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Exploring Uncharted Sources of Rare Actinomycetes for the Discovery of Innovative Antimicrobial Compounds to Combat Multidrug-Resistant Bacterial Infections

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ABSTRACT

Infectious diseases remain the leading cause of death worldwide, with approximately 10 billion fatalities, accounting for 25% of all major deaths annually. This is largely attributed to the increasing number of infections in hospitals, particularly among patients who are vulnerable due to pre-existing conditions or injuries. Recently, there has been a significant increase in the prevalence of multidrug-resistant bacteria, which are responsible for severe healthcare-associated infections in hospital settings. The development of resistance to antibiotics such as aminoglycosides, β-lactams, and methicillin has posed significant risks to human health, as treating infections caused by these resistant strains becomes increasingly difficult. Initially, vancomycin was used to address methicillin-resistant *Staphylococcus aureus*; however, the emergence of vancomycin-resistant *S. aureus* in hospitals has complicated treatment options. In addition, bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* have attracted increasing concern due to their status as dangerous pathogens. The rise of penicillin-resistant *Streptococcus pneumoniae* also presents a serious challenge, especially for immunocompromised or elderly individuals. The increasing emergence of resistant strains has made the development of new antibiotics increasingly difficult. Actinomycetes, especially *Streptomyces* species, continue to be a rich source of new antimicrobial agents, with over two-thirds of the most commonly used antibiotics derived from actinomycetes. *Streptomyces* possesses remarkable biosynthetic capabilities that allow it to produce antibiotics, which face minimal competition from other microbial genera. This review examines the rise of multidrug-resistant bacteria and the potential for controlling these infections using effective antimicrobial agents derived from rare actinomycetes. In conclusion, the pursuit of novel antibiotics remains a central goal in many research efforts, making the exploration of new habitats for antibiotic discovery a critical priority.

Keywords: Streptomycin, Gentamicin, Multidrug-resistant, *Streptomyces*, Actinomycetes

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Introduction

Antibiotic-resistant pathogens are emerging as a critical global health threat, with their prevalence expected to rise in the future. As new antibiotics are introduced, they inevitably face the challenge of resistance development over time. The high resistance observed in these pathogens is influenced by their microbial origin and their natural ecological role [1, 2]. In intensive care units, the most critically ill patients are often infected by antibiotic-resistant bacteria, which are widespread and pose a significant risk to human health worldwide [3]. The global health crisis is primarily driven by excessive antibiotic use and the lack of development of new antibiotics, leading to the continuous spread of resistant bacteria. The creation of new antibiotics to combat antibiotic-resistant bacteria is crucial for advancing medical treatments, preventing high-risk infections, and improving survival rates [2]. Without effective antibiotics, even minor surgeries or injuries can become life-threatening, requiring extended

hospital stays. Researchers in medicinal chemistry have worked diligently to either modify existing antibiotics or develop new ones derived from novel strains sourced from unexplored environments, focusing on promising isolates. Their efforts have resulted in the creation of fourth and third-generation β-lactams and macrolides, as well as numerous other effective antibiotics [4]. Despite the development of antibiotic analogs with high inhibitory potential, the shortage of safe and effective antibiotics for multi-drug-resistant (MDR) bacterial and fungal isolates in hospitals remains a major issue [5]. This review aims to address the rise in microbial resistance and the potential of secondary metabolites from rare actinomycetes, isolated from unexplored habitats, to combat these challenges.

