Jundishapur J Microbiol. 2026 July; 19(7): e168261 https://doi.org/10.5812/jjm-168261

Published Online: 2026 May 31 **Research Article**

# Antimicrobial Susceptibility of Oral Actinomycetes from HIV-Infected Patients

Aliakbar Bakhtiari [1], Golshid Javdani Shahedin [4], Reza H. Hosseini Doust [3], Ramin Mazaheri Nezhad Fard [2,*]

[1] Department of Microbiology, Islamic Azad Tehran Medical Sciences University, Tehran, Iran
[2] Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Microbiology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[4] Department of Quality Control, Production and Research Complex, Pasteur Institute of Iran, Tehran, Iran

[*] ***Corresponding Author:*** Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Email: r-mazaherinf@sina.tums.ac.ir

**Received:** 24 November, 2025; **Revised:** 2 April, 2026; **Accepted:** 24 April, 2026

## Abstract

**Background:** Actinomycetes can colonize the oral cavity of HIV-infected patients and may cause opportunistic infections. The use of commonly prescribed antimicrobials to treat these infections may contribute to the development of antimicrobial resistance (AMR).

**Objectives:** This study aimed to assess the antimicrobial susceptibility of actinomycetes isolated from the oral cavities of HIV-infected patients in Tehran, Iran. The susceptibility of the isolates to the essential oils (EOs) of thyme and ajwain was also evaluated.

**Methods:** Samples were previously collected from 200 HIV-infected participants and 85 healthy participants using oral swabs and inoculated into thioglycolate media. The isolated bacteria were characterized phenotypically and genotypically. The antimicrobial susceptibility of the isolates to 14 antimicrobials was then assessed. In addition, the susceptibility of the isolates to the EOs of thyme and ajwain was evaluated.

**Results:** Overall, actinomycetes were identified in 6.5% of HIV-infected patients and 3.5% of healthy controls. Most isolates were multidrug resistant (MDR) to amoxicillin, clindamycin, erythromycin, nalidixic acid, penicillin G, and tetracycline but were susceptible to gentamicin, ciprofloxacin, amikacin, linezolid, trimethoprim-sulfamethoxazole, and nitrofurantoin. All isolates were also susceptible to the herbal EOs.

**Conclusions:** The findings demonstrated that the Actinomycetes isolates were MDR to several antimicrobials, including some listed by the World Health Organization (WHO) as first-line agents for treating major bacterial infections. This study highlights the importance of early diagnosis and appropriate treatment of Actinomycetes-associated infections and further suggests that the EOs of thyme and ajwain may be considered therapeutic options alongside conventional antimicrobial regimens.

*Keywords:* Actinomycetes, Human Immunodeficiency Virus, Thyme, Ajwain, Antimicrobial Susceptibility Testing

## 1. Background

Based on cumulative statistics from the Iranian Ministry of Health during 1986 - 2011, 23,153 people with human immunodeficiency virus (HIV) were registered in Iran; of these, 91.5% were men, 8.5% were women, and nearly 46.5% were in the 25 - 34-year age group. Of these individuals, 3,053 later acquired acquired immunodeficiency syndrome (AIDS), and 4,311 died. Based on the epidemiological model of the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO), which uses a special formula for the "estimation of real cases based on recorded cases," 80,000 HIV-positive people are estimated to live in Iran. However, unofficial estimates suggest that this number may be as high as 120,000 (1, 2). Individuals with AIDS are highly susceptible to opportunistic infections, including those caused by



**How to Cite:** Bakhtiari A, Javdani Shahedin G, H. Hosseini Doust R, Mazaheri Nezhad Fard R. Antimicrobial Susceptibility of Oral Actinomycetes from HIV-Infected Patients. Jundishapur J Microbiol. 2026;19(7):e168261. doi: https://doi.org/10.5812/jjm-168261

actinomycetes, because HIV impairs humoral and cellular immune mechanisms.

Actinomycetes are prokaryotes that exist as free-living or saprophytic microorganisms and sometimes form symbiotic relationships with plants. These microorganisms can be isolated from various environmental ecosystems, such as soil, and constitute a significant component of the human body microflora. These bacteria include important genera such as *Actinomyces*, *Nocardia*, *Streptomyces*, and Micromonospora. Among these bacteria, *Streptomyces* is particularly important, accounting for nearly 60% of all actinomycetes. Genera within the actinomycetes, such as *Actinomyces* and *Nocardia*, can cause various infectious diseases, including actinomycosis, nocardiosis, granulomatosis, multiple abscesses, fibrosis, central nervous system infections, and disseminated infections. Furthermore, bacteria such as *Actinomyces* spp. can directly or indirectly cause oral and dental diseases (3, 4). However, treatment outcomes in patients with HIV are often suboptimal.

For example, the frequency of nocardiosis in HIV-positive patients has increased from 0.3% to 1.85%. Moreover, the adverse effects of current antimicrobials have raised serious public health concerns. Therefore, interest in herbal medicine for the treatment of infections in HIV-positive patients and patients with AIDS has increased in recent years. Thyme and ajwain are two widely studied medicinal herbs with antimicrobial properties. These herbs are widely used in Iranian cuisine and traditional medicine.

Thyme, or Shirazi thyme (*Zataria multiflora*), belongs to the order Lamiales and the family Lamiaceae and is primarily cultivated in Southwestern Asia. The dried, powdered leaves of the plant are commonly used as a spice for culinary, ornamental, and medicinal purposes (5). The major chemical constituents of thyme include various EOs, flavonoids, phenolic acids, and triterpenes. The EOs of thyme are primarily composed of thymol and carvacrol, which exhibit strong antimicrobial and anti-inflammatory activities. These flavonoids have antioxidant, anti-inflammatory, and antimicrobial properties (6). Other primary components of thyme include triterpenes, tannins, and saponins. These compounds have medicinal benefits (7).

