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Comparison of culture and molecular techniques for microbial community characterization in infected necrotizing pancreatitis

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ABSTRACT

Background: Infected necrotizing pancreatitis is associated with significant morbidity and mortality. Peripancreatic fluid cultures may fail to identify all the infecting organisms. The aim of this study was to compare the bacterial biome of peripancreatic fluid from infected necrotizing pancreatitis patients using 16S ribosomal RNA (rRNA) DNA deep sequencing and quantitative polymerase chain reaction (qPCR) targeting the 16S rRNA gene *versus* standard laboratory culture.

Materials and methods: Peripancreatic fluid was collected during operative or radiologic intervention and samples sent for culture. In parallel, microbial DNA was extracted, qPCR targeting the 16S rRNA gene and 16S rRNA PCR amplification followed by Illumina deep sequencing were performed.

Results: Using culture techniques, the bacterial strains most frequently identified were gram-negative rods (*Escherichia coli*, *Klebsiella pneumoniae*) and *Enterococcus*. Samples in which culture results were negative had copy numbers of the 16S rRNA gene close to background in qPCR analysis. For samples with high bacterial load, sequencing results were in some cases in good agreement with culture data, whereas in others there were disagreements, likely due to differences in taxonomic classification, cultivability, and differing susceptibility to background contamination. Sequencing results appeared generally unreliable in cases of negative culture where little microbial DNA was input into qPCR sequencing reactions.

Conclusions: Both sequencing and culture data display their own sources of bias and potential error. Consideration of data from multiple techniques will yield a more accurate view of bacterial infections than can be achieved by any single technique.

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1. Background

Severe acute pancreatitis (SaP) occurs in ≈25% of patients with acute pancreatitis [1]. The resultant systemic inflammatory response typically occurs in the first 10 days following onset of illness and is associated with multi organ system failure [2]. In the second phase, an anti-inflammatory response is initiated and the septic complications that arise often lead to significant morbidity and mortality [3]. The degree of pancreatic necrosis in SaP correlates with increased mortality [1], and ≈30%–40% of SaP patients will develop superinfection of pancreatic necrosis [4]. Infected necrotizing pancreatitis (INP) remains an extremely difficult disease process to treat with mortality rates approaching 50% [3] and ≈80% of deaths arising as a result of septic complications [5].

Symptoms of INP include fever, leukocytosis, and clinical deterioration. Bacterial translocation from the gastrointestinal tract, including polymicrobial aerobic and anaerobic bacteria, naturally serve as the infectious pathway for INP [6]. Diagnosis of INP is typically based on clinical suspicion along with aspiration of the necrotic area or intraoperative sampling of infected fluid or tissue during operative debridement. Samples are sent for gram stain, aerobic, and anaerobic cultures in the hope of most appropriately tailoring antibiotic therapy. A series of 104 patients with suspected infected pancreatitis reported infection in 49% of the patients, with positive gram stains in 54 of 58 infected aspirates, and positive cultures in all 58. In this series, 86% of the infected necrosis isolated only one organism, the most common species including *Klebsiella*, *E coli*, and *Staphylococcus aureus* [5].

Despite advances in care, treatment of pancreatic abscess and INP remains difficult. Antibiotic penetration to pancreatic tissue may be limited because of the absence of capillary blood flow, which is often lacking in necrotic pancreatic tissue [7]. Similarly, necrotic, devitalized pancreatic tissue lacks immunologic mechanisms to prevent proliferation of bacteria and fungi [8]. As a means of identifying microbial infection of necrotic pancreatic tissue, standard culture techniques may fail to identify the full range of infecting organisms due to limitations in available laboratory culture conditions [9]. Most notably, anaerobic bacteria that commonly frequent the gastronintestinal-tract can be particularly difficult to culture in the laboratory.

Over the past decade, deep-sequencing techniques have been introduced as a means to perform high throughput analysis of bacterial identification. Using polymerase chain reaction (PCR) to amplify the 16S ribosomal RNA (rRNA) gene followed by low cost massively parallel sequencing, these techniques have been applied to the study of the human microbiome and are revealing in exquisite detail the taxonomic diversity of the human associated microbiota [10,11]. Despite the great promise of next generation sequencing technology, artifacts and biases can greatly influence the observed microbial community composition. For example, a recent study found that samples taken from different studies show distinct patterns of clustering suggesting that factors associated with how experiments are performed can obscure biological signals in metagenomic datasets [12]. The aims of the present study were to ascertain the viability of using "*next-generation*" deep-sequencing to examine the microbial flora present in INP samples and compare the results directly to those obtained using standard laboratory culture techniques.

2. Methods

2.1. Patient identification and sample collection

This study was performed as a prospective analysis over a 1-y period (2011–2012). Permission for this study was obtained from the Carolinas Medical Center Institutional Review Board (Charlotte, NC). Patients were identified through communication with medical and surgical teams within hospital intensive care units and wards. Patients with known severe INP were eligible for inclusion and were followed prospectively during their hospital course. Patients were excluded from the study if they had prior drainage procedures, or a previous laparotomy for pancreatic debridement and drainage.

If the attending physician suspected the patient to have superinfection of INP and require operative treatment or drainage, patients were consented for participation in the study. Surgical pancreatic debridement was performed using either laparoscopic or open techniques. At the time of intervention, samples of suspected-infected pancreatic tissue or fluid were sent to the Department of Microbiology (CMC, Charlotte, NC) for gram stain, aerobic, anaerobic, and fungal culture. In parallel, a second sample was procured and snap frozen in liquid nitrogen. Alternatively, samples were collected from drainage catheters placed for sampling of peripancreatic fluid. These samples were collected at the time of drain placement under a sterile technique. Patients were excluded from the study if they had prior drainage or surgical procedures, or an open abdominal wound.

2.2. Sample preparation

DNA was extracted from samples according to the protocol described in Appendix D of the Qiagen QIAamp DNA Mini and Blood Mini handbook (Qiagen, Germantown, MD), with the modification of using 100 μL Qiagen ATL buffer at step D3 and 100 μL Qiagen AL buffer at step D4. Bacterial pellets were resuspended in lysozyme enzyme buffer (20 mg/mL of lysozyme, 20 mM of Tris-HCl, 2 mM of ethylenediaminetetraacetic acid, and 1.2% of Triton) and the extracted DNA was quantitated and stored at −20°C.

2.3. Quantitative PCR

Quantitative PCR (qPCR) was performed using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) and the MyiQ Real-Time PCR System (Bio-Rad Laboratories). Universal primers targeting the 16S rRNA gene were used to measure total bacterial abundance [13]. Primers targeting the 23S rRNA gene of *Pseudomonas aeruginosa* were used to measure the presence of that species [14]. Thermal cycling conditions were as follows: an initial denaturation step at 95°C for 5 min followed by 40 cycles of 95°C for 15 s, and 57°C for 50 s.

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