

Diagnostic Value of Metagenomic Next-Generation Sequencing in Refractory Osteomyelitis: A Retrospective Study

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Abstract

Background

Refractory osteomyelitis is a persistent and recurrent bone infection in which conventional microbiological culture often shows limited sensitivity and a prolonged turnaround time. Metagenomic next-generation sequencing (mNGS) enables comprehensive, culture-independent pathogen detection, but its clinical value in refractory osteomyelitis remains unclear.

Objective

To compare the diagnostic performance of mNGS and conventional culture in refractory and typical osteomyelitis, focusing on pathogen detection, identification of polymicrobial infection, diagnostic efficiency, and impact on antimicrobial therapy.

Methods

This retrospective study included 150 patients with osteomyelitis who underwent surgery between January 2021 and September 2023 (42 refractory, 108 typical). Intraoperative deep-tissue or purulent specimens were analyzed by both mNGS and conventional culture. Pathogen detection, polymicrobial infection identification, turnaround time, inter-method agreement, and treatment modifications were compared.

Results

mNGS detected pathogens in significantly more patients than culture in refractory (83.3% vs. 28.6%) and typical (56.5% vs. 16.7%) osteomyelitis (both $P < 0.001$), and identified polymicrobial infections in 92.31% vs. 11.54% (refractory) and 80.85% vs. 14.89% (typical) of cases (both $P < 0.001$). Turnaround time was shorter with mNGS than with culture (35.0 ± 2.6 h vs. 68.2 ± 4.9 h; $P < 0.001$). mNGS prompted antimicrobial treatment modification in 61.9% of refractory versus 37.0% of typical patients ($P < 0.001$).

Conclusions

Compared with conventional culture, mNGS improves pathogen detection, polymicrobial infection identification, and diagnostic efficiency, and substantially influences antimicrobial treatment decisions, supporting its role as a valuable adjunct for the individualized management of refractory osteomyelitis.

Introduction

Osteomyelitis is an infectious disease caused by bacterial, fungal, or other microbial invasion of bone tissue.[1] Osteomyelitis remains a challenging orthopedic infection despite advances in surgical debridement, antimicrobial therapy, and bone reconstruction [2]. Chronic osteomyelitis is notoriously difficult to eradicate because impaired local vascularity, sequestrum formation, and bacterial biofilms sustain persistent or recurrent infection [3, 4]. A subgroup of patients experiences continued infection or repeated relapse despite standardized debridement and systemic antimicrobial therapy and is classified as having refractory osteomyelitis [5]. These patients typically require multiple surgical interventions and prolonged antimicrobial treatment, imposing a substantial clinical and healthcare burden.

Refractory osteomyelitis is thought to harbor a more complex microbiological profile than typical osteomyelitis. Repeated surgery and prolonged antibiotic exposure can reshape the local microbial environment and promote biofilm formation, potentially increasing the frequency of polymicrobial infections and of infections caused by low-virulence or uncommon pathogens [6–8]. Accurate pathogen identification is therefore a prerequisite for targeted antimicrobial therapy and for successful reconstruction within systematic management strategies [9]. Conventional culture, the current standard, has important limitations: its yield is reduced by prior antibiotic exposure, low-virulence organisms, and specimen-related factors [10, 11], and anaerobes, mycobacteria, and fungi often require specialized conditions and prolonged incubation, delaying targeted treatment [12–14]. Metagenomic next-generation sequencing (mNGS) detects microbial nucleic acids without predefined targets, simultaneously identifies diverse microorganisms [15–17], and, because it does not depend on microbial viability, may be particularly valuable for polymicrobial, rare, or culture-negative infections [18–20].

However, previous studies of mNGS in osteomyelitis have mainly evaluated overall diagnostic performance, whereas its clinical utility in refractory osteomyelitis remains insufficiently characterized. This study therefore compared mNGS with conventional culture in refractory and typical osteomyelitis, focusing on pathogen detection, polymicrobial infection identification, diagnostic turnaround time, and the impact of mNGS findings on antimicrobial treatment decisions.

