	Doxycycline
Extended-spectrum cephalosporins	Cephradine
	Ceftriaxone

Figure 3. Antibiotic susceptibility profiles of the endophytic bacteria. Endophytic bacteria isolated from (a) Thankuni, (b) Neem, (c) Joba, (d) Aparajita, and (e) Snake plant were assessed for their sensitivity against 14 different antibiotics. "Red" indicates resistance, "Yellow" indicates intermediate, and "Green" indicates the sensitivity (legend box at the right) of the isolate against the used antibiotics. VAN: Vancomycin; RAD: Cephradin; PEN: Penicillin; KAN: Kanamycin; GEN: Gentamycin; ERY: Erythromycin; DOX: Doxycycline; CTR: Ceftriaxone; CIP: Ciprofloxacin; COT: Cotrimoxazole; CLI: Clindamycin; CHL: Chloramphenicol; AMX: Amoxicillin; AMP: Ampicillin.

According to the conditions, bacteria that were found to be resistant to the antibiotics used are shown in Table 2. Thankuni-associated bacteria T1, T3, T5, T6, T7, T8, T9, and T10 were identified as multidrug-resistant (MDR), while T17 and T18 exhibited extensive drug resistance (XDR). Among Neem-associated bacteria, N1, N2, N5, N6, N7, N10, N11, and N13 were categorized as MDR, whereas N8 and N15 demonstrated XDR. Within Joba-associated bacteria, J4, J5, J7, J9, J11, J12, J17, J20, J21, J22, J23, J24, J25, J28, J29, J31, J32, J33, and J34 were identified as MDR, while J13, J14, J18, J19, and J27 exhibited XDR patterns. Aparajita-associated bacteria A5, A6, A9, and A16 were classified as MDR, whereas A11 showed XDR characteristics. Lastly, Snake plant-associated bacteria S9 and S24 were identified as MDR, while S17 displayed XDR traits.

The distribution of bacterial populations across various plant species, along with the categorization into non-MDR, MDR, and XDR strains, is presented in Figure 4 and Figure 5. Among the total 113 endophytic isolates, 53 exhibited multi-drug resistance (MDR), with 12 demonstrating an alarming level of resistance, qualifying as extremely drug-resistant (XDR). Notably, the highest proportion of bacterial isolates originated from Joba specimens (31%), followed by the Snake plant (24%), Aparajita (16%), Thankuni (16%), and Neem (13%), respectively.

Upon scrutiny of resistance profiles, snake plant-associated bacteria exhibited the highest prevalence of non-multidrug resistance (non-MDR) at 89%, rendering it the least resistant among the studied plants. Conversely, Joba-associated bacteria showcased the highest multidrug resistance (MDR) at 54%, indicating elevated resistance levels. Moreover, extreme drug resistance (XDR) was notably pronounced in Neem-associated bacteria at 13%, designating Neem as the plant source harboring the highest level of extreme resistance. In contrast, the snake plant exhibited the lowest XDR percentage at 4%.

3.4. Identification of Antibiotic-Resistant Endophytic Isolates

The utilization of the 16S rRNA gene sequence serves as an invaluable tool for

Table 2. List of multi-drug resistant (MDR) and extremely-drug resistant (XDR) endophytic bacteria isolated from medicinal plants.

Source	Multi-drug resistant (MDR)	Extremely drug-resistant (XDR)
Thankuni-associated bacteria	T1, T3, T5, T6, T7, T8, T9, T10	T17, T18
Neem-associated bacteria	N1, N2, N5, N6, N7, N10, N11, N13, N14	N8, N15
Joba-associated bacteria	J4, J5, J7, J9, J11, J12, J17, J20, J21, J22, J23, J24, J25, J28, J29, J31, J32, J33, J34	J13, J14, J18, J19, J27
Aparajita-associated bacteria	A5, A6, A9, A16	A11
Snake plant-associated bacteria	S9, S24	S17

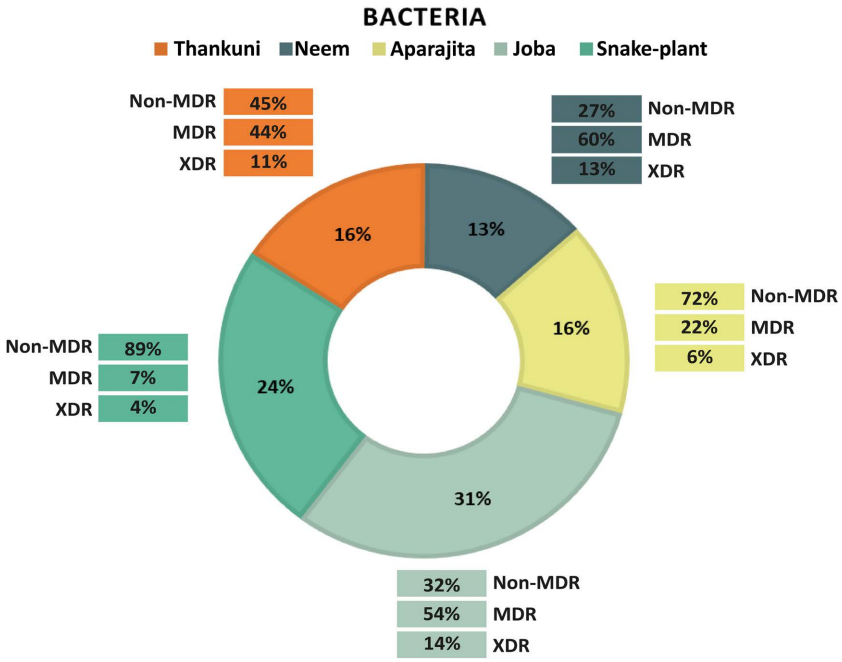

Figure 4. The distribution of enodophytic isolates and their corresponding resistance profiles across five distinct medicinal plants. Endophytic bacteria obtained from the medicinal plants exhibited a diverse array of resistance patterns. Joba, Snake plant, Aparajita, Thankuni, and Neem contributed 31%, 24%, 16%, 16%, and 13% of isolates, respectively. The resistance profiles for each plant species are as follows: Joba-associated bacteria exhibited 32% non-MDR, 54% MDR, and 14% XDR. Snake plant displayed 89% non-MDR, 7% MDR, and 4% XDR. Aparajita demonstrated 72% non-MDR, 22% MDR, and 6% XDR. Thankuni presented 45% non-MDR, 44% MDR, and 11% XDR. Neem showcased 27% non-MDR, 60% MDR, and 13% XDR. Resistance categorizations were determined based on specific criteria: MDR indicates non-susceptibility to ≥1 agent in ≥3 antimicrobial categories, while XDR denotes non-susceptibility to ≥1 agent in all but ≤2 categories.