Results and Discussion

Antibiotics and resistance issues

Antibiotics have played a transformative role in modern society, significantly contributing to fields such as biotechnology, pharmacology, and industry, primarily by treating human microbial infections. These compounds are secondary metabolites produced by microorganisms and plants [6]. The discovery of antibiotics heavily relies on microbial secondary metabolites, with natural products being central to their development. Consequently, the search for new antibiotics has shifted toward rare genera of actinomycetes from conventional habitats or, more innovatively, to discovering novel isolates from unusual environments. The rationale behind this approach is that these isolates may yield new bioactive compounds. Untapped and unconventional environmental habitats are viewed as potential reservoirs of rare actinomycetes, which may produce new and potent antimicrobial agents [7]. Over the past few decades, efforts to isolate actinomycetes from traditional environments have resulted in the rediscovery of known compounds. To overcome this, pretreatment techniques are used to reduce the dominance of gram-negative bacteria, thereby allowing rare bacteria to thrive. Numerous actinomycete isolates, primarily obtained from soil, have been screened for novel secondary metabolites, leading to the depletion of some commercially viable antibiotics. Nonetheless, there is an urgent need to identify new antimicrobial agents from actinomycetes to address the growing resistance of pathogenic microbes in hospitals worldwide [8]. Additionally, there is an increasing demand for more efficient enzymes in the pharmaceutical industry [9, 10]. The development of antibiotics fueled by microbial secondary metabolites has encouraged the medical and scientific communities to intensify their efforts to discover new compounds for commercial antibiotic production, initiating the golden age of antibiotic discovery. This era led to the production of life-saving antibiotics, including streptomycin, vancomycin, and rifamycin [11].

The growing antibiotic resistance crisis presents a serious threat to human life and the entire medical sector, with several infectious diseases re-emerging as significant risks. In clinical settings, the antibiotics used today are derived from microbial secondary metabolites or their derivatives. However, the rise of microbial resistance mechanisms, primarily driven by the presence of resistance genes, has complicated the development of new antibiotics. These resistance genes, found in microbial environments, are easily transferred between microbes via mechanisms such as transformation, conjugation, and transduction [12]. The development of new antibiotics is a challenging and labor-intensive process, hindered by factors such as the rediscovery of existing compounds, low yields, high production costs, and concerns over the safety or stability of compounds. As a result, a new strategy for antibiotic discovery must focus on sourcing novel microorganisms with unique chemical and bioactive secondary metabolites, as well as adopting advanced scientific techniques for purifying and producing these compounds [12, 13].

Multidrug-resistant Enterobacteriaceae

The global rise of multidrug-resistant Enterobacteriaceae poses a significant health threat, with countries like Saudi Arabia facing an escalating number of resistant cases [14]. While regions such as Europe and the US have implemented comprehensive monitoring systems for antibiotic resistance, other parts of the world remain far behind in these efforts. Carbapenem resistance, in particular, is a critical issue worldwide. This resistance primarily affects Gram-negative bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii, either through intrinsic properties or by acquiring transferable carbapenemase-encoding genes [15]. The most widespread and effective carbapenemases include KPC, VIM, IMP, NDM, and OXA-48, all of which are responsible for the significant hydrolysis of carbapenem antibiotics [16].

Among these pathogens, *Klebsiella pneumoniae* is a leading cause of hospital-acquired infections, particularly among cancer patients, with a high mortality rate [17]. This pathogen causes a variety of infections, including

pneumonia, urinary tract infections, and bloodstream infections, with mortality rates reaching up to 50% in immunocompromised individuals. The challenge of treating carbapenem-resistant infections has made Klebsiella pneumoniae and similar bacteria a growing concern in healthcare environments, with increasing difficulty in managing these cases [15, 18, 19].

Carbapenems are a crucial treatment option for infections caused by bacteria resistant to other classes of antibiotics, owing to their broad-spectrum activity and stability. However, their overuse has led to the growing issue of carbapenem resistance in Gram-negative bacteria, including Enterobacterales, which now represent a significant public health threat [20]. Treatment of infections caused by carbapenem-resistant bacteria remains extremely challenging and is associated with higher mortality rates [21].

To prevent the further spread of antimicrobial resistance, continuous monitoring and molecular identification of resistance mechanisms are essential. These efforts will help avoid the widespread endemic presence of resistant bacteria in healthcare settings [22]. Over time, many bacteria have adapted to resist various antibiotics, and multidrug-resistant (MDR) organisms, which are resistant to at least one agent from three or more categories of antimicrobial drugs, have become more prevalent [23]. Infection control measures are essential to limit the transmission of these resistant pathogens within healthcare settings, and evidence-based practices must be applied consistently to minimize risks to patients, staff, and visitors.