Materials and Methods

Study Design and Population

Consecutive patients clinically diagnosed with osteomyelitis who underwent surgical treatment between January 2021 and September 2023 were evaluated. Inclusion criteria were: age 18–80 years; diagnosis consistent with clinical criteria for osteomyelitis; availability of intraoperative deep-tissue or purulent specimens for both conventional culture and mNGS; complete clinical, imaging, and laboratory records; and at least 12 months of postoperative follow-up. Exclusion criteria were: absence of either culture or mNGS results; history of malignant tumors; chronic infection originating outside the musculoskeletal system; and incomplete clinical or follow-up data.

The diagnosis of osteomyelitis was based on a positive culture or mNGS result from intraoperative deep-tissue or purulent specimens and/or histopathological examination with special staining confirming pathogenic microorganisms in infected tissue, and was jointly confirmed by orthopedic surgeons and infectious disease specialists. Refractory osteomyelitis was defined as persistent or recurrent infection requiring two or more infection-related debridement procedures within 12 months despite standardized surgical debridement and systemic antimicrobial therapy. Typical osteomyelitis was defined as infection controlled after a single debridement procedure, with no clinical or imaging evidence of recurrence during at least 12 months of follow-up. Clinicodemographic data were collected, including sex, age, body mass index, disease history, lifestyle habits, infection site, clinical symptoms, surgical method, and laboratory parameters (C-reactive protein, procalcitonin, interleukin-6, and erythrocyte sedimentation rate).

Sample Collection and Preprocessing

During surgical debridement, at least 2–3 independent deep-tissue or purulent specimens were obtained under sterile conditions from necrotic bone, infected granulation tissue, or medullary purulence, avoiding contact with superficial tissues and sinus openings, and were transported to the microbiology laboratory within 2 h.

Culture Protocol

Culture Protocol

Conventional microbiological testing comprised aerobic, anaerobic, fungal, and mycobacterial cultures using standard laboratory procedures. Organisms were identified by MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing was performed using the VITEK-Compact II system (bioMérieux SA, Marcy-l'Étoile, France) or the BD M50 system (BD, Franklin Lakes, NJ, USA) and interpreted according to Clinical and Laboratory Standards Institute criteria.

Metagenomic Next-Generation Sequencing Protocol

After host depletion, nucleic acids were extracted and quantified. DNA libraries were prepared using the Hieff NGS C130P2 OnePot II DNA Library Prep Kit for MGI (Yeasen Biotechnology) and sequenced on the MGISEQ-200 platform. Raw sequencing data were quality-filtered with Trimmomatic (v0.36), and human reads were removed by alignment to the human reference genome (hs37d5) using Bowtie2 (v2.2.6). Remaining reads were aligned to a microbial genome database containing bacterial, fungal, viral, and parasitic sequences. Microorganisms were identified with Kraken (v2.0.7), and species abundance was estimated with Bracken (v2.5.0). For *Mycobacterium*, *Nocardia*, and *Legionella pneumophila*, detection of ≥ 1 species-specific read was considered positive; for other bacteria, fungi, viruses, and parasites, ≥ 3 non-overlapping reads were required. Organisms detected in negative controls were excluded according

to predefined background-control criteria. Pathogens meeting these thresholds were retained and reported together with drug-resistance genes, virulence factors, and other relevant data.

Definitions and Outcome Measures

All pathogens detected by either mNGS or culture were collectively considered the patient's pathogen spectrum. Results were classified as complete, partial, or negative detection according to whether all, some, or no pathogens were identified. Polymicrobial infection was defined as identification of ≥ 2 clinically relevant pathogens, and monomicrobial infection as identification of 1 clinically relevant pathogen. Primary outcomes were pathogen detection and polymicrobial infection identification by mNGS versus conventional culture in refractory and typical osteomyelitis. Secondary outcomes included diagnostic turnaround time, agreement between the two methods, mNGS sequencing characteristics (pathogen read counts and relative abundance), and the impact of mNGS findings on antimicrobial treatment.

