e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(8); 1358-1363

Original Research Article

Optimizing Antibiotic Therapy in Perforation Peritonitis Using Rapid Whole Genome Sequencing

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Received: 01-05-2026 / Revised: 15-06-2026 / Accepted: 21-07-2026

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Conflict of interest: Nil

Abstract

Background: Perforation peritonitis is a major surgical emergency associated with polymicrobial infection, antimicrobial resistance, sepsis, postoperative morbidity, and mortality. Conventional culture may delay pathogen identification and antimicrobial susceptibility reporting, potentially prolonging inappropriate empirical therapy. Rapid whole genome sequencing (rWGS) offers earlier and broader microbial detection with resistance profiling.

Objectives: To compare rWGS with conventional culture for detection of polymicrobial peritonitis, microbial profile, antimicrobial resistance, and to assess its utility in optimizing antibiotic therapy and clinical outcomes.

Materials and Methods: This prospective observational study included 70 patients with perforation peritonitis undergoing surgery. Intra-abdominal fluid was divided for parallel rWGS and conventional culture. Pathogen detection, polymicrobial infection, resistance patterns, turnaround time, antibiotic modification, surgical site infection (SSI), ICU admission, and mortality were evaluated.

Results: Conventional culture detected organisms in 58 patients (82.9%), whereas rWGS identified organisms in all 70 patients (100.0%). Polymicrobial infection was detected in 48.6% by culture and 68.6% by rWGS. Mean reporting time was significantly shorter with rWGS than culture (33.9 ± 6.0 vs 77.0 ± 12.1 hours; $p<0.001$). Antibiotic therapy was modified in 62.9% of patients following rWGS findings. SSI occurred in 48.6%, ICU admission in 65.7%, and mortality in 15.7%.

Conclusion: rWGS provided superior pathogen detection, improved recognition of polymicrobial infection, and substantially faster reporting, thereby supporting earlier antimicrobial optimization in perforation peritonitis.

Keywords: Perforation Peritonitis, Rapid Whole Genome Sequencing, Antimicrobial Resistance, Polymicrobial Infection, Antibiotic Therapy, Surgical Site Infection.

DOI: 10.25258/ijcpr.18.8.203

Introduction

Perforation of a hollow gastrointestinal viscus allows gastrointestinal contents and microorganisms to enter the peritoneal cavity, producing secondary peritonitis that may progress to sepsis, septic shock and multiorgan dysfunction. Digestive tract perforation with peritonitis remains an important surgical emergency, with reported mortality of approximately 8–25%.

A recent cohort of 831 patients also demonstrated a predominance of Gram-negative organisms and substantial variation in antimicrobial susceptibility, reinforcing the importance of appropriate empirical therapy. [1] Timely microbiological information is therefore central to antimicrobial selection. In an experimental study using blood samples spiked

with common sepsis-causing bacterial isolates, nanopore sequencing identified pathogens after two hours of incubation and detected clinically relevant resistance genes after further sequencing; the reported turnaround time for information supporting antibiotic selection was approximately 7–9 hours. [2] Nanopore sequencing of positive blood cultures has similarly enabled rapid identification of pathogens, antimicrobial-resistance genes and plasmids. [3] Rapid next-generation sequencing in septic patients has also demonstrated broader pathogen detection than conventional blood culture and provided microbiological information earlier in the clinical course. [4]

Clinical evidence further supports sequencing-guided treatment. In 50 patients with blood diseases and serious infections, nanopore-targeted sequencing detected pathogens in 90% compared with 32.6% by blood culture; antibiotic adjustment based on sequencing findings was followed by a 93.02% anti-infective treatment response. [5] Management of peritonitis nevertheless continues to depend on adequate source control together with appropriately selected and optimized antimicrobial therapy, particularly in resistant nosocomial infection. [6]

Antimicrobial resistance further complicates empirical selection. Among 624 Gram-negative isolates evaluated in an Italian surveillance study, predicted therapeutic coverage differed substantially among tested agents, including against meropenem-resistant isolates. [7] Antimicrobial de-escalation is an important stewardship principle, but its application requires careful clinical and microbiological assessment. [8] Plasma microbial cell-free DNA sequencing has also demonstrated utility for pathogen detection in critically ill patients with sepsis. [9] Secondary peritonitis and intra-abdominal sepsis remain an important global surgical problem for which improved systemic therapeutic strategies are required. [10] In an Indian Tier-II setting, evaluating rWGS alongside conventional culture may provide locally relevant evidence on microbial detection, resistance patterns and antibiotic optimization. This forms the rationale for the present study.

