Introduction
Antibiotic pretreatment negatively impacts the likelihood of recovering viable organisms in culture. [1,2] Molecular methods that rely on nucleic acid amplification technology (NAAT) may offer an advantage in the detection of pathogens collected after initiation of antimicrobial treatment. PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) offers a diagnostic approach with proven capacity for detection of pathogenic organisms from "culture negative" clinical samples obtained from patients on antibiotics for treatment of presumed infection.[3] Improving pathogen detection is an important goal for both patient management and institutional antimicrobial stewardship. We performed a prospective evaluation of PCR/ESI-MS for detection of bacteria in sterile specimens that were obtained from patients after initiation of antibiotics.

Methods
All sterile fluid and tissue clinical specimens submitted for microbiologic testing from patients on antibiotics during four 2 week time periods between October, 2012 and December, 2013 were included in this study. Specimens from immunocompromised patients were NOT included.

Enrollment
127 patients met the inclusion criteria and were included in the analysis. All of the specimens submitted were examined by conventional aerobic and anaerobic culture as well as PCR/ESI-MS.

Table 1. Specimens: Sterile fluids vs. OR tissues & fluids

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Surgical (OR)</th>
<th>Sterile Aspiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>Fluids</td>
<td>21</td>
<td>71</td>
</tr>
<tr>
<td>N</td>
<td>54</td>
<td>73</td>
</tr>
</tbody>
</table>

Results

The sterile clinical specimens submitted for culture and PCR/ESI-MS testing included pleural & synovial fluids; bone and brain tissues; abscess drainage; ascites; vitreous humor, heart valves, and chest wall tissue. Table 2. Days of treatment (DOT) by culture & PCR result

<table>
<thead>
<tr>
<th>DOT</th>
<th>Culture-POS</th>
<th>Culture*</th>
<th>ESI-MS</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>17</td>
<td>5</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>3-7</td>
<td>8</td>
<td>0</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>&gt;8</td>
<td>9</td>
<td>0</td>
<td>23</td>
<td>6</td>
</tr>
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</table>

Table 3. Culture Negative Results stratified by DOT (N=85)

<table>
<thead>
<tr>
<th>DOT</th>
<th>Culture-POS</th>
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<th>ESI-MS</th>
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<tr>
<td>&lt;2</td>
<td>49</td>
<td>26</td>
<td>5</td>
<td>0.759</td>
</tr>
<tr>
<td>3-7</td>
<td>40</td>
<td>8</td>
<td>32</td>
<td>0.118</td>
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<td>&gt;8</td>
<td>38</td>
<td>6</td>
<td>29</td>
<td>0.110</td>
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Based on Cohen’s Kappa statistic, there was poor overall agreement between culture and PCR/ESI-MS (κ=0.283); but for patients treated for < 48 hours, the agreement was extremely strong (κ=0.759). In contrast, there was very poor agreement between culture and PCR/ESI-MS tests for patients treated for 3-7 days, or > 7 days (κ=0.118, and 0.110, respectively).

Discussion
This is the first prospective examination of NAAT (specifically PCR/ESI-MS) vs. culture with sterile specimens obtained following initiation of antibiotics. Our results suggest that PCR/ESI-MS offers an advantage over conventional culture in detection of bacteria after initiation of antibiotics in patients treated for more than 48 hours. However, in patients treated with < 48 hours of antibiotics, PCR/ESI-MS offers little advantage over culture. A diagnostic algorithm for specimens obtained from patients on antibiotic treatment was developed based on our findings (Figure 1).

References

Figure 1. Diagnostic Algorithm for Specimens Submitted from Antibiotic Exposed Patients

Suggested Clinical Microbiology Diagnostic Algorithm for Specimens Obtained after Initiation of Antibiotics

Is patient receiving antibiotics? (YES: RECOMMEND EXTENDED-INCUBATION for specimens collected from patients after more than 2 days of antibiotic treatment.)

No Growth

Growth

No organsisms recovered in Culture.

ID Bacteria recovered in culture.

Infection suspected? (REQUEST NAAT testing of clinical specimens.)

No

YES

RECOMMEND PCR/ESI-MS

STOP

Table 4. Cohen's Kappa Results stratified by DOT

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