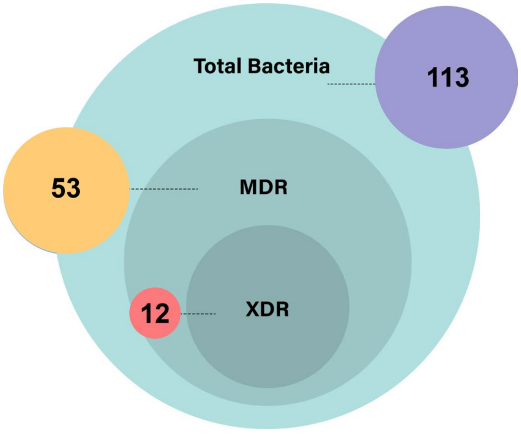

Figure 5. Quantification of total bacteria, MDR, and XDR bacteria derived from isolated endophytic bacterial strains. Among the 113 bacteria isolated from the Thankuni, Neem, Aparajita, Joba, and Snake plant, a total of 53 strains exhibited multi-drug resistance (MDR), with 12 of them categorized as extremely-drug resistant (XDR). The determination of MDR and XDR strains adheres to specific criteria: MDR signifies non-susceptibility to ≥1 agent in ≥3 antimicrobial categories, while XDR denotes non-susceptibility to ≥1 agent in all but ≤2 categories.

identifying microbial genera that elude conventional biochemical profiling [32]. Consequently, PCR-amplified products from each of the 53 resistant endophytic isolates underwent Sanger sequencing. Post-sequencing, data retrieval was facilitated through "Geneious Prime" software, followed by sequence analysis against NCBI's 16S rRNA gene reference sequences using BLAST. *In silico* sequence hybridization compared 16S rRNA gene sequences from previously deposited bacteria in NCBI with those from the present study's isolates, with BLAST results tabulated in Table S2. The diversity of resistant isolates was visually represented in a pie chart (Figure 6).

An analysis of the 53 identified multidrug-resistant bacteria revealed *Bacillus* as the predominant genus, constituting 47% of the total. *Staphylococcus* accounted for 21%, making it the second most prevalent genus among the multidrug-resistant strains, while Klebsiella represented a substantial portion at 11%. The distribution of remaining genera included *Corynebacterium* (5%), *Enterococcus* (5%), *Enterobacter* (2%), *Exiguobacterium* (2%), *Pantoea* (2%), and *Mammaliicoccus* (2%), with a combined 2% for other genera. This distribution underscores the significance of *Bacillus*, *Staphylococcus*, and *Klebsiella* as major contributors to multidrug resistance.

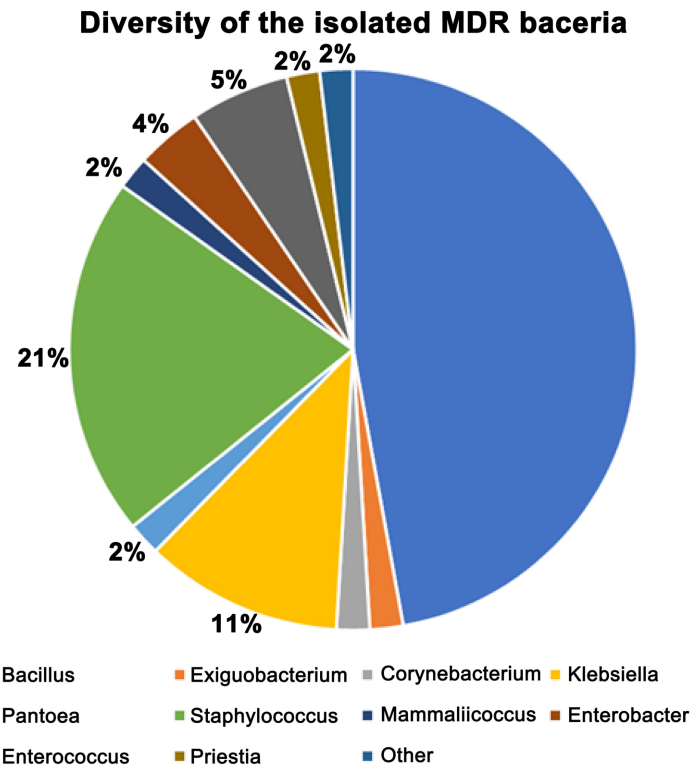

Figure 6. MDR resistance profile across the genus of the isolated endophytes. This figure illustrates the differential antibiotic resistance patterns observed among different bacterial genera. The distribution of antibiotic resistance among 53 multi-drug resistant (MDR) bacteria reveals prominent resistance across various genera. *Bacillus* (47%), *Staphylococcus* (21%), and *Klebsiella* (11%) are the predominant contributors to MDR, showcasing notable levels of resistance against diverse antibiotics.

Further analysis of sequencing data revealed genus-specific antibiotic resistance profiles, depicted in the phylogenetic tree (Figure 7). Phylogenetic tree analysis of multidrug-resistant (MDR) bacteria, defined as resistant to three or more antibiotic classes, delineated three distinct lineages. Lineage 1 comprised the majority of bacteria (43), suggesting a substantial prevalence within this lineage. In contrast, Lineage 3 was represented by a solitary bacterium, T5, indicating a comparatively unique or less prevalent lineage. Notably, Lineage 2, consisting of 9 bacteria, was associated with the Joba plant, with all members exhibiting resistance against more than three antibiotic classes. This distinctive resistance profile highlights Lineage 2's potentially elevated level of antibiotic resistance complexity compared to other lineages.

