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Clinical and Histopathological Assessment of Actinomycosis in Patients with Medication-Related Osteonecrosis of the Jaw

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Title Page

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Abstract

Objectives: This study aimed to evaluate the clinical characteristics, risk factors, and treatment outcomes of medication-related osteonecrosis of the jaw (MRONJ) in a large patient cohort, with a specific focus on the prevalence and impact of *Actinomyces* colonization.

Materials and Methods: Among 115 patients with a history of antiresorptive medication use who were followed between January 2012 and June 2014, 70 patients fulfilling the diagnostic criteria for MRONJ were included in this retrospective study. Demographic data, primary systemic diseases, medication-related variables, surgical interventions, adjunctive treatments, and treatment outcomes were analyzed. Histopathological and/or microbiological evaluations were performed to determine the presence of *Actinomyces* colonization in necrotic bone specimens. Treatment success was defined as complete mucosal healing and absence of infection at the 6-month follow-up.

Results: The cohort consisted of 42 females (60%) and 28 males (40%) with an overall mean age of 59.59 ± 13.1 years. Notably, the mean age of female patients (55.12 ± 13.28) was significantly lower than that of males (66.29 ± 9.31) (p=0.0001). Prostate cancer was the most frequent primary malignancy (31.4%), whereas lung cancer was the least common (4.3%). A statistically significant correlation was found between the presence of MRONJ and the duration of zoledronate therapy (p=0.045). *Actinomyces* colonization was identified in 21 patients (30%), and a significant relationship was observed between the primary oncological diagnosis and the incidence of *Actinomyces* (p=0.031), with the highest prevalence of *Actinomyces* colonies found in breast cancer patients (33.3%, n=7). Among the *Actinomyces*-positive group, 16 patients underwent surgical intervention (debridement or sequestrectomy) combined with long-term targeted antibiotic therapy, achieving a 71.4% success rate. Recurrence was observed in 2 patients who required secondary surgical procedures.

Conclusion: *Actinomyces* colonization appears to be a clinically relevant histopathological and microbiological finding in MRONJ, particularly in oncological patients. Although it was not

independently associated with most clinicopathological variables, its identification may support multidisciplinary treatment planning in patients with infected, persistent, or advanced lesions.

Clinical relevance:
Histopathological and microbiological evaluation for *Actinomyces* may support individualized and multidisciplinary management of MRONJ, especially in patients with persistent infection, delayed healing, or advanced disease.

Keywords: Bisphosphonates; Osteonecrosis of the Jaw; Actinomycosis; Oral Surgery; zoledronate.

Highlights

- In MRONJ lesions, exposed necrotic bone may harbor multiple bacterial colonies; however, *Actinomyces* species are among the most frequently reported microorganisms associated with necrotic jawbone specimens.
- The role of *Actinomyces* in MRONJ remains controversial: it may contribute directly to chronic infection and biofilm formation, or it may represent secondary colonization facilitated by systemic disease, impaired bone remodeling, and local tissue breakdown.
- Identification of *Actinomyces* may have clinical implications, as prolonged antimicrobial therapy combined with appropriate surgical management may support resolution in selected patients with actinomycotic osteonecrosis.
- This study evaluates the possible relationship between *Actinomyces* colonization and primary disease status, medication type, duration and route of administration, MRONJ stage, lesion localization, and treatment outcomes.

Introduction

Bisphosphonates and other antiresorptive agents are widely used in the management of osteoporosis, malignancy-related bone metastases, multiple myeloma, and other disorders affecting bone metabolism. By suppressing osteoclastic activity, these agents reduce bone resorption and contribute to the prevention of skeletal-related complications. However, medication-related osteonecrosis of the jaw (MRONJ), which may develop in association with antiresorptive and antiangiogenic therapies, is now recognized as one of the most challenging complications in oral and maxillofacial surgery. In recent years, the term “bisphosphonate-related osteonecrosis of the jaw” has largely been replaced by the broader term “medication-

related osteonecrosis of the jaw," reflecting the involvement not only of bisphosphonates but also of denosumab and several antiangiogenic agents in this clinical entity [1].

Although the pathogenesis of MRONJ has not yet been fully elucidated, it is considered a multifactorial condition involving suppressed bone remodeling, local infection, disruption of oral mucosal integrity, altered immune responses, dental interventions, poor oral hygiene, and microbial colonization. Local oral factors, particularly tooth extraction, prosthetic trauma, periodontal disease, and pre-existing infection, have been identified as important triggers for bone exposure and secondary infection. In a retrospective observational study, poor oral hygiene was detected in a substantial proportion of patients with MRONJ; however, whether poor oral hygiene represents a direct etiological factor or an accompanying condition that facilitates disease progression remains uncertain [2].

The role of microbial colonization in MRONJ lesions has attracted increasing attention in recent years. The oral cavity contains a complex microbial ecosystem composed of numerous bacterial species, and disruption of this ecological balance may contribute to biofilm formation, particularly on exposed necrotic bone surfaces. Current microbiological evidence suggests that MRONJ should not be regarded solely as a sterile necrotic process related to impaired bone metabolism. Rather, in many cases, MRONJ lesions are accompanied by polymicrobial infection, anaerobic bacteria, and particularly *Actinomyces* species [3]. Therefore, histopathological and microbiological assessments are important complements to clinical and radiological evaluation in the comprehensive diagnosis and management of MRONJ.

Actinomyces species are gram-positive, filamentous, anaerobic or microaerophilic bacteria that may be present as commensals in the normal oral flora. Under physiological conditions, they exhibit low pathogenicity; however, they may become pathogenic when mucosal barriers are disrupted, local tissue resistance is reduced, or necrotic bone surfaces are exposed. For this reason, *Actinomyces* colonies in MRONJ lesions may represent not only secondary colonization but also a biologically relevant microbial component capable of contributing to chronic inflammation and delayed healing. In a large retrospective study, *Actinomyces* colonies were reported to be significantly more frequent in MRONJ cases than in non-medication-related osteonecrosis of the jaw [4].

Kivelä-Rajamäki et al. compared 98 cases of MRONJ with 93 cases of non-medication-related osteonecrosis and found that *Actinomyces* colonies were detected in 85% of MRONJ cases and

51% of non-medication-related osteonecrosis cases. The same study reported that *Actinomyces* colonies were predominantly associated with necrotic bone surfaces and that acute inflammation was more prominent in MRONJ tissues. These findings suggest that the presence of *Actinomyces* may not merely reflect incidental contamination, but may have clinical relevance in biofilm formation and in the maintenance of the inflammatory response on necrotic bone surfaces [4].

Histopathological studies have also provided important evidence regarding the relationship between MRONJ and actinomycosis. In a 22-case MRONJ series published by Santana et al. in 2025, osteomyelitis was identified in 82% of cases, whereas actinomycosis was detected in 36.4%. The authors further reported that MRONJ most commonly affected the mandible and was characterized histopathologically by necrotic lamellar bone, empty osteocyte lacunae, and microbial colonization [5]. These findings underline the value of histopathological examination not only for confirming the diagnosis of MRONJ but also for identifying associated microbial pathologies that may influence clinical management.

Nevertheless, whether *Actinomyces* plays a primary role in MRONJ pathogenesis or represents secondary colonization of already necrotic bone remains a matter of debate. In a study comparing MRONJ with non-medication-related osteonecrosis of the jaw from clinical and radiological perspectives, the presence of *Actinomyces* colonies was not found to be an independent predictor of recurrence or healing status [6]. This suggests that *Actinomyces* alone may not fully explain the pathogenesis or clinical course of MRONJ; however, it remains an important factor within the microbial and inflammatory microenvironment of the disease.

