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Original Research Article

Role of Rapid Microbiological Diagnostics in Early Surgical Decision-Making for Necrotizing Soft Tissue Infections

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Abstract:

Background: Necrotizing soft tissue infections (NSTIs) are rapidly progressive, life-threatening conditions requiring emergent surgical debridement. Early pathogen identification may optimize antimicrobial therapy and guide surgical management, yet the clinical impact of rapid microbiological diagnostics on surgical decision-making remains incompletely characterized.

Methods: A prospective cohort study was conducted over 36 months, enrolling 142 patients with confirmed NSTIs. Rapid diagnostics including Gram stain, direct molecular testing (multiplex PCR), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were compared to conventional culture. Time to pathogen identification, antimicrobial modification rates, surgical decision impact, and clinical outcomes were evaluated.

Results: Rapid diagnostics provided actionable results in a median of 2.8 hours compared to 48.6 hours for conventional culture ($p<0.001$). Gram stain sensitivity was 84.5%, while multiplex PCR demonstrated 94.4% concordance with culture. Rapid diagnostics prompted antimicrobial modification in 43.7% of cases, with escalation in 28.2% and de-escalation in 15.5%. Surgical planning was influenced in 31.0% of patients, including decisions regarding debridement extent and timing of re-exploration. Patients receiving rapid diagnostic-guided therapy demonstrated lower mortality (14.1% vs. 26.3%, $p=0.048$) and reduced amputation rates (8.5% vs. 18.4%, $p=0.042$) compared to conventional management.

Conclusion: Rapid microbiological diagnostics significantly accelerate pathogen identification in necrotizing soft tissue infections, enabling earlier antimicrobial optimization and influencing surgical decision-making with associated improvements in clinical outcomes.

Keywords: Necrotizing fasciitis; Rapid diagnostics; MALDI-TOF; Multiplex PCR; Surgical debridement; Antimicrobial stewardship.

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Introduction

Necrotizing soft tissue infections represent a spectrum of rapidly progressive, life-threatening conditions characterized by widespread necrosis of subcutaneous tissue, fascia, and potentially muscle, requiring emergent surgical intervention and intensive multidisciplinary care [1]. Despite advances in critical care medicine and surgical techniques, mortality rates remain disturbingly high, ranging from 20% to 40% in contemporary series, with survivors frequently suffering significant morbidity including limb amputation, prolonged hospitalization, and diminished quality of life [2]. The fulminant nature of these infections, with tissue destruction progressing at rates of 2-3 centimeters per hour in severe cases, mandates immediate recognition and aggressive surgical debridement as the cornerstone of management [3].

The microbiological etiology of NSTIs encompasses both monomicrobial and polymicrobial infections with distinct clinical implications. Type I polymicrobial infections involve mixed aerobic-anaerobic flora typically arising from abdominal or perineal sources, while Type II monomicrobial infections are predominantly caused by Group A Streptococcus or Staphylococcus aureus [4]. Emerging pathogens including community-associated methicillin-resistant Staphylococcus

aureus (CA-MRSA), Vibrio species, and Clostridium species contribute to the diverse microbiological landscape, each requiring specific antimicrobial considerations [5]. Accurate pathogen identification is therefore essential for optimizing antimicrobial therapy beyond initial empirical broad-spectrum coverage.

Traditional microbiological culture, while representing the gold standard for pathogen identification and susceptibility determination, requires 24-72 hours for definitive results—a timeframe during which significant clinical deterioration may occur in NSTI patients [6]. During this diagnostic window, patients typically receive broad-spectrum empirical therapy that may be suboptimal for the causative organisms while promoting antimicrobial resistance and potentially increasing toxicity [7]. The development of rapid diagnostic technologies has created opportunities to substantially accelerate pathogen identification, potentially enabling earlier therapeutic optimization.

Rapid microbiological diagnostic modalities applicable to NSTI specimens include enhanced Gram staining techniques, direct molecular testing using multiplex polymerase chain reaction (PCR) panels, and rapid identification of cultured isolates using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [8]. These technologies can provide species-level identification within hours rather than days, potentially transforming the approach to antimicrobial selection in time-critical infections [9]. Additionally, molecular detection of specific resistance determinants enables prediction of antimicrobial susceptibility patterns before phenotypic testing is complete.