Ajwain (*Trachyspermum ammi*), also known as ajowan, ajowan caraway, bishop's weed, carom, and thymol seeds, belongs to the order Apiales and the family Apiaceae (8) and is mostly cultivated in Asia (9). The plant leaves and seed-like fruits are used as spices for culinary, medicinal, and preservative purposes. The EO of ajwain fruits primarily contains thymol, γ-terpinene, p-cymene, and terpenoids with antimicrobial, anti-inflammatory, antifungal, and anti-*Trichophyton activities*.

## 2. Objectives

Given the importance of oral infections in HIV-infected patients and the lack of antimicrobial susceptibility schemes for oral actinomycetes in Iran, this study aimed to evaluate the susceptibility of actinomycetes isolated from the oral cavity of HIV-infected patients in Tehran, Iran, to 14 commonly used antimicrobial agents and two traditional herbal essential oils (EOs).

## 3. Methods

### 3.1. Samples

This study included 285 oral samples. Of these, 200 samples were collected from HIV-infected participants with CD4 lymphocyte counts of 200 cells $\mu L^{-1}$ or greater and no recent history of antimicrobial or antifungal use before sampling. In addition, 85 samples were collected from healthy participants who met similar inclusion criteria. Samples were previously collected from the oral cavities of the participants, including lesions, gums, tooth grooves, periodontal areas, and periapical areas, at the Southern Health Center, Tehran University of Medical Sciences, Tehran, Iran (unpublished data).

### 3.2. Isolation and Phenotypic Identification

Briefly, samples were cultured on blood agar (BA) and brain-heart infusion agar (BHIA) (Merck, Germany) and incubated aerobically and anaerobically using type-A gas packs at 37°C for at least 7 days in a shaker incubator. For *Nocardia* spp., isolates were subcultured onto paraffin agar and incubated for 14 days. Suspected colonies were then stained using the Gram and Ziehl-Neelsen methods. All isolates were further identified using biochemical assays and confirmed using molecular methods, including polymerase chain reaction (PCR) and nucleotide sequencing targeting 16S rRNA genes.

Amplification was performed using the universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-TAC GGG TAC CTT GTT ACG ACT T-3'). Edited sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank) under accession numbers PQ621735.1, PQ621739.1, PQ6333383.1, PQ634341.1, PQ634379.1, PQ634397.1, and PQ634819.1.

### 3.3. Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing (AST) of the isolates was performed against 14 commonly used antimicrobial agents, including amikacin, amoxicillin, amoxicillin-clavulanic acid (co-amoxiclav), ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, nalidixic acid, nitrofurantoin, penicillin G, rifampin, tetracycline, and trimethoprim-sulfamethoxazole (cotrimoxazole), as well as the EOs of two medicinal herbs, thyme and ajwain.

### 3.4. Kirby-Bauer Method

In this study, the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Merck, Germany) was used to assess the antimicrobial susceptibility of the isolates to the selected antimicrobials, based on guidelines from the Clinical and Laboratory Standards Institute (CLSI, USA). Antimicrobial disks were provided by Padtan Teb, Iran. Results were recorded as susceptible (S), intermediately resistant (I), or resistant (R), based on the literature and CLSI-reported zone-diameter breakpoints.

The CLSI document M24, Susceptibility Testing of Mycobacteria, *Nocardia*, and Other Aerobic Actinomycetes, provides standardized guidelines for susceptibility testing of *Nocardia* spp., primarily based on the broth microdilution method. However, standardized disk diffusion breakpoints are not available for several genera of actinomycetes in this study, such as *Streptomyces*, *Nocardiopsis*, and Saccharopolyspora. In the absence of specific breakpoints, interpretive criteria were adopted from available CLSI guidance, where applicable, and from previously published studies on aerobic actinomycetes. These interpretations were applied cautiously. Based on these susceptibility categorizations, MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial classes.

### 3.5. Agar Well Diffusion Method

First, reference strains of *Staphylococcus aureus* PTCC 1917, *Klebsiella pneumoniae* PTCC 1859, *Acinetobacter baumannii* PTCC 1919, and *Enterococcus faecium* PTCC 1821 were cultured on the surface of agar plates with 6 - 8-mm punched wells. A suspension of the isolated actinomycetes in brain-heart infusion (BHI) broth was then transferred into the wells. Plates were incubated at 37°C for 24 hours (2, 10).

### 3.6. Checkerboard Assay

The checkerboard assay was used to investigate possible synergistic or antagonistic effects of various combinations of antimicrobials with the herbal EOs. These interactions were assessed using the fractional inhibitory concentration (FIC) index, calculated as $FIC_A + FIC_B$ = FIC index. In general, FIC values of 0.5 or less indicated synergistic effects, values between 0.5 and 1 indicated additive effects, values between 1 and 4 indicated indifferent effects, and values of 4 or greater indicated antagonistic effects.

In this study, the EO of each herb, *Z. multiflora* and *T. ammi*, was assessed in various combinations with five antimicrobials: amikacin, ciprofloxacin, gentamicin, linezolid, and nalidixic acid. Therefore, 10 experimental groups, consisting of 5 antimicrobials × 2 EOs, were assessed in the checkerboard assay. Standard strains of *N. asteroides* and *S. griseus* were used as positive controls (11).

### 3.7. Preparation of the Essential Oils

In this study, standard EOs of *Z. multiflora* and *T. ammi* were purchased from Barij Essence, Iran. All EOs were provided with certificates of chemical analysis. Dimethyl sulfoxide (DMSO) was used as the solvent for the EOs. A series of solubility trials was conducted to determine the optimal concentrations for complete dissolution of the EOs in DMSO. Then, 10 µL of each *Z. multiflora* and *T. ammi* EO was transferred into sterile tubes, mixed with 900 µL of DMSO, and vortexed until a clear solution formed. These stock solutions were stored until use.

