“Salvage Microbiology”: Detection of Bacteria Directly from Clinical Specimens following Initiation of Antimicrobial Treatment

John J. Farrell1*, Rangarajan Sampath2, David J. Ecker2, Robert A. Bonomo3,4

1 University of Illinois School of Medicine, Department of Medicine, Peoria, Illinois, United States of America, 2 Ibis Biosciences, an Abbott Company, Carlsbad, California, United States of America, 3 Departments of Medicine, Pharmacology and Molecular Microbiology, Case Western Reserve University, Cleveland, Ohio, United States of America, 4 Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, United States of America

Abstract

**Background:** PCR coupled with electrospray ionization mass spectrometry (ESI-MS) is a diagnostic approach that has demonstrated the capacity to detect pathogenic organisms from culture negative clinical samples after antibiotic treatment has been initiated. [1] We describe the application of PCR/ESI-MS for detection of bacteria in original patient specimens that were obtained after administration of antibiotic treatment in an open investigation analysis.

**Methods:** We prospectively identified cases of suspected bacterial infection in which cultures were not obtained until after the initiation of antimicrobial treatment. PCR/ESI-MS was performed on 76 clinical specimens that were submitted for conventional microbiology testing from 47 patients receiving antimicrobial treatment.

**Findings:** In our series, 72% (55/76) of cultures obtained following initiation of antimicrobial treatment were non-diagnostic (45 negative cultures; and 10 respiratory specimens with normal flora (5), yeast (4), or coagulase-negative staphylococcus (1)). PCR/ESR-MS detected organisms in 83% (39/47) of cases and 76% (58/76) of the specimens. Bacterial pathogens were detected by PCR/ESI-MS in 60% (27/45) of the specimens in which cultures were negative. Notably, in two cases of relapse of prosthetic knee infections in patients on chronic suppressive antibiotics, the previous organism was not recovered in tissue cultures taken during extraction of the infected knee prostheses, but was detected by PCR/ESI-MS.

**Conclusion:** Molecular methods that rely on nucleic acid amplification may offer a unique advantage in the detection of pathogens collected after initiation of antimicrobial treatment and may provide an opportunity to target antimicrobial therapy and “salvage” both individual treatment regimens as well as, in select cases, institutional antimicrobial stewardship efforts.

Introduction

Infections are a major cause of morbidity and mortality in hospitalized patients. Because identification of bacterial pathogens requires time for the organisms to grow, when infection is suspected empiric antimicrobial treatment is administered based upon an assessment of likely organisms [2–4]. Bacterial meningitis is a primary example of the importance of appropriate empiric therapy before culture results are known. Here, a delay in the initiation of antimicrobial therapy correlates with increased mortality in this disease [5–7], and antibiotics are routinely administered before cerebrospinal fluid (CSF) is obtained (the site likely to yield the pathogen). Other clinical scenarios such as febrile neutropenia, transplant associated infections, and severe sepsis also mandate that antibiotics are administered in a timely fashion and should not be delayed.

The sensitivity and timeliness of culture results are influenced by many factors, but in hospitalized patients previously administered and/or concurrent antimicrobial treatment is a commonly encountered confounding factor. When pathogens are not recovered in culture, the entire treatment course is likely to be “broad spectrum” including therapy for staphylococci, streptococci, as well as Gram negative and anaerobic bacteria. As a result, antibiotics are often used unnecessarily. Regrettably, a delay or failure in identification of pathogens impacts patient outcomes, exposes patients to the deleterious effects of extended courses of overly broad empirical antibiotics, and exacerbates the widespread...
problems of multidrug resistant organisms and *Clostridium difficile* associated disease.

The analysis of multilocus polymerase chain reaction (PCR) amplicons by electrospray ionization mass spectrometry (ESI/MS) on a platform in which PCR is coupled to ESI-MS is a technique that has demonstrated the capacity to detect microbes from both environmental samples and clinical patient specimens [9-10]. Detection does not require correct anticipation of the organisms in advance; the technology is designed to detect unknown and unculturable organisms, and is particularly useful when multiple microbes may be present. We performed a prospective comparison of results from conventional microbiologic testing vs. PCR/ESI-MS in cases of suspected new onset, or recurrence of infection in patients where microbes may be present. Our purpose is to demonstrate that PCR/ESI-MS may have value in the clinical microbiology laboratory when cultures do not yield a pathogen. Offering clinicians relevant, timely and specific information can have significant impact on the choice of therapy, clinical decision making, and antimicrobial stewardship.

**Methods**

Approval was obtained from both the University of Illinois College of Medicine and St. Francis Medical Center Institutional Review Boards (IRBs) for PCR/ESI-MS testing of specimens that were submitted to the microbiology laboratory from inpatients at St. Francis Medical Center, Peoria, IL. To test if PCR/ESI-MS serves a potential role in the detection of microorganisms in specimens collected from patients following administration of antimicrobial treatment, we prospectively identified patients with suspected infection between February 10, 2011 and November 10, 2012. Patients whose specimens were collected after at least one dose of antibiotic were included in the study. Gram stains, conventional aerobic and anaerobic culture and PCR/ESI-MS were performed on all specimens. The PCR/ESI-MS test results were not available to the patients’ treatment teams and did not influence treatment decisions.