Statistical Analysis

Normality of data distribution was assessed with the Shapiro–Wilk test. Normally distributed data are expressed as mean $\pm$ standard deviation, and non-normally distributed data as median and interquartile range (IQR). The kappa test assessed agreement in the distribution of positive results between mNGS and culture in each group, and the chi-square test compared other variables between groups. All statistical analyses were performed with SPSS for Windows, and $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics

Of 164 patients initially screened, 14 were excluded, leaving a final cohort of 150 patients (42 refractory, 108 typical; Fig. 1). The two groups were comparable in age, sex, BMI, and most comorbidities and lifestyle factors; refractory patients more often reported pain and had higher C-reactive protein levels, and the distribution of surgical methods differed between groups (Table 1).

Table 1

Comparison of patient characteristics between the refractory and typical osteomyelitis groups

Characteristic	Refractory osteomyelitis group (n = 42)	Typical osteomyelitis group (n = 108)	P-value
Age (years)	47.00 [35.50–57.50]	53.00 [40.00–63.00]	0.119
Sex			
Male	35 (83.3)	81 (75)	0.274
Female	7 (16.7)	27 (25.0)	
BMI (kg/m ²)	20.76 ± 1.54	21.35 ± 1.69	0.196
Comorbidity			
Hypertension	9 (21.4)	20 (18.5)	0.685
Diabetes	8 (19.4)	10 (9.3)	0.098
Hepatic or renal diseases	1 (2.4)	2 (1.9)	1.000
Immune system diseases	2 (4.8)	1 (1.0)	0.189
Lifestyle habits			
Smoking	4 (9.5)	11 (10.2)	0.856
Drinking	1 (2.4)	3 (2.8)	0.668
Affected side			
Left	25 (59.5)	59 (50)	0.288
Right	17 (40.5)	59 (50)	
Clinical symptoms			
Pain	13 (30.1)	13 (12.0)	0.006
Red and swollen	21 (50.0)	63 (58.3)	0.356
Sinus tract	12 (28.6)	25 (23.1)	0.489
Location			
Tibia	15 (35.8)	51 (47.2)	0.202
Ankle	5 (11.9)	6 (5.6)	0.322
Knee	4 (9.5)	3 (2.8)	0.184
Femur	2 (4.8)	11 (10.2)	0.461

Characteristic	Refractory osteomyelitis group (n = 42)	Typical osteomyelitis group (n = 108)	P-value
Foot	2 (4.8)	5 (4.6)	0.692
Others	14 (33.3)	32 (29.6)	0.659
Laboratory test results			
CRP (mg/L)	8.67 [6.11–36.72]	6.36 [0.499–8.63]	< 0.001
PCT (ng/mL)	0.033 [0.02–0.058]	0.026 [0.02–0.037]	0.360
IL-6 (pg/mL)	5.63 [3.02–22.90]	3.00 [1.50–8.67]	0.01
ESR (mm/h)	31.00 [14.00–67.00]	20.50 [6.00–41.00]	0.16
Surgical method			
Ilizarov	35 (83.3)	76 (70.37)	0.121
Masquet	7 (16.6)	12 (11.1)	0.230
Other	0 (0)	20 (18.5)	< 0.001
Skin flap	10 (23.8)	18 (16.7)	0.104

Data are presented as n (%), mean $\pm$ SD, or median [range].

mNGS Data Characteristics

The mean relative abundance of pathogens was significantly higher in typical than in refractory osteomyelitis (41.77% vs. 30.11%; $P < 0.001$), whereas pathogen read counts were similarly skewed and did not differ between groups ($P = 0.595$; Fig. 2).

Osteomyelitis Detection Time

mNGS reduced detection time by approximately half relative to culture, both overall and within each group (35.0 ± 2.6 h vs. 68.2 ± 4.9 h; $P < 0.001$; Table 2).

Table 2
Comparison of osteomyelitis detection time between mNGS and culture

Refractory osteomyelitis (n = 42)	mNGS, h	Culture, h	P-value
	35.4 ± 2.1	68.4 ± 4.8	< 0.001
Typical osteomyelitis (n = 108)	34.9 ± 2.7	68.1 ± 4.9	< 0.001
Total osteomyelitis (n = 150)	35.0 ± 2.6	68.2 ± 4.9	< 0.001

Pathogen Detection Numbers

mNGS detected a broader pathogen spectrum and higher per-pathogen counts than culture for most organisms, most notably fungi, streptococci, and *Enterococcus faecalis*; per-pathogen counts are presented in Table 3.