### 3.8. Cultivation of Bacteria on Culture Media

The isolated actinomycetes were cultured on Mueller-Hinton agar for 24 hours. Colonies were regularly assessed for purity to prevent contamination. The broth macrodilution technique was used to assess the minimum inhibitory concentration (MIC) of the EOs against the isolated actinomycetes.

### 3.9. Assessment of the Minimum Inhibitory Concentration of the Plant Essential Oils

First, a series of EO dilutions was prepared in 96-well microplates from a stock solution at a concentration of 0.99 µL $mL^{-1}$. Then, 100 µL of the bacterial suspension adjusted to the 0.5 McFarland standard was added to each well. Plates were incubated at 37°C for 24 hours, and the MICs of the EOs were assessed. The MIC was defined as the lowest concentration of EO in the first well with no visible turbidity.

### 3.10. Assessment of the Minimum Bactericidal Concentration of the Plant Essential Oils

To assess the minimum bactericidal concentration (MBC) of the EOs, 10-µL samples were collected from the wells immediately before and after the MIC well and subcultured onto BA plates. After 24 hours of incubation at 37°C, plates showing no visible bacterial growth were recorded. The MBC was defined as the lowest EO concentration that resulted in no growth on subcultures.

### 3.11. Assessment of the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Antimicrobials Against Actinomycetes

In this study, five antimicrobial agents, amikacin, ciprofloxacin, gentamicin, linezolid, and nalidixic acid, were used to assess their MICs and MBCs against the isolated actinomycetes. Briefly, bacterial suspensions were adjusted to the 0.5 McFarland standard and inoculated into 96-well microplates containing serial dilutions of the antimicrobials. The microplates were then incubated at 37°C for 24 hours, and the MIC and MBC of each antibiotic were calculated. To assess the MBC, 10 µL of the bacterial dilution from the well before the MIC well and 10 µL from the well after the MIC well were cultured on BA plates. After 24 hours of incubation at 37°C, plates were examined, and the antibiotic concentration without bacterial growth or colony production was reported as the MBC of the antibiotics for the isolated actinomycetes.

### 3.12. Statistical Analysis

In this case-control study, data were analyzed using SPSS version 26 (IBM, USA). The Shapiro-Wilk test was used to assess the normality of the distribution. Categorical variables were compared using the chi-square test or Fisher exact test, and continuous variables were analyzed using analysis of variance (ANOVA). A P value of ≤ 0.05 was considered statistically significant.

## 4. Results

The AST was performed on 19 Actinomycete isolates from HIV-infected participants, including 6 strains of *Streptomyces*, 6 strains of *N. farcinica*, 6 strains of *Nocardiopsis* (including 2 strains of *N. alba*), and 1 strain of Saccharopolyspora, as well as 3 isolates from healthy participants, including 1 strain each of *Streptomyces*, *N. flavescens*, and *Nocardiopsis*. These isolates were tested against 14 commonly used antimicrobial agents. The antimicrobials included amikacin, amoxicillin, amoxicillin-clavulanic acid, ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, nalidixic acid, nitrofurantoin, penicillin G, rifampin, tetracycline, and trimethoprim-sulfamethoxazole (cotrimoxazole) (Table 1).

As shown in Table 1, all isolates were MDR, exhibiting resistance to at least 5 antimicrobial agents. Specifically, 100% of the isolates were resistant to penicillin G, amoxicillin, erythromycin, tetracycline, and clindamycin. In contrast, consistent susceptibility was observed to nitrofurantoin, trimethoprim-sulfamethoxazole, linezolid, ciprofloxacin, and gentamicin, with all isolates classified as susceptible. Furthermore, amikacin demonstrated high efficacy, as all isolates were susceptible to this agent except for 2 isolates, *N. farcinica* and *Streptomyces* sp., which showed intermediate resistance. For nalidixic acid, most isolates were resistant; however, 2 exceptions, *Nocardiopsis alba* and *N. flavescens*, showed full susceptibility. Variable responses were observed for rifampin, as the isolates showed susceptibility, resistance, or intermediate resistance to the drug. Similarly, amoxicillin-clavulanic acid produced variable susceptibility patterns, with a few isolates susceptible and most others resistant.

The results of the well diffusion assay, in which microbial supernatants from 19 Actinomycete strains from HIV-positive patients were assessed against 4 pathogens, *S. aureus* PTCC 1917, *K. pneumoniae* PTCC 1859, *A. baumannii* PTCC 1919, and *E. faecium* PTCC 1821, revealed clear zones of growth inhibition on Mueller-Hinton agar. This finding indicated that microbial supernatants containing secreted metabolites had inhibitory activity against these pathogens. All 19 Actinomycete strains showed inhibitory activity against the pathogens, with the strongest effect consistently observed against *S. aureus* PTCC 1917. *Nocardiopsis alba* showed the strongest broad-spectrum activity, producing inhibition zones up to 25 mm against *S. aureus* PTCC 1917, whereas the weakest activity was generally observed against *E. faecium* PTCC 1821.

Using the checkerboard assay, investigation of the synergistic or antagonistic characteristics of the 5 selected antimicrobials, gentamicin, ciprofloxacin, amikacin, linezolid, and nalidixic acid, and the EOs, *Z. multiflora* and *T. ammi*, against 22 Actinomycete strains, including 19 from HIV-infected and 3 from healthy participants, revealed that most combinations were synergistic, whereas 3 showed additive interactions (FIC = 0.74) (Tables 2 and 3). In the checkerboard assay, significant differences in the mean FIC were reported among the 10 experimental groups using ANOVA ($F = 3.62$; $P = 0.00032$). In addition, the Tukey honestly

