Etiological diagnosis of bloodstream infections: novel approaches

Prof. Gilbert GREUB, MD PhD

Director of the Institute of Microbiology of the University of Lausanne, Head of diagnostic microbiology at the University Hospital Center, Lausanne, Switzerland
Conflict of interests

In Lausanne, we are using a BD-Kiestra full lab automated system, the Bruker MALDI-TOF, the BD-FX blood cultures automated system, the Vitek phenotypic & AST assay, the GenXprt, the BD-Max, the Luminex and DSX serology automates, the Scholzen incubators, several Hamilton robots, the ABI 7900 real-time systems, the Fragment analyzer, the MiSeq sequencer, …
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Relationships with industry

• Research agreement with SUEZ-ONDEO (France)
• Research agreement with BD (USA) \(\rightarrow\) Phoenix / BD-Kiestra
• Part of the Scientific advisory board of «Resistell» since April 2018
• Research agreement with Resistell (in preparation)
• Co-funder of JeuPRO (game Krobs)
Blood cultures

Culture on agar

10 µl

1st quadrant

2nd quadrant

3rd quadrant

4th quadrant

Culture in a broth

8 - 10 ml
Volume and yield of blood culture

% Relative Yield

ml

5 10 15 20 25 30 35 40 45 50 55 60

0 10 20 30 40 50 60 70 80 90 100
Clinical significance of *Staphylococcus epidermidis* isolates from blood cultures

<table>
<thead>
<tr>
<th>N of sets positive</th>
<th>N of sets obtained</th>
<th>% Significant</th>
<th>% Contaminate</th>
<th>% Indeterminate</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>97</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>95</td>
<td>3</td>
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<tr>
<td>2</td>
<td>2</td>
<td>60</td>
<td>3</td>
<td>37</td>
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<td>3</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>75</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Adapted from Weistein et al. Clin Infect Dis 1997; 24 : 584-602
Blood cultures: more frequent pathogens

- **Gram positive bacilli**: 1%
- **Gram negative cocci**: 0.1%
- **Gram negative bacilli**: 62%

Number of positive blood cultures:

1. *Escherichia coli* 28.6%
2. *Staphylococcus aureus* 42%
3. *Coagulase negative staphylococci* 13.60%
4. *Pseudomonas aeruginosa* 10.09%
5. *Klebsiella pneumoniae* 8.52%
6. *Enterococcus faecalis* 7.11%
7. *Enterobacter spp.* <5%
8. *Proteus spp.*
9. *Haemophilus influenzae group* 83%
10. *Citrobacter spp.*
11. *Enterococcus faecium* 83%
12. *Streptococcus anginosus/milleri* 94%
14. *Streptococcus pneumoniae*
15. *Morganella morganii*
16. *Serratia marcescens*
17. *Stenotrophomonas maltophilia*
18. *Acinetobacter spp.*
19. *Streptococcus alpha hemolytic*
20. *Acinetobacter spp.*

**Total**: n=16682
Blood cultures

To improve diagnosis: use a pellet

Centrifugation steps
Blood cultures

Escherichia coli

Staphylococcus capitis

Streptococcus dysgalactiae
## Applications of the pellet

### Authors (year)

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Concordant ID at species level</th>
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</thead>
<tbody>
<tr>
<td>Prod’hom (2010)</td>
<td>122</td>
<td>78% (GN: 89%, GP: 72%)</td>
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<tr>
<td>Prod’hom (unpublished)</td>
<td>314</td>
<td>85% (GN: 89%, GP: 83%)</td>
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</tbody>
</table>

### Excellent accuracy

- n=122
- 69(57%) > 2.0
- 96(79%) > 1.7

1 discordant

*S. caprae vs S. pasteuri*
## Many recent studies (>50)

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Concordant ID at species level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kok (2011)</td>
<td>507</td>
<td>74.8% (GN: 91.4%, GP: 67.7%)</td>
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<tr>
<td>Schubert (2011)</td>
<td>500</td>
<td>86.5% (GN 89.8%, GP 86.3%)</td>
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<td>March-Rossello (2013)</td>
<td>100</td>
<td>~98% (GN: 97.3%, GP: 98.4%)</td>
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<tr>
<td>Clerc (2013)</td>
<td>202</td>
<td>86.7% (only GN)</td>
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<tr>
<td>Rodrig-Sanchez (2014)</td>
<td>1084</td>
<td>63.9% (81.4% after extraction)</td>
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<tr>
<td>Jakovlev (2015)</td>
<td>152</td>
<td>81.9% (up to 89.3%)</td>
</tr>
<tr>
<td>Randazzo (2016)</td>
<td>266</td>
<td>65.8% (up to 77.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>77.8%</td>
</tr>
</tbody>
</table>
Pre-MALDI-TOF era

- **Gram + cocci**
  - arranged in chains

- **Gram + cocci**
  - arranged in clusters

- **Gram - bacilli**

Post-MALDI-TOF era

- **E. faecium**

- **S. aureus**

- **E. coli**

**Overnight incubation**

**Blood cultures**

**Before 2009**

**After 2009**

**CLINICAL IMPACT ?**

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Impact of MALDI-TOF: retrospective study

