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Detection of Anaerobic pathogens from Culture Negative Clinical Specimens by PCR & Electrospray Ionization Mass Spectrometry after initiation of Antimicrobial Treatment

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Introduction

Anaerobic bacteria are common pathogens in soft tissue and pulmonary infections, yet reports of anaerobic infections are increasingly rare. We suspect multiple factors are at play in the declining reports of anaerobic infections:

1. Clinical specimens are commonly obtained after initiation of empiric antimicrobial treatment.
2. Timely plating and incubation of anaerobic cultures is a challenge for many medical centers:

- Delivery of specimens to the lab and triage delays within the lab are common.
- Setting of anaerobic cultures is not offered 24 x 7 in many microbiology labs.

In addition to the timeliness of setting cultures, the sensitivity of anaerobic culture results is affected by previously administered and/or concurrent antimicrobial treatment. In hospitalized patients, most initial empiric antimicrobial regimens have broad anti-anaerobic activity. When cultures are obtained following initiation of antibiotics, empiric antimicrobial treatment with anti-anaerobic activity may be continued even if cultures are negative, if suspicion of infection remains high even when cultures are negative.

Empiric treatment for anaerobic pathogens impacts patient outcomes, exposes patients to the deleterious effects of extended courses of overly broad empirical antibiotics, exacerbates the widespread problem of multidrug resistant organisms (particularly VRE), and increases the risk of *Clostridium difficile* infection. [1] In a prospect evaluation of PCR/ESI-MS vs. conventional culture, PCR/ESI-MS identified pathogens in 60% of "culture negative" samples that were obtained following initiation of antibiotic treatment.[2] We present our results from a prospective examination of 14 cases of suspected anaerobic infection in patients on antimicrobial treatment at the time of sample collection.

Our purpose is to demonstrate that PCR/ESI-MS may be a valuable addition to the clinical microbiology laboratory, especially 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.

Materials & Methods

We conducted a prospective evaluation of anaerobic culture vs PCR/ESI-MS among inpatients with suspected anaerobic infection. Clinical specimens from 14 cases were submitted for testing. All specimens were obtained after at least one dose of antibiotic treatment had been administered.

Culture: Gram stain and conventional aerobic and anaerobic cultures were performed in the St. Francis Medical Center (Peoria, IL) clinical microbiology laboratory. Anaerobic cultures were plated and incubated in a 498 L SHEL Lab Bactron II anaerobic chamber.

PCR/ESI-MS: PCR/ESI-MS on the Ibis PLEX-ID™ platform (Ibis Biosciences, Carlsbad, CA) was performed on all specimens. We followed the PCR/ESI-MS protocol previously described using a commercial test kit (BAC Detection Assay, Abbott Molecular) [3,4].

Cases

Case 1: 47-year-old diabetic female smoker with acute RUL necrotizing pneumonia that progressed to a cavitary lung abscess despite 5 days of azithromycin and 11 days of treatment with ceftriaxone and vancomycin.

Case 2: 50-year-old previously healthy man with left frontal brain abscess. Cultures taken in the OR during surgical drainage of abscess after 48 hours of antibiotic treatment with ceftriaxone, metronidazole, and vancomycin.

Case 3: 64-year-old obese man with infected right prosthetic knee. Cultures taken in the OR during surgical drainage of infected TKA after one dose of cefazolin administered as routine pre-operative surgical prophylaxis.

Case 4: 50-year-old diabetic with chronic avascular necrosis of the right hip and suspected superimposed acute right hip infection (no hardware). Cultures taken in the OR after one day of IV ceftriaxone and vancomycin.

Case 5: 72-year-old woman with right cranial epidural abscess following surgical resection of large right parietal meningioma. Cultures taken in the OR during drainage of epidural abscess after one day of ceftriaxone and vancomycin.

Case 6: 78-year-old previously healthy woman was given Piperacillin/tazobactam for one day prior to ultrasound drainage of 8cm liver abscess.

Case 7: 38-year-old woman on clindamycin following tooth extraction had aerobic & anaerobic swabs of submental abscess taken in O.R. during I&D.

Case 8: 33-year-old man with left ankle fracture S/P two stage external fixation converted to internal fixation of distal tibial pilon fracture complicated by wound dehiscence with exposed hardware. Cultures of infected ankle tissue taken in OR following one week of treatment with oral cephalexin.

Case 9: 49-year-old quadriplegic man with foul-smelling left hip wound with exposed bone. Patient received six days of treatment with vancomycin and piperacillin/tazobactam prior to surgical debridement of wound.

Case 10: 50-year-old woman with necrotizing pneumonia following lobectomy for right upper lobe lung cancer. Specimens of right pleural fluid were obtained in the OR after five days of treatment with empiric vancomycin, meropenem and trimethoprim/sulfamethoxazole.

Case 11: 25-year-old woman with left ankle abscess that developed following conversion from internal fixation to external fixation of left ankle fracture despite more than a month of antibiotic treatment with oral Clindamycin.

Case 12: 84-year-old diabetic man with infection of the head of the right 1st metatarsal. All antibiotics were held for 5 days prior to bone biopsy procedure. Specimens are samples of the infected bone obtained in the OR.

Case 13: 29-year-old man with community-acquired pneumonia and a large loculated left pleural effusion S/P left thoracentesis. Following treatment with levofloxacin x 48 hours, thoracentesis was performed that yielded 900 mL of olive green fluid, which was submitted for Gram stain, cultures and cytology.

