Comparison of chromogenic and selective media for the detection of *S. aureus* and *P. aeruginosa* in respiratory samples from CF patients


MRSA Reference Laboratory,
Université Libre de Bruxelles - Hopital Erasme – Belgium

* Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute, Université catholique de Louvain

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Background
Chronic bacterial infections contribute to a decline in lung function

**S. aureus (SA)**
- 44% colonisation and infection in Belgium (multicentric study)\(^1\)
- 4.2% (0-23%) small colony variants (SCV) ~ underdetection?

**P. aeruginosa (PA)**
- 20-70% in Belgium (multicentric study)\(^2\)
- Abnormal phenotypes (mucoid, SCV)

**SCV ~ persistent infection**

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Objectives
To compare the performance of chromogenic media with conventional media for the detection and the presumptive identification of S. aureus and P. aeruginosa

- normal phenotype
- variants phenotypes (SCV, mucoid)
Methods
Materials
- 159 respiratory samples from 64 CF patients in a CF reference centre
- COL, HAEM, MAN, MAC and BCSA usual plates
- SAID and PAID chromogenic plates
- Incubated at 35°C for 5 days, examined daily

Presumptive identification
- SAID: suspect colonies (characteristic growth, morphology and colour)
- PAID: all

Phenotypic identification
- SA: coagulase
- PA: oxidase, ADH, 42°, Kligler
Genotypic confirmation
- SA: *nuc* and *mecA* genes, 16S rRNA
- PA (if atypical biochemical profile): 16S rRNA sequencing

Antimicrobial susceptibility testing
- Normal SA: Vitek2
- SCV-SA: disk diffusion, Vancomycin agar screen
- PA: disk diffusion
Results
**Results**

### Prevalence of *S. aureus* and *P. aeruginosa* colonization

<table>
<thead>
<tr>
<th></th>
<th>Number of sputum samples&lt;sup&gt;a&lt;/sup&gt; (n=159)</th>
<th>Number of isolates</th>
<th>Number of CF patients&lt;sup&gt;b&lt;/sup&gt; (%) (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. aureus</strong></td>
<td>48</td>
<td>79</td>
<td>33 (51.6)</td>
</tr>
<tr>
<td>- MRSA*</td>
<td>11</td>
<td>6 (9.4)</td>
<td></td>
</tr>
<tr>
<td>- SA-SCV</td>
<td>21</td>
<td>11 (17.2)</td>
<td></td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>72</td>
<td>133</td>
<td>33 (51.6)</td>
</tr>
<tr>
<td>- mucoid</td>
<td>54</td>
<td>19 (29.7)</td>
<td></td>
</tr>
<tr>
<td>- PA-SCV</td>
<td>33</td>
<td>19 (29.7)</td>
<td></td>
</tr>
<tr>
<td><strong>SA+PA</strong></td>
<td>22</td>
<td></td>
<td>17 (26.6)</td>
</tr>
</tbody>
</table>

<sup>*All meca* +</sup>

- **Note:** SA= *S. aureus*; PA= *P. aeruginosa*; SCV= small colony variant; CF= cystic fibrosis
- **a**Mean sputum samples per patient = 2.6; **b**Median age = 30 years (range 5-60)
Analytical performance of MAN and SAID media for *S. aureus* detection

<table>
<thead>
<tr>
<th>Medium</th>
<th>MAN</th>
<th>SAID</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of SA isolates* (N=79)</td>
<td>64</td>
<td>71</td>
</tr>
<tr>
<td>-SCV (n=21)</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>81.0</td>
<td>89.9</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>77.1</td>
<td>67.2</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>85.9</td>
<td>92.0</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>70.3</td>
<td>61.2</td>
</tr>
</tbody>
</table>

Note: CF= cystic fibrosis; SA= all *S. aureus*; SCV= small colony variant

* 2 only recovered Colombia blood agar
Results

Morphological aspect of SCV *S. aureus*

>24h

>24h

>24h

>48h
## Results

### Antimicrobial resistance profile of SA (n=79) isolates

<table>
<thead>
<tr>
<th>Antimicrobials*</th>
<th>PEN</th>
<th>FOX</th>
<th>VAN</th>
<th>CIP</th>
<th>GEN</th>
<th>SXT</th>
<th>LIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-SA (%)</td>
<td>82.4</td>
<td>9.8a</td>
<td>0</td>
<td>23.5</td>
<td>5.9</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>(n=58)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCV-SA (%)</td>
<td>76.0</td>
<td>28.6a</td>
<td>0</td>
<td>42.9</td>
<td>9.5</td>
<td>95.2</td>
<td>0</td>
</tr>
<tr>
<td>(n=21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: N-SA= normal phenotype *S.aureus*; SCV= small colony variant