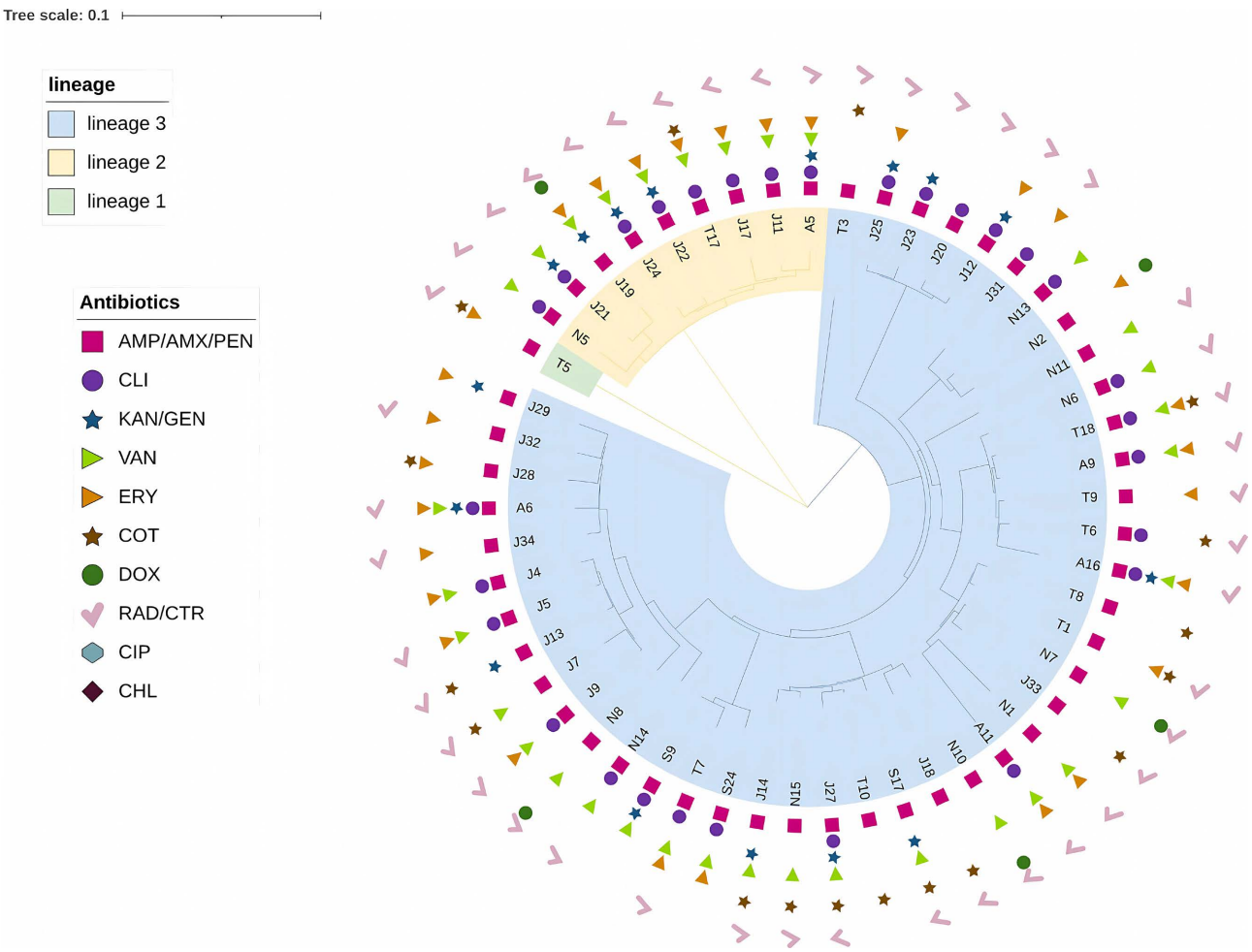

Figure 7. The phylogenetic tree illustrating the evolutionary relationships among the antibiotic-resistant endophytic bacteria. The phylogenetic tree depicted here illustrates the relationships among antibiotic-resistant endophytic bacteria, with three distinct lineages delineated by different colors. Furthermore, 10 different symbols are employed to represent 10 distinct classes of antibiotics, as elucidated in the legend box. Isolates demonstrating resistance to various antibiotics are dispersed around the tree, reflecting their resistance profiles across different antibiotic classes. This visual representation provides valuable insights into the evolutionary relationships among the resistant bacteria and their patterns of antibiotic resistance. VAN: Vancomycin; RAD: Cephradine; PEN: Penicillin; KAN: Kanamycin; GEN: Gentamycin; ERY: Erythromycin; DOX: Doxycycline; CTR: Ceftriaxone; CIP: Ciprofloxacin; COT: Cotrimoxazole; CLI: Clindamycin; CHL: Chloramphenicol; AMX: Amoxicillin; AMP: Ampicillin.

4. Discussion

The introduction of the first antibiotic, penicillin, in 1940 marked the beginning of a new era in global antibiotic therapy, offering potent remedies for the prevailing diseases of that time. However, the overuse and misuse of antibiotics, coupled with the emergence of new bacterial strains, have led to the development of antibiotic resistance. Antibiotic resistance presents a global concern, as numerous pathogenic organisms have developed resistance to one or more types of antibiotics [33]. Indeed, one of the top three major threats to public health in the twenty-first century, according to the World Health Organization, is antibiotic resistance [34]. Methicillin resistance in *Staphylococcus aureus* (MRSA) stands as one of the most prominent examples of AMR and has been associated with elevated mortality rates worldwide each year [35].

Similar to the ubiquity of bacteria, antibiotic resistance is widely distributed, and resistance genes are found in water, the environment, animals, and people. These resistance genes are transmitted both inside and across these reservoirs, with the proportional contributions of the different transmission pathways varying according to the resistance elements and bacterial species involved [36]. Although several studies have identified risk factors for acquiring, infecting, or colonizing with ARB, precise measurement of transmission pathways and their relative significance is necessary given the worldwide urgency to lower the frequency of antibiotic resistance [37]. The present study comprehensively examined the antibiotic resistance profiles of endophytic bacteria isolated from five distinct medicinal plant species. Our findings emphasize the significant prevalence of antibiotic resistance among endophytic bacteria associated with medicinal plants, highlighting the potential implications for human health and ecosystem dynamics.

Medicinal plants are widely used in traditional medicine, and endophytic bacteria play a role in the health of these plants [9]. Studying antibiotic resistance in endophytic bacteria ensures the safety of medicinal plants, as antibiotic-resistant strains may have implications for the efficacy of traditional remedies. If these bacteria harbor antibiotic-resistant genes, there is a risk of transmitting these traits to pathogenic bacteria in the human or animal microbiome. This transfer could contribute to the wider problem of antimicrobial resistance, supported by evidence of antibiotic resistance genes transferring from soil bacteria to clinical pathogens [25].

Our research aimed to investigate the antibiotic resistance profiles of endophytic bacteria isolated from five different medicinal plants—Thankuni, Neem, Aparajita, Joba, and Snakeplant. The isolation process yielded a total of 113 endophytic bacteria, each associated with a specific plant species.

Antibiotic susceptibility testing revealed varying degrees of resistance among the isolated endophytic bacteria. The disk diffusion method enabled the assessment of susceptibility to 14 different antibiotics across 10 distinct categories. Our results demonstrated that all bacterial isolates exhibited some level of antibiotic resistance, with a notable prevalence of multidrug-resistant (MDR) and

extensively drug-resistant (XDR) strains. The comparative analysis of antibiotic resistance among different plant species revealed significant variability in resistance profiles. Joba-associated bacteria exhibited the highest MDR prevalence, while Neem-associated bacteria displayed the highest XDR prevalence. These findings highlight the importance of considering plant host specificity in understanding antibiotic resistance dynamics within plant-microbe ecosystems.

The emergence of MDR and XDR strains among endophytic bacteria from diverse plants emphasizes the global challenge of antibiotic resistance and prompts further investigation into the specific factors driving resistance in these bacterial communities. Understanding the genetic basis of resistance in endophytic bacteria is crucial for developing effective strategies to mitigate the spread of resistant strains.