Table 3
Pathogen counts detected by mNGS and culture

Pathogens	mNGS in refractory osteomyelitis	Culture in refractory osteomyelitis	mNGS in typical osteomyelitis	Culture in typical osteomyelitis	Total positive results: mNGS vs. culture, n
<i>Staphylococcus aureus</i>	16	13	25	20	41:33
Coagulase-negative <i>Staphylococcus</i>	3	0	8	6	11:6
<i>Streptococcus</i>	3	0	8	1	11:1
Fungus	1	0	3	0	4:0
<i>Enterococcus faecalis</i>	7	1	6	1	13:2
<i>Mycobacterium tuberculosis</i>	0	0	2	2	2:2
<i>Escherichia coli</i>	3	3	2	1	5:4

Consistency Between mNGS and Culture Results

Agreement between mNGS and culture was poor in refractory ($\kappa = 0.021$, $P = 0.749$) and fair in typical ($\kappa = 0.27$, $P < 0.001$) osteomyelitis. The overall detection rate with mNGS was 83.3% in refractory and 56.5% in typical osteomyelitis, versus 28.6% and 16.7% with culture (both $P < 0.001$; Table 4).

Table 4
Distribution of mNGS and culture positive results in refractory and typical osteomyelitis

Method		Culture		
mNGS	Refractory osteomyelitis	Complete detection	Partial detection	Negative
	Complete detection	9	17	9
	Partial detection	0	3	0
	Negative	3	0	1
	Typical osteomyelitis	Complete detection	Partial detection	Negative
	Complete detection	12	17	32
	Partial detection	0	13	0
	Negative	6	0	28

Distribution of Polymicrobial and Monomicrobial Infections

In the overall population, 73 patients (48.7%) had polymicrobial infections, 49 (32.7%) had monomicrobial infections, and 28 (18.7%) had negative results. The proportion of polymicrobial infections was significantly higher in refractory than in typical osteomyelitis (61.9% vs. 43.5%; $P < 0.001$; Fig. 3).

Detection of Polymicrobial and Monomicrobial Infections

mNGS detected polymicrobial infections in 86.3% of affected patients versus 13.7% with culture ($P < 0.001$), with a similar advantage in both subgroups. For monomicrobial infections, mNGS was superior in typical osteomyelitis (88.2% vs. 38.2%; $P < 0.001$) but not in refractory disease (80.0% vs. 66.7%; $P = 0.602$; Table 5).

Table 5
Comparison of detective ability for mixed and single infections

Group	Detection method	Mixed infection, n (%)	P-value	Single infection, n (%)	P-value
Refractory osteomyelitis	mNGS	24 (92.31)	< 0.001	12 (80.00)	0.602
	culture	3 (11.54)		10 (66.67)	
Typical osteomyelitis	mNGS	38 (80.85)	< 0.001	30 (88.23)	< 0.001
	culture	7 (14.89)		13 (38.24)	
Total osteomyelitis	mNGS	63 (86.30)	< 0.001	32 (65.31)	0.001
	culture	10 (13.70)		23 (46.94)	

Impact of mNGS Results on Antimicrobial Treatment

Antimicrobial regimens were modified in 61.9% of refractory versus 37.0% of typical patients ($P < 0.001$; Fig. 4).

Discussion

This study evaluated the diagnostic value of mNGS in refractory osteomyelitis relative to typical osteomyelitis and conventional culture, and three findings stand out. First, refractory osteomyelitis was associated with a more complex microbial profile, with a higher proportion of polymicrobial infections. Second, mNGS markedly outperformed culture in pathogen detection and cut turnaround time by approximately half, particularly for polymicrobial infections. Third, mNGS results changed antimicrobial treatment decisions more often in refractory than in typical osteomyelitis. Together, these findings position mNGS as a valuable complement to culture for the individualized management of refractory osteomyelitis.