Actinomycetes and their natural products

Actinomycetes are a diverse group of bacteria that include both filamentous and non-filamentous forms, characterized by their unique ability to produce a wide range of secondary metabolites. These gram-positive, aerobic bacteria have high guanine and cytosine content in their DNA, often exhibiting a G+C ratio of up to 80% [24]. Some species also demonstrate anaerobic respiration, such as Actinomyces, or parasitic relationships with plants and animals [25]. Actinomycetes are widely distributed in terrestrial and marine environments, often in symbiotic relationships with plants and animals. They are abundant in soil and marine ecosystems, with numbers varying according to the type of soil or marine habitat [24]. This group is renowned for its remarkable biosynthetic capabilities, producing a variety of secondary metabolites with significant biological activities.

Out of the approximately 22,000 known microbial secondary metabolites, actinomycetes are responsible for the production of about 70%, followed by fungi at 20%, and the genus *Bacillus* at 7% [7]. Actinomycetes are highly valued in biotechnology and industry due to their ability to synthesize a wide range of bioactive compounds, including antibiotics, as well as antitumor, immunosuppressive, antioxidant, and anti-inflammatory agents.

The actinobacteria phylum encompasses six classes, 18 orders, and 63 families, with numerous genera such as *Streptomyces*, *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora*, and *Actinoplanes*. The *Streptomyces* genus, in particular, holds significant importance due to its role in producing the majority of commercially important antibiotics [26, 27].

Rare Actinobacteria

The study of rare actinomycetes and non-*Streptomyces* Actinobacteria from unexplored environments has garnered significant interest recently [6]. These organisms, which often belong to unique genera other than *Streptomyces*, are less commonly isolated through standard methods. Multiple research efforts have been devoted to developing specialized techniques to obtain these rare strains from diverse locations. For example, Aly and El-Sabbagh [28] employed various approaches, including applying wet and dry heat, special media, and the inclusion of substances like phenol, antibiotics, and calcium carbonate to enhance growth conditions. Their study resulted in the isolation of 50 rare actinomycetes from Nile River sediments, which exhibited notable antifungal, antibacterial, and anticancer activity, as well as the production of numerous antibiotics. Other rare actinomycete genera, such as *Actinoplanes*, *Actinomadura*, *Microbispora*, *Micropolyspora*, *Microtetraspora*, *Mycobacterium*, *Nocardiopsis*, *Nocardia*, *Promicromonospora*, *Rhodococcus*, *Saccharomonospora*, *Saccharopolyspora*, *Streptosporangium*, *Thermoactinomyces*, *Thermomonospora*, and *Thermopolyspora*, have also been identified in Chinese lake sediments [29].

A group of antibiotics called lynamicins was discovered in *Marinispora* sp., a rare actinomycete, which showed effective antibacterial properties against various gram-positive and gram-negative bacteria, including MRSA and vancomycin-resistant *E. faecium*. Other rare genera like *Actinomadura*, *Isoptericola*, *Microbispora*, *Nocardia*, *Nonomuraea*, and *Rhodococcus* were isolated from mangrove soils and plants in China, and notably, Actinoalloteichus was found in solar salterns for the first time [6]. Numerous bioactive compounds have been

sourced from these genera, including *Nonomuraea*, *Actinoalloteichus*, *Pseudonocardia*, *Saccharothrix*, and *Actinosynnema* [6]. One such compound was isolated from *Nocardiopsis alba* found in mangrove soil, which demonstrated significant antioxidant activity. In Brazil, 14 new species of rare actinomycetes were found in mangrove sediments, belonging to genera such as *Brevibacterium*, *Dermabacter*, *Kytococcus*, *Microbacterium*, *Nesterenkonia*, and *Rothia* [7]. Furthermore, marine sediments, especially from great ocean depths, are highly regarded as rich sources of rare actinomycetes, with isolates found at depths of 5000 meters in the Atlantic Ocean [25].

A new species, *Streptomyces mangrovi* sp. nov., and another closely related to *S. albogriseolus* NRRL B-1305, were isolated from a mangrove soil sample in Haikou, China. Both isolates exhibited strong antimicrobial and antioxidant properties [30]. Bahamdain *et al*. [31] also documented rare actinomycetes from the Al-Lith Hot Spring Area in Saudi Arabia, showing moderate activity against several human bacterial pathogens.