**Table 1.** Antimicrobial Susceptibility Test of the Actinomycetes Isolated from All Participants to 14 Antimicrobials Using the Disk Diffusion Method [a]

| Isolates | FM (25 µg) | SXT (25 µg) | AMC (20 µg) | LZ (30 µg) | CC (30 µg) | AN (30 µg) | CP (50 µg) | GM (15 µg) | NA (30 µg) | RA (5 µg) | TE (30 µg) | E (15 µg) | AMX (25 µg) | PEN (10 IU) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HIV-infected participants** | | | | | | | | | | | | | | |
| *Nocardia farcinica* | S | S | S | S | R | S | S | S | R | S | S | R | R | R |
| *Streptomyces* sp. | S | S | R | S | R | S | S | S | R | R | S | R | R | R |
| *Nocardia farcinica* | S | S | R | S | R | S | S | S | R | R | R | R | R | R |
| *Streptomyces* sp. | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Nocardia farcinica* | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Streptomyces* sp. | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Nocardiopsis alba* | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Streptomyces* sp. | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Nocardia farcinica* | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Nocardia farcinica* | S | S | R | S | R | S | S | S | R | R | R | R | R | R |
| *Nocardia farcinica* | S | S | R | S | R | I | S | S | R | R | R | R | R | R |
| *Streptomyces* sp. | S | S | R | S | R | I | S | S | R | R | R | R | R | R |
| *Nocardiopsis* sp. | S | S | R | S | R | S | S | S | R | R | R | R | R | R |
| *Nocardiopsis* sp. | S | S | R | S | R | S | S | S | R | R | R | R | R | R |
| *Nocardiopsis* sp. | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Streptomyces* sp. | S | S | S | S | R | S | S | S | R | S | R | R | R | R |
| Saccharopolyspora sp. | S | S | R | S | R | S | S | S | R | R | R | R | R | R |
| *Nocardiopsis* sp. | S | S | R | S | R | S | S | S | R | I | R | R | R | R |
| *Nocardiopsis alba* | S | S | S | S | R | S | S | S | S | I | R | R | R | R |
| **Healthy participants** | | | | | | | | | | | | | | |
| *Streptomyces* sp. | S | S | R | S | R | S | S | S | R | R | R | R | R | R |
| *Nocardia flavescens* | S | S | R | S | R | S | S | S | S | I | R | R | R | R |
| *Nocardiopsis* sp. | S | S | R | S | R | S | S | S | R | I | R | R | R | R |

[a] Abbreviations: FM, nitrofurantoin; SXT, trimethoprim-sulfamethoxazole; AMC, amoxicillin-clavulanic acid; LZ, linezolid; CC, clindamycin; AN, amikacin; CP, ciprofloxacin; GM, gentamicin; NA, nalidixic acid; RA, rifampin; TE, tetracycline; E, erythromycin; AMX, amoxicillin; PEN, penicillin G. All antimicrobial units are in micrograms. For nalidixic acid and nitrofurantoin, no basic values are specified in CLSI, and the specified values are based on the experiences of various researchers. Linezolid levels are not listed in CLSI.

significant difference test was used to more accurately identify significant differences between groups. Synergistic effects were observed for combinations of antimicrobials such as linezolid and amikacin with thyme. Other comparisons showed no statistically significant differences ($P > 0.05$). In this study, the separate effects of antimicrobials and EOs on FIC were significant ($P < 0.05$); however, the interactive effects of antimicrobials and EOs on FIC were not statistically significant ($P = 0.0813$). Furthermore, the Tukey honestly significant difference test was used for pairwise comparison of the mean FIC among the 5 antimicrobials, calculated as the mean of the combinations with the 2 EOs. The mean FIC of linezolid differed significantly from those of the other antimicrobials, including amikacin, ciprofloxacin, gentamicin, and nalidixic acid.

## 5. Discussion

In this study, the antimicrobial susceptibility of 19 Actinomycetes isolates from the oral cavity of HIV-infected participants and 3 additional isolates from healthy participants was assessed against various concentrations of 14 antimicrobials, including amikacin, amoxicillin, amoxicillin-clavulanic acid, ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, nalidixic acid, nitrofurantoin, penicillin G, rifampin, tetracycline, and trimethoprim-sulfamethoxazole, using the Kirby-Bauer method. Although nitrofurantoin is primarily used for urinary tract infections and is not routinely prescribed for systemic or oral infections, it was included to provide a broader AMR profile. Therefore, the reported susceptibility should be interpreted cautiously and does not necessarily imply clinical applicability for oral Actinomycetes infections. Furthermore, the MIC and MBC values of these isolates for 5 antimicrobials (amikacin, ciprofloxacin, gentamicin, linezolid, and nalidixic acid) were assessed.

**Table 2.** Summary of Fractional Inhibitory Concentration Values for the Combinations of Antimicrobials with *Z. Multiflora*

| No. | Isolate | Gentamicin | Ciprofloxacin | Linezolid | Amikacin | Nalidixic Acid |
|---|---|---|---|---|---|---|
| **1** | *Nocardia farcinica* | 0.5 | 0.5 | 0.5 | 0.749 | 0.495 |
| **2** | *Streptomyces* sp. | 0.496 | 0.496 | 0.497 | 0.5 | 0.498 |
| **3** | *Nocardia farcinica* | 0.5 | 0.5 | 0.498 | 0.499 | 0.495 |
| **4** | *Streptomyces* sp. | 0.5 | 0.5 | 0.497 | 0.5 | 0.499 |
| **5** | *Nocardia farcinica* | 0.479 | 0.479 | 0.497 | 0.499 | 0.490 |
| **6** | *Streptomyces* sp. | 0.5 | 0.5 | 0.498 | 0.499 | 0.495 |
| **7** | *Nocardiopsis alba* | 0.499 | 0.499 | 0.5 | 0.749 | 0.495 |
| **8** | *Streptomyces* sp. | 0.496 | 0.496 | 0.495 | 0.5 | 0.495 |
| **9** | *Nocardia farcinica* | 0.5 | 0.5 | 0.498 | 0.5 | 0.498 |
| **10** | *Nocardia farcinica* | 0.5 | 0.5 | 0.497 | 0.499 | 0.498 |
| **11** | *Nocardia farcinica* | 0.475 | 0.475 | 0.497 | 0.499 | 0.499 |
| **12** | *Streptomyces* sp. | 0.47 | 0.47 | 0.498 | 0.5 | 0.495 |
| **13** | *Nocardiopsis* sp. | 0.475 | 0.475 | 0.498 | 0.499 | 0.490 |
| **14** | *Nocardiopsis* sp. | 0.5 | 0.5 | 0.497 | 0.5 | 0.498 |
| **15** | *Nocardiopsis* sp. | 0.496 | 0.495 | 0.497 | 0.499 | 0.498 |
| **16** | *Streptomyces* sp. | 0.499 | 0.5 | 0.498 | 0.499 | 0.490 |
| **17** | Saccharopolyspora sp. | 0.499 | 0.498 | 0.5 | 0.748 | 0.498 |
| **18** | *Nocardiopsis* sp. | 0.48 | 0.475 | 0.497 | 0.5 | 0.490 |
| **19** | *Nocardiopsis alba* | 0.499 | 0.5 | 0.49 | 0.499 | 0.498 |
| **20** | *Streptomyces* sp. | 0.49 | 0.496 | 0.0498 | 0.499 | 0.490 |
| **21** | *Nocardia flavescens* | 0.499 | 0.5 | 0.499 | 0.499 | 0.495 |
| **22** | *Nocardiopsis* sp. | 0.5 | 0.5 | 0.497 | 0.499 | 0.490 |

