

Original Research Article

MRI Diagnosis and Targeted Resistance-Gene PCR in Diabetic Foot Osteomyelitis: A Prospective Pilot Study

Imad Kh. Resen^{1*}, Zamazam Hussein Shummar², Saradiq Mudhafar Jebur³, Ahlam Gareeb Nhaer⁴, Zaid Ali Hussein⁵¹Dijlah University College, Baghdad, Iraq²Dijlah University College, Baghdad, Iraq³Dijlah University College, Baghdad, Iraq⁴College of Science, Wasit University, Wasit, Iraq⁵Department of Radiology, Al-Zahraa Teaching Hospital, Wasit Health Directorate, Wasit, Iraq**Article History**

Received: 03.07.2026

Accepted: 27.08.2026

Published: 03.09.2026

Journal homepage:<https://www.easpublisher.com>**Quick Response Code**

Abstract: Objective: To evaluate the diagnostic performance of magnetic resonance imaging (MRI) for diabetic foot osteomyelitis (DFO) and to examine the feasibility and turnaround advantage of targeted resistance-gene polymerase chain reaction (PCR) as an adjunct to conventional microbiology. **Methods:** This prospective single-center pilot study included 80 consecutive adults with type 2 diabetes and suspected deep foot infection or osteomyelitis. All participants underwent standardized 1.5-T MRI and reference-standard classification based on histopathology when available, with bone culture and blinded multidisciplinary adjudication used as supportive or alternative evidence when histology was unavailable or nondiagnostic. Targeted PCR assessed *mecA*, *blaCTX-M*, *blaTEM*, *blaSHV*, *blaKPC*, *blaNDM*, and *blaOXA-48*. MRI diagnostic estimates were calculated from a patient-level 2 × 2 table and reported with 95% confidence intervals. PCR-specific analyses used available valid molecular results, and 90-day clinical outcomes were descriptive. **Results:** DFO was confirmed in 46/80 participants (57.5%). MRI yielded 91.3% sensitivity (95% CI, 79.2–97.6), 82.4% specificity (65.5–93.2), 87.5% positive predictive value, 87.5% negative predictive value, and 87.5% accuracy. The positive and negative likelihood ratios were 5.17 and 0.11, respectively. Conventional culture recovered pathogens in 38/46 confirmed DFO cases (82.6%) and required 62.4 ± 9.3 hours. Valid PCR results were available for 60 participants; predefined resistance determinants were detected in 28/60 (46.7%), most frequently *mecA*, *blaCTX-M*, and *blaNDM*. Molecular and corresponding phenotypic resistance results showed 85.0% agreement (Cohen's κ = 0.71). PCR results were available in approximately 5 hours, about 57.4 hours earlier than final culture on average. Antimicrobial modification after PCR availability and before the final culture report occurred in 22/60 tested participants. Eleven amputations occurred during 90-day follow-up; these outcomes were exploratory and descriptive. **Conclusion:** MRI showed high diagnostic performance in this clinically enriched cohort, particularly a low negative likelihood ratio. Targeted resistance-gene PCR did not replace culture or establish bone infection, but it provided selected resistance information substantially earlier. The findings support a complementary diagnostic pathway in which MRI defines anatomical bone involvement, culture establishes organism identity and phenotype, and targeted PCR may shorten the interval to resistance-informed treatment decisions.

Keywords: Diabetic Foot, Osteomyelitis, Magnetic Resonance Imaging, Polymerase Chain Reaction, Antimicrobial Resistance; Bone Culture.

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

1. INTRODUCTION

Diabetes-related foot infection is among the most consequential complications of diabetes because the apparent surface lesion may underestimate the depth

and anatomical extent of disease. Once infection reaches bone, treatment becomes longer, surgical decision-making becomes more difficult, and the risk of limb loss increases, particularly in patients with neuropathy,

*Corresponding Author: Imad Kh. Resen
Department of Radiology, Dijlah University College, Baghdad, Iraq

peripheral arterial disease, or delayed presentation [1, 2]. Diabetic foot osteomyelitis (DFO) therefore represents a practical diagnostic threshold rather than simply another stage of soft-tissue infection.

No single test resolves the diagnostic problem in every patient. Clinical examination remains fundamental, and the probe-to-bone test can increase suspicion in an appropriate clinical context, although its performance depends on ulcer depth, disease prevalence, and examiner technique [3]. Inflammatory markers may support the diagnosis but cannot independently confirm or exclude bone infection [4]. Plain radiography is inexpensive and remains an appropriate first imaging step, yet early osteomyelitis may precede visible cortical destruction or periosteal reaction [1-5].

MRI is the preferred advanced imaging modality when DFO remains uncertain. It depicts marrow replacement, cortical integrity, sinus tracts, abscesses, and the full extent of soft-tissue involvement [5, 6]. The difficulty is specificity. Reactive marrow edema, Charcot neuro-osteoarthropathy, trauma, and postoperative change can overlap with infection on fluid-sensitive sequences [6, 7]. Diffusion-weighted imaging (DWI) and contrast-enhanced imaging may improve tissue characterization in selected cases, but acquisition differences and region-of-interest methodology still prevent adoption of a universal apparent diffusion coefficient threshold [5-8]. MRI is therefore most persuasive when interpreted as a structured anatomical pattern anchored to the ulcer or sinus tract rather than as an isolated signal abnormality.

Microbiological testing answers a different question. Bone culture remains the preferred method for identifying the causative organism and guiding antimicrobial therapy, although yield may fall after previous antibiotic exposure, inadequate sampling, or devitalized tissue [9-11]. Conventional culture also requires time. During that interval, clinicians often rely on broad empirical therapy. Targeted molecular assays may shorten access to selected resistance information by detecting predefined determinants such as *mecA*, *blaCTX-M*, or *blaNDM* [10-14]. Their limitation is equally important: a resistance gene neither proves osteomyelitis nor replaces organism identification and complete phenotypic susceptibility testing.