Our findings highlight the importance of continued surveillance of antibiotic resistance percentages in diverse ecological niches. Responsible antibiotic use, coupled with in-depth research into the mechanisms driving resistance in endophytic bacteria, will be essential in addressing the escalating issue of antibiotic resistance. Future studies should also explore the potential risks associated with the traditional use of medicinal plants hosting these resistant strains, considering the varying resistance percentages observed in this research.

Furthermore, our study identified *Bacillus*, *Staphylococcus*, and *Klebsiella* as the predominant genera among multidrug-resistant bacteria. These findings align with previous studies [38] [39] highlighting the importance of these genera in clinical and environmental settings. The prevalence of these genera in our study suggests their potential role as reservoirs of antibiotic resistance genes within medicinal plant ecosystems.

The phylogenetic analysis provided insights into the evolutionary relationships among antibiotic-resistant endophytic bacteria. Three distinct lineages were delineated, with varying distributions of resistant bacteria across the lineages. Interestingly, Lineage 2 was associated with the Joba plant and exhibited resistance against multiple antibiotic classes, suggesting a potentially unique resistance profile within this lineage.

5. Conclusion

In conclusion, our study provides valuable insights into the antibiotic resistance profiles of endophytic bacteria associated with medicinal plants. The prevalence of multidrug-resistant strains, coupled with the diverse resistance profiles across plant species, emphasizes the complexity of antibiotic resistance within plant-microbe systems. Future research efforts should focus on elucidating the genetic mechanisms underlying antibiotic resistance in endophytic bacteria and developing targeted intervention strategies to mitigate the spread of resistance in both clinical and environmental settings.

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Authors' Contributions

Bonoshree Sarkar: Investigation, analysis, writing—original draft. Afroza Sultana: Investigation, analysis, writing—original draft. Nabila Nawar Binti: Funding acquisition, writing—review & editing. Farhana Tasnim Chowdhury: Funding acquisition, writing—review & editing. Sadia Afrin: Investigation, writing—review & editing. Mohammad Fahim: Investigation, writing—review & editing. Taibur Rahman: Supervision, resources, funding acquisition, writing—review & editing. Atiqur Rahman: Conceptualization, supervision, resources, funding acquisition, writing—review & editing.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary

Table S1. Antibiotic susceptibility of endophytic bacteria from Thankuni (a), Neem (b), Aparajita (c), Joba (d), and Snakeplant (e) as determined by zone of inhibition diameters.

Bacteria No.	PEN (10 µg) ZOI (mm)	RAD (25 µg) ZOI (mm)	CLI (2 µg) ZOI (mm)	ERY (15 µg) ZOI (mm)	KAN (30 µg) ZOI (mm)	AMP (25 µg) ZOI (mm)	CIP (5 µg) ZOI (mm)	GEN (10 µg) ZOI (mm)	DOX (30 µg) ZOI (mm)	AMX (30 µg) ZOI (mm)	VAN (30 µg) ZOI (mm)	CTR (30 µg) ZOI (mm)	CHL (30 µg) ZOI (mm)	COT (25 µg) ZOI (mm)
							(a)							