The MIC and MBC values of the EOs of *Z. multiflora* and *T. ammi* against these Actinomycete isolates were also investigated. The bacterial isolates were exposed to each antimicrobial with various concentrations of *Z. multiflora* or *T. ammi* EOs, and FIC values were then calculated. The finding that all isolates were resistant to penicillin G, amoxicillin, erythromycin, tetracycline, and clindamycin should be interpreted carefully. Actinomycetes such as *Nocardia*, *Streptomyces*, and *Nocardiopsis* are known to naturally possess resistance mechanisms as part of their biological characteristics. Because several members of this group produce antimicrobial compounds, they commonly carry self-protection genes that confer resistance to various antimicrobial classes. Resistance to β-lactam antibiotics in these bacteria has been associated with β-lactamase production, structural characteristics of the cell wall, and alterations in penicillin-binding proteins. In addition, resistance to macrolides, lincosamides, and tetracyclines has been associated with ribosomal modification and efflux systems (12-16). Therefore, the high resistance rates observed in this study may partly reflect the intrinsic characteristics of these organisms rather than exclusively acquired resistance resulting from antimicrobial exposure.

Compared with previous reports on actinomycetes isolated from HIV-infected patients, the resistance profile in this study appears relatively high, particularly for β-lactams and macrolides. Similar resistance patterns have been reported in clinical *Nocardia* isolates from Europe and Asia, where high resistance to penicillin and erythromycin has been documented. However, the consistent susceptibility to amikacin, linezolid, and trimethoprim-sulfamethoxazole in this study is consistent with global reports, in which these agents remain effective treatment options. These similarities suggest that the observed resistance profile may reflect intrinsic resistance mechanisms and global antimicrobial use patterns rather than a purely local phenomenon (10, 12, 15).

In a 6-month study by Eshraghi et al. (17) on 100 patients with periodontal infections who showed signs of gingivitis and periodontitis, isolates of *A. viscosus* and *A. naeslundii* were identified. In a study by Abtahi et al. in Arak, Iran, in 2019, the prevalence of nocardiosis in patients with pulmonary infections was investigated, and an infection rate of 4.32% was reported (18). In a 2018 study by Khatibi et al. (19) on the pathogenicity of filamentous bacteria in denture-associated stomatitis, a condition affecting 24% - 60% of denture wearers, the primary causative agents were identified as *Candida*

**Table 3.** Summary of Fractional Inhibitory Concentration Values for the Combinations of Antimicrobials with *T. Ammi*

| No. | Isolate | Gentamicin | Ciprofloxacin | Linezolid | Amikacin | Nalidixic Acid |
|---|---|---|---|---|---|---|
| 1 | *Nocardia farcinica* | 0.499 | 0.499 | 0.0627 | 0.312 | 0.364 |
| 2 | *Streptomyces* sp. | 0.495 | 0.495 | 0.377 | 0.499 | 0.497 |
| 3 | *Nocardia farcinica* | 0.499 | 0.499 | 0.497 | 0.498 | 0.364 |
| 4 | *Streptomyces* sp. | 0.499 | 0.499 | 0.497 | 0.499 | 0.498 |
| 5 | *Nocardia farcinica* | 0.48 | 0.48 | 0.0377 | 0.498 | 0.5 |
| 6 | *Streptomyces* sp. | 0.499 | 0.499 | 0.497 | 0.498 | 0.364 |
| 7 | *Nocardiopsis alba* | 0.499 | 0.499 | 0.499 | 0.312 | 0.364 |
| 8 | *Streptomyces* sp. | 0.495 | 0.495 | 0.495 | 0.499 | 0.497 |
| 9 | *Nocardia farcinica* | 0.499 | 0.499 | 0.497 | 0.499 | 0.497 |
| 10 | *Nocardia farcinica* | 0.499 | 0.499 | 0.377 | 0.498 | 0.497 |
| 11 | *Nocardia farcinica* | 0.48 | 0.48 | 0.377 | 0.498 | 0.498 |
| 12 | *Streptomyces* sp. | 0.48 | 0.48 | 0.497 | 0.499 | 0.364 |
| 13 | *Nocardiopsis* sp. | 0.48 | 0.48 | 0.497 | 0.498 | 0.5 |
| 14 | *Nocardiopsis* sp. | 0.499 | 0.499 | 0.377 | 0.499 | 0.497 |
| 15 | *Nocardiopsis* sp. | 0.496 | 0.496 | 0.377 | 0.498 | 0.497 |
| 16 | *Streptomyces* sp. | 0.499 | 0.499 | 0.497 | 0.498 | 0.364 |
| 17 | Saccharopolyspora sp. | 0.499 | 0.499 | 0.499 | 0.312 | 0.497 |
| 18 | *Nocardiopsis* sp. | 0.48 | 0.48 | 0.377 | 0.499 | 0.5 |
| 19 | *Nocardiopsis alba* | 0.499 | 0.499 | 0.489 | 0.498 | 0.497 |
| 20 | *Streptomyces* sp. | 0.49 | 0.49 | 0.497 | 0.498 | 0.5 |
| 21 | *Nocardia flavescens* | 0.499 | 0.499 | 0.498 | 0.498 | 0.364 |
| 22 | *Nocardiopsis* sp. | 0.5 | 0.5 | 0.496 | 0.498 | 0.364 |