MRI and resistance-gene PCR are consequently complementary rather than competing tests. MRI addresses the presence and anatomical extent of suspected bone infection; molecular testing may reduce the delay to selected resistance information while conventional culture establishes the final microbiological phenotype. This prospective pilot study evaluated the diagnostic performance of MRI in clinically suspected DFO and explored the feasibility, agreement, and turnaround time of targeted resistance-gene PCR within the same diagnostic pathway.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

We conducted a prospective, single-center observational pilot study with a diagnostic-accuracy component and an exploratory assessment of short-term management outcomes. Consecutive participants were recruited at Al-Zahraa Teaching Hospital, Wasit, Iraq, from 1 February to 31 July 2025, and 90-day follow-up was completed by 31 October 2025. The diagnostic component was designed in accordance with STARD 2015 reporting principles [20], and the 2023 IWGDF/IDSA recommendations for suspected diabetes-related foot infection [1]. Because molecular testing was not assigned randomly, treatment modification and amputation outcomes were treated as exploratory and were not interpreted as evidence of a causal treatment effect.

2.2 Participants and Eligibility

Adults aged 18 years or older with type 2 diabetes were eligible when they presented with a foot ulcer and clinical suspicion of deep infection or osteomyelitis. Eligibility required at least one of the following: a positive probe-to-bone test, exposed bone, an ulcer persisting for more than four weeks, or clinical evidence that infection extended beyond superficial soft tissue. Exclusion criteria were an absolute MRI contraindication, acute Charcot neuro-osteoarthropathy without clinical evidence of infection, previous major amputation of the affected limb, an unrelated systemic infection likely to confound inflammatory or microbiological findings, or failure to obtain material adequate for reference-standard classification.

The cohort included 80 participants as a pragmatic pilot sample. The study was intended to provide preliminary estimates of MRI sensitivity and specificity and to examine the feasibility of incorporating rapid targeted resistance testing into the local pathway; it was not powered for definitive comparative-effectiveness conclusions. Baseline data included age, sex, duration of diabetes, glycated hemoglobin when available, previous antimicrobial exposure, ulcer duration and size, probe-to-bone result, peripheral arterial disease status, renal function, and final medical or surgical management.

2.3 MRI Acquisition

All participants underwent plain radiography followed by 1.5-T MRI of the affected foot or ankle using a Philips Achieva system (Philips Healthcare, Best, the Netherlands) with an 8-channel dedicated extremity coil. The protocol included multiplanar T1-weighted imaging, fluid-sensitive fat-suppressed imaging, axial DWI with apparent diffusion coefficient maps, and fat-suppressed T1-weighted imaging before and after intravenous gadolinium when contrast administration was clinically appropriate and not contraindicated. DWI used b values of 0 and 800 s/mm² with diffusion gradients in three

orthogonal directions. When administered, a macrocyclic gadolinium-based contrast agent was given

at 0.1 mmol/kg after review of renal function and prior contrast reactions.

Table 1: MRI acquisition and interpretive framework for suspected diabetic foot osteomyelitis

Sequence	Plane	Purpose	Core parameters	Interpretive role	Principal limitation
T1-weighted spin echo	Axial, coronal, sagittal	Anatomy and marrow replacement	TR 500–700 ms; TE 10–20 ms; small FOV; thin sections	Confluent low T1 marrow signal contiguous with an ulcer or sinus tract supports osteomyelitis	Mild or patchy signal change may be reactive and is not diagnostic alone
T2-weighted FS or STIR/SPAIR	Axial and coronal	Marrow edema and soft-tissue extent	TR 3000–4000 ms; TE 80–100 ms	Shows edema, cellulitis, sinus tracts, and fluid collections	High fluid-sensitive signal alone cannot reliably distinguish infection from Charcot change
DWI with ADC maps	Axial	Adjunctive tissue characterization	b = 0 and 800 s/mm ² ; three orthogonal diffusion directions	Restricted diffusion may support active infection or abscess	No universal ADC cutoff was imposed because values vary with acquisition and ROI placement
T1-weighted FS, pre-contrast	Axial and coronal	Baseline for enhancement assessment	Matched geometry with post-contrast acquisition	Reference for true enhancement	Uneven fat suppression may mimic or obscure enhancement
T1-weighted FS, post-contrast	Axial, coronal, sagittal	Abscess, devitalized tissue, sinus tract, enhancement pattern	Gadolinium 0.1 mmol/kg when not contraindicated	Rim enhancement and nonenhancing necrotic tissue assist surgical mapping	Enhancement is not specific for infection
GRE or susceptibility-sensitive sequence	Axial, optional	Gas, hemorrhage, or susceptibility foci	Short TE; flip angle 20–30°	May increase conspicuity of susceptibility-related signal loss	Signal void is nonspecific and requires radiographic or CT correlation

2.4 MRI Interpretation

Two fellowship-trained musculoskeletal radiologists, each with more than five years of post-training experience, independently reviewed all MRI examinations. They were blinded to culture, PCR, histopathology, treatment decisions, and 90-day outcomes. MRI was classified as positive for osteomyelitis when confluent T1 marrow replacement occurred in an anatomical distribution related to the ulcer or sinus tract and was accompanied by at least one supportive feature: cortical disruption, a sinus tract reaching bone, an intraosseous or adjacent abscess, or convincing diffusion restriction. Marrow edema on fluid-sensitive images without corresponding T1 replacement was considered indeterminate rather than automatically positive. Discordant reader interpretations were resolved by consensus before the final MRI classification entered the diagnostic analysis. Reader-level data were not sufficiently preserved to calculate an independent interobserver κ statistic.

2.5 Specimen Collection and Conventional Microbiology

Bone specimens were obtained whenever clinically feasible, consistent with guidance favoring bone-based microbiology in suspected DFO [1-11]. When bone biopsy was not possible, deep tissue was collected after cleansing and surgical debridement; superficial swabs were excluded. Specimens were divided under sterile conditions for direct microscopy, conventional culture, histopathology when bone was available, and molecular testing. Gram staining was performed immediately. Aerobic and anaerobic cultures were processed using standard hospital laboratory procedures, organisms were identified by conventional phenotypic and biochemical methods, and antimicrobial susceptibility testing was interpreted according to Clinical and Laboratory Standards Institute criteria current during the study period. Culture results were accepted only when specimen identity, incubation controls, and susceptibility quality-control requirements were satisfied.