These rare actinomycetes have been retrieved from extreme environments like deserts, caves, hot springs, marine ecosystems, and sandy soils. They are increasingly being investigated for their ability to produce valuable bioactive substances and natural products, particularly antibiotics. Actinomycetes from genera such as *Nocardiopsis*, *Micromonospora*, *Salinispora*, and *Pseudonocardia* have demonstrated the production of a wide range of antibiotics and other bioactive compounds [27]. For instance, Xinghaiamine A, extracted from *Streptomyces xinghaiensis*, exhibited antibacterial activity against *Acinetobacter baumannii* and *S. aureus*, as well as potent cytotoxic effects against cancer cell lines [30]. A review by Aly *et al*. [32] highlighted extreme habitats as untapped sources for discovering new actinomycetes with antimicrobial properties. Another study by Aly *et al*. [33] isolated *Streptomyces griseorubens* from the Saman Region Cave in Saudi Arabia, which showed significant antimicrobial activity against multidrug-resistant bacterial strains.

Secondary metabolites produced by Actinomycetes

Actinomycetes are known for producing volatile substances like geosmin and methyl iso-borneol, which contribute to the distinctive "earthy" smell [25]. These microorganisms are major producers of clinically important antibiotics, accounting for about two-thirds of the known antibiotics, out of a total of more than 10,000 identified compounds. Each strain of actinomycetes possesses the genetic potential to produce multiple secondary metabolites, with some strains capable of generating up to 20 distinct bioactive compounds [34]. These compounds are used for various medical purposes, including as antibiotics, anticancer agents, immune modulators, and treatments for tuberculosis [7]. **Table 1** lists some of the clinically significant antibiotics produced by *Streptomyces* and other rare actinomycete genera. Since the discovery of streptomycin in *Streptomyces griseus*, many other antibiotics have been developed, including carbapenems (e.g., cephalosporins), macrolides (e.g., erythromycin), ansamycins (e.g., rifampicin), glycopeptides (e.g., vancomycin), and tetracyclines (e.g., demelocyclin) [35]. Other commercially valuable antibiotics, such as daptomycin, lincomycin, isocoumarins, streptorubin B, pyrrole-2-carboxamide, acetyltryptamine, fervenulin, and neomycin, have also been discovered from *Streptomyces strains* [11]. Furthermore, anticancer drugs, such as anthracyclines (e.g., aclarubicin, daunomycin, doxorubicin), peptides (e.g., bleomycin, actinomycin D), aureolic acids (e.g., mithramycin), enediynes (e.g., neocarzinostatin), antimetabolites (e.g., pentostatin), and mitomycins, have been isolated from various actinomycete genera [34].

Table 1. Secondary metabolites produced by *Streptomyces* and rare actinomycetes

Isolate	Active material	Active isolate	Activity	References
Old *Streptomyces* genera	Axenomycins Aminoglycosides Dunaimycins Amphotericin B Cyclo (L-leucyl-l-prolyl) Chloramphenicol Tetracycline Musacin C	*Streptomyces lisandri Streptomyces diastatochromogenes* *Streptomyces nodosus* *Streptomyces* sp. KH614 *Streptomyces venezuelae* *Streptomyces aureofaciens Streptomyces griseovirdis*	Antibacterial	[36] [37] [38-40]
Recent *Streptomyces* genera	Asenjonamides A–C	*Streptomyces asenjonii*	Antibacterial	[41]
	Actinomycins V, X, and D	*Streptomyces antibioticus*	Antibacterial	[42]
	UND	*Streptomyces thermolilacinus*	Antibacterial	[43]

	Lucensomycin	*Streptomyces plumbeus*	Antifungal	[44]
Other *Streptomyces* genera	Gentamicin	*Micromonospora purpurea* var. *violaceae*	Antibacterial	[45]
	Rifamycin	*Amycolatopsis mediterranei*	Antibacterial	[46]
	Erythromycin	*Saccharopolyspora erythraea*	Antibacterial	[47]
	Lassomycin	*Lentzea kentukyensis*	Antituberculr	[48]
	Difluostatin A	*Micromonospora rosaria*	Antibacterial	[49]
	Salinosporamide A	*Salinispora tropica*	Anticancer, Antimalarial	[50]
	Atrop-abyssomicin C and proximicin A	*Verrucosispora maris*	Antitubercular, Antitumor	[51]