Material and Methods

Study Design: This was a prospective observational study in patients of perforation peritonitis who were undergoing surgical management.

Study Setting: The study was conducted in the Department of General Surgery, Subharti Hospital, a tertiary care teaching institute.

Study Population and Sample Size: Patients with diagnosis of perforation peritonitis who were planned for surgery were screened for eligibility. All eligible patients presenting during the study period were recruited. The final study population consisted of 70 patients.

Inclusion criteria: Patients diagnosed with perforation peritonitis and planned for surgical intervention were included.

Exclusion Criteria: Patients who had been treated with antibiotics in the previous two weeks were excluded.

Sample Collection and Investigations: Intra-operatively, intra-abdominal fluid was collected from the site of perforation and other suspected contaminate areas of the peritoneal cavity. Each sample was split into two parts for parallel processing by rapid whole genome sequencing (rWGS) and conventional culture. rWGS Libraries were prepared and sequenced from DNA extracted from peritoneal fluid. The generated reads were mapped to reference genomes or assembled to identify the microbes. We performed variant calling and annotation to identify causative organisms and antimicrobial-resistance determinants. Quality control procedures were performed prior to reporting the results.

Appropriate media for routine microbiological analysis including blood agar, MacConkey agar and chocolate agar were inoculated with the samples. Organisms were identified by colony morphology, gram stain and biochemical character. Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion, broth dilution and/or E-test methods. Interpretation of susceptibility was according to CLSI or EUCAST criteria.

Outcome Assessment: We evaluated organism detection, polymicrobial infection, antimicrobial-resistance patterns, turnaround time, appropriateness of empirical therapy, antibiotic modification, SSI, ICU admission, length of hospital stay and mortality.

Ethical Consideration: Informed consent was obtained from all the participants. Patient data confidentiality and anonymization were guarantyd and ethics committee approval was obtained before the study initiation.

Statistical Analysis: Categorical variables were expressed as frequency and percentage and continuous variables were described as mean ± SD. The McNemar test, paired-samples t-test and chi-square test were used as appropriate. P-values were used to determine statistical significance.

Results

Table 1: Culture Characteristics among Patients with Perforation Peritonitis (n= 70).

Variable	Category	Frequency (n)	Percentage (%)	Cumulative Percentage (%)
Culture Polymicrobial	No	36	51.4	51.4
	Yes	34	48.6	100.0
Detection by Culture	No	12	17.1	17.1
	Yes	58	82.9	100.0
MDR (Culture)	No	36	51.4	51.4
	Yes	34	48.6	100.0

Organisms were isolated by conventional culture in 58 out of 70 patients (82.9%) and 12 patients (17.1%) had no growth (Table 1). Polymicrobial infection was found in 34 cases (48.6%). MDR organisms were also identified in 34 patients (48.6%), showing a considerable burden of complex and resistant intra-abdominal infection in the study population.

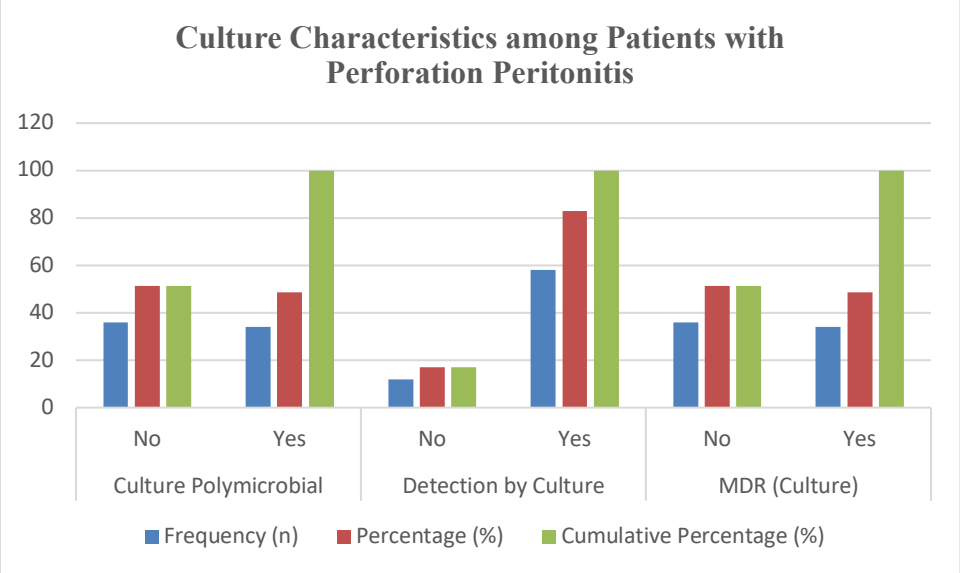

Figure 1: Culture Characteristics among Patients with Perforation Peritonitis (n= 70).