2.6 Targeted Resistance-Gene PCR

Genomic DNA was extracted from bone or deep-tissue material using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, including tissue pretreatment. DNA concentration and purity were assessed by NanoDrop spectrophotometry, and an extraction control was included in each batch. The targeted panel evaluated mecA, blaCTX-M, blaTEM, blaSHV, blaKPC, blaNDM,

and blaOXA-48. Each PCR run included a positive control, a negative extraction control, and a no-template control, and results were accepted only when controls behaved as expected. The assay was used as a rapid screen for selected resistance determinants and was not interpreted as a stand-alone diagnosis of osteomyelitis or as a replacement for complete phenotypic susceptibility testing.

Table 2: Microbiological and molecular workflow

Stage	Method	Target turnaround	Primary output	Clinical role	Main limitation
1	Gram stain and direct microscopy	<1 hour	Gram reaction and cellular morphology	Immediate preliminary information	No definitive identification or susceptibility result
2	Aerobic and anaerobic culture with AST	48–72 hours	Organism identification and phenotypic susceptibility	Definitive antimicrobial selection	Yield may decrease after prior antibiotics or inadequate sampling
3	Targeted resistance-gene PCR	4–6 hours	Presence of selected resistance determinants	Earlier resistance-informed information	Restricted to predefined genes and does not prove bone infection

2.7 Reference Standard and Adjudication

The reference standard was defined before data analysis. Histopathological evidence of acute or chronic osteomyelitis, including inflammatory infiltration, bone necrosis, or both, established definite DFO. A positive bone culture supported the diagnosis and identified the causative organism but was not required when histopathology was diagnostic. When histopathology was unavailable or nondiagnostic, an independent three-member adjudication panel consisting of an infectious-disease physician, an orthopedic or diabetic-foot surgeon, and a musculoskeletal radiologist reviewed bone culture, operative findings, ulcer-to-bone continuity, and clinical follow-up. The panel was blinded to the initial MRI index-test classification, and disagreements were resolved by consensus. Deep-tissue culture alone was not considered definitive proof of bone infection.

Final reference-standard classification was assigned at patient level as positive, negative, or indeterminate. Histopathology-positive cases were classified as DFO positive. Histopathology-negative cases were classified as negative unless compelling discordant evidence required reassessment by the panel. Cases without adequate histology were classified by adjudication. Indeterminate cases were prespecified for exclusion from the primary accuracy analysis; none remained indeterminate in the supplied final analytic dataset.

2.8 Study Outcomes

The primary outcome was MRI diagnostic performance for DFO, expressed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive and negative

likelihood ratios, and diagnostic odds ratio against the predefined reference standard. PCR-specific exploratory outcomes were turnaround time, agreement between detected resistance determinants and the corresponding phenotypic resistance profile, and the proportion of tested participants whose antimicrobial regimen was modified after PCR availability but before the final culture report. Clinical outcomes included conservative treatment, debridement, minor amputation, major amputation, and 90-day amputation status. MRI and PCR were not combined into a single index test because they address different clinical questions.

2.9 Statistical Analysis

Anonymized data were analyzed using IBM SPSS Statistics, version 28. Distributional normality was assessed with the Shapiro–Wilk test. Continuous variables were summarized as mean $\pm$ standard deviation or median with interquartile range, as appropriate, and categorical variables as counts and percentages. A patient-level 2×2 table was constructed for MRI against the reference standard. Diagnostic estimates were reported with 95% confidence intervals; exact binomial intervals were used for proportions.

Agreement between PCR-detected resistance determinants and the corresponding phenotypic resistance profile was assessed among participants with both valid results using percentage agreement and Cohen's κ . κ values were interpreted as slight (≤ 0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), or almost perfect (0.81–1.00). Turnaround times were summarized descriptively. Because dispersion data for PCR turnaround were unavailable, no formal between-method significance test was performed. Ninety-day outcomes were descriptive because

molecular testing was not randomly assigned and coherent non-overlapping exposure groups were not available for valid causal comparison. No statistical imputation was performed; available-case denominators are reported for each outcome.

2.10 Missing and Technically Inadequate Data

Missing, failed, and technically inadequate observations were documented before analysis. MRI examinations with severe motion, incomplete coverage, or nondiagnostic artifact were prespecified for exclusion from the MRI accuracy analysis. Participants with failed PCR amplification, insufficient DNA, or invalid controls remained eligible for the MRI analysis but were excluded from PCR-specific concordance and turnaround analyses. No statistical imputation was performed because of the pilot sample size.

2.11 Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board of the Wasit University College of Medicine

approved the protocol before recruitment (Approval No. WUM-2025-018, dated 20 January 2025). All participants provided written informed consent for imaging, specimen collection, and use of anonymized clinical data. Direct identifiers were removed before analysis, and access to the study database was restricted to the research team.

3. RESULTS

3.1 Participant Flow and Analytic Population

Eighty consecutive participants were enrolled and included in the descriptive cohort. The predefined reference standard classified 46 participants as having DFO (57.5%; exact 95% CI, 45.9–68.5) and 34 as not having osteomyelitis. All 80 participants had a final reference-standard classification in the supplied analytic dataset and were included in the primary MRI accuracy analysis. PCR-specific analyses were restricted to the 60 participants with a valid molecular result; 20 had invalid or uninterpretable PCR results. Figure 1 summarizes the analytic pathways and denominators.