*albicans* and filamentous bacteria, particularly *Actinomyces* spp. Results showed that actinomycetes were isolated from 5 of 15 control samples (33.3%) and 11 of 15 patient samples (73%). Findings from the study by Abbasian et al. (20) demonstrated that combined treatment with intravenous antimicrobials, such as imipenem, trimethoprim-sulfamethoxazole, and amikacin, along with oral medications, was effective in treating disseminated nocardiosis. Khoroushi et al. (21) reported that the MICs of bell pepper and eggplant skin were 250 mg $mL^{-1}$ for *Streptococcus mutans* and 125 mg $mL^{-1}$ for *S. sobrinus* and *S. sanguinis*. For eggplant caps, the MIC was 500 mg $mL^{-1}$ for *S. mutans* and *S. sobrinus* and 125 mg $mL^{-1}$ for *S. sanguinis*. Findings by Zakerbostanabad and colleagues indicated that the MIC and MBC of ginger extract against *Actinomyces* spp. were 0.02 and 0.04 mg $mL^{-1}$, respectively. Therefore, ginger extract could be used in the preparation of antimicrobial mouthwashes.

In this study, 19 actinomycete isolates from the oral cavity of HIV-infected participants and 3 actinomycete isolates from the oral cavity of control participants were exposed to pathogenic bacteria, including *S. aureus* PTCC 1917, *K. pneumoniae* PTCC 1859, *A. baumannii* PTCC 1919, and *E. faecium* PTCC 1821, to investigate their antimicrobial characteristics. The results showed that all 22 Actinomycetes isolates exhibited antimicrobial characteristics in the presence of pathogenic bacteria, producing clear zones of inhibition on Mueller-Hinton agar. Although this assay was not the primary aim of the study, it provided complementary insight into the antimicrobial potential of Actinomycetes isolates. In a study by Ebadi et al. (22), 52 Actinomycete isolates were characterized. Isolate 28 showed an Rf value similar to that of gentamicin, whereas isolates 4 and 34 showed Rf values similar to that of streptomycin. The 16S rRNA genes of the isolates were sequenced, revealing that isolate 28 shared 99.93% similarity with *S. youssoufiensis* and isolate 4 shared 99.93% similarity with *S. cyaneofuscatus*. Selvin et al. (23) investigated the role of *Streptomyces* Btl7 in the biosynthesis of antibacterial agents from Dendrilla nigra. These researchers reported an MIC of 44 µg $mL^{-1}$ and an MBC of 88 µg $mL^{-1}$.

In a study by Zakerbostanabad and colleagues on the antimicrobial characteristics of actinomycetes isolated from Iranian deserts against *S. aureus*, *K. pneumoniae*, *A. baumannii*, and *E. faecium*, 22 of 300 isolates showed antimicrobial activity against these bacteria. Isolates from the Lut Desert in Central Iran generally showed

better antimicrobial characteristics. Results of the study by Nasri et al. (24) demonstrated that, among 51 isolates of halophilic *Actinomyces* spp., 3 isolates produced active antimicrobial metabolites against *Bacillus cereus*, *S. aureus*, and *C. albicans*, whereas *Pseudomonas aeruginosa* and *Escherichia coli* were resistant to these metabolites. The isolated bacteria were identified as *S. flavidofoscus* HBUM 17405, *N. dansvili* OK-22, and *Actinomyces* Nd28. Soofiani and colleagues characterized *Streptomyces* isolates from the soil of East Azerbaijan Province, Iran, and found that 44 of 310 Actinomycete isolates showed antibacterial activity against *Shigella flexneri* ATCC 1290, *Listeria monocytogenes* ATCC 3390, *B. cereus* ATCC 1431, *E. coli* ATCC 1399, and *K. pneumoniae* ATCC 1234.

Another study by Ezeonwumelu et al. (25) was conducted on actinomycetes experimentally isolated from the oral cavity of HIV-positive patients. They reported that trimethoprim-sulfamethoxazole had the weakest inhibitory activity against *S. aureus* but was most effective against *E. coli* ATCC 2592 and *P. aeruginosa* ATCC 27853. A study by Bandari et al. (26) on cytotoxic compounds of *Actinomyces* from the Persian Gulf showed that 15 *Actinomyces* isolates produced protease enzymes, and the anticancer characteristics of the isolates against 2 malignant leukemia cell lines were demonstrated at high concentrations. In a study by Mirsonbol et al. (11) on the antimicrobial activity of *S. tendae* 944 against *B. cereus*, *P. aeruginosa*, *Salmonella Typhimurium*, *Proteus mirabilis*, *S. aureus*, and Micrococcus luteus using the agar well diffusion method, the findings revealed that *S. tendae* strain 944 had strong antimicrobial activity against all investigated pathogens.