Table 2: Antibiotic Therapy Profile among Patients with Perforation Peritonitis (n = 70).

Variable	Category	Frequency (n)	Percentage (%)
Appropriate Empirical Therapy	No	44	62.9
	Yes	26	37.1
Modified after Rwgs	No	26	37.1
	Yes	44	62.9
Escalation	No	26	37.1
	Yes	44	62.9

Table 2 demonstrates that empirical antibiotic therapy was appropriate in only 26 patients (37.1%), whereas 44 patients (62.9%) received initially inappropriate therapy. 44 (62.9%) cases required antibiotic modification after rWGS results. The rate of patients with treatment escalation was similar, highlighting the clinical benefit of antimicrobial optimization using rWGS.

Table 3: Postoperative Outcomes among Patients with Perforation Peritonitis (n= 70).

Variable	Category	Frequency (n)	Percentage (%)	Cumulative Percentage (%)
SSI	No	36	51.4	51.4
	Yes	34	48.6	100.0
ICU Admission	No	24	34.3	34.3
	Yes	46	65.7	100.0
Mortality	No	59	84.3	84.3
	Yes	11	15.7	100.0

Table 3 shows the postoperative results in patients with perforation peritonitis. Surgical site infection was found in 34 patients (48.6%). ICU admission was needed by 46 patients (65.7%). Mortality was observed in 11 cases (15.7%), whereas 59 patients (84.3%) survived, reflecting the considerable postoperative morbidity associated with severe perforation peritonitis.

Table 4: Association between Final Polymicrobial Diagnosis and Surgical Site Infection among Patients with Perforation Peritonitis (n = 70).

Final Polymicrobial Diagnosis	SSI: No	SSI: Yes		P-Value
No	22	0		
Yes	14	34	48	< 0.001
Total	36	34	70	

Statistical test: χ^2 test.

Table 4 shows a significant association between polymicrobial infection and surgical site infection. Among 48 patients with a final polymicrobial diagnosis, 34 (70.8%) developed SSI, whereas none of the 22 non-polymicrobial cases developed SSI. This association was statistically significant (chi-square test, p<0.001) indicating a significantly higher risk of SSI with a polymicrobial infection.

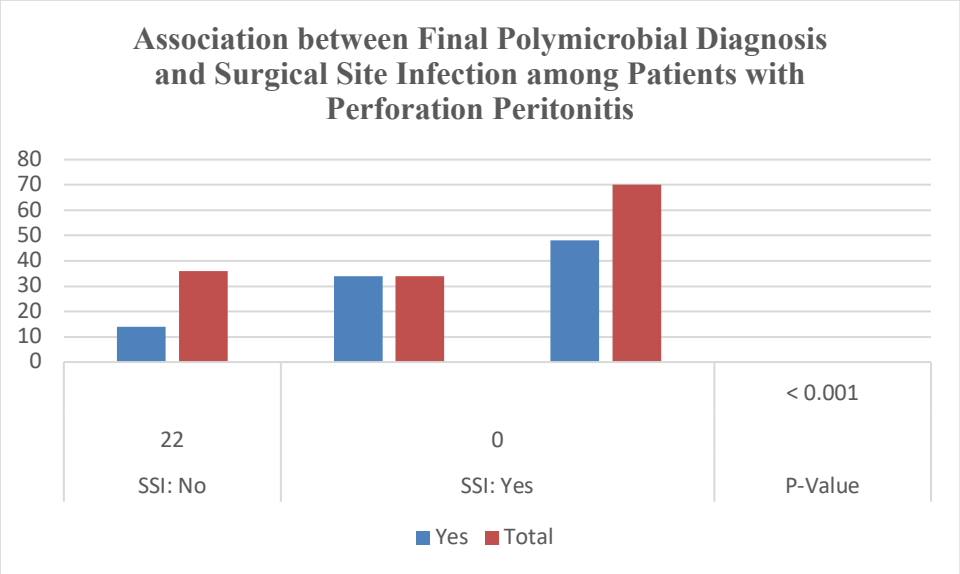

Figure 2: Association between Final Polymicrobial Diagnosis and Surgical Site Infection among Patients with Perforation Peritonitis (n = 70).