In this context, evaluating *Actinomyces* colonization in patients with MRONJ may provide valuable insights into disease pathogenesis, clinical progression, and treatment response. In particular, clarifying the relationships between primary systemic disease, type of medication, route and duration of drug administration, MRONJ stage, lesion localization, and histopathological evidence of actinomycosis may contribute to more individualized clinical management. Therefore, the aim of the present study was to retrospectively evaluate the histopathological presence of *Actinomyces* in patients who developed osteonecrosis of the jaw following antiresorptive therapy and to investigate the association between actinomycosis and clinicodemographic variables, medication-related factors, lesion stage, and anatomical localization.

Materials and Methods

Study Design and Ethical Approval

This retrospective observational study was conducted in accordance with the ethical approval obtained from the Ethics Committee of Hacettepe University Faculty of Medicine, dated January 21, 2015, with the approval number GO 15/03. The study was based exclusively on previously recorded clinical files, radiological data, histopathological reports, microbiological records, and infectious disease consultation notes. No new intervention was performed for the purpose of this study. All procedures were conducted in accordance with the principles of the Declaration of Helsinki and its later amendments. As this was a retrospective study based on existing patient records, no additional patient intervention was performed for study purposes. Written informed consent was obtained from all patients during routine clinical follow-up and treatment procedures. The consent included permission for the use of anonymized clinical, radiological, histopathological, microbiological, and treatment-related data for future scientific research and publication purposes.

Patient Selection and Data Collection

A total of 115 patients with a history of antiresorptive medication use were screened. Among these patients, 70 who met the diagnostic criteria for MRONJ were included in the final analysis. Of these 70 patients, 21 were identified as having actinomycosis based on histopathological and/or microbiological examination.

The following variables were recorded from patient files and MRONJ follow-up forms: age, sex, systemic disease, underlying oncological, rheumatological, or osteoporotic condition, history of chemotherapy or radiotherapy, type and name of antiresorptive medication, route of administration, duration of drug exposure, MRONJ stage, lesion localization, presence of pathological fracture, histopathological evidence of actinomycosis, presence of other microorganisms, and treatment protocols applied for actinomycosis.

Clinical and Radiological Assessment

All patients underwent detailed medical and dental history taking, followed by clinical examination. Symptoms, clinical findings, and dental or surgical procedures were recorded using standardized follow-up forms prepared for patients receiving antiresorptive therapy.

Patients were classified according to the staging system proposed by the American Association of Oral and Maxillofacial Surgeons for medication-related osteonecrosis of the jaw [7]. Patients without exposed necrotic bone but with nonspecific clinical or radiological findings were categorized as Stage 0. Patients with exposed necrotic bone without clinical evidence of infection were classified as Stage 1. Patients with exposed necrotic bone accompanied by pain, edema, or signs of infection were classified as Stage 2. Cases presenting with advanced findings such as osteolysis extending beyond the alveolar bone, pathological fracture, or extraoral fistula were classified as Stage 3.

All patients were evaluated using panoramic radiography. Computed tomography was additionally performed in patients requiring detailed assessment of sequestrum margins, suspected pathological fracture, or persistent infection. Radiological findings were used to support clinical staging and to guide treatment planning.

Surgical Approach and Biopsy Procedure

Treatment decisions were made according to MRONJ stage, clinical presentation, radiological findings, and the presence of infection. Patients were managed with follow-up, biopsy, debridement, or sequestrectomy when indicated. Before dental procedures, patients were consulted with the relevant medical departments according to their systemic conditions. In accordance with consultation recommendations, tooth extractions were performed under antibiotic prophylaxis when necessary, and primary closure was achieved whenever possible.

In patients scheduled for debridement, sequestrum borders were first assessed radiologically. When sequestrum margins were clearly defined, debridement and biopsy were performed during the same surgical session. In cases with unclear sequestrum borders or acute infection, antibiotic therapy was first administered, and deep bone biopsy was performed after infection control had been achieved. In patients presenting with acute infection, antibiotic therapy was administered before definitive surgical intervention according to clinical need. Therefore, pre-biopsy antimicrobial exposure was not fully standardized in all patients. However, the presence

of *Actinomyces* was assessed not only by microbiological culture but also by histopathological identification of characteristic bacterial colonies.

During biopsy, the mucosa was elevated and superficial necrotic bone was removed. Deep bone samples were then obtained using sterile surgical instruments. Specimens collected for histopathological examination were placed in pathology containers containing 10% formaldehyde. In patients undergoing sequestrectomy, the removed sequestrum was sent for pathological examination, while samples obtained from the underlying deep bone were submitted for anaerobic culture and direct microscopic evaluation.

Histopathological Evaluation

All histopathological examinations were performed in the Department of Medical Pathology, Faculty of Medicine, Hacettepe University. The specimens were first examined macroscopically in terms of size, shape, and surface characteristics. They were then fixed in 10% buffered formaldehyde solution and decalcified in 10% formic acid solution until the bone tissue was sufficiently softened.

After routine tissue processing, 4-µm-thick sections were obtained from paraffin blocks. The sections were stained with hematoxylin and eosin and examined under light microscopy. When necessary, additional Periodic acid–Schiff, Grocott methenamine silver, and Gram staining methods were used to better visualize *Actinomyces* colonies [8].Histopathological evaluation focused on the presence of osteonecrosis, neutrophilic infiltration, and *Actinomyces* colonies. Globoid bacterial colonies composed of filamentous rod-like structures with a radiating arrangement within bone tissue, occasionally accompanied by sulfur granules, were considered compatible with actinomycosis (Fig. 1,2,3,4,5)

Infectious Disease Consultation and Antibiotic Therapy

Patients with histopathological or microbiological evidence of actinomycosis were referred to the Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Hacettepe University. Detailed systemic examinations and medical history assessments were performed, and patients were evaluated clinically and through laboratory parameters for evidence of infection.

During follow-up, complete blood count, erythrocyte sedimentation rate, C-reactive protein, liver function tests, and renal function tests were monitored regularly. Patients with advanced disease requiring parenteral antibiotic therapy were hospitalized. These patients received intravenous ampicillin–sulbactam therapy for at least two weeks. According to clinical and laboratory response, patients were switched to oral antibiotic therapy after an average of approximately three weeks of parenteral treatment.

Oral antibiotic therapy was planned with amoxicillin–clavulanic acid, and the treatment duration was generally approximately six months. The decision to discontinue antibiotic therapy was made jointly by the oral and maxillofacial surgery and infectious diseases departments. Patients without actinomycosis or other microbial colonization were followed with standard antibiotic therapy and subsequently included in routine clinical follow-up after clinical improvement.

Statistical Analysis

Descriptive statistics were used to summarize the data. Continuous variables were expressed as mean ± standard deviation or median with minimum–maximum values, depending on the distribution of the data. Categorical variables were presented as numbers and percentages. The normality of continuous variables was assessed using the Shapiro–Wilk test. Normally distributed continuous variables were compared using the independent samples t-test, whereas non-normally distributed continuous variables were compared using the Mann–Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Fisher's exact test was preferred when the expected cell count was less than 5. A p-value of <0.05 was considered statistically significant.

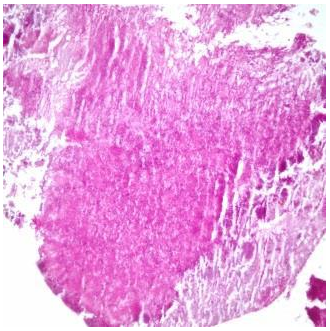


Figure 1. *Actinomyces* colonies. Globoid, purple-colored bacterial colonies with a fibrillar appearance are characteristic of *Actinomyces* colonies (H&E, ×400).