T1	6 (R)	15 (R)	32 (S)	12 (R)	15 (I)	6 (R)	33 (S)	17 (S)	15 (I)	6 (R)	15 (I)	21 (I)	13 (I)	6 (R)
T2	6 (R)	20 (I)	29 (S)	22 (I)	17 (I)	22 (S)	30 (S)	22 (S)	25 (S)	8 (R)	18 (S)	21 (I)	23 (S)	26 (S)
T3	6 (R)	25 (S)	20 (S)	17 (I)	17 (I)	6 (R)	30 (S)	19 (S)	24 (S)	6 (R)	17 (S)	21 (I)	23 (S)	6 (R)
T4	6 (R)	20 (I)	18 (I)	22 (I)	17 (I)	23 (S)	29 (S)	21 (S)	25 (S)	7 (R)	15 (I)	19 (R)	25 (S)	21 (S)
T5	6 (R)	13 (R)	21 (S)	8 (R)	22 (R)	6 (R)	25 (S)	24 (S)	26 (S)	6 (R)	16 (I)	6 (R)	24 (S)	7 (R)
T6	6 (R)	16 (R)	6 (R)	17 (I)	15 (I)	6 (R)	23 (S)	19 (S)	22 (S)	6 (R)	17 (S)	20 (I)	24 (S)	6 (R)
T7	6 (R)	9 (R)	6 (R)	6 (R)	20 (S)	6 (R)	27 (S)	19 (S)	16 (S)	6 (R)	6 (R)	17 (R)	22 (S)	20 (S)
T8	6 (R)	23 (S)	22 (S)	20 (I)	19 (S)	12 (R)	25 (S)	19 (S)	21 (S)	6 (R)	17 (S)	6 (R)	22 (S)	7 (R)
T9	6 (R)	12 (R)	25 (S)	6 (R)	18 (S)	6 (R)	24 (S)	18 (S)	24 (S)	6 (R)	18 (S)	18 (R)	23 (S)	25 (S)
T10	6 (R)	24 (S)	20 (S)	23 (S)	20 (S)	6 (R)	19 (I)	16 (S)	26 (S)	6 (R)	15 (I)	9 (R)	25 (S)	7 (R)
T11	6 (R)	21 (S)	6 (R)	20 (I)	19 (S)	6 (R)	24 (S)	15 (S)	17 (S)	6 (R)	16 (I)	27 (S)	26 (S)	28 (S)
T12	11 (R)	20 (I)	25 (S)	20 (I)	21 (S)	13 (R)	24 (S)	20 (S)	17 (S)	6 (R)	17 (S)	20 (I)	24 (S)	22 (S)
T13	18 (R)	30 (S)	28 (S)	25 (S)	19 (S)	27 (R)	32 (S)	24 (S)	28 (S)	7 (R)	17 (S)	29 (S)	24 (S)	33 (S)
T14	17 (R)	19 (I)	28 (S)	19 (I)	19 (S)	11 (R)	25 (S)	22 (S)	23 (S)	6 (R)	17 (S)	25 (S)	22 (S)	21 (S)
T15	14 (R)	6 (R)	24 (S)	27 (S)	23 (S)	6 (R)	22 (S)	19 (S)	28 (S)	6 (R)	24 (S)	26 (S)	31 (S)	36 (S)
T16	24 (R)	23 (S)	19 (I)	26 (S)	24 (S)	18 (R)	26 (S)	23 (S)	33 (S)	6 (R)	22 (S)	21 (R)	33 (S)	29 (S)
T17	6 (R)	8 (R)	6 (R)	6 (R)	14 (I)	6 (R)	20 (I)	16 (S)	13 (I)	6 (R)	6 (R)	25 (S)	23 (S)	23 (S)
T18	6 (R)	8 (R)	6 (R)	6 (R)	13 (R)	7 (R)	22 (S)	14 (I)	13 (I)	6 (R)	6 (R)	24 (S)	23 (S)	20 (S)
							(b)							
N1	6 (R)	14 (R)	20 (I)	13 (R)	21 (S)	21 (S)	22 (S)	22 (S)	18 (S)	6 (R)	14 (R)	9 (R)	25 (S)	11 (I)
N2	6 (R)	27 (S)	6 (R)	11 (R)	23 (S)	13 (I)	23 (S)	20 (S)	10 (R)	6 (R)	16 (I)	17 (S)	22 (S)	26 (S)
N3	6 (R)	29 (S)	20 (I)	27 (S)	19 (S)	7 (R)	30 (S)	21 (S)	16 (S)	8 (R)	18 (I)	7 (R)	27 (S)	32 (S)
N4	6 (R)	17 (I)	21 (S)	17 (I)	21 (S)	21 (S)	30 (S)	20 (S)	22 (S)	6 (R)	17 (I)	8 (R)	16 (I)	35 (S)
N5	6 (R)	16 (R)	6 (R)	22 (I)	19 (S)	11 (R)	24 (S)	19 (S)	16 (S)	6 (R)	14 (R)	19 (S)	22 (S)	26 (S)
N6	6 (R)	18 (I)	21 (S)	17 (I)	23 (S)	11 (R)	27 (S)	25 (S)	15 (I)	6 (R)	13 (R)	6 (R)	22 (S)	25 (S)
N7	6 (R)	20 (I)	20 (I)	20 (I)	20 (S)	19 (S)	30 (S)	21 (S)	9 (R)	6 (R)	14 (R)	7 (R)	20 (S)	23 (S)
N8	6 (R)	20 (I)	15 (I)	18 (I)	22 (S)	15 (R)	24 (S)	19 (S)	10 (R)	6 (R)	14 (R)	10 (R)	23 (S)	25 (S)
N9	6 (R)	14 (R)	20 (I)	22 (I)	21 (S)	8 (R)	20 (I)	19 (S)	15 (I)	6 (R)	16 (I)	6 (R)	24 (S)	17 (S)
N10	6 (R)	15 (R)	17 (I)	21 (I)	20 (S)	6 (R)	19 (I)	19 (S)	14 (I)	6 (R)	14 (R)	6 (R)	24 (S)	16 (S)
N11	6 (R)	16 (R)	19 (I)	20 (I)	21 (S)	10 (R)	18 (I)	18 (S)	18 (S)	6 (R)	14 (R)	6 (R)	24 (S)	24 (S)
N12	6 (R)	18 (I)	23 (S)	23 (S)	18 (S)	13 (I)	20 (I)	21 (S)	16 (S)	6 (R)	15 (I)	19 (S)	21 (S)	23 (S)
N13	6 (R)	17 (I)	7 (R)	25 (S)	25 (S)	19 (S)	22 (S)	22 (S)	20 (S)	6 (R)	13 (R)	21 (S)	22 (S)	22 (S)
N14	6 (R)	16 (R)	14 (R)	20 (I)	17 (I)	21 (R)	17 (I)	18 (S)	16 (S)	6 (R)	13 (R)	13 (R)	23 (S)	24 (S)
N15	6 (R)	13 (R)	20 (I)	21 (I)	21 (S)	10 (R)	19 (I)	20 (S)	13 (I)	6 (R)	14 (R)	6 (R)	24 (S)	6 (R)