Table 5: Distribution of Organisms Isolated by Culture (n = 58)

Organism	Total (n)	Percentage (%)
Escherichia coli	39	67.2
Klebsiella pneumoniae	15	25.9
Pseudomonas aeruginosa	7	12.1
Bacteroides fragilis	18	31.0
Enterococcus faecalis	14	24.1

The most common organism isolated was Escherichia coli seen in 39 of 58 culture positive patients (67.2%) (Table 5). The predominantly enteric polymicrobial pattern was Bacteroides fragilis (31.0%), Klebsiella pneumoniae (25.9%), Enterococcus faecalis (24.1%) and Pseudomonas aeruginosa (12.1%).

Table 6: Overall Comparison of Culture and rWGS in Patients with Perforation Peritonitis (n = 70).

Parameter	Culture	rWGS	p-value
Detection, n (%)	58 (82.9)	70 (100.0)	<0.001
Polymicrobial, n (%)	34 (48.6)	48 (68.6)	<0.001
Time (hours), Mean ± SD	77.0 ± 12.1	33.9 ± 6.0	<0.001

Table 6 highlights the significant advantage of rWGS over traditional culture in the diagnostic performance. Organism detection was achieved in 100.0% of patients with rWGS versus 82.9% with culture, while polymicrobial infection was identified in 68.6% versus 48.6%, respectively. rWGS also substantially reduced reporting time (33.9 ± 6.0 vs 77.0 ± 12.1 hours; p<0.001 for all comparisons).

Discussion

In this cohort, organisms were cultured by conventional culture in 58/70 (82.9%) patients. Polymicrobial growth and multidrug resistance were shown in 34/70 (48.6%) each. Likewise Wang et al. (2025) [1] described a large resistant enteric burden of 831 patients and recovered 694 isolates,

503 gram negative organisms. Bassetti et al. (2024) [7] In 624 Gram-negative ICU isolates high resistance pressure was observed, cefiderocol susceptibility was between 87.7% and 100%. Sensitivity 68.1%, specificity 63.2% (Wang et al., 2021) [9] plasma mcf DNA. Conversely, Zhang et al (2023) [5] reported blood-culture positivity of only 32.6%, which was far lower than culture detection of 82.9% in the present study.

Empirical therapy was appropriate in only 26/70 patients (37.1%), whereas 44/70 (62.9%) required modification after rWGS and the same proportion underwent escalation. Similarly, Zhang et al. (2023) [5] changed antimicrobial therapy in 43 patients by sequencing and had a response rate of 93.02% (40/43). However, with nanopore sequencing, clinical resistance data can be generated in 7–9 h allowing earlier therapeutic intervention (Ali et al 2024) [2]. Taxt et al. (2020) [3] detected all the predefined resistance genes and plasmids within one hour of sequencing. De Waele et al. (2020) [8] also pointed out the necessity of microbiologically guided de-escalation. SSI 34/70 (48.6%), ICU admission 46/70 (65.7%), death 11/70 (15.7%) De Waele et al (2023) showed that nosocomial peritonitis is associated with multidrug resistance, ICU care and significant mortality, and therefore requires source control and appropriate antimicrobial therapy. High morbidity occurred 6 post-operatively. This was further supported by Clements et al. (2021) [10] who showed that secondary peritonitis and intra-abdominal sepsis were the main causes of morbidity and mortality even with improved critical care. Wang et al. (2021) [9] 199 critical ill patients. 94 patients with sepsis supporting the systemic burden of infection. In cohort 50 patients, Zhang et al. (2023) [5] reported no infection-related deaths after the change of treatment in the standard sequence.