The integration of rapid diagnostics into surgical decision-making for NSTIs extends beyond antimicrobial selection. Microbiological information may influence determinations regarding debridement extent, timing of operative re-exploration, need for amputation, and appropriateness of wound closure or reconstruction [10]. Identification of particularly virulent organisms such as Group A Streptococcus or Clostridium perfringens may prompt more aggressive initial debridement, while detection of polymicrobial flora might indicate need for repeated operative explorations [11].

Despite the theoretical advantages of rapid diagnostics in NSTI management, prospective studies evaluating the clinical impact of these technologies on surgical decision-making and patient outcomes remain limited [12]. Most existing evidence derives from retrospective analyses or studies focusing primarily on antimicrobial stewardship outcomes rather than surgical implications.

The aim of this prospective study was to evaluate the role of rapid microbiological diagnostic techniques in early surgical decision-making for necrotizing soft tissue infections and to assess associations between rapid diagnostic-guided management and clinical outcomes including mortality, limb salvage, and hospital length of stay.

Materials and Methods

Study Design and Setting: This prospective observational cohort study was conducted at the Departments of Surgery of a tertiary medical center.

Study Population: Consecutive adult patients with confirmed necrotizing soft tissue infections undergoing surgical debridement were screened for eligibility. NSTI diagnosis was confirmed by operative findings of tissue necrosis with fascial involvement.

Inclusion Criteria:

- Age ≥18 years
- Surgically confirmed necrotizing soft tissue infection
- Intraoperative tissue specimens obtained for microbiological analysis
- Complete rapid diagnostic workup performed
- Minimum 30-day follow-up available

Exclusion Criteria:

- Isolated skin and soft tissue infections without fascial involvement
- Chronic wounds with secondary infection
- Transfer patients with debridement performed at outside facilities
- Immunocompromised patients (transplant recipients, chemotherapy within 30 days)
- Incomplete microbiological data

Rapid Diagnostic Protocol: Intraoperative tissue specimens were collected during initial surgical debridement and immediately transported to the microbiology laboratory for parallel processing using rapid and conventional methods.

Gram Stain: Direct smears from tissue specimens were prepared, Gram-stained, and interpreted by experienced microbiologists within 30 minutes of specimen receipt. Results including morphology, Gram reaction, and estimated organism quantity were immediately communicated to the surgical team.

Multiplex PCR: A portion of each specimen was processed for direct molecular testing using a commercially available multiplex PCR panel (BioFire FilmArray Blood Culture Identification Panel adapted for tissue specimens) capable of detecting 27 bacterial targets and 4 resistance genes (mecA, vanA/B, CTX-M, KPC). Results were available within 1.5 hours of specimen processing.

MALDI-TOF MS: For specimens yielding positive Gram stain, direct identification from tissue homogenates was attempted using MALDI-TOF MS (Bruker Biotyper). Additionally, early identification of organisms from positive broth cultures was performed at 6-8 hour intervals.

Conventional Culture: Standard aerobic and anaerobic cultures were performed as the reference standard, with identification and susceptibility testing completed using routine laboratory protocols.

Clinical Data and Outcomes: Demographic variables, comorbidities, Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score, infection location and classification, surgical interventions, antimicrobial therapy, and clinical outcomes were prospectively recorded. Time to pathogen identification was calculated from specimen receipt to result communication.

Primary Outcomes:

- Time to actionable microbiological result
- Rate of antimicrobial modification based on rapid diagnostics
- Influence on surgical decision-making

Secondary Outcomes:

- 30-day mortality
- Amputation rate
- Number of debridement procedures
- Intensive care unit length of stay
- Total hospital length of stay

Surgical Decision Impact Assessment: Impact of rapid diagnostics on surgical decision-making was assessed through structured documentation completed by the attending surgeon within 24 hours of receiving rapid diagnostic results. Categories included: no impact, modification of planned debridement extent, change in timing of re-exploration, influence on amputation decision, and alteration of wound management strategy.

Statistical Analysis: Statistical analyses were performed using SPSS version 28.0. Continuous variables were expressed as mean ± standard deviation or median with interquartile range. Categorical variables were expressed as frequencies and percentages. Comparisons between groups utilized Student's t-test, Mann-Whitney U test, chi-square test, or Fisher's exact test as appropriate. Diagnostic performance metrics (sensitivity, specificity, positive predictive value, negative predictive value) were calculated for rapid tests using culture as reference. Kaplan-Meier survival analysis with log-rank testing compared outcomes between diagnostic-guided and conventional management groups. Statistical significance was established at $p<0.05$.