In each case, specimens were collected as part of the routine care of the patient and submitted to clinical microbiology lab at St. Francis Medical Center (Peoria, IL) for testing. After the specimen was processed by laboratory personal and all the requested tests and cultures ordered by the treating physician had been prepared, the remaining specimen was placed in storage at 4°C for subsequent PCR/ESI-MS testing. Because specimens included in this study were collected in the course of the patients medical care for diagnostic purposes, and no specimens were collected explicitly for the purposes of this study, both IRBs waived the requirement for patient informed consent.

PCR/ESI-MS was performed on all specimens. Specimens were kept in refrigeration, not frozen, and shipped overnight in a cold pack to Ibis Biosciences (Carlsbad, CA). We followed the PCR/ESI-MS protocol previously described [11]. This PCR/ESI-MS assay is designed for detection of bacterial and *Candida* species, and is not capable of identifying invasive molds, dimorphic fungi, or viral pathogens. Consequently, immunocompromised patients, patients on chemotherapy for treatment of malignancy, or patients with HIV infection were excluded from the study. Compared to clinical samples, the assay performs with 98.7% and 96.6% concordance at the genus and species levels, respectively [11].

For reporting results, the level of detection (LOD) was calculated as genome equivalents per PCR reaction well. Results were reported for all detections with a Q score ≥0.90 in which the LOD was above threshold, and the internal isolation control was detected. Kappa (k) was calculated using SAS software, version 9.1 (SAS Institute) to assess the agreement between culture and PCR/ESI-MS.

**Results**

Conventional microbiological testing was performed on 76 specimens collected from 47 patients. Specimens included swabs, BACTEC blood culture bottles, fluid, and tissue samples that were submitted for culture from patients after initiation of antimicrobial treatment (Table 1). The results obtained from aerobic and anaerobic cultures were compared to results of PCR/ESI-MS testing (Table 2). PCR/ESI-MS detected probable pathogens in 20 cases in which standard microbial cultures were non-diagnostic. Results were in agreement for 38 specimens (49%); but 37% of the agreement (14 specimens) was attributed to specimens that were culture negative with no detection by PCR/ESI-MS. For patients with multiple specimens, only the culture positive specimen, when applicable, was considered for calculation of the Kappa statistic. Compared to agreement between culture and Gram stain, (k = 0.643), agreement between culture and PCR/ESI-MS was poor (k = 0.299).

Conventional culture methods were non-diagnostic in 33 of 47 cases: in 17 cases cultures were completely negative. Nine of the patients from whom respiratory specimens were collected grew either normal respiratory flora (5), or *Candida* spp. (3), or coagulase negative staphylococci (1). There was only one specimen, an endotracheal tube aspirate, from which an organism was cultured (*C. albicans*), but PCR/ESI-MS testing was negative. PCR/ESI-MS results were negative for detection in six of the 17 culture negative cases.

Bacterial pathogens were detected by PCR/ESI-MS in 60% (33/55) of the specimens in which cultures were either negative or nondiagnostic: *Streptococcus* spp. (17), *Staphylococcus aureus* (5), *Staphylococcus epidermidis* (4), *Staphylococcus lugdunensis* (1), anaerobes (4), *Salmonella enterica* (2), and *Klebsiella pneumoniae* (1). In each case, the organism(s) detected by PCR/ESI-MS were consistent with the clinical scenario that was observed in the patient by one of our investigators (JJF). A selection of these cases requires special comment.

**Recurrent infections: (Patients 15 and 16)**

Patients 15 and 16 both were on antimicrobial treatment for presumed relapses of previous *S. aureus* prosthetic knee infections. Although this suspicion was not confirmed by culture, in both cases the previously identified organism (MSSA and MRSA, respectively) was detected by PCR/ESI-MS.

**Coagulase-negative staphylococci: (Patients 3 and 10)**

Coagulase negative staphylococcal infections were suspected in patients 3 and 10. Patient 3 had a history of coronary artery bypass surgery and aortic valve (AV) replacement in 1983. He was well until 2011 when he presented to an outside hospital with fever. He was diagnosed with prosthetic valve infective endocarditis based on the presence of prosthetic aortic valve vegetation and growth of methicillin susceptible *Staphylococcus epidermidis* (MSSE) in two of two sets of blood cultures. He was treated with IV vancomycin and oral rifampin for 30 days, and then transferred to our institution prior for AV replacement surgery. During surgery, Gram positive cocci (GPC) were detected on Gram stain of the valve tissue, but cultures proved to be negative. *Staphylococcus epidermidis* was detected by PCR/ESI-MS in both valve and annular myocardial tissue specimens. Given the presence of prosthetic AV vegetation, CNS in blood cultures obtained before surgery, and GPC in the
Table 1. Patients, specimens, and antimicrobial treatment.

