Rapid Sepsis Microbiologic Diagnosis: A New World

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For available slides:
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Advances in Bacterial/Fungal Identification
Advances in Bacterial/Fungal Identification

Special thanks to Ronald Humphries, PhD, U of Arizona for allowing me to borrow some of his slides.
Traditional and newer methods of diagnosis in microbiology

• Microscopy and culture
• Identification – phenotypic
• Antimicrobial susceptibility – phenotypic
• Newer phenotypic based tests
Advantages of traditional methods

- Can be catch-all and diagnose mixed infections
- Established sensitivity and methods
- Living organisms can be recovered
Disadvantages of traditional methods

• Labour-intensive and perceived as expensive
• Clearly relatively slow
• A ‘skilled’ activity – operator dependent
• Results may not have a definite identification
Ideal technology

- Performed at bedside
- Very rapid (<30 min)
- Highly accurate:
  - What is the organism?
  - What is the organism resistant & susceptible to?

What we have in short term:
- Rapid ID & AST (hrs from positive culture)
- Rapid ID (hrs from blood draw)
- Rapid ID & resistance genes (hrs from blood draw)
### Key differentiating features of rapid microbiologic technologies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen source</td>
<td>Positive BC, direct blood, other</td>
</tr>
<tr>
<td>Time frame of result</td>
<td>Hrs: 0-1, 1-6, 6-12, 12-24, &gt;24</td>
</tr>
<tr>
<td>Clinical target</td>
<td>Septic shock, sepsis, serious infection</td>
</tr>
<tr>
<td>ID technology</td>
<td>Various forms of PCR, NGS, MALDI-TOF, FISH, MR, DNA bioparticles, gold nanoparticle microarray</td>
</tr>
<tr>
<td>AST technology</td>
<td>Genotype vs phenotype</td>
</tr>
</tbody>
</table>
Current approaches to diagnosing BSI

Time to result is key in diagnosing BSIs leading to sepsis. This remains a challenge with current treatment methodologies.

**Blood culture is the gold standard...**
- Blood culture remains the gold standard in microbial diagnosis of sepsis.
- Blood cultures are used to detect the presence (or lack) of bacteria or fungi in the blood, to identify the type present and the guided treatment.
- With blood culturing, bacteria or fungi must grow to a sufficient quantity before a result can be detected and identified.

**...Often followed by MALDI or PCR for ID**
- MALDI-TOF is a type of mass spectrometry.
- Applied to the analysis of positive blood cultures, it can rapidly identify the causative pathogen (both bacterial and fungal) and some antimicrobial resistances.
- Blood culture followed by MALDI-TOF represents the current state of the art.

1-6 days  
1-4 hours
Matrix Assisted Laser Desorption Ionization – Time of Flight/Mass Spectrometry or MALDI-TOF/MS
MALDI-TOF MS: History

- Developed in 1980s by Karas and Hillenkamp
- Detection of large molecules using TOF by Tanaka and Yoshida
- Introduction of matrix compounds to analyze large molecules
- First commercial instrument developed by Shimadzu
- First commercial database developed by Anagnostec (1998)
- Shimadzu scientist receives Nobel Prize in Chemistry
  - Kiochi Tanaka (2002)
- Technology in use in Europe for >10 years.
MALDI-TOF SPECTRA

Escherichia coli

Bacillus subtilis

Candida albicans

Aspergillus fumigatus

Courtesy of bioMérieux
Biochemical to MALDI-TOF Bacterial Identification

• Most significant advance in Clinical Microbiology (Bacteriology) in 30 years!