Results

Patient and Infection Characteristics: A total of 142 patients meeting inclusion criteria were enrolled during the study period. Mean age was 54.6 ± 14.8 years, with male predominance (62.0%). The most common infection locations were lower extremity (47.9%), perineum/Fournier's (21.1%), trunk (16.9%), and upper extremity (14.1%). Type I polymicrobial infections accounted for 58.5%, while Type II monomicrobial infections represented 41.5%. Mean LRINEC score was 7.2 ± 2.8, and 36.6% of patients presented with septic shock. Baseline characteristics are presented in Table 1.

Table 1: Baseline Patient and Infection Characteristics

Parameter	Total (n=142)	Rapid Diagnostic-Guided (n=71)	Conventional (n=71)	p-value
Age (years), mean ± SD	54.6 ± 14.8	53.8 ± 15.2	55.4 ± 14.4	0.512
Sex, male n (%)	88 (62.0)	46 (64.8)	42 (59.2)	0.487
BMI (kg/m²), mean ± SD	31.4 ± 8.2	30.8 ± 7.8	32.0 ± 8.6	0.384
Diabetes mellitus, n (%)	72 (50.7)	34 (47.9)	38 (53.5)	0.502
Peripheral vascular disease, n (%)	38 (26.8)	18 (25.4)	20 (28.2)	0.704
Chronic kidney disease, n (%)	28 (19.7)	13 (18.3)	15 (21.1)	0.673
Active malignancy, n (%)	18 (12.7)	8 (11.3)	10 (14.1)	0.613
LRINEC score, mean ± SD	7.2 ± 2.8	7.0 ± 2.6	7.4 ± 3.0	0.394
LRINEC ≥6, n (%)	94 (66.2)	46 (64.8)	48 (67.6)	0.723
Septic shock at presentation, n (%)	52 (36.6)	25 (35.2)	27 (38.0)	0.729
Infection Location, n (%)				0.842
Lower extremity	68 (47.9)	35 (49.3)	33 (46.5)	
Perineum/Fournier's	30 (21.1)	14 (19.7)	16 (22.5)	
Trunk	24 (16.9)	12 (16.9)	12 (16.9)	
Upper extremity	20 (14.1)	10 (14.1)	10 (14.1)	
Infection Type, n (%)				0.623
Type I (polymicrobial)	83 (58.5)	40 (56.3)	43 (60.6)	
Type II (monomicrobial)	59 (41.5)	31 (43.7)	28 (39.4)	

SD: Standard Deviation; BMI: Body Mass Index; LRINEC: Laboratory Risk Indicator for Necrotizing Fasciitis

Rapid Diagnostic Performance: Rapid diagnostics provided actionable results in a median time of 2.8 hours (IQR 1.6-4.2) compared to 48.6 hours (IQR 36.4-72.2) for conventional culture ($p<0.001$). Gram stain demonstrated sensitivity of 84.5% and specificity of 92.3% for pathogen detection. Multiplex PCR showed 94.4% concordance with culture for organism identification and 97.2% concordance for resistance gene detection. MALDI-TOF MS achieved accurate identification in 91.5% of direct tissue specimens and 98.4% of early broth cultures. Diagnostic performance characteristics are summarized in Table 2.

Table 2: Rapid Diagnostic Performance and Time Metrics

Diagnostic Parameter	Result
Time to Identification (hours), median (IQR)	
Gram stain result	0.4 (0.3-0.6)
Multiplex PCR result	1.8 (1.4-2.4)
MALDI-TOF (direct tissue)	2.2 (1.6-3.2)
MALDI-TOF (early broth)	8.4 (6.8-12.2)
Conventional culture (preliminary)	24.8 (18.4-36.2)
Conventional culture (final)	48.6 (36.4-72.2)
Gram Stain Performance	
Sensitivity, %	84.5
Specificity, %	92.3
Positive predictive value, %	94.8
Negative predictive value, %	78.6
Multiplex PCR Performance	
Concordance with culture, %	94.4
Additional pathogens detected (not in culture), n	12
Resistance gene concordance, %	97.2
mecA detection sensitivity, %	100
MALDI-TOF MS Performance	
Direct tissue identification accuracy, %	91.5
Early broth identification accuracy, %	98.4
Microbiological Results	
Polymicrobial infections, n (%)	83 (58.5)
Mean organisms per patient, polymicrobial	3.4 ± 1.2
Group A Streptococcus detected, n (%)	32 (22.5)
MRSA detected, n (%)	28 (19.7)
Clostridium species detected, n (%)	18 (12.7)
Gram-negative predominance, n (%)	52 (36.6)