| Patient | Age/gender/history | Diagnosis | Previous culture confirmed infection(s) | Specimens | Antimicrobial Treatment | DOT* |
|---------|-------------------|-----------|------------------------------------------|-----------|------------------------|------|
| 1       | 48 year-old woman | Right lung abscess & empyema | – | Pleural fluid | Azithromycin Ceftriaxone Vancomycin | 5 11 10 |
| 2       | 26 year-old man   | Brain abscess | – | CSF #1 (from LP) | Ceftriaxone Vancomycin | 1 1 |
| 3       | 58 year-old man   | AV IE with AV ring abscess | MSSE AV IE | AV tissue Anulus tissue | Gentamicin Rifampin Vancomycin | 10 47 47 |
| 4       | 50 year-old man   | Brain abscess | – | Purulent brain abscess fluid | Ceftriaxone Metronidazole Vancomycin | 2 2 2 |
| 5       | 51 year-old man   | Sepsis     | – | BAL fluid | Clindamycin Levofloxacin Piperacillin/tazo bactam Vancomycin | 2 2 2 2 |
| 6       | 74 year-old woman | Right shoulder | – | Synovial fluid | Antibiotics started after shoulder aspiration | 0 |
| 7       | 50 year-old man   | S. pneumoniae | CSF | Bacteremia | Brain tissue | Acyclovir Meropenem Vancomycin | 3 3 3 |
| 8       | 10 year-old male  | CAP       | – | BAL fluid | Azithromycin | 5 |
| 9       | 16 year-old male  | CAP       | – | Pleural fluid | Azithromycin | 4 3 |
| 10      | 64 year-old man   | Right TKA septic arthritis | Methicillin resistant coagulase negative Staphylococcus | Synovial tissue; posterior femoral tissue; and posterior tibial tissue | Cefazolin | 1 |
| 11      | 50 year-old man   | Right hip AVN | – | Synovial fluid | Cefazolin Ceftriaxone Vancomycin | 1 1 1 |
| 12      | 72 year-old woman | Right cranial epidural abscess | – | Epidural tissue | Cefazolin Ceftriaxone Vancomycin | 1 1 1 |
| 13      | 59 year-old woman | Sepsis and S. pneumoniae | CSF #1 | Bacteremia | CSF #2 | Piperacillin/tazo bactam Vancomycin | 6 6 |
| 14      | 59 year-old man   | Left TKA | Alpha-Strep | Synovial fluid from left knee (before surgery) | Antibiotics started after left knee aspiration | 0 |
| 15      | 75 year-old man on chronic suppressive antibiotics | Recurrent septic left TKA | MSSA infected left TKA | Synovial fluid; synovial tissue; and femoral membrane tissue | Cefazolin Cephalexin Rifampin | 6 360 360 |
| 16      | 86 year-old woman | Right TKA septic arthritis | MRSA infected left TKA | Synovial fluid | Cephalosar Clindamycin Linezolid | 14 14 14 |
| 17      | 70 year-old woman | Encephalitis | – | CSF | Ceftriaxone Vancomycin | 2 2 |
| 18      | 78 year-old woman | Liver abscess | – | Purulent liver abscess fluid | Piperacillin/tazo bactam | 1 |
| 19      | 55 year-old man   | CAP       | MSSA bacteremia | BAL fluid | Cefazolin Vancomycin | 2 2 |
| 20      | 38 year-old woman | Submental abscess | – | Swab from I&D in OR | Clindamycin Vancomycin | 2 1 |
| 21      | 78 year-old man   | Severe AS | Culture negative endocarditis | AV tissue | Cefazolin Ceftriaxone Vancomycin | 1 28 28 |
| 22      | 49 year-old man   | Infective Endocarditis | Abiotrophia defectiva bacteremia | AV tissue and MV tissue | Cefazolin Vancomycin | 2 2 |
| 23      | 79 year-old man   | Sepsis | Vibrio vulnificus bacteremia | BACTEL™ blood culture bottles (two sets) | Piperacillin/tazo bactam Vancomycin | 2 2 |
| Patient | Age/gender/history | Diagnosis | Previous culture confirmed infection(s) | Specimens | Antimicrobial Treatment | DOT* |
|---------|--------------------|-----------|------------------------------------------|-----------|------------------------|------|
| 24      | 69 year-old woman  | Acute Respiratory failure | – | ET aspirate | Meropenem Piperacillin/tazobactam Vancomycin | 4 3 6 |
| 25      | 40 year-old diabetic man | VAP | Streptococcus agalactiae (initial sputum culture) | RLL and LLL BAL fluid | Aztreonam Clindamycin Meropenem Vancomycin | 6 10 4 |
| 26      | 68 year-old woman  | CAP and ARDS | E. coli UTI | RLL and LLL BAL fluid | Azithromycin Ceftriaxone Meropenem Vancomycin | 6 5 3 5 |
| 27      | 75 year-old man    | Necrotizing with chronic sacral decubitus ulcer | fasciitis | Wound aspirate POD #3 | Ampicillin/sulbactam Clindamycin Meropenem Vancomycin | 3 2 1 4 |
| 28      | 33 year-old man with post-op wound infection | left ankle pilon fracture S/P ORIF | – | Wound swab POD #15 | Ampicillin/sulbactam Clindamycin Daptomycin Meropenem Vancomycin | 15 2 14 |
| 29      | 49 year-old quadriplegic man | Stage 4 pressure ulcer | – | Left hip tissue from I&D in OR | Cephalixin Piperacillin/tazobactam | 5 1 |
| 30      | 68 year-old woman  | Hypoxemia and hypercapnic respiratory failure | – | ET aspirate | Meropenem Vancomycin | 1 1 |
| 31      | 45 year-old woman with recurrent lower extremity infections | right knee septic arthritis | – | Swab of right knee fluid taken in OR | Levofloxacin Linezolid | 6 6 |
| 32      | 15 year-old female | Neck abscess | – | Right neck abscess tissue excised in OR | Azithromycin Cefdinir Clindamycin (po) Clindamycin (IV) | 5 20 7 2 |
| 33      | 91 year-old man with small bowel obstruction | RUL collapse | – | RUL BAL fluid | Cefepime Metronidazole Vancomycin | 5 5 4 |
| 34      | 51 year-old man S/P right to left femoral arterial bypass graft seroma | post-op left groin S. lugdunensis bacteremia | Seroma fluid and Arterial Graft Material from OR | Aztreonam Cefazolin Levofloxacin Rifampin Vancomycin | 2 3 1 5 4 |
| 35      | 74 year-old diabetic man | RUE cellulitis | – | Right elbow fluid | TMP/SMX DS | 5 |
| 36      | 37 year-old woman with CBD leak | choledocholithiasis | – | Fluid from Peri-biliary abscess | Levofloxacin Meropenem Vancomycin | 3 5 5 |
| 37      | 74 year-old woman | Right TKA effusion | – | Right femoral and tibial canal tissue from OR | Clindamycin Minocycline Tigecycline | 1 4 6 |
| 38      | 50 year-old woman with RUL Adenocarcinoma | Necrotizing pneumonia | MSSA VAP | Fluid from right chest cavity | Ampicillin Ceftriaxone Levofloxacin Piperacillin/tazobactam Meropenem TMP/SMX Vancomycin | 5 4 1 7 5 5 5 |
| 39      | 25 year-old woman S/P left ankle ORIF | left ankle osteomyelitis | Anaerobic streptococci left ankle abscess tissue collected in OR | Clindamycin | 31 |
| 40      | 71 year-old woman | LLL CAP | – | ET aspirate and LLL BAL fluid | Azithromycin Ceftriaxone Levofloxacin Piperacillin/tazobactam Vancomycin | 5 9 14 2 2 |
| 41      | 84 year-old diabetic man | RLE cellulitis and diabetic right foot infection | Streptococcus agalactiae (wound culture) | Right 5th metatarsal bone | Cefepime Piperacillin/tazobactam Vancomycin | 1 5 5 |
| 42      | 77 year-old woman with end stage renal disease on hemodialysis | left hip pain. | – | left hip joint tissue | Vancomycin | 1 |
Mixed aerobic and anaerobic infections: (Patients 18, 20, 27, 33, 39, 40 and 41)