  • **Rapid and cost effective identification of bacteria directly from isolated colonies and positive culture bottles based on protein biomarkers**

    • Protein biomarkers measured are highly expressed proteins responsible for housekeeping functions, such as ribosomal (16S) and transcription/translation factor proteins

**FASTER, BETTER, CHEAPER, BUT NOT PERFECT!**
Conventional ID vs MALDI

• Saturday noon after a very long Friday night, Dr. Kumar (the patient) is admitted feeling very cranky
• Sunday noon, febrile with a WBC of 13 so blood cultures drawn
• Monday, 12pm, Dr. K’s blood culture flags positive
• Bottle removed, gram stain /culture prepared
• Gram negative rods seen, floor called at 1:10pm
• 3pm – Dr. K started on Ceftriaxone….15 hrs later

• Tuesday, 10:30am *P. aeruginosa* identified
• Floor called 10:45am
• Dr. K started on Pip/tazo at noon….sadly he dies 2 days later in septic shock in ICU

• MALDI ID would have seen me on appropriate anti-Pseudomonal therapy 20-24 hours earlier
Times for Standard Blood Culture and Sensitivities
Impact of MALDI-TOF MS
Study from Methodist Hospital, Huston TX

- Intervention arm (Gram Negative Bacilli):
  - Integrated rapid ID with active antimicrobial stewardship
  - **Results called to infectious diseases pharmacist 24/7**
  - Pharmacist recommends de-escalation or adjustment of therapy based on the rapid ID
  - Time to adjusted therapy was significantly reduced by 31 hrs.

![Time to therapy adjustment graph](https://via.placeholder.com/150)

CATSS: Impact of correction of IA antimicrobials on survival

<table>
<thead>
<tr>
<th>Time</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-12 hrs</td>
<td>55</td>
</tr>
<tr>
<td>12-24 hrs</td>
<td>115</td>
</tr>
<tr>
<td>24-36 hrs</td>
<td>90</td>
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<tr>
<td>&gt;36 hrs</td>
<td>167</td>
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</table>
Twenty-Three

No Test Is Perfect

• **E. coli Vs. Shigella**
  - Very closely related and cannot be differentiated

• **Streptococcus pneumoniae Vs. Streptococcus mitis group**
  - Very closely related, new databases can give a definitive ID
  - Differentiate by Bile solubility or optichin disk

• **Bordetella pertussis Vs. Bordetella bronchiseptica**
  - Very closely related and cannot be differentiated
  - Rarely cultured

• **Stenotrophomonas maltophilia Vs. Pseudomonas hibiscola, Ps. gentculata, Ps. betelli**
  - Very closely related and cannot be differentiated
  - Biochemical ID required

• **Acinetobacter baumanii-calcoaceticus complex** (A. baumanii, A. calcoaceticus, A. genospecies 3, A. genospecies 13):
  - Species differentiation can be difficult.
Molecular diagnostic tests - basic concepts of nucleic acid based tests

- The main purpose of amplification (e.g. PCR) is to make a huge number of copies of a gene.
- Does not require viable organisms
- Can be very sensitive – but contamination issues
- For rapidity requires a known target gene sequence
- Therefore can miss unknowns
- Molecular testing in diagnostic microbiology is usually restricted to detection (qualitative), and genomic antimicrobial sensitivity (ie PCR based).
- Ability to detect unknown or multiple organisms problematic – cannot replace standard C+S at this time; therefore, some pathogens may still take days or weeks and require reference/research laboratory facilities
Current diagnostic molecular tests- advantages

- Rapid diagnosis some potential close to bedside
- Culture- negative samples both non-culturable, or slow- growing, fastidious organisms
- Antibiotic susceptibility (mostly genomic)
Current Molecular Diagnostic Tests - Disadvantages