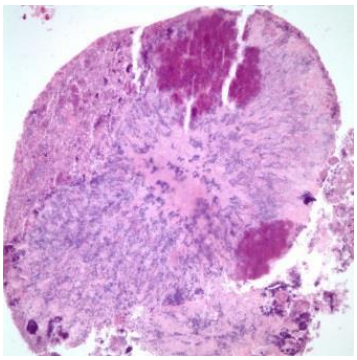


Figure 2. *Actinomyces* colonies highlighted by Periodic acid–Schiff staining. *Actinomyces* colonies are accentuated by magenta staining with Periodic acid–Schiff (PAS, ×400).

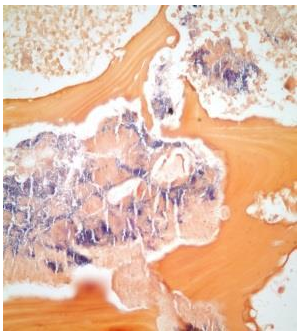


Figure 3. *Actinomyces* colonies observed with Gram staining. *Actinomyces* colonies are visualized using Gram staining (×400).

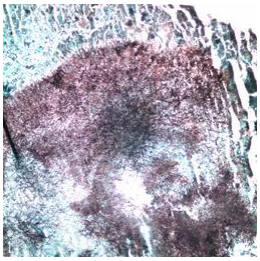


Figure 4. *Actinomyces* colonies stained with Grocott methenamine silver. *Actinomyces* colonies appear black with Grocott methenamine silver (GMS) staining.

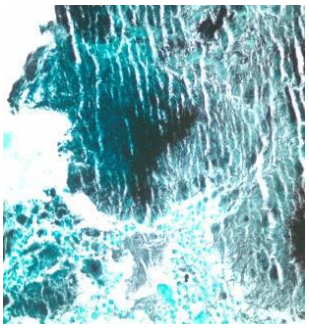


Figure 5. More prominent fibrillar appearance of *Actinomyces* colonies. The fibrillar structure of *Actinomyces* colonies becomes more clearly visible with Grocott methenamine silver staining (GMS, ×400).

Results

After reviewing the follow-up forms and radiological records of 115 patients included in the study, 70 patients who met the diagnostic criteria were diagnosed with MRONJ.

Table 1. Distribution of Patients by Sex and Age Analysis

Sex	**Number (n)**	**Percentage (%)**	**Age Range (Min–Max)**	**Mean Age (±SD)**	**p**
Female	42	60	22–80	55.12 ± 13.28	**0.0001***
Male	28	40	47–82	66.29 ± 9.31	
Total	70	100	22–82	59.59 ± 13.1	

- $p < 0.05$

In the cohort of 70 patients diagnosed with MRONJ, the female population [42 patients (60%)] was higher than the male population [28 patients (40%)]. Statistical analysis revealed that the mean age of female patients (55.12 ± 13.28 years) was significantly lower than that of male patients (66.29 ± 9.31 years) (p=0.0001),(Table 1; Fig. 6).

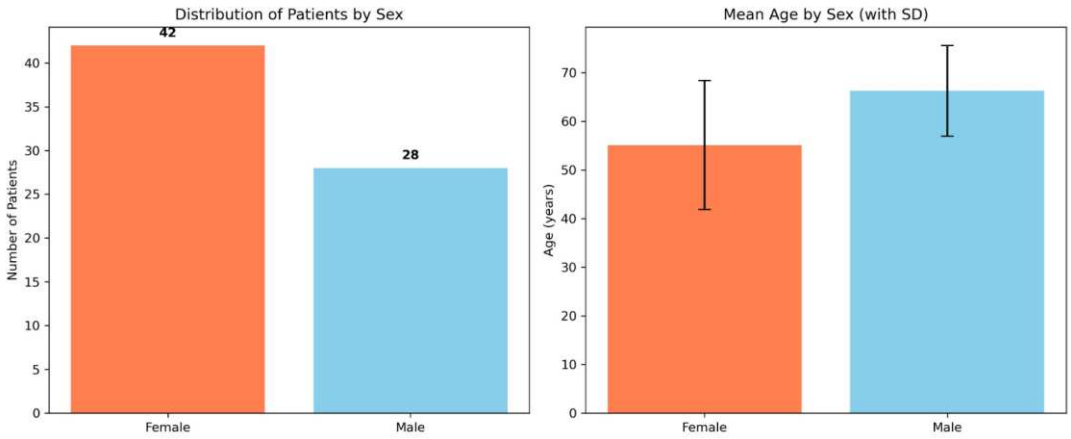


Figure 6. Distribution of Patients by Sex and Age Analysis

Table 2. Distribution of Patients According to the Type of Medication Used

Medication Type / Active Ingredient	**Route of Administration**	**Number (n)**	**Percentage (%)**
Zoledronate	IV	67	95.7
Alendronate	Oral	2	2.9
Ibandronate	Oral	1	1.4
Total		70	100

**Relationship between duration of zoledronate use and the presence of MRONJ; $p < 0.05$.*

The majority of patients in the study group [95.7% (n=67)] were receiving zoledronate, a third-generation intravenous nitrogen-containing antiresorptive agent. The number of patients using oral agents was considerably low, with 2 patients (2.9%) receiving alendronate and 1 patient (1.4%) receiving ibandronate (Table 2; Fig. 7).

Table 3. Duration of Zoledronate Use According to MRONJ Status

MRONJ status	Number of patients (n)	Duration of zoledronate use, months median (min–max)	p-value
Present	70	24 (4–180)	**0.045***
Absent	45	24 (1–72)	

Statistical analysis showed a significant relationship between the presence of MRONJ and the duration of zoledronate use, including the median exposure interval (p=0.045). This finding suggests that the risk of osteonecrosis increases significantly with longer drug exposure(Table 3).

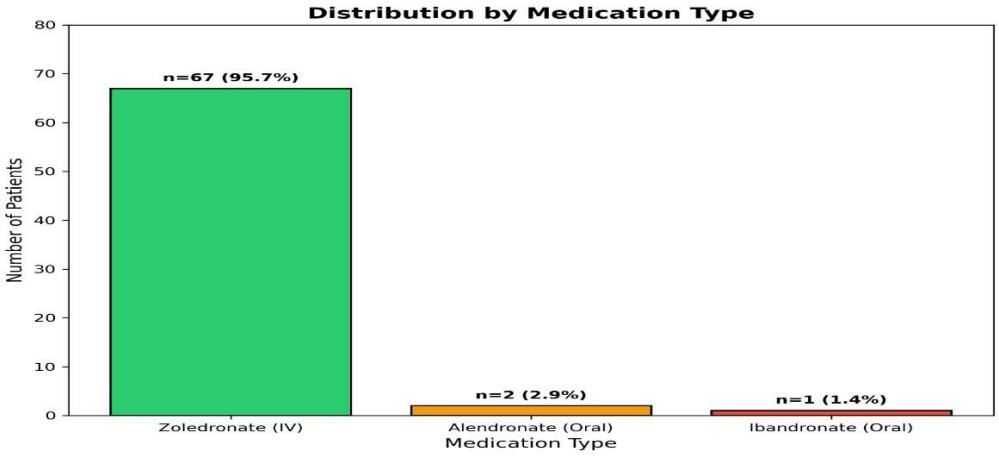


Figure 7. Distribution of Patients According to the Type of Medication Used

Table 4. Distribution of Primary Diagnosis and Its Relationship with *Actinomyces* Colonization

Primary Disease	**Number of Patients**	**Percentage (%)**	***Actinomyces* (+)**	**%**	***Actinomyces* (–)**	**%**	**p-value**
Prostate cancer	22	31.4	6	28.6	16	32.7	
Breast cancer	19	27.1	7	33.3	12	24.5	
Multiple myeloma	7	10	0	0	7	100	**0.031***
Osteoporosis	7	10	1	4.8	6	12.2	
Renal cancer	5	7.1	2	9.5	3	6.1	
Lung cancer	3	4.3	0	0	3	6.1	
Other	7	10	5	23.8	2	4.1	
Total	70	100	21	100	49	100	

- $p < 0.05$

Among the followed patients, MRONJ was most frequently observed in patients with prostate cancer as the primary disease [22 patients (31.4%)] and least frequently in patients with lung cancer [3 patients (4.3%)]. Pathological and microbiological examinations revealed *Actinomyces* colonization in 21 of the 70 patients with MRONJ.