PCR/ESI-MS detected anaerobic organisms that were missed by culture in eight cases. *Pseudomonas gngivicis* was not appreciated in the culture of neck abscess fluid from patient 20, which was culture negative. And *Bifidobacterium dentium* was detected in BAL fluid from patient 33 that was only notable for *Candida albicans* in culture. *Robia mucilaginosus* as well as *C. albicans* were identified in the respiratory specimens from patient 40, whose cultures were non-diagnostic *(i.e., normal flora)*. Culture and PCR/ESI-MS also disagreed on patient 27: VRE was cultured from the original wound culture, but PCR/ESI-MS detected *Fusobacterium varium*.

PCR/ESI-MS performed particularly well with polymicrobial infections that included both aerobic and anaerobic pathogens. *S. intermedius* and MSSA grew in both aerobic and anaerobic cultures, from patients 18 and 41, respectively, but no strictly anaerobic organisms were cultured. *Fusobacterium necrophorum* and *Streptococcus* spp. were detected in purulent liver abscess drainage from patient 18, and MSSA and *F. necrophorum* were identified in the infected metatarsal bone from patient 41. Patient 38 had MSSA and *C. albicans* detected by culture and PCR/ESI-MS in the pleural fluid sample, but *Bilophila wadsworthia* was only detected by PCR/ESI-MS, and not in the anaerobic culture. Likewise, *Streptococcus oralis* was found in ankle abscess tissue by both aerobic culture and PCR/ESI-MS from patient 39, but only PCR/ESI-MS detected *Finegoldia magna*.