Refractory osteomyelitis represents a distinct infection state defined by persistent microbial complexity. Repeated surgical interventions, prolonged antimicrobial exposure, and biofilm formation can alter the local microbial ecosystem, favoring the coexistence of multiple microorganisms and the enrichment of low-abundance or difficult-to-culture pathogens [11–13]. Consistent with this view, our refractory group showed a significantly higher prevalence of polymicrobial infection (61.9% vs. 43.5%). Because conventional culture identifies only a fraction of this spectrum, incomplete pathogen characterization may contribute to treatment failure and recurrence[21]. Comprehensive pathogen identification should therefore be regarded as an integral component of managing refractory osteomyelitis, guiding targeted antimicrobial therapy and infection control.

Previous studies have demonstrated the diagnostic value of mNGS across infectious diseases, including bone and joint infections[22]. The advantage of mNGS was most evident in polymicrobial infection, where it detected 86.3% of affected patients versus 13.7% with culture, and it provided results approximately twice as fast. The poor agreement between methods in refractory osteomyelitis ($\kappa = 0.021$) should not be interpreted as a limitation of mNGS; it reflects their fundamentally different detection principles. Culture requires viable organisms and can be compromised by prior antibiotic exposure, slow-growing organisms, and polymicrobial competition, whereas mNGS detects microbial nucleic acids regardless of viability and can simultaneously identify difficult-to-culture pathogens. For clinicians, this means that mNGS can reveal pathogens missed by culture, enabling earlier and broader antimicrobial coverage in a disease where delayed or incomplete treatment carries a high cost.

mNGS also had direct therapeutic relevance: antimicrobial regimens were modified in 61.9% of refractory patients, significantly more often than in typical osteomyelitis (37.0%). This suggests that mNGS provides actionable information precisely when culture fails to fully characterize complex infections[22]. However, mNGS cannot replace culture, because antimicrobial susceptibility testing remains essential

for treatment selection. A combined strategy that integrates the rapid, broad detection of mNGS with culture-based susceptibility data, and that interprets mNGS findings alongside clinical manifestations, imaging, and surgical observations to distinguish true pathogens from contaminants, is likely to confer the greatest clinical benefit in refractory osteomyelitis.

This study has several limitations. The retrospective, single-center design may introduce selection bias and limit generalizability. mNGS does not provide antimicrobial susceptibility results, and treatment modification does not necessarily translate into improved outcomes. Finally, we evaluated diagnostic performance and treatment decisions rather than long-term prognosis. Multicenter prospective studies are warranted to validate the clinical effectiveness, cost-effectiveness, and impact of mNGS-guided management on long-term outcomes in refractory osteomyelitis.

Conclusions

Refractory osteomyelitis is characterized by a more complex pathogen profile and a higher prevalence of polymicrobial infection than typical osteomyelitis. Compared with conventional culture, mNGS demonstrated superior pathogen detection, polymicrobial infection identification, and diagnostic efficiency, and provided clinically relevant information that influenced antimicrobial treatment decisions. These findings support mNGS as a valuable complementary diagnostic approach for refractory osteomyelitis, particularly in patients with recurrent infection, suspected polymicrobial involvement, or negative culture results. Prospective multicenter studies are needed to determine its impact on long-term clinical outcomes.

Data Availability

The data presented in this study are available on request from the corresponding author. Requests will be reviewed against ethical, scientific, regulatory, and legal requirements and should be directed to shenhao7212@sina.com.

Declarations

Competing Interests

The authors declare no conflicts of interest.

Financial Support

The authors declare no financial support.

Ethical Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Shanghai Sixth People's Hospital (protocol code NO.2025-KY-503(K)).

Consent to Participate

Written informed consent to participate in the study was obtained from all participants.

Author Contribution

X.M. and H.S. conceived and designed the study. X.M. and H.W. developed the methodology. H.W. and Z.R. performed the software-related work. X.M., P.H., and H.S. performed validation. X.M. and J.T. conducted the formal analysis. X.M. and G.H. performed the investigation. X.M. and P.H. provided resources. H.W. and H.L. curated the data. X.M. wrote the original draft of the manuscript. X.M. and B.W. reviewed and edited the manuscript. X.M. and Z.R. prepared the visualizations. H.S. and P.H. supervised the study. X.M. administered the project. All authors read, reviewed, and approved the final manuscript.