- Rapid tests can detect specific organisms only
- Usually only genomic resistance potential based on known gene sequences
- Do not provide a catch-all in a realistic timeframe
- Incremental cost to current practice as as standard C+S has to be maintained
- Clinical impact not well understood
<table>
<thead>
<tr>
<th>Company</th>
<th>Product or Platform</th>
<th>Status</th>
<th>Technology</th>
<th>Time to Result (approx.)</th>
<th>Specimen Type</th>
<th>Pathogen ID</th>
<th>Genetic Resistance Markers</th>
<th>Full AST</th>
<th>Host Response</th>
<th>Biomarker/Other</th>
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</thead>
<tbody>
<tr>
<td>BioFire</td>
<td>FilmArray</td>
<td>On Market</td>
<td>RT-PCR</td>
<td>1 hour</td>
<td>Blood culture</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Luminex</td>
<td>Verigene</td>
<td>On Market</td>
<td>Gold nanoparticle microarray</td>
<td>2 hours</td>
<td>Blood culture</td>
<td>Yes</td>
<td>Yes (MRSA)</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>GenMark</td>
<td>ePlex</td>
<td>On Market</td>
<td>PCR</td>
<td>1.5 hours</td>
<td>Blood culture</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<td>Accelerate Diagnostics</td>
<td>PhenoTest</td>
<td>On Market</td>
<td>FISH</td>
<td>1.5 hours; 6-7 hours (AST)</td>
<td>Blood culture</td>
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<td>Xpert SA/MRSA</td>
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<td>RT-PCR</td>
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<td>On Market</td>
<td>PCR</td>
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<td>AMI-PCR</td>
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<td>Cognitor Minus</td>
<td>Development</td>
<td>EGTA</td>
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<td>Blood culture</td>
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<td>Roche Molecular</td>
<td>SeptiFast</td>
<td>On Market</td>
<td>RT-PCR</td>
<td>4-6 hours</td>
<td>Whole blood</td>
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<td>Roche GeneWeave</td>
<td>Smarticate</td>
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<td>DNA bioparticles</td>
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<td>Seegene</td>
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<td>T2 Biosystems</td>
<td>T2Bacteria</td>
<td>On Market</td>
<td>Magnetic Resonance</td>
<td>3-5 hours</td>
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<td>Immunexpress</td>
<td>SeptiCyte</td>
<td>On Market</td>
<td>Gene expression</td>
<td>3.5-5 hours</td>
<td>Whole blood</td>
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<td>Karius</td>
<td>Karius Test</td>
<td>On Market</td>
<td>NGS</td>
<td>&gt;24 hours</td>
<td>Whole blood</td>
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<td>PathoQuest</td>
<td>iDect</td>
<td>On Market</td>
<td>NGS</td>
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<td>Whole blood</td>
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<td>O- linea</td>
<td>ASTriD</td>
<td>Development</td>
<td>Rolling Circle Isothermal Amp.</td>
<td>4 hours for ID; 10 hours AST</td>
<td>Whole blood</td>
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<td>Yes</td>
<td>Yes</td>
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<td>Qvella</td>
<td>FAST-ID BSI</td>
<td>Development</td>
<td>Multiplex PCR</td>
<td>&lt;1 hour</td>
<td>Whole blood</td>
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<td>No</td>
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<tr>
<td>DNAe</td>
<td>LiDia</td>
<td>Development</td>
<td>PCR and semiconductor chip</td>
<td>3 hours</td>
<td>Whole blood</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Inflammatix</td>
<td>HostDx Sepsis</td>
<td>Development</td>
<td>Gene expression profile</td>
<td>N/A</td>
<td>Whole blood</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td>Cytovalle</td>
<td>NA</td>
<td>Development</td>
<td>White Blood Cell morphology</td>
<td>&lt;10 minutes</td>
<td>Whole blood</td>
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<td>Yes</td>
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<td>Abionic</td>
<td>abioSCOPE</td>
<td>Development</td>
<td>PSP Biomarker - Immunoassay</td>
<td>5 minutes</td>
<td>Whole blood</td>
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<td>No</td>
<td>No</td>
<td>Yes</td>
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<td>Sphingotec</td>
<td>Nexus IB10</td>
<td>Development</td>
<td>Bio-ADM-Immunoassay</td>
<td>20 minutes</td>
<td>Whole blood</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Beckman Coulter</td>
<td>DxA 900</td>
<td>FDA filing</td>
<td>Monocyte morphology</td>
<td>&lt;5 Minutes</td>
<td>Whole blood</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Direct from Blood Tests

**IRIDICA**
- **Step 1:** Sample prep
  - Lysis
  - Nucleic acid purification

- **Step 2:** PCR
  - Multiplex broad-range PCR
    - Target: 16S and 23S rRNA genes

- **Step 3:** Species identification
  - Purification
  - Mass spectrometry

- Ideal total time and batch size: 6 hours, 6 samples per run

**SeptiFast**
- **Step 1:** Sample prep
  - Lysis
  - Nucleic acid extraction

- **Step 2:** PCR
  - Multiplex target specific real-time PCR/in-situ hybridization
    - Target: ITS regions between the 16S and 23S