Among MRONJ patients with *Actinomyces* colonization, the highest frequency was observed in patients with breast cancer [7 patients (33.3%)]. No *Actinomyces* growth was detected in patients diagnosed with multiple myeloma or lung cancer. A statistically significant relationship was found between oncological diagnosis and the frequency of *Actinomyces* colonization (p=0.031).(Table 4; Fig. 8)

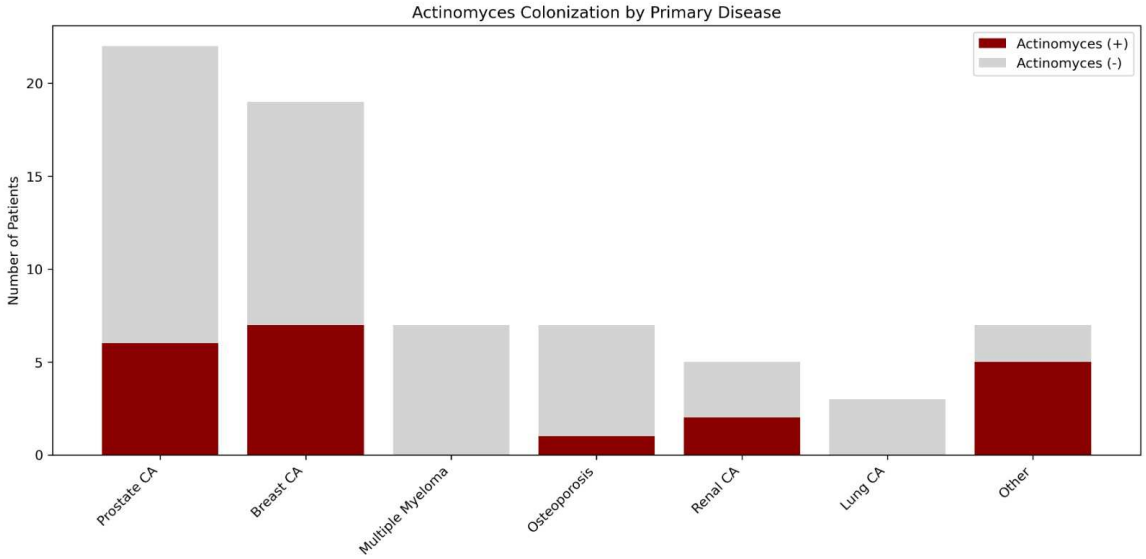


Figure 8. Distribution of Primary Diagnosis and Its Relationship with *Actinomyces* Colonization

Table 5. Relationship Between Route of Medication Administration and *Actinomyces*

Route of Administration	**Number of Patients**	***Actinomyces* (+)**	**Percentage (%)**	**p-value**
IV	67	18	85.7	0.402
Oral	3	3	14.3	
Total	70	21	100	

Of the 21 patients with *Actinomyces* colonization, 18 patients (85.7%) had received IV medication and 3 patients (14.3%) had received oral medication. Among the 67 patients who received IV medication, *Actinomyces* was detected in 18 patients. No statistically significant relationship was found between the route of administration and the presence of *Actinomyces* (p=0.402).(Table 5; Fig. 9)

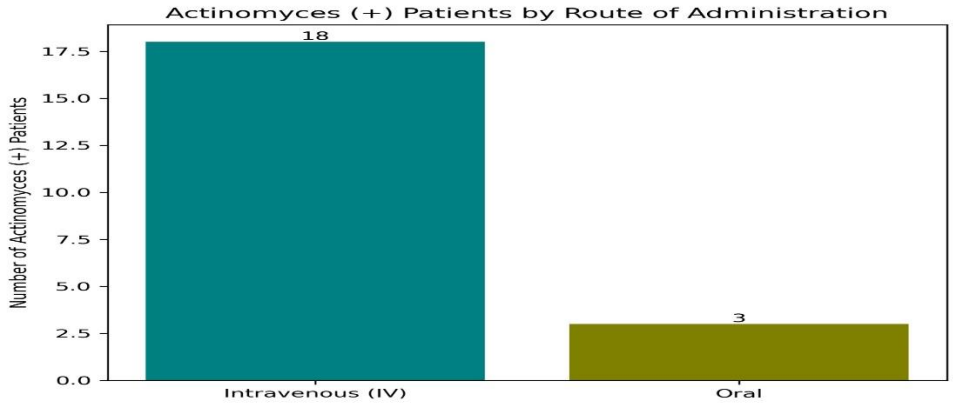


Figure 9. Relationship Between Route of Medication Administration and *Actinomyces*,

Table 6. Relationship Between Medication Type and *Actinomyces* in Patients with MRONJ

Medication Type	**Number of Patients**	**Percentage (%)**	***Actinomyces* (+)**	**%**	***Actinomyces* (–)**	**%**	**p-value**
Zoledronate	67	95.7	21	100	46	93.9	0.549
Alendronate	2	2.9	0	0	2	4.1	
Ibandronate	1	1.4	0	0	1	2.0	
Total	70	100	21	100	49	100	

Actinomyces colonies were observed only in patients receiving zoledronate. No *Actinomyces* colonization was detected in patients receiving alendronate or ibandronate. No statistically significant relationship was found between medication type and the frequency of *Actinomyces* colonization (p=0.549),(Table 6; Fig. 10)

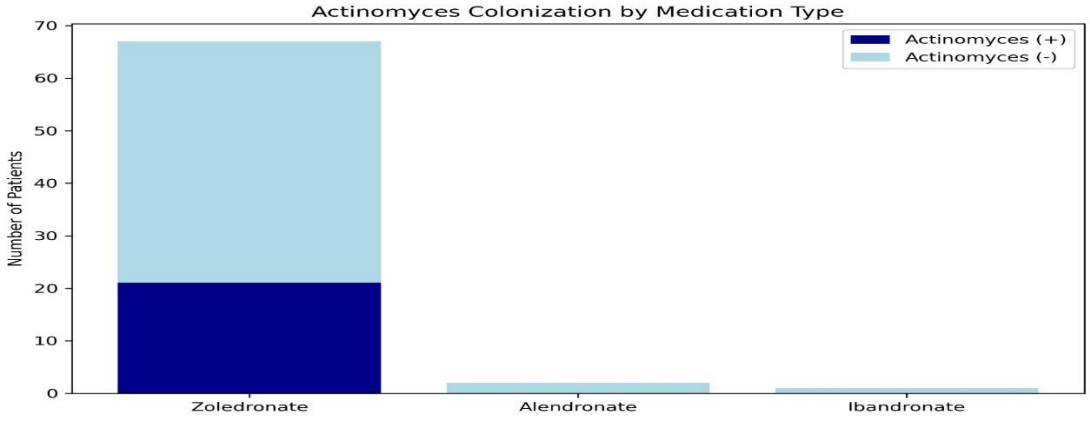


Figure 10. Relationship Between Medication Type and *Actinomyces* in Patients with MRONJ