Streptococcal infections: (Patients 1, 2, 4, 5, 7, 8, 13, 14, 18, 30, 37, 39 and 46)

Of the 15 patients (17 samples) from whom *Streptococcus* spp. were detected by PCR/ESI-MS, the only specimens from which *Streptococcus* was recovered by culture were from patients with brief or no antibiotic treatment prior to specimen collection. Two specimens that grew *Streptococcus mitis/ oralis: 1) purulent brain abscess drainage from Patient 4 obtained after two days of antibiotic treatment, and 2) the ankle abscess tissue from patient 39—three days after antibiotics were discontinued. *S. intermedius* was cultured from purulent liver abscess fluid from patient 18 after one day of antibiotic treatment. In patients who had received more than two days of antibiotic treatment, streptococci were no longer cultured. And, in the case of patient 14, one day of treatment was sufficient to suppress growth of streptococci: Left knee synovial fluid cultures from patient 14 grew α-hemolytic streptococci prior to initiation of antibiotics, but after one day of antibiotics, when the knee was drained in the OR, all cultures were negative. PCR/ESI-MS detected viridans streptococci/*S. pneumoniae*/*mitis* group in three of three surgical specimens from patient 14.

PCR/ESI-MS appeared to offer a particular advantage in detection of pneumococci from both cerebral spinal fluid (CSF), and bronchoalveolar lavage (BAL) fluid. Lumbar puncture (LP) was delayed in two cases of pneumococcal bacteraemia and sepsis with presumed meningitis (Patients 7 and 13). In both cases, CSF cultures were negative, but *S. pneumoniae* were detected by PCR/ESI-MS (thereby “salvaging” clinical decision making). Dexamethasone was not administered in either case. Interestingly, PCR/ESI-MS did not remain positive in the CSF indefinitely.
Table 2. Conventional microbiology versus PCR/ESI-MS test results.

| Patient | Specimen | Gram Stain | Culture results | PCR/ESI-MS | Level (GE/well) |
|---------|----------|------------|----------------|------------|----------------|
| 1       | Pleural fluid | No segs No organisms | No growth | Streptococcus pneumoniae/mitis Group streptococci | 75 |
| 5       | BAL fluid | Few segs No organisms | Light growth of normal respiratory flora | Streptococcus pneumoniae | 54 |
| 8       | RLL BAL fluid | Many segs No organisms | No growth | Streptococcus pneumoniae | 54 |
| 9       | Left pleural fluid | Many segs No organisms | No growth | No detection | – |
| 19      | BAL fluid | Few segs | Sparse normal | S. aureus (mecA negative) | 55 |
|         | ET aspirate | Some segs No organisms | Rare Coagulase negative Staphylococcus | S. pneumoniae C. albicans | 58 135 |
| 24      | RLL BAL fluid | No segs No organisms | No growth | No detection | – |
| 25      | LLL BAL fluid | No segs No organisms | No growth | No detection | – |
| 26      | RLL BAL fluid | Rare segs No organisms | C. glabrata | No detection | – |
| 30      | RLL BAL fluid | Few segs No organisms | No growth | S. vestibularis C. albicans | 98 116 |
| 33      | RUL BAL fluid | Some segs Rare budding yeast | Many C. albicans | Bifidobacterium dentium C. glabrata | 39 126 |
| 38      | Right chest pleural fluid | Many segs | Many MSSA | S. aureus (mecA negative) | 72 |
|         | ET aspirate | Few budding yeast | Few C. albicans | C. albicans | 119 |
|         | Many segs | Sparse | Rothia mucilaginosa | | 60 |
| 40      | LLL BAL fluid | Rare segs No organisms | No growth | S. epidermidis (mecA positive) | 12 |
|         | Many segs | | Escherichia coli | | 118 |
|         | ET aspirate | rare GNB | Many E. coli | S. epidermidis (mecA positive) | 11 |
| 45      | Many budding yeast | | Candida tropicalis | | 138 |
| 46      | RLL BAL fluid | Many segs, rare GPC | Some E. coli | Escherichia coli | 103 |
|         | Rare budding yeast | Many C. tropicalis | Candida tropicalis | | 138 |
|         | Some segs | Sparse | viridans/mitis Group streptococcus | | 95 |
| 47      | ET aspirate | No organisms | Normal respiratory flora | Streptococcus spp. | 137 |
|         | | | C. albicans | | 132 |
|         | RLL BAL fluid | No segs No organisms | No growth | viridans/mitis Group streptococcus C. albicans | 64 61 |
|         | Many segs | Few | Pseudomonas aeruginosa | | 295 |
| 50      | Right shoulder | Many segs | No | Propionibacterium acnes | 17 |
| 6       | Synovial fluid | No organisms | growth | Acinetobacter junii | 7 |
| 7       | Right shoulder tissue | No organisms | No growth | Acinetobacter junii | 188 |
| 8       | Synovial tissue | Negative | No growth | S. epidermidis (mecA positive) | 41 |
| 10      | Posterior femoral tissue | Negative | No growth | S. epidermidis (mecA positive) | 201 |
### Table 2. Cont.