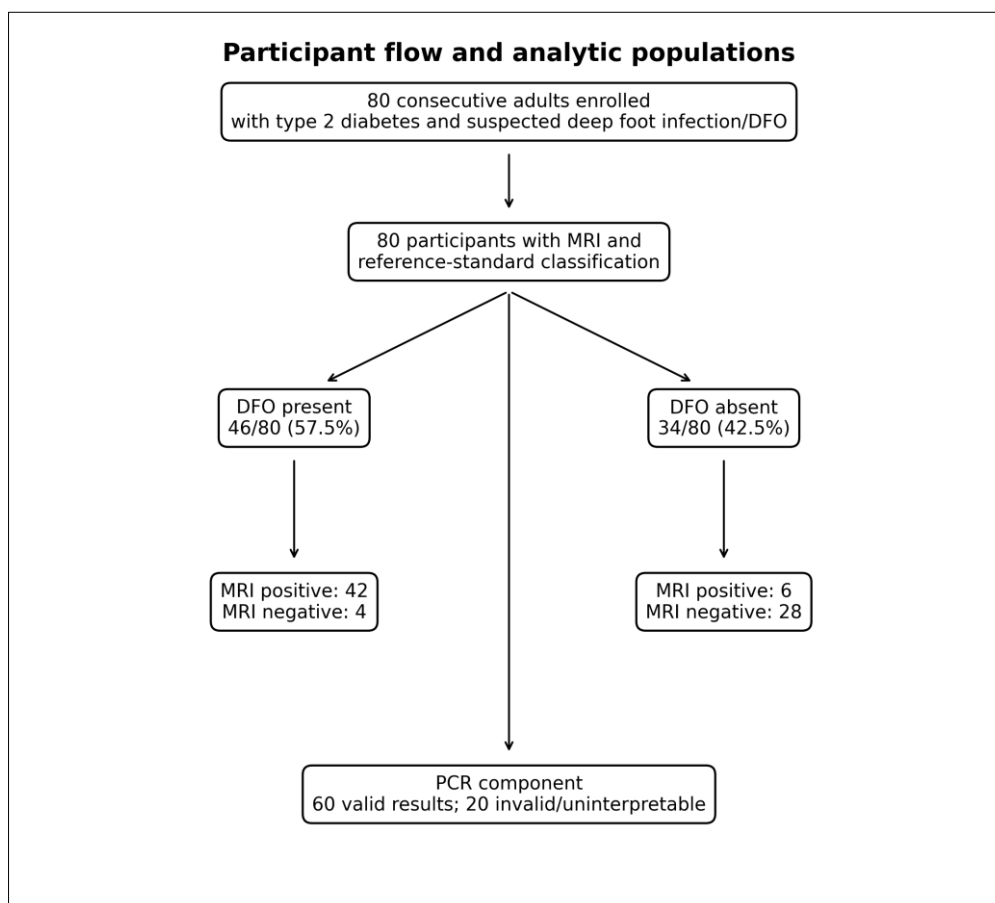


Figure 1: Participant flow and analytic populations. All 80 enrolled participants entered the primary MRI diagnostic-accuracy analysis; PCR-specific analyses used the 60 valid molecular results

3.2 Baseline Characteristics

The mean participant age was 58.3 ± 9.6 years, and 47/80 (58.8%) were men. Mean diabetes duration was 12.1 ± 6.8 years. Most ulcers had persisted for more

than four weeks (64/80, 80.0%), and 49/80 participants (61.2%) had a positive probe-to-bone test. Baseline characteristics are shown in Table 3.

Table 3: Baseline characteristics of the study cohort

Characteristic	Overall cohort (n = 80)
Age, years	58.3 ± 9.6
Male sex	47 (58.8%)
Female sex	33 (41.2%)
Duration of diabetes, years	12.1 ± 6.8
Ulcer size, cm	3.4 ± 1.2
Ulcer duration >4 weeks	64 (80.0%)
Positive probe-to-bone test	49 (61.2%)
Reference-standard DFO	46 (57.5%)

3.3 Diagnostic Performance of MRI

MRI correctly identified 42 of 46 reference-standard-positive cases and 28 of 34 reference-standard-negative cases. Four examinations were false negative and six were false positive (Table 4). Sensitivity was 91.3% (95% CI, 79.2–97.6), specificity 82.4% (65.5–

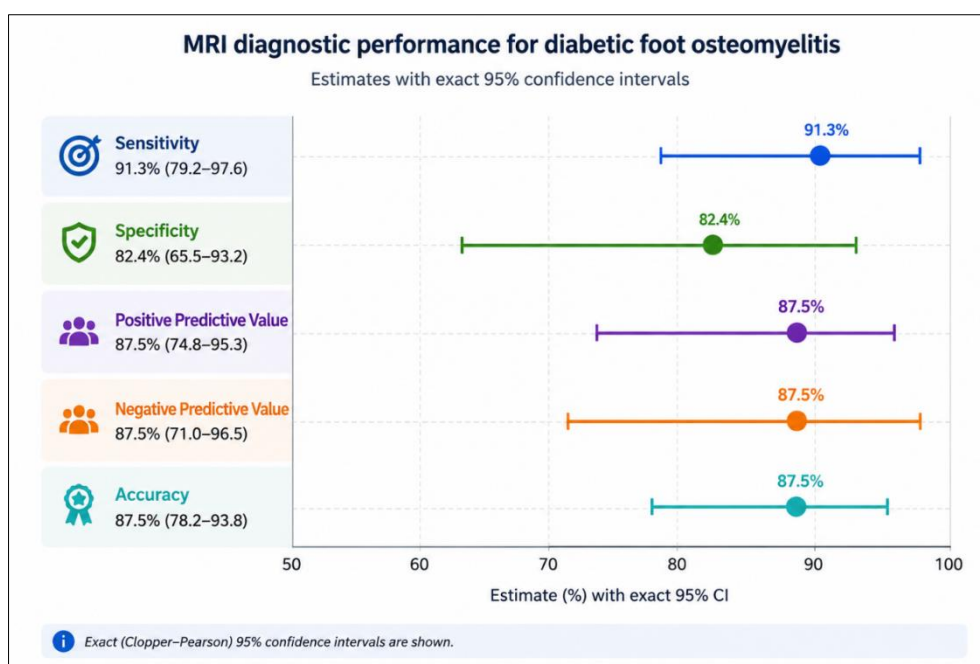
93.2), and overall accuracy 87.5% (78.2–93.8). The PPV and NPV were both 87.5%. The positive likelihood ratio was 5.17 (95% CI, 2.49–10.75), the negative likelihood ratio 0.11 (0.04–0.27), and the diagnostic odds ratio 49.0 (12.7–189.5) (Table 5; Figure 2).