In a 2003 study by Sweeney et al. (27), the antibacterial activity of linezolid was assessed against a range of bacterial strains, including vancomycin-susceptible and vancomycin-resistant *E. faecalis*, methicillin-susceptible and methicillin-resistant *S. aureus*, penicillin-susceptible and methicillin-resistant *E. faecalis*, penicillin-sensitive and methicillin-resistant *S. pneumoniae*, *E. coli*, and *K. pneumoniae*. Among 35 antimicrobials assessed in combination with linezolid, the FIC analysis revealed synergistic effects with 6 antibiotics, including amoxicillin, erythromycin, imipenem, sparfloxacin, teicoplanin, and tetracycline, whereas antagonistic effects were observed with ofloxacin and sparfloxacin. Detailed demographic characteristics are provided in the Supplementary Data (Supplementary Text S1). The lack of strict matching between the groups may represent a potential confounding factor. In addition, the lack of standardized disk diffusion breakpoints for several Actinomycetes genera represents a methodological limitation and may affect the interpretation of susceptibility categories.

### 5.1. Conclusions

Overall, AST of the isolated actinomycetes in this study revealed frequent AMR patterns among the bacterial isolates. Moreover, the MDR profiles of all Actinomycetes isolates highlighted possible difficulties or failures in the effective treatment of these infections. Notably, any delay or failure in the treatment of infections in patients with HIV/AIDS can result in death. Therefore, AST profiling of microbial infections in HIV-infected individuals can contribute to improved infection management and longer patient survival.

The findings also demonstrated that the isolates were MDR to various commonly used antimicrobials, including amoxicillin, clindamycin, erythromycin, nalidixic acid, penicillin G, and tetracycline. Some of these agents are listed by the WHO as first-line medicines for major bacterial infections. In addition, the reported AMR to previously effective antimicrobials such as co-amoxiclav increases concerns about the rapid AMR development of actinomycetes. In conclusion, the findings highlight the importance of early diagnosis and treatment of infections caused by actinomycetes and suggest that thyme and ajwain EOs may have potential as adjunctive agents. However, further in vivo and clinical studies are needed to verify their therapeutic applicability. Because this was the first study of its kind in Iran, further studies on the prevalence of actinomycetes in HIV-infected patients and the AST of these bacteria are strongly recommended.

## Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

## Footnotes

**AI Use Disclosure:** The authors declare that no generative AI tools were used in the creation of this article.

**Authors' Contribution:** All authors contributed to the study design, execution, data analysis, and manuscript preparation. All authors read and approved the final manuscript and take responsibility for its accuracy and integrity.

**Conflict of Interests Statement:** The authors do not declare any conflicts of interests for this study.

**Data Availability:** The data that support the findings of this study are available from the corresponding author upon reasonable request. Due to privacy and ethical restrictions related to HIV-positive participants, the data are not publicly available.

**Ethical Approval:** The current study was completely carried out based on the Declaration of Helsinki for ethical principles of medical research studies involving humans (https://www.wma.net/policies-post/wma-declaration-of-helsinki). This study was approved by the Ethics Committee of the Islamic Azad Medical Sciences University (approval no. IR.IAU.PS.REC.1402.385, issued 28/08/2023).

**Funding/Support:** No funding was received for this study.

**Informed Consent:** All the participants in this study (patients and controls) received information on the study objectives and benefits and were provided with written informed consent. All personal information of the participants was archived strictly confidential.