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Figures

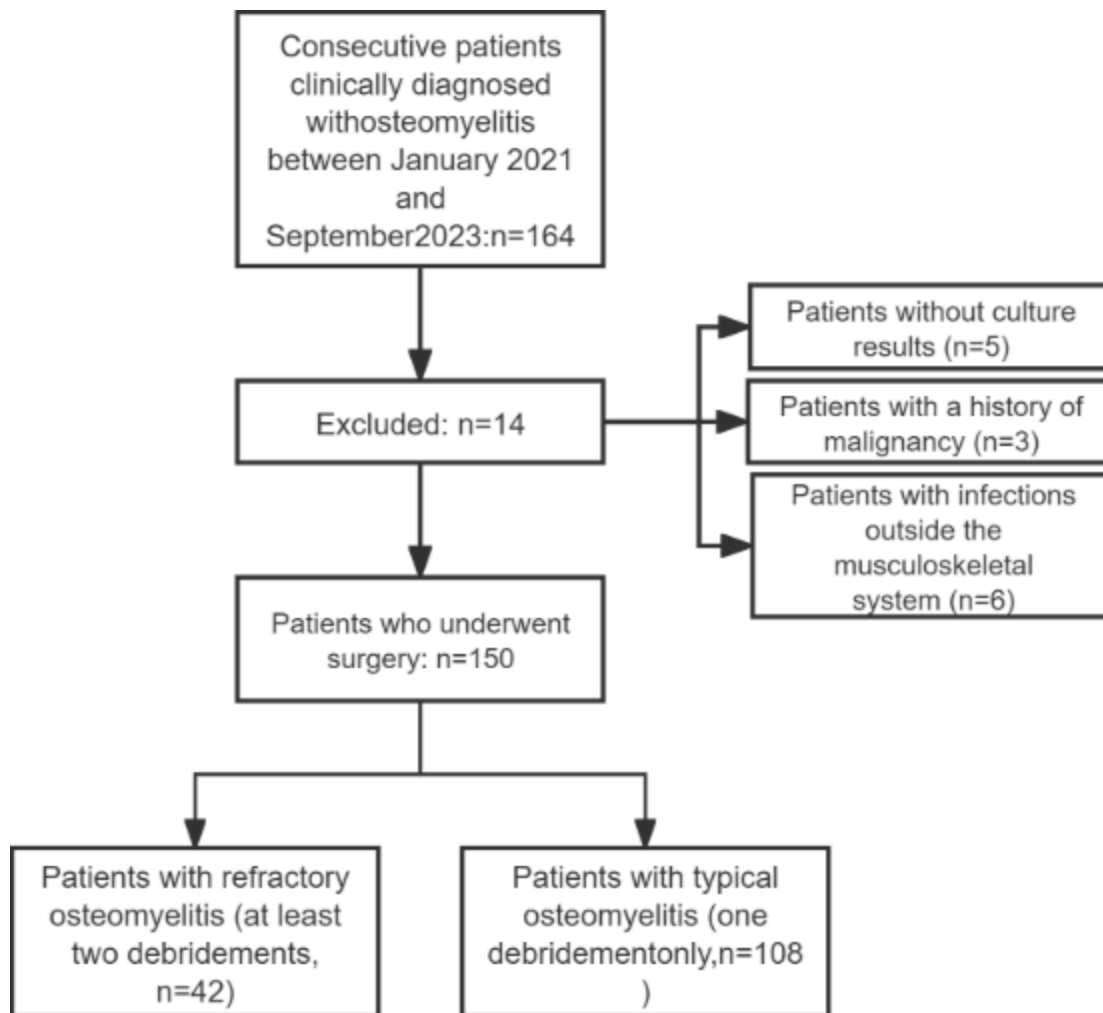


Figure 1

Patient selection flowchart

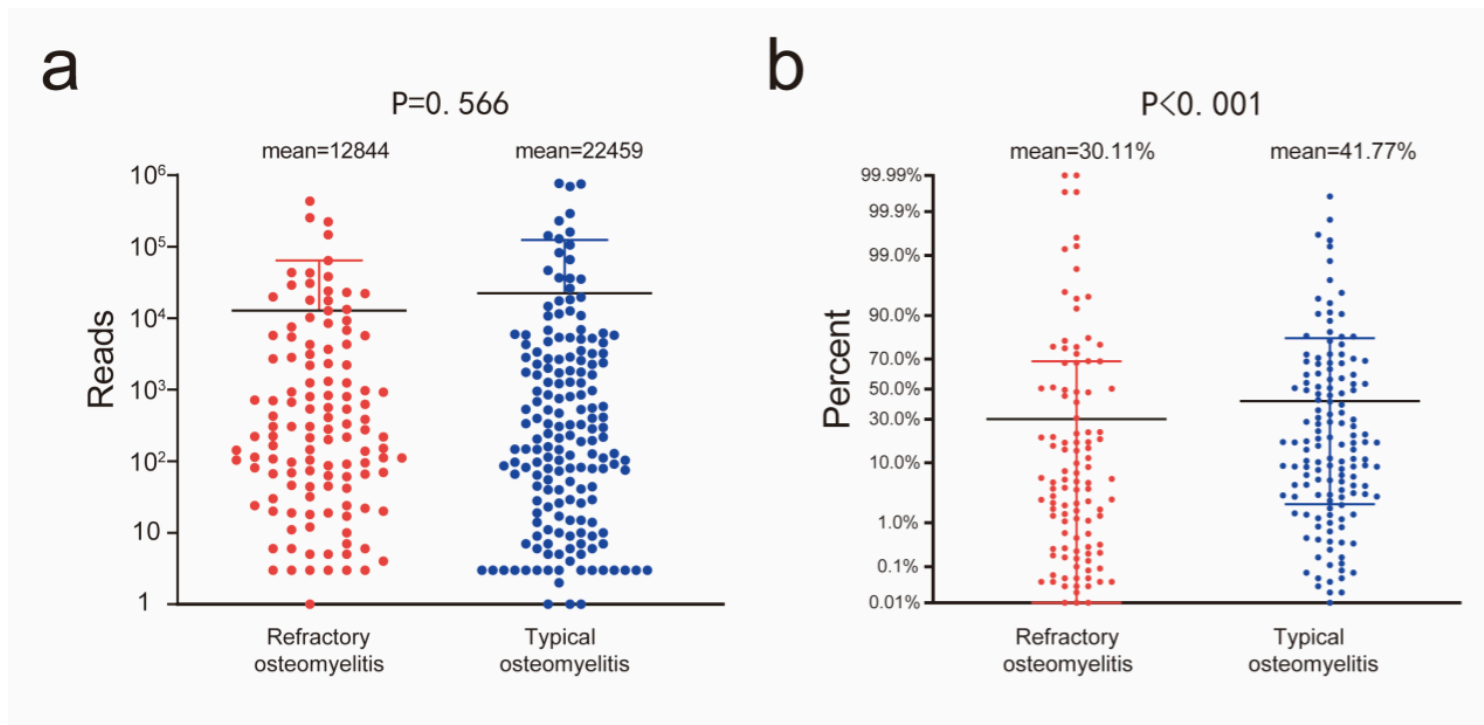


Figure 2

Distribution of mNGS readings and relative abundance of bacterial species in refractory and common osteomyelitis. a: Readings. b: Relative abundance

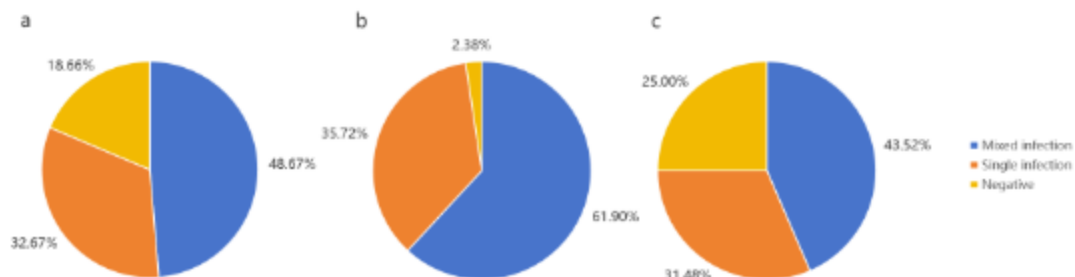


Figure 3

Proportion of osteomyelitis patients with mixed infection, single infection, and negative results. a: Overall population. b: Refractory osteomyelitis group. c: Typical osteomyelitis group