- Time to identification
  1h35 with MALDI-TOF (versus 25h43 without)

Clinical impact:
- 20/157 results led to a treatment change (in adults)
- 1/40 results led to a treatment change (in children)
- New blood cultures (n=2)
- Catheter removal (n=4)
- Additional investigations (n=3)
- Documented sample exchange (n=1)
- Exclude a contamination

Martiny  et al. Clin Microbiol Infect 2013
Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the Clinical Management of Patients With Gram-negative Bacteremia: A Prospective Observational Study

Olivier Clerc,1 Guy Prod’hom,2 Christelle Vogne,2 Alain Bizzini,2 Thierry Calandra,1 and Gilbert Greub1,2

1Infectious Diseases Service and 2Institute of Microbiology, Lausanne University Hospital Center and University of Lausanne, Switzerland
### Impact of the Sequential Reporting

<table>
<thead>
<tr>
<th>Impact</th>
<th>N</th>
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</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>42 (20.8)</td>
</tr>
<tr>
<td>Streamlining</td>
<td>16 (7.9)</td>
</tr>
<tr>
<td>Spectrum broadening</td>
<td>16 (7.9)</td>
</tr>
<tr>
<td>Introduction of empirical antibiotic therapy</td>
<td>10 (5.0)</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>71 (35.1)</td>
</tr>
<tr>
<td>Streamlining</td>
<td>22 (10.9)</td>
</tr>
<tr>
<td>Spectrum broadening</td>
<td>31 (15.3)</td>
</tr>
<tr>
<td>Introduction of focused empirical antibiotic therapy</td>
<td>18 (8.9)</td>
</tr>
</tbody>
</table>

**Impact of MALDI-TOF on empirical antibiotic therapy in 35% of bacteremia**
A prospective randomized trial

- MALDI-TOF subgroup 1
- Conventional subgroup 1
- MALDI-TOF *E. coli*/Klebsiella spp.
- Conventional *E. coli*/Klebsiella spp.

p<0.001 (Log-rank test)

Cumulative optimal treatment vs. Hours since blood culture draw
Blood cultures

Antibiotic susceptibility

Starting from a bacterial pellet

Inhibition diameter

MIC

Before 2012

24h

From isolated colonies

After 2012

Starting from a bacterial pellet

4-6h

PCR MRSA (on blood culture pellet)

Blood cultures

- **Gram staining**: > 100% accurate, ≤1h
- **MALDI-TOF MS**: > 99% accurate, ≤1h
- **Antibiotic susceptibility testing**: > 99% accurate, ~6h
- **POCT-PCR MRSA**
  - Sensitivity: 99%
  - Specificity: 100%, 2-3h
Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.


Clin Microbiol Infect. 2017
Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.
Clin Microbiol Infect. 2017
Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.
Clin Microbiol Infect. 2017
Genomic of medical importance
Institutional project since 2012

For selected cases:
- Pathogenicity
- Clinical picture
- Outbreak
- Specific AB resistance
- Need of diagnostic tools

Goal: results in 48 to 72 hours

Clients:
- microbiologists
- medical doctors

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Novel approaches for the etiological diagnosis of bacteremia

Blood culture pellet
  + Gram
  + MALDI-TOF
  + Rapid PCR tests
  + Rapid biochemical tests
  + Automated AST (Vitek, Phoenix)
  + Atomic force microscopy
  + Genomics (taxogenomics, virulome, resistome)

Directly from blood (FISH, PCRs, Microarray)
Acknowledgement to all the Diagnostic Lausanne team

Biologist and medical doctors

Claire BERTELLI, Dominique BLANC, Alix COSTE, Antony Croxatto, Philippe HAUSER, Katia JATON, Frédéric LAMOTH, Pascal MEYLAN, Imaculée NAHIMANA, Onya OPOTA, Trestan PIOLONNEL, Guy PROD’HOM, Florian TAGINI

Laboratory technicians

Christian DURUSSEL
Sébastien AEBY, Ali ALIU, Caroline BÄNNINGER, Janka BARANYAI, Anne BERTSCHY, Robert BEGUIN, René BROUILLET, Sylvie CAILLON BOUCHEZ, Lara CAMPA VAUTHEY, Sarah CHAPPUIS, Catherine CONUS, Myriam CORTHESY, Borislav DEMCIK, Tina DI FAZIO MIGNANIELLO, Fabienne DI PAOLA, Julie DUCROT, Danielle DULON, France DUSSERRE, Lone EL HOUSS, Shklqim GIZHA, Claudine GOSTELY, Nancy GUTMANN, Alice KUEMIN, Alexandra KÜNTZER, Virginie MARTIN, Silvana MARRA, Carole MASSONNET, Zahera NASERI, Marie-Anne PAGE, Dominique PILLOUD, Carine PINTER PONT, Valérie PONNAZ LINIOUBLI, Josiane RICHARD, Anna RUEGGER, Maria SENRA ORTIZ, Laurence SIMON, Albert SOLER, and Jolanda VONLANTHEN
Thank you

Institute of Microbiology of the University of Lausanne