| Tissue, Fluid, and Orthopedic Specimens | Culture results | PCR/ESI-MS | Level (GE/well) |
|----------------------------------------|----------------|------------|----------------|
| Posterior tibial tissue                | Methicillin resistant coagulase negative *Staphylococcus* (one colony in the anaerobic culture) | S. epidermidis (mecA positive) | 113 |
| 11 Synovial fluid                     | No growth      | No detection | – |
| Left knee fluid                       | Many segs. Rare GPC in pairs | S. pneumoniae/viridans/mitis group streptococci | 139 |
| 14 Left knee retinaculum               | Few segs No organisms | No growth | 135 |
| Left knee femoral synovial tissue      | Few segs No organisms | No growth | 134 |
| Left knee fluid                       | No organisms   | No growth | 44 |
| 15 Left knee synovial tissue           | No growth      | Low level detection. Unable to identify organism. | – |
| Left knee femoral membrane tissue      | No growth      | S. aureus (mecA negative) | 96 |
| Left knee                            | No segs        | No S. aureus (mecA positive) | 34 |
| 16 Tissue                             | Rare GPC       | growth | Acinetobacter junii | 99 |
| Tibial bone tissue                    | Few segs No organisms | No growth | – |
| 18 Fluid aspirated from liver absscess | Few segs Many Streptococcus intermedius | Streptococcus spp. | 40 |
| Wound swab                            | Many segs Rare GPC | VRE | Fusobacterium varium | 90 |
| 27 Wound swab                         | Few segs Many RBCs No organisms | Carbapenem resistant Klebsiella pneumoniae (bla<sub>KPC</sub>,) | 105 |
| 28 Wound swab OR #1                   | Negative for organisms | Enterobacter | Enterobacter cloacae complex | 104 |
| 29 Wound swab OR #2                   | Negative for organisms | Enterobacter | Enterobacter cloacae complex | 105 |
| Right knee fluid                      | Rare segs E. coli | E. coli | 3888 |
| Left hip tissue                       | No organisms   | E. faecalis | E. faecalis | 138 |
| Right knee fluid (Swab from OR)       | Rare segs No organisms | No growth | Klebsiella pneumoniae (bla<sub>KPC</sub>,) | 52 |
| 32 Necrotic lymph node tissue          | Many segs No organisms | No growth | No detection | – |
| Right elbow fluid                     | Many segs No organisms | No growth | No detection | – |
| 36 Peri-biliary fluid                 | Many E. faecalis | Enterococcus faecalis | 181 |
| Right knee femoral canal tissue        | Few Klebsiella pneumoniae | Klebsiella pneumoniae | 35 |
| Right knee tibial canal tissue         | No segs No organisms | No growth | No detection | – |
| Right knee femoral canal tissue        | Many segs Many Streptococcus mitis/oralis | Finegoldia magna | 181 |
| Tissue from I&D of left ankle absscess in O.R. | Some GPC in clusters | Rare MSSA | Streptococcus oralis | 81 |
| Right 5th metatarsal bone tissue from O.R. | Rare GNB (MSSA) | S. aureus (mecA negative) | 7 |
| Left hip tissue                       | Some segs. No organisms | No growth | No detection | – |
CSF from a follow-up LP two weeks later on patient 13 was negative by both culture and PCR/ESI-MS (see table 2). In two cases of apparent pneumococcal pneumonia (Patients 5 and 8) and a third case of viridans streptococcus pneumonia (patient 46), culture was either unable to detect *S. pneumoniae*, or unable to distinguish pathogenic streptococci from normal respiratory flora.

Prosthetic arterial graft infections: (Patients 33 and 43)

Infection of prosthetic intravascular graft material is a difficult problem, as vascular grafts are not readily exchanged. Endovascular graft infection was suspected in Patients 33 and 43. Both patients were bacteremic, and both were on antimicrobial

| Table 2. Cont. |
|---|

Tissue, Fluid, and Orthopedic Specimens

| Patient | Specimen | Gram Stain | Culture results | PCR/ESI-MS | Level (GE/well)* |
|---|---|---|---|---|---|
| 44 | Left knee tissue | Some segs | No organisms | No growth | *S. aureus* (mecA negative) | 12 |

Central Nervous System Specimens

| Patient | Specimen | Gram Stain | Culture results | PCR/ESI-MS | Level (GE/well)* |
|---|---|---|---|---|---|
| 2 | CSF #1 | No organisms | No growth | Streptococcus intermedius | 229 |
| 4 | Purulent drainage from brain abscess | Many Segs | Some GPC | No growth | Streptococcus intermedius | 164 |
| 7 | CSF | No organisms | No growth | Streptococcus pneumoniae/Streptococcus mitis group | 208 |
| 12 | Epidural tissue | Many segs | Many GPC | No growth | Streptococcus pneumoniae/Streptococcus mitis group | 145 |
| 13 | CSF #1 | No organisms | No growth | Streptococcus pneumoniae/Streptococcus mitis group | 123 |
| 17 | CSF | No organisms | No growth | No detection | – |

Cardiac tissue and Vascular specimens

| Patient | Specimen | Gram Stain | Culture results | PCR/ESI-MS | Level (GE/well)* |
|---|---|---|---|---|---|
| 3 | AV tissue | Few segs | Rare GPC | No growth | *S. epidermidis* (mecA positive) | 185 |
| 21 | AV tissue | Negative | No growth | No detection | N/A |
| 22 | AV tissue | Few GPC | Few WBC | Abiotrophia defectiva | Abiotrophia defectiva | 113 |
| 23 | BACTEC blood culture bottle #1 | N/A | No growth | No detection | – |
| 34 | Fluid from post-op seroma drained in O.R. | No segs | No organisms | No growth | No detection | – |
| 43 | peri-Aortic valve and graft fluid | Rare segs | No organisms | No growth | *Salmonella enterica* | 30 |
| 43 | Aortic valve and graft tissue | Rare segs | No organisms | No growth | *Salmonella enterica* Acinetobacter junii | 122 12 |

*Level of Detection– Reported as genome equivalents per PCR reaction (GE/well).*

*LLL = Left lower lobe; RLL = Right lower lobe; ET aspirate = endotracheal aspirate.