Table 7. Relationship Between Sex and *Actinomyces* in Patients with MRONJ

Sex	**Number of Patients**	**Percentage (%)**	***Actinomyces* (+)**	**%**	***Actinomyces* (–)**	**%**	**p-value**
Male	28	40	9	42.9	19	38.8	0.794
Female	42	60	12	57.1	30	61.2	
Total	70	100	21	100	49	100	

Of the 21 patients with *Actinomyces* colonization, 9 (42.9%) were male and 12 (57.1%) were female. Although *Actinomyces* colonization was more frequently observed in female patients, no statistically significant relationship was found between sex and *Actinomyces* colonization (p=0.794),(Table 7; Fig. 11)

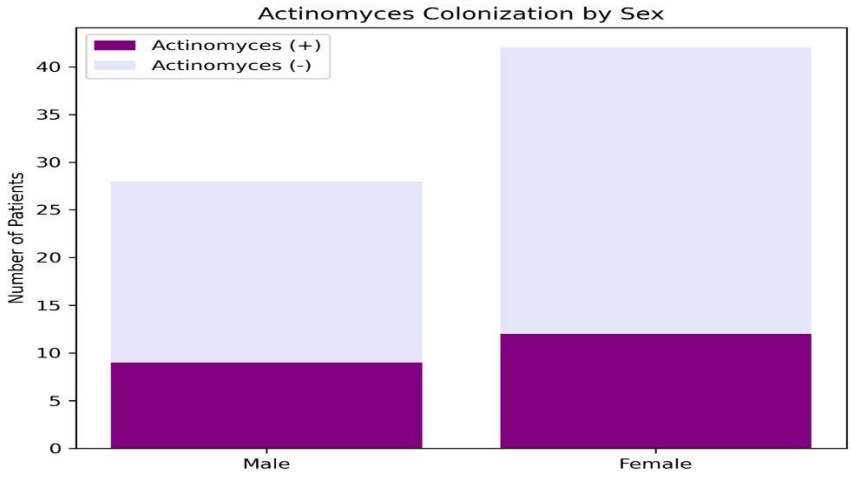


Figure 11. Relationship Between Sex and *Actinomyces* in Patients with MRONJ

Table 8. Relationship Between Fracture and *Actinomyces* in Patients with MRONJ

Fracture Status	**Number of Patients**	**Percentage (%)**	***Actinomyces* (+)**	**%**	***Actinomyces* (–)**	**%**	**p-value**
Present	4	5.7	3	14.3	1	2.0	0.078
Absent	66	94.3	18	85.7	48	98.0	
Total	70	100	21	100	49	100	

Fracture was observed in 4 of the 70 patients with MRONJ (5.7%). Among the 21 patients with *Actinomyces* colonization, 3 patients (14.3%) had a fracture. Of the 4 patients with fracture,

Actinomyces was isolated in 3 patients (75%). However, no statistically significant relationship was found between fracture and the presence of *Actinomyces* (p=0.078),(Table 8; Fig. 12).

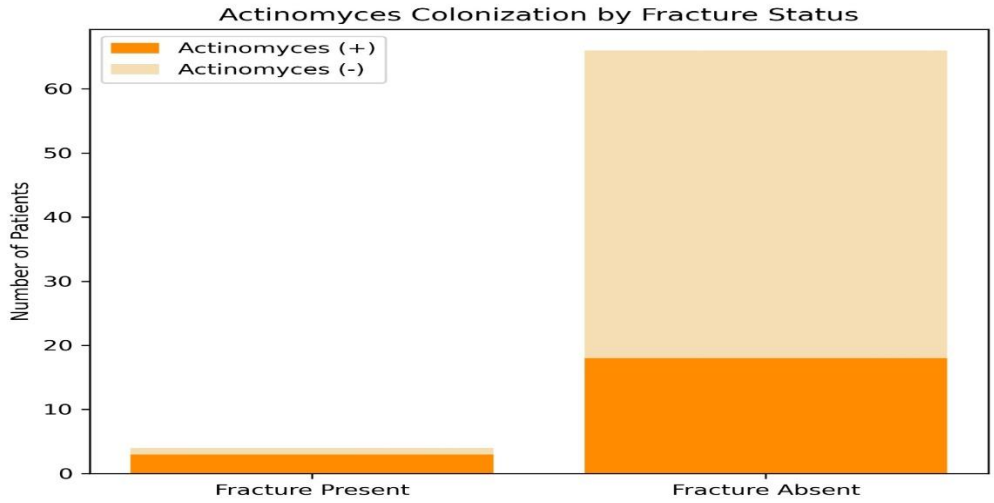


Figure 12. Relationship Between Fracture and *Actinomyces* in Patients with MRONJ

Table 9. Relationship Between MRONJ Stage and *Actinomyces*

MRONJ Stage	**Number of Patients**	**Percentage (%)**	***Actinomyces* (+)**	**%**	***Actinomyces* (–)**	**%**	**p-value**
Stage 1	20	28.6	5	23.8	15	30.6	0.270
Stage 2	20	28.6	4	19.1	16	32.7	
Stage 3	30	42.9	12	57.1	18	36.7	
Total	70	100	21	100	49	100	

Among the 70 patients with MRONJ, 30 patients (42.9%) were diagnosed with Stage 3 disease, 20 patients (28.6%) with Stage 2 disease, and 20 patients (28.6%) with Stage 1 disease. *Actinomyces* colonization was most frequently observed in Stage 3 patients [12 patients (57.1%)]. However, no statistically significant relationship was found between MRONJ stage and the presence of *Actinomyces* (p=0.270),(Table 9; Fig.13)

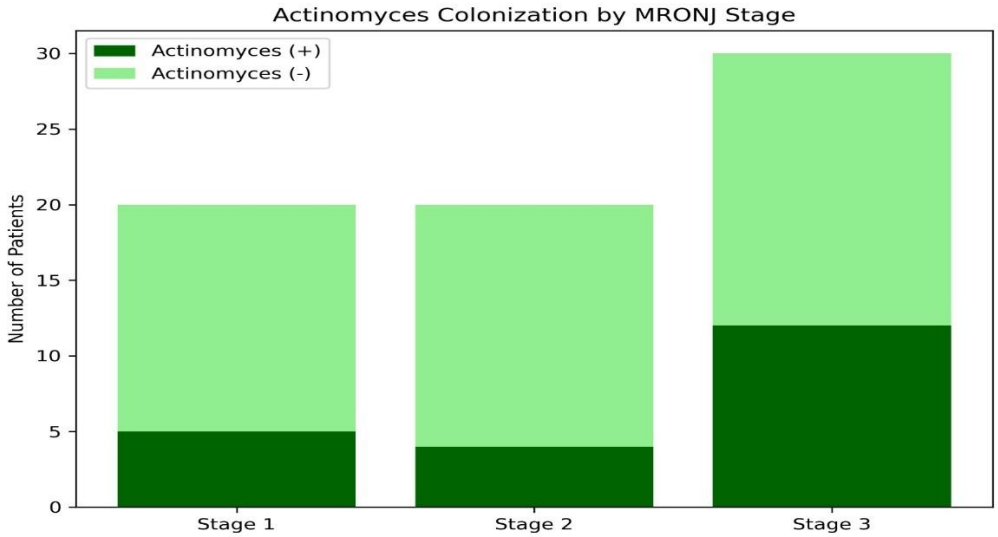


Figure 13. Relationship Between MRONJ Stage and *Actinomyces*

Table 10. Relationship Between MRONJ Localization and *Actinomyces*

MRONJ Localization	**Number of Patients**	**Percentage (%)**	***Actinomyces* (+)**	**%**	***Actinomyces* (–)**	**%**	**p-value**
Maxillary anterior–premolar region	12	17.1	4	19.0	8	16.3	
Maxillary posterior region	11	15.7	1	4.8	10	20.4	
Mandibular anterior–premolar region	11	15.7	4	19.0	7	14.3	0.349
Mandibular posterior region	36	51.4	12	57.1	24	49.0	
Total	70	100	21	100	49	100	