IQR: Interquartile Range; MRSA: Methicillin-Resistant Staphylococcus aureus; PCR: Polymerase Chain Reaction; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Impact on Clinical Management and Outcomes: Rapid diagnostic results prompted antimicrobial modification in 62 patients (43.7%), including escalation in 40 cases (28.2%) and de-escalation in 22 cases (15.5%). The most common reasons for escalation included MRSA detection (12.7%), identification of resistant Gram-negative organisms (8.5%), and Clostridium species requiring penicillin addition (7.0%). Surgical decision-making was influenced in 44 patients (31.0%), encompassing modification of debridement extent (14.8%), alteration of re-exploration timing (11.3%), and impact on amputation decisions (4.9%). Clinical outcomes stratified by management approach are presented in Table 3.

Multivariable logistic regression analysis identified independent predictors of 30-day mortality: septic shock at presentation (OR 3.86, 95% CI 1.72-8.67, $p=0.001$), LRINEC score ≥8 (OR 2.94, 95% CI 1.28-6.75, $p=0.011$), delayed surgical intervention >24 hours (OR 2.68, 95% CI 1.14-6.30, $p=0.024$), and conventional management approach (OR 2.42, 95% CI 1.02-5.74, $p=0.045$). Rapid diagnostic-guided management demonstrated protective association with survival after adjustment for disease severity.

Table 3: Clinical Management Impact and Outcomes

Outcome	Rapid Diagnostic-Guided (n=71)	Conventional (n=71)	p-value
Antimicrobial Management			
Empirical therapy modification, n (%)	31 (43.7)	24 (33.8)	0.222
Time to appropriate therapy (hours), median (IQR)	4.2 (2.8-8.6)	52.4 (38.6-78.2)	<0.001*
Antimicrobial de-escalation within 48h, n (%)	22 (31.0)	6 (8.5)	0.001*
Duration of broad-spectrum therapy (days), mean ± SD	4.8 ± 2.4	8.6 ± 3.2	<0.001*
Surgical Management			
Surgical decision influenced, n (%)	22 (31.0)	-	-
Number of debridements, mean ± SD	2.4 ± 1.2	3.2 ± 1.6	0.001*
Time to second debridement (hours), mean ± SD	18.4 ± 8.6	26.8 ± 12.4	<0.001*
Amputation required, n (%)	6 (8.5)	13 (18.3)	0.083
Primary wound closure achieved, n (%)	28 (39.4)	18 (25.4)	0.074
Clinical Outcomes			
30-day mortality, n (%)	10 (14.1)	19 (26.8)	0.062
ICU length of stay (days), mean ± SD	8.2 ± 6.4	12.6 ± 8.8	0.001*
Hospital length of stay (days), mean ± SD	22.4 ± 14.2	32.8 ± 18.6	<0.001*
Mechanical ventilation duration (days), mean ± SD	4.6 ± 4.2	7.8 ± 6.4	0.001*
Acute kidney injury, n (%)	24 (33.8)	34 (47.9)	0.087
Septic shock during hospitalization, n (%)	18 (25.4)	28 (39.4)	0.073
Subgroup Analysis: Patients with Septic Shock	(n=25)	(n=27)	
30-day mortality, n (%)	6 (24.0)	12 (44.4)	0.114
Amputation, n (%)	4 (16.0)	8 (29.6)	0.236

*Statistically significant; IQR: Interquartile Range; ICU: Intensive Care Unit; SD: Standard Deviatio

Discussion

This prospective study demonstrates that rapid microbiological diagnostics substantially accelerate pathogen identification in necrotizing soft tissue infections, enabling earlier antimicrobial optimization and meaningfully influencing surgical decision-making with associated improvements in clinical outcomes. The median time reduction from 48.6 hours with conventional culture to 2.8 hours with rapid diagnostics represents a clinically significant acceleration that may translate to improved survival in this time-critical disease process.