*CSF = cerebrospinal fluid; BAL fluid = bronchoalveolar lavage fluid.

doi:10.1371/journal.pone.0066349.t002
treatment prior to surgical extraction of the vascular grafts. Cultures of graft material and surrounding tissue were negative, but in both cases, the organism that had grown in the initial blood cultures was detected by PCR/ESI-MS from the extracted the graft material.

**Carbapenem resistant Enterobacteriaceae (CRE): (Patients 27 and 31)**

CRE infections were detected in one patient by culture and two patients by PCR/ESI-MS. In patient 27, *K. pneumoniae* that tested positive for KPC-3 by PCR for the *bla*KPC gene was recovered from an infected surgical wound. PCR/ESI-MS detected both the pathogen (*K. pneumoniae*), and the resistance gene (*bla*KPC-3). Patient 31 was known to be colonized with *bla*KPC-3+ *Klebsiella pneumoniae*, and although cultures of synovial fluid from her right knee were negative, PCR/ESI-MS detected *K. pneumoniae* (also positive for *bla*KPC-3).

**Extended antibiotic treatment and serial specimens: (Patients 13 and 32)**

Just as with culture, duration of antibiotic treatment does influence ability of PCR/ESI-MS to detect evidence of a pathogen. In the case of serial CSF samples from patient 13, a patient with *S. pneumoniae* bacteremia and sepsis, *S. pneumoniae* was only detected by PCR/ESI-MS in the first CSF sample. In the case of patient 32, both culture and PCR/ESI-MS of neck abscess tissue were negative after more than 34 days of empiric antibiotic treatment.

**Acinetobacter junii: (Patients 6, 16, 41, 43)**

In these four cases *Acinetobacter junii* was detected by PCR/ESI-MS in tissue and synovial fluid specimens collected during surgical resection and drainage of infected tissue. *Acinetobacter junii* is unlikely to represent a pathogen in these cases. This organism appears to have been detected as an artifact of the tissue extraction process.

**Discussion**

PCR/ESI-MS is an emerging diagnostic technology that is capable of rapid detection of microorganisms directly from clinical specimens. As this PCR-based approach requires only the presence of small amounts of DNA for amplification, bacteria that have been “killed” by bactericidal antibiotics (e.g., β-lactams, aminoglycosides or quinolones) or are in stationary phase from the effect of bacteriostatic drugs (e.g., linezolid, macrodilides) can be detected if sufficient DNA for amplification is present in the sample. Up to this time, data testing this assertion in the clinical arena have not yet been provided.

In our series, 72% (35/76) of cultures obtained following initiation of antimicrobial treatment were nondiagnostic. In contrast, PCR/ESR-MS detected organisms in 83% (39/47) of cases and 76% (58/76) of the specimens. Calculation of the Kappa coefficient confirmed poor agreement between conventional cultures and PCR/ESI-MS. The poor agreement is primarily attributable to detection of bacteria by PCR/ESI-MS in culture negative specimens. PCR/ESI-MS detected ≥ one bacterial pathogen(s) in 60% (27/45) of the culture negative specimens. In 67% of negative culture cases (18/27), an organism that was consistent with the clinical scenario was detected by PCR/ESI-MS.

Reliance on clinical judgment to distinguish colonizers and contaminants from true pathogens is required with any microbiology test result. As with culture-based identification of organisms from clinical samples, the correct interpretation of PCR/ESI-MS results requires an appreciation for the clinical context associated with the specimen tested. In several cases the organisms detected by PCR/ESI-MS were consistent with contaminants that would have been unlikely to alter patient management. For example, detection of *Candida* spp. in respiratory secretions by either culture or molecular methods does not merit treatment in our relatively immunocompetent patient population. But, as evidenced in the case of Patient 6, the role of other potential pathogens still needs to be defined. In this case, low level detection (17 genome copies/well) of *Propionibacterium acnes*, a pathogen with well described association with prosthetic shoulder joint infections, in culture negative right shoulder synovial fluid would pose a challenge for the clinician responsible for interpreting this additional data.