Continued

						(c)								
A1	17 (R)	18 (I)	25 (S)	24 (S)	23 (S)	14 (R)	29 (S)	20 (S)	30 (S)	25 (R)	18 (S)	26 (S)	26 (S)	30 (S)
A2	30 (S)	18 (I)	25 (S)	23 (S)	21 (S)	26 (R)	25 (S)	20 (S)	32 (S)	28 (R)	20 (S)	25 (S)	25 (S)	25 (S)
A3	36 (S)	23 (S)	25 (S)	25 (S)	18 (S)	25 (R)	25 (S)	19 (S)	30 (S)	35 (S)	16 (I)	25 (S)	24 (S)	25 (S)
A4	30 (S)	28 (S)	26 (S)	30 (S)	19 (S)	30 (S)	30 (S)	20 (S)	26 (S)	32 (S)	21 (S)	30 (S)	20 (S)	28 (S)
A5	6 (R)	8 (R)	6 (R)	11 (R)	18 (S)	6 (R)	30 (S)	12 (R)	19 (S)	8 (R)	6 (R)	26 (S)	26 (S)	18 (S)
A6	6 (R)	12 (R)	6 (R)	6 (R)	11 (R)	6 (R)	25 (S)	13 (I)	15 (I)	10 (R)	6 (R)	22 (I)	22 (S)	25 (S)
A7	8 (R)	14 (R)	25 (S)	22 (I)	16 (I)	10 (R)	24 (S)	15 (S)	30 (S)	20 (R)	15 (I)	25 (S)	24 (S)	30 (S)
A8	45 (S)	21 (S)	30 (S)	33 (S)	18 (S)	25 (R)	22 (S)	17 (S)	40 (S)	46 (S)	20 (S)	24 (S)	29 (S)	23 (S)
A9	6 (R)	10 (R)	6 (R)	13 (R)	24 (S)	8 (R)	30 (S)	15 (S)	20 (S)	10 (R)	7 (R)	28 (S)	27 (S)	19 (S)
A10	40 (S)	30 (S)	32 (S)	32 (S)	23 (S)	26 (R)	32 (S)	22 (S)	33 (S)	46 (S)	16 (I)	30 (S)	30 (S)	30 (S)
A11	7 (R)	9 (R)	7 (R)	7 (R)	18 (S)	20 (R)	30 (S)	15 (S)	20 (S)	7 (R)	7 (R)	12 (R)	22 (S)	20 (S)
A12	14 (R)	23 (S)	15 (I)	21 (I)	17 (I)	20 (R)	31 (S)	14 (I)	28 (S)	20 (R)	16 (I)	16 (I)	15 (I)	34 (S)
A13	36 (S)	30 (S)	20 (I)	20 (I)	19 (S)	26 (R)	26 (S)	22 (S)	23 (S)	36 (S)	18 (S)	20 (I)	25 (S)	25 (S)
A14	26 (I)	26 (S)	18 (I)	19 (I)	15 (I)	7 (R)	25 (S)	11 (R)	30 (S)	21 (R)	17 (S)	27 (S)	13 (I)	32 (S)
A15	24 (I)	25 (S)	15 (I)	32 (S)	17 (I)	22 (R)	30 (S)	15 (S)	30 (S)	30 (S)	15 (I)	20 (I)	20 (S)	35 (S)
A16	6 (R)	12 (R)	6 (R)	6 (R)	12 (R)	6 (R)	23 (S)	15 (S)	13 (I)	8 (R)	6 (R)	24 (S)	20 (S)	16 (S)
A17	29 (S)	34 (S)	17 (I)	40 (S)	20 (S)	18 (R)	30 (S)	16 (S)	40 (S)	30 (S)	19 (S)	19 (I)	6 (R)	42 (S)
A18	11 (R)	15 (R)	26 (S)	24 (S)	22 (S)	14 (R)	25 (S)	18 (S)	34 (S)	20 (R)	21 (S)	23 (S)	26 (S)	40 (S)
						(d)								
J1	23 (I)	24 (S)	6 (R)	26 (S)	19 (S)	18 (R)	30 (S)	21 (S)	28 (S)	13 (R)	20 (S)	25 (S)	30 (S)	20 (S)
J2	40 (S)	34 (S)	30 (S)	34 (S)	25 (S)	28 (R)	32 (S)	20 (S)	35 (S)	50 (S)	19 (S)	30 (S)	24 (S)	28 (S)
J3	6 (R)	23 (S)	26 (S)	24 (S)	18 (S)	11 (R)	28 (S)	18 (S)	25 (S)	12 (R)	21 (S)	11 (R)	28 (S)	20 (S)
J4	24 (I)	17 (I)	6 (R)	6 (R)	16 (I)	12 (R)	25 (S)	19 (S)	19 (S)	6 (R)	12 (R)	25 (S)	21 (S)	24 (S)
J5	40 (S)	12 (R)	6 (R)	7 (R)	15 (I)	25 (R)	26 (S)	20 (S)	26 (S)	7 (R)	10 (R)	30 (S)	21 (S)	22 (S)
J6	10 (R)	22 (S)	25 (S)	18 (I)	27 (S)	8 (R)	30 (S)	20 (S)	28 (S)	13 (R)	16 (I)	28 (S)	25 (S)	25 (S)
J7	10 (R)	15 (R)	20 (I)	21 (I)	15 (I)	6 (R)	27 (S)	21 (S)	30 (S)	17 (R)	12 (R)	13 (R)	24 (S)	6 (R)
J8	32 (S)	24 (S)	25 (S)	23 (S)	17 (I)	35 (S)	30 (S)	17 (S)	32 (S)	50 (S)	13 (R)	30 (S)	27 (S)	27 (S)
J9	6 (R)	15 (R)	6 (R)	6 (R)	15 (I)	20 (R)	26 (S)	20 (S)	20 (S)	11 (R)	6 (R)	29 (S)	25 (S)	25 (S)
J10	13 (R)	21 (S)	28 (S)	23 (S)	19 (S)	14 (R)	30 (S)	19 (S)	30 (S)	16 (R)	17 (S)	26 (S)	15 (R)	27 (S)
J11	6 (R)	9 (R)	6 (R)	10 (R)	17 (I)	7 (R)	30 (S)	13 (I)	18 (S)	9 (R)	6 (R)	25 (S)	21 (S)	15 (I)
J12	25 (I)	13 (R)	6 (R)	6 (R)	7 (R)	17 (R)	16 (I)	15 (S)	21 (S)	6 (R)	18 (S)	15 (R)	23 (S)	20 (S)
J13	12 (R)	8 (R)	20 (I)	15 (I)	16 (I)	6 (R)	22 (S)	10 (R)	25 (S)	12 (R)	15 (I)	8 (R)	20 (S)	6 (R)
J14	8 (R)	8 (R)	20 (I)	15 (I)	16 (I)	6 (R)	25 (S)	12 (R)	26 (S)	11 (R)	14 (R)	13 (R)	18 (S)	6 (R)
J15	32 (S)	21 (S)	25 (S)	21 (I)	15 (I)	23 (R)	26 (S)	18 (S)	28 (S)	35 (S)	16 (I)	25 (S)	27 (S)	26 (S)
J16	15 (R)	23 (S)	25 (S)	20 (I)	16 (I)	15 (R)	28 (S)	21 (S)	30 (S)	20 (R)	17 (S)	23 (S)	25 (S)	22 (S)
J17	6 (R)	12 (R)	6 (R)	6 (R)	15 (I)	6 (R)	30 (S)	16 (S)	16 (S)	9 (R)	7 (R)	32 (S)	28 (S)	20 (S)
J18	11 (R)	15 (R)	20 (I)	21 (I)	15 (I)	7 (R)	23 (S)	17 (S)	26 (S)	13 (R)	20 (S)	13 (R)	23 (S)	6 (R)