*PEN, penicillin; CEF, cefoxitin; VAN, vancomycin; CIP, ciprofloxacin; GEN, gentamicin; SXT, cotrimoxazole; LIN, linezolid

**a All *mecA* +
## Analytical performance of MAC and PAID media for *P. aeruginosa* detection

<table>
<thead>
<tr>
<th></th>
<th>MAC</th>
<th>PAID</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of PA isolates*</td>
<td>101</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>- Mucoid (n=54)</td>
<td>53</td>
<td>49</td>
<td>p=0.025</td>
</tr>
<tr>
<td>- SCV (n=33)</td>
<td>18</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>75.9</td>
<td>88.7</td>
<td>p=0.013</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>64.5</td>
<td>90.8</td>
<td></td>
</tr>
<tr>
<td>PPV (%)</td>
<td>78.9</td>
<td>92.9</td>
<td></td>
</tr>
<tr>
<td>NPV (%)</td>
<td>60.5</td>
<td>85.6</td>
<td></td>
</tr>
</tbody>
</table>

Note: CF= cystic fibrosis; PA= all *P. aeruginosa*; SCV= small colony variant

* 2 only recovered on Haemophilus agar; 1 only on *Burkholderia cepacia* selective agar
Morphological aspect of normal phenotype *P. aeruginosa*

Results

>48h
Morphological aspects of mucoid *P. aeruginosa*

Results

- MAC
- PAID

>48h

>48h
Morphological aspect of SCV *P. aeruginosa*

Results

- COL
  - >48h
- MAC
  - >48h
- PAID
  - >48h
### Antimicrobial resistance profile of PA (n=133) isolates

<table>
<thead>
<tr>
<th>Antimicrobials *</th>
<th>MER</th>
<th>AKN</th>
<th>TOB</th>
<th>ATM</th>
<th>CAZ</th>
<th>CFP</th>
<th>TZP</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-PA (%) (n=46)</td>
<td>17.8</td>
<td>33.3</td>
<td>13.3</td>
<td>22.2</td>
<td>28.9</td>
<td>22.2</td>
<td>6.7</td>
<td>20.0</td>
</tr>
<tr>
<td>SCV-PA (%) (n=33)</td>
<td>29.4</td>
<td>44.1</td>
<td>23.5</td>
<td>38.2</td>
<td>41.2</td>
<td>35.3</td>
<td>20.6</td>
<td>29.4</td>
</tr>
<tr>
<td>Mucoid PA (%) (n=54-10)a</td>
<td>25.0</td>
<td>38.6</td>
<td>11.0</td>
<td>27.2</td>
<td>31.8</td>
<td>38.6</td>
<td>22.7</td>
<td>18.2</td>
</tr>
</tbody>
</table>

*Note: N-PA= normal phenotype *P. aeruginosa*; SCV= small colony variant

* MER, meropenem; AKM, amikacin; TOB, tobramycin; ATM, aztreonam; CAZ, ceftazidime; CFP, cefepime; TZP, piperacillin-tazobactam; CIP, ciprofloxacin

*a* not available for 10 isolates
Conclusions
Prevalence of *S. aureus* and *P. aeruginosa* among CF patients: 52%
- SA-SCV: 18%; MRSA: 10%
- PA-SCV and mucoid PA: 30%

PAID chromogenic media demonstrated better performances than conventional media
- For detection of PA isolates
- For recovery of SCV
- But translucent colonies should be identified to exclude PA

SAID had limited ability to differentiate SA from the polymicrobial flora in CF sputa (PPV=61.2%)

*S. aureus* and *P. aeruginosa* SCVs were more resistant to antimicrobials than normal phenotype
Acknowledgements
Results

Achromobacter xylosoxyidans plated onto MAC and PAID