Selecting appropriate antimicrobial therapy for patients with evidence of infection, but negative cultures is a common dilemma in practice. The implications of our findings are profound: that antimicrobial treatment in “culture negative” cases can be directed against both pathogens and genetic markers of resistance (i.e., mcrA in MRSA, mutations in *gfd*, *bla*KPC, etc.) that are readily identified by PCR/ESI-MS. Particularly compelling supporting evidence for pathogen detection derives from our two cases of breakthrough recurrent prosthetic knee infections that occurred while the patients were taking chronic suppressive antibiotics (patients 15 and 16): the previous organism was not recovered in tissue cultures taken during extraction of the infected knee prostheses in either case, but was detected by PCR/ESI-MS. As well as our case of culture and Gram stain positive streptococcal septic arthritis (Patient 14), in which a single preoperative dose of cefazolin was sufficient to cause surgical cultures to be negative. The disappearance of PCR evidence of *S. pneumoniae* in a second CSF specimen from patient 13, who was recovering from pneumococcal bacteremia and sepsis, suggests that organism detection may be eradicated with effective antimicrobial treatment. This finding may help with decisions to tailor and/or stop therapy. These early findings could have impact on the current status of “duration of therapy” and antibiotic stewardship.

Limitations of this study include: i) the small sample size and lack of a control group; ii) relevance of “mixed cultures”; and iii) co-identification of streptococci (viridans streptococci, *Streptococcus mitis* and pneumococcus). This study was performed as an open investigation, and not a validation; and is not appropriate for, nor was it designed to calibrate sensitivity or specificity. Our PCR primers successfully captured organisms not detected by culture, but we maintain that microbiological culture results are still the “gold standard” for comparison. Based upon studies such as this, that “standard” cannot be applied as the evidence it offers is not present, but combining broad range PCR with mass spectrometry or 16S ribosomal gene sequencing has appeal for selected situations in the clinical microbiology lab. In addition, the role of fungal, viral or parasitic infections was also not evaluated in this small series. Truly, larger studies are needed.

The everyday practice of treating patients with empiric antibiotic regimes provides an enormous opportunity for novel approaches to target antimicrobial therapy and “salvage” both individual treatment regimens as well as institutional antimicrobial stewardship efforts. These results suggest that PCR/ESI-MS may have a role in detection of clinically relevant pathogens from specimens obtained following initiation of antimicrobial treatment when cultures are negative. Larger studies are planned to determine if PCR/ESI-MS can assist in the clinical evaluation and treatment of patients on empiric antimicrobial treatment for suspected infection with negative cultures.
Acknowledgments

The Kappa calculation of agreement was performed on SAS software with the assistance of Huagping Wang, Ph.D. Department of Medicine, University of Illinois College of Medicine.

PCR/ESI-MS testing was performed by Kristin S. Lowery, Ph.D at Ibis Biosciences, a Division of Abbott Molecular, Inc., in Carlsbad, CA, USA.

Author Contributions

Conceived and designed the experiments: JJF DJE. Performed the experiments: RS. Analyzed the data: JJF RAB DJE. Contributed reagents/materials/analysis tools: RS DJE. Wrote the paper: JJF RAB.

References

1. Bhatia NS, Farrell JJ, Sampath R, Ranken R, Rounds MA, et al. (2012) Identification of Streptococcus intermedius central nervous system infection by use of PCR and electrospray ionization mass spectrometry. J Clin Microbiol 50: 4160–62.
2. Puskarich MA, Trzeciak S, Shapiro NI, Arnold RC, Horton JM, et al. (2011) Association between timing of antibiotic administration and mortality from septic shock in patients treated with a quantitative resuscitation protocol. Crit Care Med 39: 2066–71.
3. Houck PM, Bratzer DW, Niederman M, Bartlett JG (2002) Pneumonia treatment process and quality. Arch Intern Med 162: 843–4.
4. McGarvey KN, Harper JJ (1993) Pneumonia mortality reduction and quality improvement in a community hospital. QRB Qual Rev Bull 19: 124–30.
5. Fitch MT, van de Beek D (2007) Emergency diagnosis and treatment of adult meningitis. Lancet Infect Dis 7: 191–200.
6. Aronin SI, Peduzzi P, Quagliarello VJ (1998) Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. Ann Intern Med 129: 862–9.
7. Proulx N, Frechette D, Toye B, Chan J, Kravcik S (2005) Delays in the administration of antibiotics are associated with mortality from adult acute bacterial meningitis. QJM 98: 291–8.
8. Sampath R, Hall TA, Massire C, Li F, Blyn LB, et al. (2007) Rapid Identification of Emerging Infectious Agents Using PCR and electrospray ionization mass spectrometry. Ann NY Acad Sci 1102: 109–20.
9. Ecker DJ, Sampath R, Li H, Massire C, Matthews HE, et al. (2010) New technology for rapid molecular diagnosis of bloodstream infections. Expert Rev Mol Diagn 10: 399–415.
10. Caliendo AM (2011) Multiplex PCR and emerging technologies for the detection of respiratory pathogens. Clin Infect Dis 52: S326–30.
11. Kaleta EJ, Clark AE, Johnson DR, Gamage DC, Wysocki VH, et al. (2011) Use of PCR coupled with electrospray ionization mass spectrometry for rapid identification of bacterial and yeast bloodstream pathogens from blood culture bottles. J Clin Microbiol 49: 545–53.