Continued

J19	6 (R)	6 (R)	20 (I)	6 (R)	11 (R)	6 (R)	20 (I)	12 (R)	12 (R)	6 (R)	6 (R)	25 (S)	20 (S)	21 (S)
J20	28 (S)	12 (R)	12 (R)	20 (I)	14 (I)	19 (R)	20 (I)	17 (S)	30 (S)	30 (S)	18 (S)	19 (I)	26 (S)	22 (S)
J21	6 (R)	12 (R)	6 (R)	15 (I)	12 (R)	6 (R)	20 (I)	13 (I)	15 (I)	8 (R)	6 (R)	26 (S)	22 (S)	20 (S)
J22	6 (R)	6 (R)	6 (R)	6 (R)	13 (R)	6 (R)	22 (S)	15 (S)	13 (I)	6 (R)	6 (R)	35 (S)	20 (S)	17 (S)
J23	22 (I)	12 (R)	9 (R)	25 (S)	9 (R)	15 (R)	17 (I)	13 (I)	25 (S)	30 (S)	16 (I)	18 (I)	25 (S)	23 (S)
J24	6 (R)	12 (R)	6 (R)	6 (R)	13 (R)	6 (R)	20 (I)	15 (S)	15 (I)	9 (R)	6 (R)	26 (S)	22 (S)	20 (S)
J25	20 (I)	13 (R)	6 (R)	6 (R)	8 (R)	15 (R)	21 (S)	14 (I)	19 (S)	30 (S)	16 (I)	15 (R)	27 (S)	18 (S)
J26	35 (S)	18 (R)	25 (S)	30 (S)	16 (I)	21 (R)	24 (S)	15 (S)	30 (S)	40 (S)	15 (I)	22 (I)	25 (S)	21 (S)
J27	6 (R)	6 (R)	6 (R)	22 (I)	6 (R)	6 (R)	21 (S)	19 (S)	25 (S)	6 (R)	12 (R)	11 (R)	21 (S)	6 (R)
J28	36 (S)	19 (I)	25 (S)	8 (R)	21 (S)	23 (R)	26 (S)	23 (S)	33 (S)	30 (S)	16 (I)	23 (S)	30 (S)	6 (R)
J29	6 (R)	20 (I)	20 (I)	6 (R)	12 (R)	6 (R)	28 (S)	21 (S)	17 (S)	20 (R)	17 (S)	25 (S)	26 (S)	24 (S)
J30	25 (I)	35 (S)	6 (R)	26 (S)	20 (S)	24 (R)	28 (S)	25 (S)	30 (S)	28 (R)	20 (S)	31 (S)	28 (S)	25 (S)
J31	18 (R)	20 (I)	6 (R)	6 (R)	20 (S)	6 (R)	28 (S)	16 (S)	40 (S)	15 (R)	20 (S)	10 (R)	15 (I)	23 (S)
J32	6 (R)	9 (R)	30 (S)	6 (R)	20 (S)	6 (R)	38 (S)	20 (S)	21 (S)	16 (R)	21 (S)	30 (S)	26 (S)	28 (S)
J33	15 (R)	15 (R)	27 (S)	27 (S)	26 (S)	13 (R)	32 (S)	26 (S)	32 (S)	21 (R)	17 (S)	18 (I)	26 (S)	6 (R)
J34	11 (R)	13 (R)	27 (S)	12 (R)	22 (S)	6 (R)	28 (S)	21 (S)	23 (S)	14 (R)	20 (S)	21 (I)	27 (S)	27 (S)
J35	40 (S)	20 (I)	24 (S)	26 (S)	20 (S)	24 (R)	25 (S)	14 (I)	30 (S)	40 (S)	15 (I)	22 (I)	25 (S)	23 (S)
							(e)							
S1	40 (S)	30 (S)	28 (S)	32 (S)	14 (I)	24 (S)	30 (S)	18 (S)	30 (S)	40 (S)	28 (S)	20 (I)	28 (S)	28 (S)
S2	14 (R)	30 (S)	32 (S)	26 (S)	20 (S)	8 (R)	30 (S)	20 (S)	40 (S)	16 (R)	18 (S)	18 (I)	30 (S)	20 (S)
S3	36 (S)	30 (S)	30 (S)	32 (S)	18 (S)	24 (S)	26 (S)	19 (S)	30 (S)	40 (S)	16 (I)	20 (I)	20 (S)	22 (S)
S4	14 (R)	27 (S)	24 (S)	36 (S)	18 (S)	10 (R)	30 (S)	18 (S)	30 (S)	20 (R)	15 (I)	27 (S)	25 (S)	16 (S)
S5	11 (R)	16 (I)	24 (S)	28 (S)	15 (I)	10 (R)	20 (I)	15 (S)	28 (S)	19 (R)	16 (I)	19 (I)	23 (S)	16 (S)
S6	32 (S)	22 (I)	21 (S)	22 (I)	15 (I)	25 (S)	25 (S)	18 (S)	29 (S)	40 (S)	17 (S)	18 (I)	25 (S)	22 (S)
S7	18 (R)	17 (I)	25 (S)	27 (S)	20 (S)	9 (R)	25 (S)	9 (R)	30 (S)	21 (R)	17 (S)	21 (S)	27 (S)	17 (S)
S8	40 (S)	21 (I)	26 (S)	27 (S)	18 (S)	24 (S)	24 (S)	18 (S)	34 (S)	42 (S)	16 (I)	22 (S)	27 (S)	21 (S)
S9	7 (R)	20 (I)	7 (R)	9 (I)	13 (R)	7 (R)	25 (S)	12 (R)	16 (S)	7 (R)	7 (R)	26 (S)	25 (S)	20 (S)
S10	16 (R)	25 (S)	24 (S)	9 (I)	19 (S)	9 (R)	29 (S)	19 (S)	26 (S)	21 (R)	16 (I)	19 (I)	27 (S)	29 (S)
S11	34 (S)	23 (S)	26 (S)	28 (S)	15 (I)	22 (S)	22 (S)	15 (S)	30 (S)	38 (S)	16 (I)	19 (I)	23 (S)	22 (S)
S12	34 (S)	23 (S)	24 (S)	26 (S)	16 (I)	18 (S)	30 (S)	16 (S)	34 (S)	34 (S)	16 (I)	21 (S)	22 (S)	22 (S)
S13	37 (S)	24 (S)	26 (S)	28 (S)	15 (I)	21 (S)	24 (S)	14 (I)	32 (S)	34 (S)	16 (I)	17 (I)	23 (S)	22 (S)
S14	20 (I)	25 (S)	12 (R)	24 (S)	18 (S)	13 (I)	20 (I)	18 (S)	24 (S)	20 (R)	16 (I)	28 (S)	24 (S)	21 (S)
S15	18 (R)	25 (S)	30 (S)	18 (I)	15 (I)	17 (S)	25 (S)	15 (S)	28 (S)	30 (S)	15 (I)	20 (I)	25 (S)	22 (S)
S16	22 (I)	23 (S)	24 (S)	29 (S)	16 (I)	12 (I)	19 (I)	16 (S)	29 (S)	24 (R)	17 (S)	20 (I)	27 (S)	20 (S)
S17	6 (R)	7 (R)	20 (I)	21 (I)	14 (I)	6 (R)	19 (I)	11 (R)	22 (S)	6 (R)	14 (R)	7 (R)	21 (S)	6 (R)
S18	12 (R)	21 (I)	24 (S)	28 (S)	16 (I)	10 (R)	21 (S)	14 (I)	27 (S)	17 (R)	15 (I)	20 (I)	24 (S)	14 (R)
S19	21 (I)	25 (S)	28 (S)	27 (S)	16 (I)	13 (I)	26 (S)	18 (S)	24 (S)	20 (R)	17 (S)	18 (I)	27 (S)	21 (S)
S20	34 (S)	22 (I)	22 (S)	24 (S)	15 (I)	23 (S)	29 (S)	15 (S)	30 (S)	18 (R)	14 (R)	20 (I)	25 (S)	20 (S)

Continued

S21	15 (R)	24 (S)	25 (S)	25 (S)	17 (I)	19 (S)	25 (S)	14 (I)	17 (S)	20 (R)	13 (R)	21 (S)	24 (S)	17 (S)
S22	33 (S)	26 (S)	25 (S)	30 (S)	18 (S)	21 (S)	27 (S)	20 (S)	32 (S)	38 (S)	18 (S)	21 (S)	27 (S)	24 (S)
S23	20 (I)	30 (S)	26 (S)	28 (S)	23 (S)	20 (S)	26 (S)	18 (S)	30 (S)	22 (R)	17 (S)	23 (S)	26 (S)	18 (S)
S24	6 (R)	30 (S)	6 (R)	10 (R)	17 (I)	8 (R)	27 (S)	15 (S)	17 (S)	9 (R)	7 (R)	17 (I)	25 (S)	20 (S)
S25	16 (R)	24 (S)	26 (S)	26 (S)	19 (S)	16 (S)	26 (S)	18 (S)	30 (S)	20 (R)	17 (S)	24 (S)	26 (S)	18 (S)
S26	18 (R)	25 (S)	27 (S)	30 (S)	18 (S)	20 (S)	26 (S)	18 (S)	31 (S)	22 (R)	19 (S)	25 (S)	26 (S)	18 (S)
S27	20 (I)	28 (S)	25 (S)	36 (S)	24 (S)	21 (S)	32 (S)	22 (S)	26 (S)	25 (R)	19 (S)	23 (S)	28 (S)	22 (S)
S28	21 (I)	28 (S)	30 (S)	36 (S)	28 (S)	22 (S)	28 (S)	20 (S)	32 (S)	32 (S)	19 (S)	29 (S)	27 (S)	20 (S)

Table S2. Identification of the isolated endophytic bacteria to genus or species level and their percent identity based on blasting our sequence data with NCBI databases.