The most frequent localization of MRONJ among the 70 patients was the mandibular posterior region (51.4%). *Actinomyces* colonization was also most commonly observed in the mandibular posterior region [12 patients (57.1%)]. The lowest frequency was observed in the maxillary posterior region [1 patient (4.8%)]. Nevertheless, no statistically significant relationship was found between MRONJ localization and the presence of *Actinomyces*

(p=0.349),(Table 10; Fig.14)

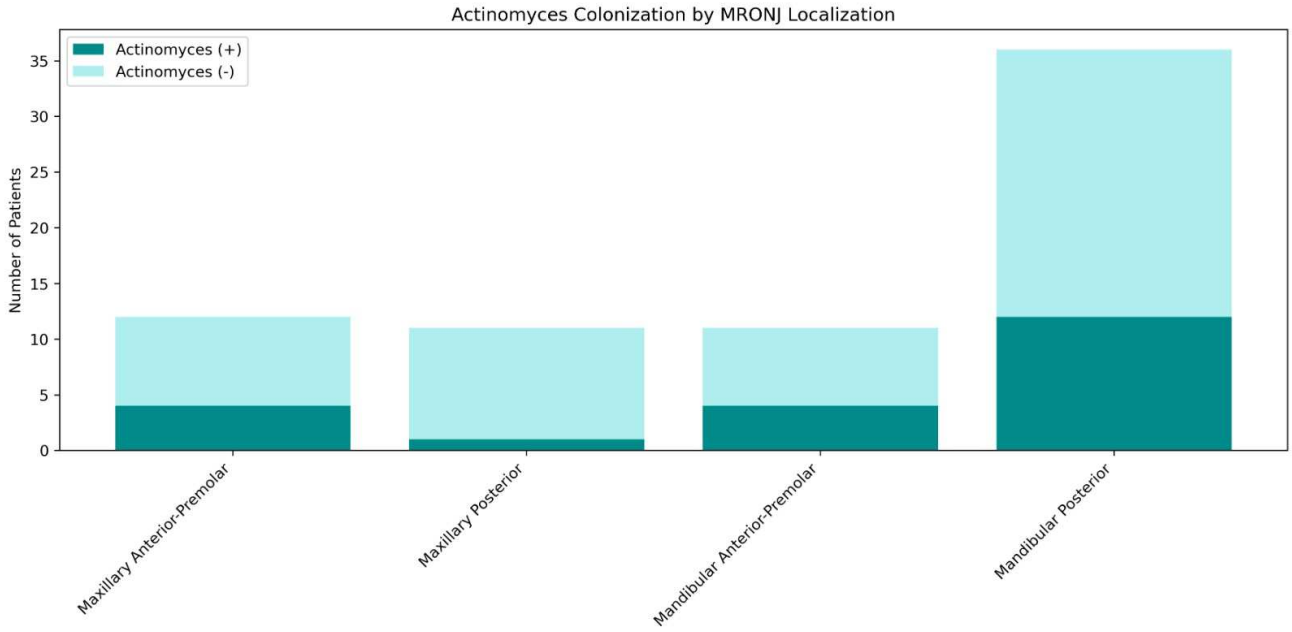


Figure 14. Relationship Between MRONJ Localization and *Actinomyces*

Table 11. General Treatment Planning of All Followed Patients

Patient Status	**n**	**(%)**	**Treatment Applied**	**n**	**(%)**
MRONJ Present	70	60.8	Antibiotics + patient information	9	12.9
			Antibiotics + debridement	29	41.4
			Treatment for *Actinomyces*	21	30.0
MRONJ Absent	45	39.2	Patient information only	45	100
Total	115	100	—		

A total of 115 patients were followed. MRONJ was observed in 70 patients (60.8%), whereas 45 patients (39.2%) showed no signs of MRONJ. Patients without MRONJ received only patient information and oral hygiene education.

In patients with MRONJ, treatment was planned according to disease stage and microbial findings. Nine patients (12.9%) received antibiotics and patient information; 29 patients (41.4%) underwent antibiotic therapy and debridement, with primary closure performed in selected cases and xeroform dressing used in some patients for secondary healing. Twenty-one patients (30%) received treatment for *Actinomyces*, consisting of approximately 1 month of hospitalization followed by 6 months of oral antibiotic follow-up. In non-resistant strains, amoxicillin–clavulanic acid was recommended at a dosage of either 3×1 or 2×1(Table 11; Fig.15)

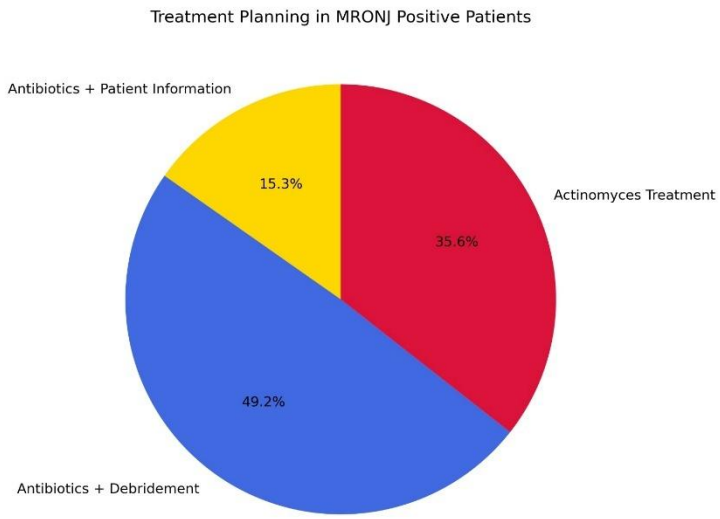


Figure 15. General Treatment Planning of All Followed Patients

Table 12. Treatment Protocols and Outcomes of Patients Treated for Actinomycosis

n	Surgical Treatment	Hospitalization	IV Treatment	Duration of Oral Antibiotics	Outcome	Oncological Diagnosis
1	Debridement 3 times	2 times	+	12 months	Healed	Breast cancer
2	Debridement	1 time	+	6 months	Healed	Breast cancer
3	Debridement	–	–	6 months	Healed	Breast cancer
4	–	–	–	6 months	Healed	Prostate cancer
5	–	1 time	+	6 months	Not healed; lymphoma recurrence	Lymphoma
6	Debridement	1 time	+	6 months	Healed	Prostate cancer
7	Debridement	1 time	+	6 months	Healed	Prostate cancer
8	Debridement 6 times	2 times	+	12 months	Healed	Breast cancer
9	Debridement	1 time	+	6 months	Healed	Thyroid cancer
10	Sequestrectomy	2 times	+	18 months	Not healed; lost to follow-up	Prostate cancer
11	Debridement + resection	1 time	+	6 months	Healed	Prostate cancer
12	Debridement	1 time	+	12 months	Healed; prosthetic rehabilitation performed	Breast cancer
13	Debridement	1 time	+	6 months	Healed	Breast cancer
14	Debridement	2 times	+	12 months	Healed; prosthetic rehabilitation performed	Thyroid cancer

15	Debridement	–	–	6 months	Healed	Renal cancer
16	Sequestrectomy	–	–	12 months	Healed	Lymphoma
17	Debridement	1 time	+	12 months	Not healed; lost to follow-up	Renal cancer
18	–	1 time	+	Irregular	Not healed	Prostate cancer
19	Debridement	–	–	6 months	Healed	Prostate cancer
20	–	–	–	6 months	Not healed; lost to follow-up	Breast cancer
21	–	–	–	–	Deceased	Nasopharyngeal cancer

When the treatment protocols of the 21 patients in whom *Actinomyces* was isolated from MRONJ sites through pathological and microbiological examinations were reviewed, 16 patients underwent surgical intervention, while medical treatment was prioritized in the remaining patients. Recurrence of *Actinomyces* infection was observed in two patients who had received zoledronate treatment due to breast cancer; therefore, multiple surgical interventions and a second hospitalization were required.