Table 4: Patient-level cross-tabulation of MRI against the reference standard

MRI classification	DFO present	DFO absent	Total
MRI positive	42	6	48
MRI negative	4	28	32
Total	46	34	80

Table 5: Diagnostic performance of MRI for diabetic foot osteomyelitis

Measure	Estimate	95% CI
Sensitivity	91.3%	79.2–97.6
Specificity	82.4%	65.5–93.2
Positive predictive value	87.5%	74.8–95.3
Negative predictive value	87.5%	71.0–96.5
Accuracy	87.5%	78.2–93.8
Positive likelihood ratio	5.17	2.49–10.75
Negative likelihood ratio	0.11	0.04–0.27
Diagnostic odds ratio	49.0	12.7–189.5

**Figure 2: MRI diagnostic performance for diabetic foot osteomyelitis with exact 95% confidence intervals for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy**

3.4 Representative MRI Findings

A representative confirmed case is shown in Figure 3. The study image demonstrates extensive marrow signal abnormality across the posterior subtalar joint with structural change and surrounding

inflammatory features, illustrating the value of interpreting marrow replacement and adjacent anatomical abnormalities together rather than relying on fluid-sensitive hyperintensity alone.



Figure 3: Sagittal MRI of the right ankle in a patient with confirmed diabetic foot osteomyelitis. STIR imaging demonstrates extensive marrow edema involving the talus and adjacent calcaneus across the posterior subtalar joint, with associated subchondral irregularity and surrounding inflammatory change. The corresponding T2-weighted image shows structural alteration and cystic erosive changes, supporting septic subtalar arthritis with contiguous osteomyelitis

3.5 Conventional Microbiology

Conventional culture recovered a pathogen in 38 of 46 participants with reference-standard-confirmed DFO, corresponding to a culture-positive yield of 82.6% (exact 95% CI, 68.6–92.2). This value is reported as culture yield rather than an independent diagnostic

sensitivity because culture contributed supportive information to the reference-standard process in some patients. Mean observed culture turnaround time was 62.4 ± 9.3 hours. *Staphylococcus aureus* was the most frequent isolate, followed by *Pseudomonas aeruginosa* and *Escherichia coli* (Table 6).

Table 6: Distribution of culture-positive microbiological findings

Culture result	n (%) among 38 positive cultures	Comment
<i>Staphylococcus aureus</i>	16 (42.1%)	Included 9 MRSA isolates
<i>Pseudomonas aeruginosa</i>	8 (21.1%)	Predominantly chronic ulcers
<i>Escherichia coli</i>	7 (18.4%)	Some isolates showed ESBL phenotype
Mixed anaerobic growth	4 (10.5%)	Observed mainly in deep necrotic infection
Other organisms	3 (7.9%)	Remaining culture-positive isolates

3.6 Targeted Resistance-Gene PCR

Valid PCR results were available for 60 of 80 participants (75.0%). Twenty specimens did not produce an interpretable molecular result because extraction or amplification failed to meet assay validity criteria and were excluded from PCR-specific analyses. At least one predefined resistance determinant was detected in 28/60 participants (46.7%; exact 95% CI, 33.7–60.0). Among the reported detected targets, *mecA* was identified in 12 participants, *blaCTX-M* in 9, and *blaNDM* in 7 (Table 7; Figure 4).

Overall agreement between molecular resistance detection and the corresponding phenotypic resistance result was 85.0% (95% CI, 73.4–92.9), with Cohen's $\kappa = 0.71$ (95% CI, 0.52–0.90), consistent with substantial agreement. Mean PCR turnaround was approximately 5 hours, providing selected resistance information about 57.4 hours earlier than conventional culture on average. Because a dispersion measure for PCR turnaround time was not available, no formal significance test comparing turnaround times was calculated.

Table 7: Distribution of selected resistance-gene findings among participants with valid PCR results

Target	Positive, n	Frequency among PCR-tested	Interpretation
<i>mecA</i>	12	20.0%	Methicillin resistance determinant
<i>blaCTX-M</i>	9	15.0%	Extended-spectrum β -lactamase determinant
<i>blaNDM</i>	7	11.7%	Metallo- β -lactamase/carbapenem resistance determinant
Any predefined marker	28	46.7%	At least one target detected