## References


1. Dehghan P, Mohammadi F, Javaheri MR, Nekoeian S. Identification of Candida species in the oral cavity of diabetic patients. *Current Medical Mycology*. 2016;**2**(2):1-0. [PubMed ID: 28681013]. [PubMed Central ID: PMC5490298]. https://doi.org/10.18869/acadpub.cmm.2.2.4.
2. Heidarian A, Dehghan P, Chadeganipour M, Tayeri K. Frequency of Candida species isolated from the oral cavity of HIV-infected patients referring to Behavioral Disease Counseling Center of Isfahan in 2017 - 2018. *Scientific Journal of Kurdistan University of Medical Sciences*. 2019;**24**(5):30-41. https://doi.org/10.29252/sjku.24.5.30.
3. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ. Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. *Clinical Microbiology Reviews*. 2006;**19**(2):259-82. [PubMed ID: 16614249]. [PubMed Central ID: PMC1471991]. https://doi.org/10.1128/CMR.19.2.259-282.2006.
4. Ferry T, Valour F, Karsenty J, Breton P, Gleizal A, Braun E, et al. Actinomycosis: etiology, clinical features, diagnosis, treatment and management. *Infection and Drug Resistance*. 2014:183. [PubMed ID: 25045274]. [PubMed Central ID: PMC4094581]. https://doi.org/10.2147/IDR.S39601.
5. Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F. Chemical composition of essential oils in Zataria multiflora Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. *Food and Chemical Toxicology*. 2010;**48**(6):1562-7. [PubMed ID: 20332011]. https://doi.org/10.1016/j.fct.2010.03.025.
6. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*. 2019;**18**(1):241-72. https://doi.org/10.1007/s11101-018-9591-z.
7. Waheed M, Hussain MB, Saeed F, Afzaal M, Ahmed A, Irfan R, et al. Phytochemical profiling and therapeutic potential of thyme (Thymus spp.): A medicinal herb. *Food Science and Nutrition*. 2024;**12**(12):9893-912. [PubMed ID: 39723027]. [PubMed Central ID: PMC11666979]. https://doi.org/10.1002/fsn3.4563.
8. Shahrajabian MH, Sun W, Cheng Q. Pharmaceutical benefits and multidimensional uses of ajwain (Trachyspermum ammi L.). *Pharmacogn Commun*. 2021;**11**(2):138-141. https://doi.org/10.5530/pc.2021.2.25.
9. Boskabady MH, Alitaneh S, Alavinezhad A. Carum copticum L.: a herbal medicine with various pharmacological effects. *BioMed Research International*. 2014;**2014**(1):569087-11. [PubMed ID: 25089273]. [PubMed Central ID: PMC4096002]. https://doi.org/10.1155/2014/569087.
10. Condas LAZ, Ribeiro MG, Muro MD, Vargas APCD, Matsuzawa T, Yazawa K, et al. Molecular identification and antimicrobial resistance pattern of seven clinical isolates of Nocardia spp. in Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*. 2015;**57**(3):251-6. [PubMed ID: 26200967]. [PubMed Central ID: PMC4544251]. https://doi.org/10.1590/S0036-46652015000300012.
11. Mirsonbol SZ, Issazadeh K, Zarrabi S, Mirpour M. Antimicrobial Activity of Streptomyces Tendae Strain 944 Isolated from the Eastern Regions of Gilan Province and Optimization of its Bioactive Compounds. *Journal of Knowledge and Health in Basic Medical Sciences*. 2019;**14**(2):38-46.
12. Hershko Y, Levytskyi K, Rannon E, Assous MV, Ken-Dror S, Amit S, et al. Phenotypic and genotypic analysis of antimicrobial resistance in Nocardia species. *Journal of Antimicrobial Chemotherapy*. 2023;**78**(9):2306-14. [PubMed ID: 37527397]. https://doi.org/10.1093/jac/dkad236.
13. M KR, C SD. Recent insights into actinobacteria research in antimicrobial resistance: a review. *Molecular Biology Reports*. 2025;**52**(1). 683. [PubMed ID: 40627029]. https://doi.org/10.1007/s11033-025-10797-5.
14. Yang S, Zhang W, Yang B, Feng X, Li Y, Li X, et al. Metagenomic evidence for antibiotic-associated actinomycetes in the Karamay Gobi region. *Frontiers in Microbiology*. 2024;**15**. 1330880. [PubMed ID: 38505550]. [PubMed Central ID: PMC10949947]. https://doi.org/10.3389/fmicb.2024.1330880.
15. Song Z, Du B, Yuan M, Shen J, Xu S, Guan W, et al. Genomic and phenotypic characterization of antimicrobial resistance in clinical Nocardia species isolates. *Frontiers in Cellular and Infection Microbiology*. 2025;**15**. 1672889. [PubMed ID: 41050760]. [PubMed Central ID: PMC12488653]. https://doi.org/10.3389/fcimb.2025.1672889.
16. Sartori M, Toppo S, Lavezzo E. Molecular resistance mechanisms to newly approved antibiotics (2017 - 2025) in WHO priority pathogens. *Frontiers in Microbiology*. 2025;**16**. 1719798. [PubMed ID: 41608681]. [PubMed Central ID: PMC12835396]. https://doi.org/10.3389/fmicb.2025.1719798.
17. Eshraghi S, Salari MH, Kadkhoda Z, Yaghmaei S. Isolation and characterization of oral Actinomyces strain from patients with periodontal disease. *J Dent Med Tehran Univ Med Sci*. 2001;**14**(3):21-9.
18. Abtahi H, Saffari M, Jourabchi A, Rafiei M. Pulmonary nocardiosis and its related factors in patients with pulmonary infection in Arak. *Feyz Journal of Kashan University of Medical Sciences*. 2003;**7**(3):87-91.
19. Khatibi M, zaker bostan abad S, Rahimi MK, semsar Asl Y. The relation between Actinomyces and denture stomatitis. *Journal of Research in Dental Sciences*. 2019;**16**(2):102-9. https://doi.org/10.29252/jrds.16.2.102.
20. Abbasian L, Dehghan Manshadi SA, Hassan Nezhad M, Masoumzadeh N, Ghaderkhani S, Keyvanfar A, et al. A Case Report of Disseminated Nocardiosis in a Patient with HIV Infection: Concurrent Liver, Pulmonary and Brain Involvements. *Archives of Clinical Infectious Diseases*. 2024;**19**(2). e140527. https://doi.org/10.5812/archcid-140527.

21. Khoroushi M, Khorasgani MR, Aliasghari A. Determination of minimum inhibitory concentration (MIC) of two plants extract on cariogenic streptococci. *Journal of Dental Medicine*. 2017;**30**(1):12-7.
22. Ebadi S. *Identification of antibiotic-producing Streptomyces species from Iranian soils using phenotypic and genotypic methods [master's thesis] [in Persian]*. Qazvin: Qazvin University of Medical Sciences; 2016.
23. Selvin J, Joseph S, Asha KRT, Manjusha WA, Sangeetha VS, Jayaseema DM, et al. Antibacterial potential of antagonistic Streptomyces sp. isolated from marine sponge Dendrilla nigra. *FEMS Microbiology Ecology*. 2004;**50**(2):117-22. [PubMed ID: 19712370]. https://doi.org/10.1016/j.femsec.2004.06.007.
24. Nasri MR, Baserisalehi M, Kurdtabar M. Characterization of Antimicrobial Metabolites Produced by Halophilic Actinomyces Isolated from Aran-Bidgol and Maharloo lakes. *Armaghane-danesh*. 2019;**24**(3):373-387.
25. Ezeonwumelu J, Ntale M, Kasozi K, Ogbonnia S, Tanayen J, Agwu E, et al. Resistance, Minimum Inhibitory and Bactericidal Concentration Profiles of Oral Bacteria from HIV/AIDS Patients in South Western Uganda. *British Journal of Medicine & Medical Research*. 2016;**18**(11):1-4. [PubMed ID: 27338010]. [PubMed Central ID: PMC4919687]. https://doi.org/10.9734/BJMMR/2016/28491.
26. Bandari Z, Mozamian E, Azarpira N. Investigating the cytotoxic effects of Persian Gulf marine Actinomycetes protease on blood cancer cell line. *Basic and Clinical Cancer Research*. 2016;**8**(4):3-14.
27. Sweeney MT, Zurenko GE. In vitro activities of linezolid combined with other antimicrobial agents against staphylococci, enterococci, pneumococci and selected gram-negative organisms. *Antimicrobial Agents and Chemotherapy*. 2003;**47**(6):1902-6. [PubMed ID: 12760865]. [PubMed Central ID: PMC155858]. https://doi.org/10.1128/AAC.47.6.1902-1906.2003.