During follow-up, oral antibiotic protocols were supported by laboratory blood parameters evaluated by the infectious diseases department. Complete healing was achieved in 15 patients after an average treatment period of 6–12 months. However, 5 patients did not respond to treatment. The main reasons for treatment failure were irregular antibiotic use, recurrence of the primary oncological disease, and poor compliance with follow-up appointments.

One patient died during follow-up due to oncological disease. Prosthetic rehabilitation was performed in two patients who achieved healing, and no complications were observed during follow-up(Table 12; Fig.16).

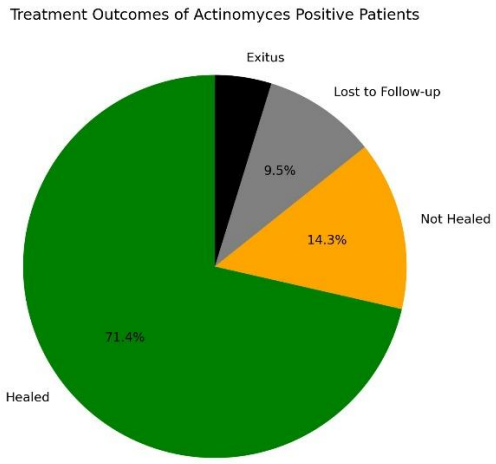


Figure 16. Treatment Protocols and Outcomes of Patients Treated for Actinomycosis

Discussion

Medication-related osteonecrosis of the jaw is not a simple and sterile necrotic process that can be explained solely by the suppression of bone turnover secondary to antiresorptive or antiangiogenic therapies. Current evidence indicates that MRONJ is a multifactorial clinicopathological condition in which impaired bone remodeling, local trauma, disruption of oral mucosal integrity, infection, poor oral hygiene, microbial colonization, and patient-related systemic factors interact with one another. In particular, when necrotic bone becomes exposed to the oral environment, it may provide a suitable surface for the oral microbiota, thereby facilitating bacterial adhesion, biofilm formation, and the persistence of chronic inflammation. Therefore, MRONJ should not be regarded merely as "medication-related bone necrosis," but rather as a dynamic disease process with infectious and microbial biofilm-related components [9–12].

In the present study, 70 of the 115 followed patients were diagnosed with MRONJ, and *Actinomyces* colonization was detected in 21 of these cases based on histopathological and/or microbiological evaluation. This finding supports the view that the microbial component of MRONJ lesions should not be overlooked. In our cohort, *Actinomyces* positivity was more frequently observed in patients with oncological primary diagnoses, advanced-stage lesions, and mandibular posterior involvement. However, except for the significant association between primary diagnosis and *Actinomyces* colonization, no statistically significant relationship was found between *Actinomyces* colonization and sex, route of drug administration, medication

type, fracture status, MRONJ stage, or lesion localization. These findings suggest that *Actinomyces* colonization should be interpreted not as an independent determinant of MRONJ severity, but rather as a clinicopathological finding associated with the chronically infected microenvironment of necrotic bone [5,6,11,12].

In this study, *Actinomyces* colonization was detected in 30% of MRONJ cases. This rate is generally consistent with recent histopathological studies. de Santana et al. evaluated 22 MRONJ cases and reported actinomycosis in 36.4% and osteomyelitis-like findings in 82% of the cases [5]. In contrast, Kivelä-Rajamäki et al. reported a considerably higher frequency of *Actinomyces* colonies in MRONJ cases, reaching 85%, and demonstrated that these colonies were predominantly associated with necrotic bone surfaces [12]. Differences in the reported rates may be attributable to variations in sampling techniques, histopathological diagnostic criteria, the use of microbiological culture or molecular methods, disease stage, and the characteristics of the study populations. Therefore, the frequency of *Actinomyces* should not be interpreted solely as a numerical colonization rate, but should instead be evaluated in conjunction with the clinical stage of MRONJ, the extent of necrotic bone exposure, and the presence of infection-related findings [5,12].

A statistically significant association was found between *Actinomyces* colonization and primary disease diagnosis in the present study. Although prostate cancer and breast cancer were the most frequent primary diagnoses among MRONJ patients, *Actinomyces* positivity was most commonly observed in patients with breast cancer. This finding should not be interpreted as evidence that a specific malignancy directly predisposes patients to *Actinomyces* colonization. A more plausible explanation is that, in oncological patients, prolonged and potent antiresorptive therapy, history of chemotherapy or radiotherapy, altered immune responses, systemic disease burden, oral hygiene status, and the need for dental interventions may collectively create a more favorable microenvironment for microbial colonization within MRONJ lesions. Recent studies have shown that MRONJ development in cancer patients is closely associated with cumulative drug exposure, the use of potent antiresorptive agents, dental infections, invasive dental procedures, and accompanying systemic factors [9–11,13]. Therefore, the significant association observed in our study between primary diagnosis and *Actinomyces* positivity should be considered not as a disease-specific microbial predisposition, but rather as the combined effect of accumulated systemic and local risk factors in oncological patients.

In the present cohort, the vast majority of patients had received intravenous zoledronate, and a statistically significant association was found between the duration of zoledronate use and the presence of MRONJ. This finding supports the concept that cumulative exposure to potent antiresorptive agents may represent an important risk determinant for MRONJ development. Zoledronate is widely used to reduce skeletal-related events, particularly in cancer patients with bone metastases; however, prolonged and repeated administration may contribute to suppression of bone remodeling, reduced healing capacity of the jawbones, and the development of necrotic bone lesions. Current studies indicate that MRONJ risk should be assessed not only according to the type of medication used, but also in relation to treatment duration, cumulative dose, the presence of dental infection, invasive dental procedures, and the patient's systemic condition [7,9,13,14]. Therefore, the association observed in our study between zoledronate exposure duration and MRONJ highlights the importance of regular oral examination, control of dental infections, and multidisciplinary risk assessment before invasive procedures, particularly in oncological patients receiving long-term antiresorptive therapy.