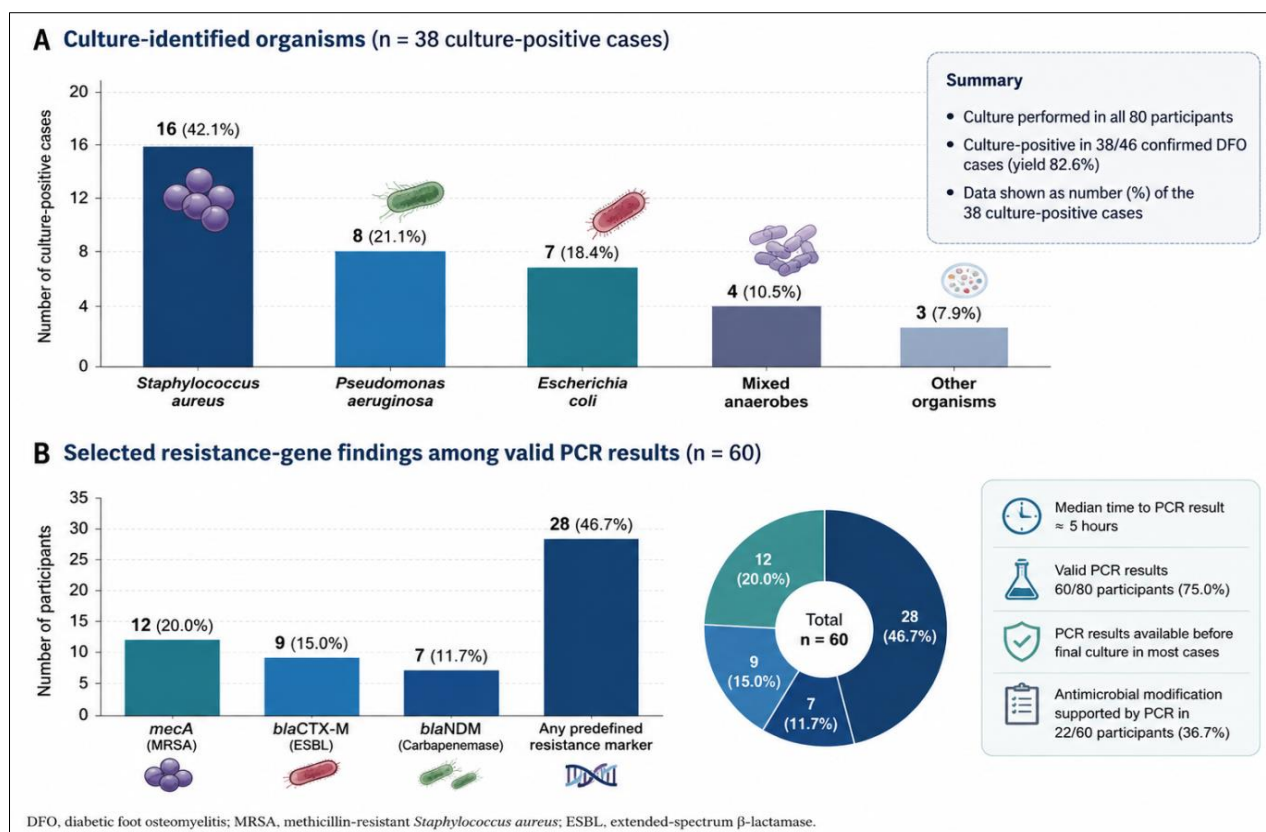


Figure 4: Culture and targeted molecular findings. (A) Distribution of organisms recovered from the 38 culture-positive cases, with *Staphylococcus aureus* as the predominant isolate (16/38, 42.1%), followed by *Pseudomonas aeruginosa* (8/38, 21.1%) and *Escherichia coli* (7/38, 18.4%). (B) Selected resistance-gene findings among the 60 participants with valid PCR results. At least one predefined resistance marker was detected in 28/60 participants (46.7%), most commonly *mecA* (12/60, 20.0%), *blaCTX-M* (9/60, 15.0%), and *blaNDM* (7/60, 11.7%). Valid PCR results were obtained in 60/80 participants (75.0%), with a turnaround time of approximately 5 hours; antimicrobial modification before the final culture report occurred in 22/60 PCR-tested participants (36.7%). DFO, diabetic foot osteomyelitis; MRSA, methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum β -lactamase

3.7 Treatment Modification and 90-Day Outcomes

Antimicrobial modification after PCR results became available and before the final culture report occurred in 22/60 PCR-tested participants (36.7%), equivalent to 22/80 (27.5%) of the full cohort. During 90 days of follow-up, 11/80 participants (13.8%) underwent amputation: eight minor amputations (10.0%) and three

major amputations (3.8%). Because molecular testing was not randomly assigned and the available dataset did not provide coherent non-overlapping exposure groups for adjusted comparison, these management and limb outcomes are presented descriptively and are not attributed causally to PCR testing (Table 8).

Table 8: Exploratory treatment and 90-day clinical outcomes

Outcome	Observed result
Antimicrobial modification before final culture	22/60 (36.7%) among PCR-tested
Any amputation by 90 days	11/80 (13.8%)
Minor amputation	8/80 (10.0%)
Major amputation	3/80 (3.8%)

4. DISCUSSION

This prospective pilot study examined two distinct but clinically connected points in the DFO pathway. MRI showed high diagnostic performance against the predefined reference standard, while targeted resistance-gene PCR provided selected resistance information substantially earlier than conventional

culture in the tested subset. The results do not support combining MRI and PCR into one diagnostic index. Their value is sequential: MRI defines the anatomical probability and extent of bone infection, culture identifies the organism and phenotypic susceptibility profile, and targeted PCR may reduce the waiting interval for selected resistance information.

DFO was confirmed in 57.5% of the cohort. This proportion should not be interpreted as population prevalence. The inclusion criteria intentionally selected patients with a high pretest probability of advanced infection, including prolonged ulceration, positive probe-to-bone testing, exposed bone, or evidence of deeper tissue involvement. Contemporary IWGDF/IDSA frameworks similarly emphasize clinical stratification before advanced imaging [1–3]. The present diagnostic estimates therefore describe performance in a clinically suspicious referral population rather than screening performance in unselected patients with diabetes.

MRI sensitivity reached 91.3%, and the negative likelihood ratio was 0.11. In this cohort, a technically adequate negative MRI therefore produced a substantial downward shift in the probability of DFO. Specificity was lower at 82.4%, which remains consistent with the known overlap between infection and noninfectious marrow abnormalities. Reactive edema, Charcot neuro-osteoarthropathy, postoperative change, and trauma can all increase fluid-sensitive marrow signal [5–8]. The study deliberately required confluent T1 marrow replacement linked anatomically to the ulcer or sinus tract and at least one supportive feature rather than treating edema alone as diagnostic. The six false-positive and four false-negative cases are a useful reminder that no sequence or isolated sign is definitive.