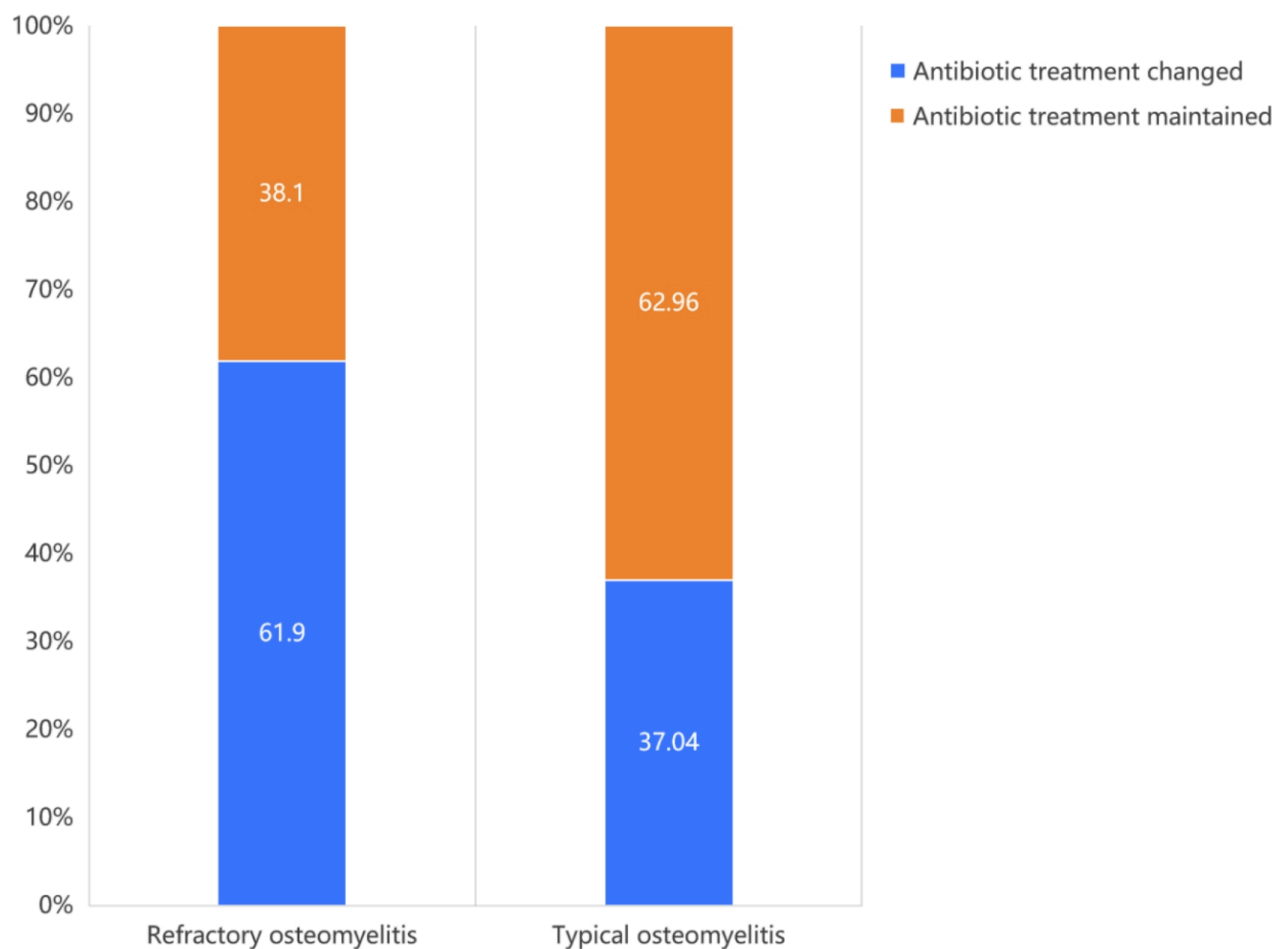


Figure 4

Rate of medication regimen adjustment after mNGS analysis in patients with osteomyelitis