In this study, most *Actinomyces*-positive cases were classified within the advanced MRONJ group, and *Actinomyces* colonization was most frequently observed in Stage 3 lesions. However, no statistically significant association was found between MRONJ stage and the presence of *Actinomyces*. This finding suggests that *Actinomyces* colonization is not an independent factor determining disease stage. Nevertheless, advanced lesions may provide more favorable conditions for colonization because of a larger exposed necrotic bone surface, fistula formation, chronic inflammation, disruption of soft tissue integrity, and a more pronounced anaerobic microenvironment. Similarly, MRONJ and *Actinomyces* positivity were most frequently observed in the mandibular posterior region in our study. The posterior mandible is considered a clinically vulnerable region for MRONJ because it is frequently exposed to tooth extractions, periodontal infections, prosthetic trauma, and high masticatory forces. Moreover, the relatively limited vascularity of the mandible compared with the maxilla may reduce healing capacity and contribute to the persistence of microbial colonization on necrotic bone surfaces. In a large-scale cohort study, Wei et al. reported that posterior mandibular tooth extraction was one of the high-risk local factors for MRONJ development, particularly in patients receiving long-term antiresorptive therapy [15]. However, the absence of a significant association between lesion localization and *Actinomyces* positivity in our study suggests that anatomical site alone is insufficient to explain microbial colonization. Thus, the findings related to disease stage and localization support the interpretation that *Actinomyces* is

more likely a component of the microbial-inflammatory process that develops within a chronically necrotic bone environment, rather than a primary cause of MRONJ [7,9,12,14,15].

One clinically important finding of this study is that patients with *Actinomyces* colonization frequently required a combined approach involving prolonged antibiotic therapy and surgical management. Surgical intervention was performed in 16 of the 21 *Actinomyces*-positive patients, and treatment protocols were tailored according to clinical status, signs of infection, the presence of necrotic bone, and systemic disease burden. A substantial proportion of patients received long-term oral antibiotic therapy following intravenous antibiotic treatment, and complete healing was achieved in 15 patients by the end of follow-up. This result suggests that, in MRONJ cases, treatment success may depend not only on the removal of necrotic bone but also on targeting the accompanying microbial-inflammatory component. Because actinomycosis can persist in chronic, infiltrative, and low-oxygen tissue environments, prolonged antibiotic therapy combined with surgical debridement is considered one of the fundamental components of conventional treatment [8]. Therefore, the detection of *Actinomyces* in MRONJ lesions should be considered clinically relevant for treatment duration, infectious disease consultation, and multidisciplinary follow-up [7,8,11].

Although most *Actinomyces*-positive patients in our study achieved healing after treatment, some cases showed an insufficient response. In non-healed cases, irregular antibiotic use, recurrence of the primary oncological disease, poor compliance with follow-up appointments, and deterioration of general systemic status were prominent factors. This finding indicates that treatment success in MRONJ is not determined solely by the surgical or antibiotic protocol applied, but also by the patient's systemic disease burden, immunological status, oncological treatment course, treatment adherence, and regular clinical follow-up. In chronic infections associated with actinomycosis, adherence to prolonged antibiotic therapy is particularly critical for infection control and prevention of recurrence. Therefore, in patients with MRONJ and concomitant *Actinomyces* colonization, treatment planning should not be limited to determining surgical margins; instead, it should be conducted through a long-term, patient-centered, multidisciplinary approach involving infectious disease specialists, oncologists, and oral and maxillofacial surgeons [7,8,11].

One of the strengths of this study is that *Actinomyces* colonization in MRONJ cases was evaluated not merely on the basis of clinical observation, but through histopathological and/or microbiological findings. Although clinical and radiological findings are often considered

sufficient for the diagnosis and staging of MRONJ, identification of accompanying microbial colonization within necrotic bone tissue may provide additional insight into the biological behavior of the disease and its response to treatment. In the present study, patient records, radiological findings, histopathological examinations, microbiological evaluations, infectious disease consultations, and treatment outcomes were assessed together. This comprehensive approach allowed *Actinomyces* to be evaluated not as an isolated pathological finding, but in relation to the clinical course of MRONJ, lesion stage, localization, primary disease status, and treatment response. In this respect, the study contributes to the current literature emphasizing the importance of microbial and inflammatory components in the clinical management of MRONJ [5,11,12].

This study has several limitations. First, because of its retrospective design, the completeness of patient records, follow-up duration, and treatment adherence could not be fully controlled by the investigators. Second, the study was conducted at a single center, and the sample size was limited, particularly for subgroup analyses. This may have reduced the statistical power to detect potential associations between *Actinomyces* colonization and variables such as medication type, route of administration, MRONJ stage, lesion localization, and fracture status. Third, the fact that the majority of patients had received zoledronate limited comparisons among different antiresorptive agents. Fourth, although the presence of *Actinomyces* was identified through histopathological and/or microbiological evaluation, advanced molecular microbiological techniques were not systematically applied in all cases. This may have limited a more detailed assessment of microbial diversity and polymicrobial biofilm structures. In addition, because of the retrospective design, pre-biopsy antimicrobial exposure, including systemic antibiotics or antiseptic mouth rinses, could not be standardized in all patients. This may have influenced microbiological culture results. Nevertheless, histopathological evidence of *Actinomyces* colonies was also included in the assessment, which may partially reduce the impact of culture-related limitations. Therefore, the present findings should be interpreted as observational data demonstrating the clinicopathological presence and potential clinical relevance of *Actinomyces* in necrotic bone tissue, rather than proving a definitive causal role of *Actinomyces* in the pathogenesis of MRONJ.

In conclusion, this study demonstrates that *Actinomyces* colonization is a noteworthy histopathological and microbiological finding in MRONJ cases. In our cohort, *Actinomyces* was more frequently observed in patients with oncological primary diagnoses, advanced-stage

lesions, and mandibular posterior involvement; however, no statistically significant association was found with clinicopathological variables other than primary diagnosis. These findings suggest that *Actinomyces* should not be regarded as a primary causal factor acting alone in MRONJ development, but rather as an important accompanying component of the chronically infected and inflammatory microenvironment of necrotic bone. From a clinical perspective, histopathological and microbiological evaluation is important in MRONJ cases presenting with persistent infection, fistula formation, recurrent drainage, advanced-stage lesions, or delayed healing. In patients with detected *Actinomyces*, a multidisciplinary approach involving infectious disease consultation, prolonged antibiotic therapy, appropriate surgical debridement, and regular follow-up may improve treatment success. Future prospective, multicenter studies with larger sample sizes and advanced molecular microbiological methods are needed to clarify the actual role of *Actinomyces* in MRONJ pathogenesis, its effect on disease progression, and its clinical significance in treatment response.

List of abbreviations

AAOMS: American Association of Oral and Maxillofacial Surgeons
CRP: C-reactive protein
CT: Computed tomography
GMS: Grocott methenamine silver
H&E: Hematoxylin and eosin
IV: Intravenous
MRONJ: Medication-related osteonecrosis of the jaw
PAS: Periodic acid–Schiff
SPSS: Statistical Package for the Social Sciences

Declarations

Ethics approval and consent to participate

This retrospective observational study was conducted in accordance with the ethical approval obtained from the Ethics Committee of Hacettepe University Faculty of Medicine, dated January 21, 2015, with the approval number GO 15/03. The study was based exclusively on previously recorded clinical files, radiological data, histopathological reports, microbiological

records, and infectious disease consultation notes. No new intervention was performed for the purpose of this study. All procedures were conducted in accordance with the principles of the Declaration of Helsinki and its later amendments.

As this was a retrospective study based on existing patient records, no additional patient intervention was performed for study purposes.
Clinical trial number: Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy and confidentiality considerations but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

AE contributed to the study conception and design, data collection, data interpretation, and manuscript drafting. SA contributed to clinical data collection, study design, and critical revision of the manuscript. ACI contributed to infectious disease evaluation, treatment planning, and interpretation of antimicrobial management. KK performed and interpreted the histopathological evaluations. OTK contributed to study supervision, clinical interpretation, and critical revision of the manuscript. SU contributed to infectious disease supervision, treatment interpretation, and critical review of the manuscript. All authors read and approved the final version of the manuscript.

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