DWI and contrast-enhanced imaging were used as adjuncts rather than as independent arbiters of infection. This is important because current evidence suggests potential incremental value for diffusion and dynamic or contrast-enhanced techniques, while also showing substantial variation in acquisition parameters, ADC measurement, and proposed thresholds [5]. The absence of a universal ADC cutoff in this study was therefore intentional. A structured interpretation based on anatomy, T1 marrow replacement, ulcer-to-bone continuity, cortical disruption, sinus tract, abscess, and concordant diffusion restriction is more transferable than a single numeric threshold.

Conventional culture was positive in 82.6% of reference-standard-confirmed DFO cases. The remaining negative cultures are clinically plausible and may reflect previous antimicrobial exposure, low organism burden, devitalized bone, transport factors, or the use of deep tissue when bone biopsy was not feasible [1–16]. *Staphylococcus aureus* was the dominant cultured organism, with *Pseudomonas aeruginosa*, *Escherichia coli*, and anaerobic growth also represented. This pattern is compatible with the polymicrobial and resistant spectrum described in chronic or previously treated diabetic foot infection [15, 16]. Culture remained indispensable because it linked organism identification to phenotypic susceptibility, a function that the targeted PCR panel could not replace.

The molecular component adds a narrower but potentially useful layer. At least one predefined resistance determinant was detected in 46.7% of participants with valid PCR results, and overall molecular–phenotypic agreement was 85.0% with substantial κ agreement. Imperfect agreement is expected. Gene detection does not ensure expression, detected DNA may not represent the dominant viable pathogen, and phenotypic resistance may arise through mechanisms outside a targeted panel [13–15]. The practical advantage in this cohort was time: selected molecular resistance information was available approximately 57 hours earlier than final conventional culture on average.

Antimicrobial modification after PCR availability and before final culture occurred in 36.7% of PCR-tested participants. This temporal sequence is clinically interesting, but it cannot establish that PCR caused the treatment change or improved outcomes. The molecular test was not randomized, and clinicians were managing patients using all available clinical, imaging, and microbiological information. The 13.8% 90-day amputation rate likewise reflects the severity of the cohort but should not be attributed to molecular testing. Peripheral arterial disease, glycemic control, renal impairment, ulcer depth, delayed presentation, debridement, revascularization, and surgical timing all influence limb loss [17–19].

The study’s main contribution is therefore not a claim that PCR improves MRI or that rapid genotyping replaces culture. It is a feasibility model for sequencing diagnostic information. MRI can answer whether and where bone appears infected; conventional microbiology can identify the pathogen and full susceptibility phenotype; targeted PCR may provide an earlier warning for selected resistance mechanisms while culture is pending. In settings where 1.5-T MRI and basic molecular capability are available, that division of labor is clinically realistic and deserves larger prospective evaluation.

5. Limitations

Several limitations narrow the interpretation. First, this was a single-center pilot cohort with 80 participants and a high pretest probability of DFO, which limits generalizability to screening populations or centers with different referral patterns. The sample size was appropriate for preliminary precision estimates but not for definitive comparative-effectiveness conclusions.

Second, histopathology was not available in every participant. The study therefore used a predefined composite pathway in which histology established definite disease when available and multidisciplinary adjudication classified cases when histology was unavailable or nondiagnostic. Although the panel was blinded to the initial MRI classification, the use of more

than one reference-standard pathway introduces potential classification variability.

Third, PCR produced valid results in only 60/80 participants. The molecular analysis was consequently an available-case analysis, and the panel was restricted to selected resistance determinants. Results cannot be extrapolated to resistance mechanisms not included in the assay. Detailed reasons for each invalid PCR result were not preserved beyond failure of extraction or amplification validity criteria.

Fourth, reader-level MRI data were insufficient to calculate an independent interobserver κ value. Final diagnostic classification was based on consensus after independent review. Exact event dates were also incomplete, preventing time-to-event analysis for 90-day outcomes.

Finally, the exploratory clinical outcomes were not randomized or adjusted for the major determinants of limb loss. The observed timing of antimicrobial modification after PCR availability is therefore descriptive and should not be interpreted as proof of treatment benefit.

6. CONCLUSION

In adults with type 2 diabetes and clinically suspected deep foot infection, 1.5-T MRI demonstrated high diagnostic performance for DFO, with 91.3% sensitivity, 82.4% specificity, and a negative likelihood ratio of 0.11. Targeted resistance-gene PCR provided selected resistance information approximately 57 hours earlier than conventional culture in participants with valid molecular results, but it did not replace culture or establish bone infection. These findings support a complementary diagnostic pathway and justify larger multicenter studies designed to test whether earlier resistance information translates into measurable treatment or limb-related benefit.

7. Declarations

Ethics approval and consent to participate: The Institutional Review Board of the Wasit University College of Medicine approved the study (WUM-2025-018; 20 January 2025). Written informed consent was obtained from all participants for imaging, specimen collection, and use of anonymized clinical data.

Author Contributions

Imad Kh. Resen: Conceptualization, methodology, investigation, radiological analysis, data interpretation, writing – original draft, and writing – review and editing.
Zaid Ali Hussein: Investigation, radiological assessment, clinical interpretation, and writing – review and editing.

Ahlam Gareeb Nhaer: Microbiological analysis, molecular testing oversight, data interpretation, and writing – review and editing.

Zamazam Hussein Shummar: Investigation, data collection, data curation, and writing – review and editing.

Saradiq Mudhafar Jebur: Data collection, data curation, literature review, and writing – review and editing.

All authors read and approved the final manuscript.

Conflict of Interest: The authors declare that they have no conflicts of interest related to this study.

Funding: This research received no external funding.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Wasit University College of Medicine (Approval No. WUM-2025-018, dated 20 January 2025). Written informed consent was obtained from all participants before enrollment, imaging, specimen collection, and use of anonymized clinical data.

Consent for Publication: Not applicable. The manuscript does not contain identifiable personal information requiring individual consent for publication.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to institutional and ethical restrictions.