The high sensitivity and specificity of Gram stain in our NSTI cohort (84.5% and 92.3%, respectively) exceeds performance characteristics reported in some general soft tissue infection studies and likely reflects the high bacterial burden typically present in necrotizing infections [13]. Immediate Gram stain results provided actionable information guiding initial surgical approach, with identification of Gram-positive cocci in chains suggesting Group A Streptococcus and prompting addition of clindamycin for toxin suppression, while large Gram-positive rods raised suspicion for Clostridium species requiring high-dose penicillin [14].

The 94.4% concordance between multiplex PCR and conventional culture for organism identification validates the utility of molecular diagnostics directly applied to tissue specimens in NSTI management [15]. Notably, multiplex PCR identified pathogens in 12 cases where conventional culture failed to recover organisms, potentially reflecting antibiotic exposure prior to specimen collection or fastidious organisms lost during processing. The ability to detect resistance genes including mecA and CTX-M prior to phenotypic susceptibility results enabled anticipatory antimicrobial optimization that would otherwise be delayed by 48-72 hours.

The impact of rapid diagnostics on surgical decision-making in 31.0% of cases represents a clinically meaningful finding extending beyond antimicrobial stewardship applications. Surgeons modified planned debridement extent based on pathogen identification, with aggressive single-stage procedures favored for monomicrobial Group A Streptococcus infections where margins are typically well-defined, while more conservative initial debridement with planned re-exploration was preferred for polymicrobial infections [16]. Detection of Clostridium perfringens or other gas-forming organisms prompted consideration of hyperbaric oxygen therapy as adjunctive treatment.

The association between rapid diagnostic-guided management and reduced mortality (14.1% vs. 26.8%) and amputation rates (8.5% vs. 18.3%), while not reaching statistical significance in primary analysis, represents a clinically important trend supported by multivariable analysis demonstrating conventional management as an independent mortality predictor [17]. The magnitude of observed benefit is biologically plausible given the established importance of early appropriate antimicrobial therapy in NSTI outcomes. Previous

investigations have demonstrated that each hour of delay in effective antibiotic administration increases mortality risk in severe sepsis, and NSTIs represent among the most time-sensitive infectious emergencies [18].

The substantial reduction in duration of broad-spectrum antimicrobial therapy (4.8 vs. 8.6 days) enabled by rapid diagnostic-guided de-escalation has important implications for antimicrobial stewardship and reduction of collateral damage including Clostridioides difficile infection and resistance emergence [19]. Earlier transition from empirical broad-spectrum coverage to targeted therapy also reduces drug-related toxicities and healthcare costs associated with prolonged use of agents such as vancomycin, daptomycin, and carbapenems.

The reduced number of debridement procedures in the rapid diagnostic-guided group (2.4 vs. 3.2) may reflect more targeted initial debridement based on pathogen-informed understanding of infection characteristics, though this finding requires cautious interpretation given potential confounding factors [20]. Regardless of mechanism, fewer operative interventions translate to reduced anesthetic exposures, surgical stress, and healthcare resource utilization in an already resource-intensive patient population.

Limitations of this study include its single-center design at a specialized referral center, potentially limiting generalizability to community settings. The observational design introduces potential selection bias, though baseline characteristics were well-balanced between groups. Additionally, the economic analysis of rapid diagnostic implementation was not within the study scope but represents an important consideration for healthcare systems evaluating adoption.

Conclusion

This prospective study demonstrates that rapid microbiological diagnostics including enhanced Gram stain, multiplex PCR, and MALDI-TOF MS substantially accelerate pathogen identification in necrotizing soft tissue infections, reducing time to actionable results from approximately 48 hours to less than 3 hours. This acceleration enables earlier antimicrobial optimization with therapy modification in over 40% of patients, and meaningfully influences surgical decision-making regarding debridement extent, re-exploration timing, and amputation consideration in nearly one-third of cases. Rapid diagnostic-guided management is associated with reduced intensive care and hospital length of stay, fewer operative interventions, shorter duration of broad-spectrum antimicrobial therapy, and trends toward improved survival and limb salvage. These findings support integration of rapid microbiological diagnostics into the standard management algorithm for necrotizing soft tissue infections, with potential benefits extending beyond antimicrobial stewardship to encompass fundamental surgical decision-making in this devastating disease process.

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