Bacteria No.	Bacterial genus, species, and strain with the greatest similarity	Accession No. with the greatest similarity	Query coverage	Maximum identity
T1	*Bacillus altitudinis* strain EN-43-07 16S ribosomal RNA gene	OR898492	100	99.7
T3	*Exiguobacterium mexicanum* strain MKSAN3 16S ribosomal RNA gene	OR452292	100	98.9
T5	*Corynebacterium qintianiae* strain MC1420 16S ribosomal RNA	NR_181082	99.76	98.6
T6	*Bacillus subtilis* isolate NRS6131 genome assembly	OX419652	100	99.5
T7	*Bacillus sonorensis* strain Isolat7 16S ribosomal RNA gene	OR264514	100	95.5
T8	*Bacillus altitudinis* strain CES-OCA-19 chromosome	CP126086	100	99.6
T9	*Bacillus subtilis* strain MBB3B9_DBT-NECAB chromosome	CP089269	99.89	99.9
T10	*Bacillus tropicus* strain T36S-23 chromosome	CP119875	100	100
T17	*Klebsiella pneumoniae* subsp. pneumoniae strain KP67 chromosome	CP101560	100	97.8
T18	*Bacillus subtilis* strain MBB3B9_DBT-NECAB chromosome	CP089269	100	97.8
N1	*Bacillus altitudinis* strain BT 62 16S ribosomal RNA gene	KJ848578	98.97	85.2
N2	*Bacillus megaterium* strain BMS4 16S ribosomal RNA gene	KC429572	98.51	95.4
N5	*Pantoea dispersa* strain YSD_J2 chromosome	CP074350	100	97.9
N6	*Bacillus halotolerans* strain HEP10A4 16S ribosomal RNA gene	KY608836	100	97.8
N7	*Bacillus altitudinis* strain ABT_AC37 chromosome	CP087276	100	98.7
N8	*Staphylococcus sp.* G2DM-49 16S ribosomal RNA gene	DQ416800	100	96.6
N10	*Bacillus cereus* strain JSNBEBT50_4E 16S ribosomal RNA gene	PP064124	99.89	92.9
N11	*Bacillus sp.* NCCP-980 gene for 16S ribosomal RNA	AB970705	100	97.8
N13	*Bacillus sp.* MB108 gene for 16S ribosomal RNA	AB536937	100	97
N14	*Mammaliicoccus sciuri* strain FDAARGOS_285 chromosome	CP022046	100	98.9
N15	*Bacillus tropicus* strain T36S-23 chromosome	CP119875	100	98.4
J4	*Staphylococcus epidermidis* strain AS-6s208 16S ribosomal RNA gene	OR899957	100	99.3
J5	*Staphylococcus epidermidis* strain PartG-Sepidermidis-RM8376	CP064359	100	99.8
J7	*Staphylococcus epidermidis* strain PartG-Sepidermidis-RM8376	CP064360	100	97.8
J9	*Staphylococcus epidermidis* strain PartG-Sepidermidis-RM8376	CP064361	100	99.6

Continued

J11	*Klebsiella pneumoniae* strain KP36482022BGR genome assembly	OY978494	100	98.3
J12	*Enterococcus sp.* strain AabL10 16S ribosomal RNA gene	OR513033	100	99.3
J13	*Staphylococcus epidermidis* strain AS-6s208 16S ribosomal RNA gene	OR899957	100	99.3
J14	*Bacillus subtilis* strain AUBS5 16S ribosomal RNA gene	PP064165	100	99.2
J17	*Klebsiella pneumoniae* isolate 152 genome assembly	OW967899	100	99.3
J18	*Bacillus sp.* (in: firmicutes) strain CpH06 16S ribosomal RNA gene	OR742178	99.65	97.6
J19	*Enterobacter asburiae* strain NJ-2 16S ribosomal RNA gene	OR064262	100	99.4
J20	*Enterococcus gallinarum* strain 27SVSFAW 16S ribosomal RNA gene,	ON514114	100	99
J21	*Enterobacter quasiroggenkampii* strain V1735 16S ribosomal RNA gene	OR454040	100	99
J22	*Klebsiella pneumoniae* strain KP36482022BGR genome assembly	OY978494	100	99.2
J23	*Enterococcus sp.* strain AabL10 16S ribosomal RNA gene	OR513033	100	99.2
J24	*Klebsiella pneumoniae* strain KP36482022BGR genome assembly	OY978494	100	98.3
J25	*Bacterium NV211* 16S ribosomal RNA gene, partial sequence.	KU561912	100	98.3
J27	*Bacillus sp.* (in: firmicutes) strain YX16-26 16S ribosomal RNA	OR394161	100	97.5
J28	*Staphylococcus taiwanensis* strain 171 16S ribosomal RNA gene	OR673552	100	98.9
J29	*Staphylococcus hominis* strain CEMTC_7276 16S ribosomal RNA gene	OR640309	99.76	98.8
J31	*Priestia megaterium* strain YX2-3 16S ribosomal RNA gene	OR394178	100	95.8
J32	*Staphylococcus hominis* strain ZG13-53 16S ribosomal RNA gene	OR243850	100	97.5
J33	*Bacillus aerius* strain V3122 16S ribosomal RNA gene	OR752027	100	99.4
J34	*Staphylococcus hominis* strain PCU7 16S ribosomal RNA gene	OR253330	99.76	98.4
A5	*Klebsiella pneumoniae* strain KP36482022BGR genome assembly,	OY978494	100	99.1
A6	*Staphylococcus petrasii* strain AM14 16S ribosomal RNA gene	ON323047	100	99
A9	*Bacillus subtilis* strain UKS56 16S ribosomal RNA gene, partial	KX953131	100	99.3
A11	*Bacillus sp.* (in: Bacteria) strain IG5(32) 16S ribosomal RNA gene,	MH595647	89.66	81.2
A16	*Bacillus subtilis* isolate NRS6131 genome assembly, chromosome:	OX419652	75.95	93.9
S9	*Lysinibacillus sp.* xfqu3 16S ribosomal RNA gen	GQ480504	100	98.4
S17	*Bacillus cereus* strain 13.1 16S ribosomal RNA gene, partial	OQ756385	100	97.9
S24	*Lysinibacillus sp.* YS11 chromosome, complete genome	CP026007	100	98.3