REFERENCES

1. Senneville É, Albalawi Z, van Asten SA, Abbas ZG, Allison G, Aragón-Sánchez J, et al. IWGDF/IDSA guidelines on the diagnosis and treatment of diabetes-related foot infections. *Clin Infect Dis*. 2024;79(2):e91-e141. doi:10.1093/cid/ciad527.
2. Senneville É, Albalawi Z, van Asten SA, Abbas ZG, Allison G, Aragón-Sánchez J, et al. Diagnosis of infection in the foot of patients with diabetes: a systematic review. *Diabetes Metab Res Rev*. 2024;40(3):e3723. doi:10.1002/dmrr.3723.
3. Monteiro-Soares M, Russell D, Boyko EJ, Jeffcoate W, Mills JL, Morbach S, et al. Guidelines on the classification of foot ulcers in people with diabetes: IWGDF 2023 update. *Diabetes Metab Res Rev*. 2024;40(3):e3648. doi:10.1002/dmrr.3648.
4. Lauri C, Noriega-Álvarez E, Chakravartty RM, Gheysens O, Glaudemans AWJM, Slart RHJA, et al. Diagnostic imaging of the diabetic foot: an EANM evidence-based guidance. *Eur J Nucl Med Mol Imaging*. 2024;51(8):2229-2246. doi:10.1007/s00259-024-06693-y.
5. Wudhikulprapan W, Phinyo P, Hadi A, Kanthawang T, Choudur HN. Diagnosing osteomyelitis in diabetic foot by diffusion-weighted imaging and dynamic contrast material-enhanced magnetic

- resonance imaging: a systematic review and meta-analysis. *Clin Radiol*. 2024;79(11):805-817. doi:10.1016/j.crad.2024.07.015.
6. Llewellyn A, Kraft J, Holton C, Harden M, Simmonds M. Imaging for detection of osteomyelitis in people with diabetic foot ulcers: a systematic review and meta-analysis. *Eur J Radiol*. 2020;131:109215. doi:10.1016/j.ejrad.2020.109215.
7. Lauri C, Tamminga M, Glaudemans AWJM, Juárez Orozco LE, Erba PA, Jutte PC, et al. Detection of osteomyelitis in the diabetic foot by imaging techniques: a systematic review and meta-analysis comparing MRI, white blood cell scintigraphy, and FDG-PET. *Diabetes Care*. 2017;40(8):1111-1120. doi:10.2337/dc17-0532.
8. Kapoor A, Page S, Lavalley M, Gale DR, Felson DT. Magnetic resonance imaging for diagnosing foot osteomyelitis: a meta-analysis. *Arch Intern Med*. 2007;167(2):125-132. doi:10.1001/archinte.167.2.125.
9. Morrison WB, Schweitzer ME, Wapner KL, Hecht PJ, Marks RM. Osteomyelitis of the foot: relative importance of primary and secondary MR imaging signs. *Radiology*. 1998;207(3):625-632. doi:10.1148/radiology.207.3.9609903.
10. Ansert EA, Tarricone AN, Coye TL, Crisologo PA, Truong D, Suludere MA, et al. Update of biomarkers to diagnose diabetic foot osteomyelitis: a meta-analysis and systematic review. *Wound Repair Regen*. 2024;32(4):366-376. doi:10.1111/wrr.13174.
11. Schechter MC, Ali MK, Risk BB, Singer AD, Santamarina G, Rogers HK, et al. Percutaneous bone biopsy for diabetic foot osteomyelitis: a systematic review and meta-analysis. *Open Forum Infect Dis*. 2020;7(10):ofaa393. doi:10.1093/ofid/ofaa393.
12. Lavery LA, Armstrong DG, Peters EJG, Lipsky BA. Probe-to-bone test for diagnosing diabetic foot osteomyelitis: reliable or relic? *Diabetes Care*. 2007;30(2):270-274. doi:10.2337/dc06-1572.
13. Morsli M, Salipante F, Magnan C, Dunyach-Remy C, Sotto A, Lavigne JP. Direct metagenomics investigation of non-surgical hard-to-heal wounds: a review. *Ann Clin Microbiol Antimicrob*. 2024;23(1):39. doi:10.1186/s12941-024-00698-z.
14. Saleem M, Moursi SA, Altamimi TNA, Alharbi MS, Alaskar AM, Hammam SAH, et al. Prevalence and molecular characterization of carbapenemase-producing multidrug-resistant bacteria in diabetic foot ulcer infections. *Diagnostics (Basel)*. 2025;15(2):141. doi:10.3390/diagnostics15020141.
15. Maity S, Leton N, Nayak N, Jha A, Anand N, Thompson K, et al. A systematic review of diabetic foot infections: pathogenesis, diagnosis, and management strategies. *Front Clin Diabetes Healthc*. 2024;5:1393309. doi:10.3389/fcdhc.2024.1393309.
16. van Asten SA, La Fontaine J, Peters EJG, Bhavan K, Kim PJ, Lavery LA. The microbiology of diabetic foot infections: a meta-analysis. *Diabetes Metab Res Rev*. 2017;33(4):e2905. doi:10.1002/dmrr.2905.
17. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med*. 2017;376(24):2367-2375. doi:10.1056/NEJMra1615439.
18. Edmonds M, Manu C, Vas P. The current burden of diabetic foot disease. *J Clin Orthop Trauma*. 2021;17:88-93. doi:10.1016/j.jcot.2021.01.017.
19. Jing C, Ralph JE, Lim J, Cathey JM, O'Neill CN, Anastasio AT. Novel treatments for diabetic foot osteomyelitis: a narrative review. *Microorganisms*. 2025;13(7):1639. doi:10.3390/microorganisms13071639.
20. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ*. 2015;351:h5527. doi:10.1136/bmj.h5527.

Cite This Article: Resen, I. K., Shummar, Z. H., Jebur, S. M., Nhaer, A. G., & Hussein, Z. A. (2026). MRI Diagnosis and Targeted Resistance-Gene PCR in Diabetic Foot Osteomyelitis: A Prospective Pilot Study. *EAS J Radiol Imaging Technol*, 8(5), 103-113. <https://doi.org/10.36349/easjrit.2026.v08i05.001>