Actinomycetes in soil ecosystems

Actinomycetes are found abundantly in both terrestrial and aquatic ecosystems, thriving as free-living saprophytic bacteria within the soil. Certain strains are capable of living inside plant, insect, or aquatic animal tissues, while a few others can cause mild diseases in plants and animals [52]. These bacteria have been isolated from diverse environments such as alkaline soils, desert terrains, and even saltpan sand under snow-capped mountains [9]. In Egyptian soils, several strains have shown significant antimicrobial activity against a range of pathogens like *S. aureus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. typhi* [53]. Jeffrey [54] reported the isolation of 62 actinomycete strains from Sarawak's soil, with *Streptomyces atrovirens* being isolated from Korean soil [55]. *Actinomycetes* from various biotopes in Punjab, India, exhibited antimicrobial activity [26], and in Domang, India, 12 out of 26 isolates (47%) showed antifungal properties, while 6 isolates (23%) demonstrated antibacterial effects [56]. Additionally, three actinomycete isolates from a Mount Everest soil sample displayed broad-spectrum activity against both gram-positive and gram-negative bacteria [57]. In Pakistan's desert, Fatima *et al*. [58] discovered 110 actinomycete strains, some of which were active against MRSA.

Aquatic environments as habitats for Actinomycetes

Actinomycetes are present in aquatic habitats, where their population density varies with environmental conditions such as temperature, salinity, pH, and nutrient availability. These organisms are commonly found in freshwater bodies like rivers, lakes, and seas, potentially being transferred from the surrounding soil [59]. Micromonospora has been identified in the sediments of streams, rivers, and lakes [25]. Xu and Jiang [29] identified Micromonospora as the dominant actinomycete (39-89%) in 12 Chinese lakes, with *Streptomyces* as the 2nd most abundant genus. In Fetzara Lake, actinomycetes exhibited inhibitory activity against both gram-positive and gram-negative bacteria [60]. Poornima *et al*. [61] isolated promising actinomycetes from shrimp pond sediments [61]. Dias *et al*. [62] collected 238 bacterial isolates from the mangrove ecosystems of Ilha do Cardoso-Cananeia, Brazil, including actinomycetes, which produced enzymes of industrial relevance, such as amylase, lipase, esterase, and protease. In a study by Raja *et al*. [63], 17 marine actinobacteria strains were isolated from mangrove rhizosphere sediments, with three isolates, SSR-2, SSR-3, and SSR-10, proving to be effective inhibitors of pathogens. Girão *et al*. [64] isolated 90 actinomycete strains from *Laminaria ochroleuca* algae, half of which inhibited the growth of *C. albicans* and *S. aureus*, and one strain showed anticancer activity against human cancer cells.

Actinomycetes from extreme environments

Extreme environments present harsh conditions that differ drastically from those that support most living organisms [65]. These conditions are ideal habitats for actinomycetes, which have adapted to thrive in areas with extreme cold, high radiation, high salinity, and extreme pH levels. Organisms from such environments often produce bioactive compounds with remarkable stability, solubility, and bioavailability [66]. For example, strains such as *Streptomyces*, *Micromonospora*, *Saccharothrix*, *Streptosporangium*, and *Cellulomonas* have been found in the Qinghai-Tibet Plateau [67], and *Micromonospora*, *Actinomadura*, and *Nocardiopsis* have been isolated from the soda saline soils of Buryatiya's salty lakes [68]. Halophilic actinomycetes have been isolated from the hypersaline soils of Jeddah, Saudi Arabia, with *Streptomyces* being predominant in alkaline and saline water sources. One novel halophilic actinomycete, *Nocardiopsis terrae*, isolated from saline water, produced the antimicrobial agent quinoline, while Zhao *et al*. [69] identified actinopolysporins A, B, and C, novel polyketides with anticancer properties from *Actinopolyspora erythraea* [69]. Arid regions are home to unexplored