Case 14: 48-year-old paraplegic man on empiric antibiotics for infected right hip pressure ulcer. Specimens of infected tissue were obtained in the OR after three days of treatment with empiric vancomycin and piperacillin/tazobactam.

Results

Patient	Specimen	Gram stain	Aerobic Culture	Anaerobic Culture	PCR/ESI-MS	LOD* (GE/well) [#]	DOT ⁺
1	Pleural Fluid	Negative for organisms	No growth	No growth	<i>S. pneumoniae</i>	75	11
2	Purulent Brain Abscess Fluid	Many segs GPC in chains	No growth	No growth	<i>S. intermedius</i>	164	2
3	Right Knee tissue	Many segs No organisms	No growth	<i>S. epidermidis</i>	<i>S. epidermidis</i>	113	1
4	Right Hip fluid	Few segs No organisms	No growth	No growth	No detection	—	1
5	Epidural Abscess Fluid	Many segs No organisms	<i>Propionibacterium acnes</i>	<i>Propionibacterium acnes</i>	<i>Propionibacterium acnes</i>	184	1
6	Liver Abscess Fluid	Many segs GPC in chains	<i>Corynebacterium</i> spp.	<i>S. intermedius</i>	<i>Streptococcus</i> spp. <i>Fusobacterium nucleatum</i>	40 58	1
7	Submental Abscess Fluid	Many segs No organisms	No growth	<i>Peptostreptococcus</i> spp.	<i>Porphyromonas gingivalis</i>	48	2
8	Left Ankle swab (collected in OR)	Many segs No organisms	<i>Enterobacter cloacae</i> complex	<i>Enterobacter cloacae</i> complex	<i>Enterobacter cloacae</i> complex	105	7
9	Left hip tissue	Many segs No organisms	<i>Enterococcus faecalis</i> <i>Escherichia coli</i>	<i>Bacteroides fragilis</i>	<i>Enterococcus faecalis</i> (+ van A) <i>Escherichia coli</i> <i>Bacteroides fragilis</i>	118 3888 2153	6
10	Pleural Fluid	Negative for organisms	<i>S. aureus</i> (MSSA)	No growth	<i>S. aureus</i> (<i>mecA</i> negative) <i>C. albicans</i> <i>Bifidobium wadsworthia</i>	72 119 15	6
11	Left Ankle tissue (collected in OR)	Negative for organisms	<i>Streptococcus mitis/oralis</i>	No growth	<i>Streptococcus oralis</i> <i>Streptococcus infantarius</i> <i>Finnegoldia magna</i>	81 58 74	31
12	Right hallux bone biopsy	Negative for organisms	<i>S. aureus</i> (MSSA)	No growth	<i>S. aureus</i> (<i>mecA</i> negative) <i>Fusobacterium nucleatum</i>	7 11	6
13	Parapneumonic effusion	Negative for organisms	No growth	No growth	<i>Fusobacterium nucleatum</i>	188	2
14	Left hip tissue	Many segs No organisms	No growth	No growth	<i>Corynebacterium striatum</i>	531	2

Results: Anaerobes were detected by culture in only 14% (2/14) of cases, but were detected by PCR/ESI-MS in 57% (8/14) cases ($\kappa = 0.222$). PCR/ESI-MS detected at least one pathogen in 13/14 cases, and detected ≥ 2 bacteria in five cases.

	Culture (%)	PCR/ESI-MS (%)	κ
Aerobic	7 (50)	11 (86)	0.429
Anaerobic	2 (14)	8 (57)	0.222

Both aerobic and anaerobic bacteria were more likely to be detected by PCR/ESI-MS than culture. Aerobic culture and PCR/ESI-MS agreed for 57% of specimens (8/14). Anaerobic culture and PCR/ESI-MS results agreed for only 1 of 6 anaerobes detected.

Conclusions

- PCR/ESI-MS demonstrated remarkable capacity for detection of anaerobic organisms that appear likely pathogens in these selected cases.
- PCR/ESI-MS clearly complements conventional cultures (both aerobic and anaerobic) when specimens are obtained following initiation of antibiotics.
- PCR/ESI-MS exhibited a unique capacity to identify multiple pathogens in cases of polymicrobial infections, possible attributable to avoiding growth competition.

References

1. Rice LB. Complex dynamics of antimicrobials in the human gastrointestinal tract. *Am Clin Climatol Assoc.* 2013; 124:123-32
2. Farrell JJ, Sampath R, Ecker DJ, Bonomo RA. Salvage Microbiology: Direct Detection of Pathogens from Clinical Specimens Following Initiation of Antimicrobial Treatment. *PLoS One.* 2013; 8(6):e66349.
3. Bhatia NS, Farrell JJ, Sampath R, Ranken R, Rounds MA, Ecker DJ, Bonomo RA. Identification of *Streptococcus intermedius* central nervous system infection by use of PCR & ESI Mass Spectrometry. 2012; *J Clin Micro* 50 (12): 4160-3.
4. Kaleta EJ, Clark AE, Johnson DR, et al. Use of PCR coupled with electrospray ionization mass spectrometry for rapid identification of bacteria and yeast bloodstream pathogens from 122 blood culture bottles. *J. Clin. Micro.* 2011; 49: 345-53.