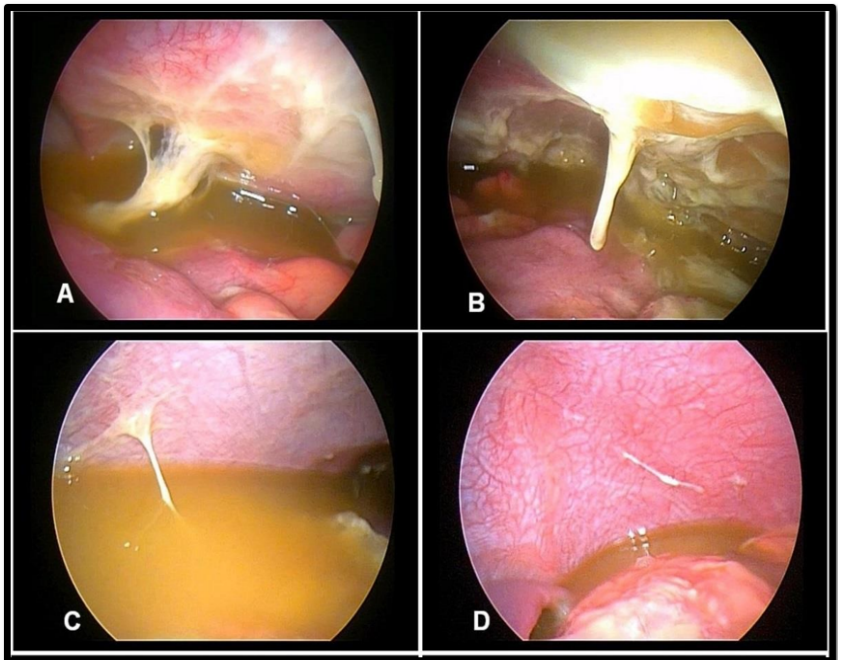

Figure 3: Purulent peritoneal fluid in A: Right iliac fossa, B: Pelvis, C: Right upper quadrant, D: Left upper quadrant

Polymicrobial infection showed a strong relationship with surgical site infection: among 48 patients with a final polymicrobial diagnosis, 34 developed SSI, whereas none of the 22 non-polymicrobial cases developed SSI; the association was statistically significant (χ^2, $p<0.001$). This was a large polymicrobial burden of 694 isolates in 831 patients with a predominance of Gram-negative organisms in digestive tract perforation described by Wang et al. (2025) [1] The association of 70.8% between SSI and polymicrobial cases found here was a unique finding as none of the comparator studies quantified the association between SSI and polymicrobial cases.

Culture showed an enteric microbial profile in majority of cases. Escherichia coli was isolated in 39/58 patients culture positive (67.2%) followed by Bacteroides fragilis in 18 (31.0%) and Klebsiella pneumoniae in 15 (25.9%). Enterococcus faecalis in 14 (24.1%) and Pseudomonas aeruginosa in 7 (12.1%). Wang et al (2025) reported similar prevalence of Gram negatives (1 Gram negative isolate out of 694 isolates from 831 patients). The most common isolated Gram negative pathogens were E. coli, K. pneumoniae and P. aeruginosa. Bassetti et al (2024) [7] assessed 624 Gram negative isolates from the ICU including P. aeruginosa, A. baumannii, K. pneumoniae and E. coli.

The polymicrobial nature of intra-abdominal infection has also been discussed by De Waele et al. (2023) [6] and Clements et al. (2021) [10]. Conversely, the studies reported did not detect B. fragilis and E. faecalis at levels as high as 31.0% and 24.1%, respectively.

Rapid whole genome sequencing was superior to conventional culture with organism detection in 70/70 patients (100.0%) versus 58/70 (82.9%), polymicrobial detection in 48/70 (68.6%) versus 34/70 (48.6%) and shorter reporting time of 33.9±6.0 versus 77.0±12.1 hours, all differences were significant at $p < 0.001$.

Zhang et al. (2023) [5] also reported 90% positivity by nanopore-targeted sequencing versus 32.6% by blood culture. Wang et al. (2021)9 reported mcfDNA sensitivity of 68.1% and specificity of 63.2%. 5–6 h and workflow (Grumaz et al. (2020) [4] in 7–9 h. Taxt et al. (2020) [3] Pathogens identified <10 min sequencing. Thus, while diagnostic superiority was uniform, turnaround for sequencing for these studies was significantly less than 33.9 ± 6.0 hours.

Conclusion

Rapid whole genome sequencing demonstrated obvious clinical utility in perforation peritonitis by enhancing pathogen detection and identification of polymicrobial infection and also significantly reducing diagnostic turnaround time in comparison to conventional culture. rWGS identified organisms in 100% of patients compared with 82.9% by culture, and polymicrobial infection in 68.6% versus 48.6%, with a reduction in reporting time from 77.0 ± 12.1 hours to 33.9 ± 6.0 hours.

These findings had therapeutic implications as 62.9% of patient's required antibiotic modification and escalation after rWGS results. High prevalence of multidrug resistance and high association of polymicrobial infection with surgical site infection indicate the need for early microbiological precision.

Appropriate treatment decisions, avoidance of inefficient empiric treatment and improved antimicrobial stewardship may be facilitated by timely genomic-guided antimicrobial optimization in patients with perforation peritonitis treated surgically with SSI in 48.6%, ICU admission in 65.7% and mortality in 15.7%.

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