actinobacteria capable of surviving xerophilic, thermophilic, halophilic, and alkaliphilic conditions, and these strains may produce novel antibiotic metabolites [35, 66]. In regions of extreme heat, thermophilic actinomycetes such as *Streptomyces*, *Micromonospora*, *Actinomadura*, and *Streptosporangium* are found in large numbers. From xerophilic actinobacteria, compounds such as Ectoin and Hydroxyectoin, with industrial and agricultural applications, have been identified [35]. Cave habitats have also yielded 47 species across 30 genera of actinobacteria [10], and Zhang *et al*. [70] reported the isolation of *Nocardioides allogilvus* sp. nov. from a Chinese karst cave. Likewise, *Streptomyces* from remote karst soil in China produced the antibiotic Xiakemycin A [67]. Despite the cold limiting bacterial growth, *Streptomyces polaris* sp. nov. and *S. septentrionalis* sp. nov. were isolated from frozen Arctic soils. Furthermore, actinomycetes have been identified in volcanic and geothermal regions where temperatures can exceed 70 °C, such as desert soils, hot springs, volcanic eruptions, and thermal industrial waste sites, making these environments rich in thermophilic actinomycetes [31, 71].

Approaches to isolating rare actinomycetes

Actinomycetes exhibit slower growth rates compared to other soil bacteria [25], and their development can be suppressed by the presence of gram-negative bacteria. To enhance the recovery of rare actinomycetes, selective isolation techniques are required [72]. Employing specific growth media, incorporating inhibitors like antibiotics, and applying physical or chemical treatments to soil samples can help decrease the abundance of competing microorganisms and fungi, thus favoring the growth of rare actinobacterial species. The frequency at which rare actinomycetes are isolated is notably lower than that of *Streptomyces* strains, which are more readily retrieved using standard methods.

Physical and chemical pretreatment methods are both beneficial for isolating various actinobacterial species [10]. While physical pretreatment is typically sufficient for isolating common actinomycetes, a combination of physical and chemical approaches is often necessary to obtain species other than *Streptomyces* [70]. Physical pretreatments, such as air drying, moist heating, dry heating, and electromagnetic radiation, have proven effective in improving the isolation of actinomycetes [10, 70]. For example, air-drying soil at 120 °C for one hour is commonly used to isolate *Dactylosporangium*, *Microbispora*, and *Streptosporangium* [67]. Additionally, Micromonospora can be selectively isolated by heating soil samples at 55-65 °C for 30 minutes. In one experiment, drying soil at 45 °C for one hour was employed for isolating various rare actinomycetes, while moist heating proved useful in removing rapidly growing bacteria, allowing the proliferation of rare actinomycetes [73].

Chemical treatments, such as adding calcium carbonate, chitin, calcium chloride, sodium chloride, phenol, SDS, and chemotactic agents, have also been shown to enhance the isolation of rare actinobacterial genera [10, 72, 74]. For example, phenol treatment has been effective in isolating *Micromonospora* (49.18%), *Streptomyces*, *Actinomadura*, *Microbispora*, and *Polymorphospora*. Fang *et al*. [74] found that pre-treating soil from Sigangli Cave, China, with heat, varying pH levels, and calcium salt supplements helped isolate rare actinobacteria. In a similar study, *Actinomadura* and *Saccharopolyspora* were isolated from cave soil samples that underwent heat treatment and selective media [33]. Moreover, soil from the Cholistan Desert, Pakistan, was treated with heat (50-55 °C for 2-16 hours) and calcium carbonate (10:1 w/w) to facilitate the isolation of rare actinomycetes [58].

Conclusion

Actinomycetes, also referred to as filamentous microorganisms, possess a prokaryotic structure and exhibit distinct growth patterns, including powdery formations, mycelial structures on both the substrate and aerial surfaces, as well as sporangia and conidial chains. These gram-positive, aerobic bacteria have DNA content reaching up to 80%. They are predominantly found in soil and aquatic ecosystems, though they may also be associated with plants and animals. Actinomycetes are recognized for their unique characteristics and their exceptional capacity to produce a variety of secondary metabolites, including antibiotics, and compounds with therapeutic properties such as antitumor, immunosuppressive, antioxidant, and anti-inflammatory effects.

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