- **Step 3:** Species identification
  - High resolution melt

- Ideal total time and batch size: 6 hours, 8 samples per run

**SeptiTest**
- **Step 1:** Sample prep
  - Human DNA lysis and degradation
  - Pathogenic lysis and nucleic acid extraction

- **Step 2:** PCR
  - Universal PCR
    - Target: 16S and 18S rRNA genes

- **Step 3:** Species identification
  - Purification
  - Sequencing

- Ideal total time and batch size: 8-10 hours, 12 samples per run

**U-dHRM**
- **Step 1:** Sample prep
  - Lysis
  - Nucleic acid extraction

- **Step 2:** PCR
  - Universal PCR
    - Target: 16S rRNA genes

- **Step 3:** Species identification
  - Digital high resolution melt

- Ideal total time and batch size: 4 hours, 1 sample per run
## Current list of pathogen and resistance markers (DNAe)

<table>
<thead>
<tr>
<th>GRAM POSITIVE</th>
<th>GRAM NEGATIVE</th>
<th>YEAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus spp.</td>
<td>Escherichia coli</td>
<td>Candida albicans/tropicalis</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Klebsiella spp.</td>
<td>Candida parapsilosis</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Enterobacter spp.</td>
<td>Candida glabrata/krusei</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Serratia marcescens</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>Proteus mirabilis</td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Acinetobacter baumannii</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>RESISTANCE MARKERS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mecA/mecC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vanA/vanB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KPC</td>
</tr>
</tbody>
</table>
Timeline of sepsis technologies relative to BC
Blood drawn, sent to lab → Blood Cx System → Flagged as positive → Gram stain & phone call → Sub-cultured to solid media → Isolate ID and AST

What’s Wrong?

- Total time: 3-5 days
- Wrong empiric therapy = 5x higher mortality
- 25-40% of patients receive ineffective empiric therapy
- Up to 60% of septic patients have negative blood cultures
- Some organisms cannot be cultured

BSI, blood stream infection

1. 2006 Crit Care Med 34:1589
2. Chest 2000:118;146-55
Ideal Approach to BSI Diagnostics

Blood drawn, sent to lab
Cultured on automated system
Flagged as positive
Gram stain performed and called
Sub-cultured to solid media
Isolate ID and susceptibility

Need ID & AST data here!
Newer Approaches to BSI Diagnostics

- Blood drawn, sent to lab
- Cultured on automated system
- Flagged as positive
- Gram stain performed and called
- Sub-cultured to solid media
- ID & AST

ID only: Rapid MALDI
AST only: Automated AST, EUCAST disk, Lifescale

ID + resistance genes: bioMerieux Biofire, Luminex Verigene, Genmark ePlex
ID + MICs: Accelerate PhenoTest BC

- Not all-inclusive!
- Nice reviews: Belkum and Dunne, JCM 2013 51:2018; Pulido et al 2013 JAC 68:2710
Approaches to Testing (to date)

**Phenotype**
“*What concentration of the drug inhibits growth of the bug?*” (i.e., MIC)

- Takes time to observe behavior
- Need lots of bug - more than in most BSIs
- Independent of “R” mechanism
- Predict S & R
- Interpret using “breakpoints”

**Genotype**
“*Is there a gene that predicts the drug won’t kill the bug?*”

- Quick
- Detects few or nonviable cells - stoichiometry issues
- Only detects targets sought
- Can’t fully predict S or R
- If detected, must assume “R”
**Multicenter Evaluation of the Accelerate PhenoTest BC Kit for Rapid Identification and Phenotypic Antimicrobial Susceptibility Testing Using Morphokinetic Cellular Analysis**


J Clin Microbiol. 2018 Jan 5

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**Gram-Positive Antibiotic Susceptibilities**

- 97.6% essential agreement (EA)
- 97.9% categorical agreement (CA)
- 1.0% very major error (VME)
- 0.7% major error (ME)
- 1.3% minor error (mE)

**Gram-Negative Antibiotic Susceptibilities**

- 95.4% essential agreement (EA)
- 94.3% categorical agreement (CA)
- 0.5% very major error (VME)
- 0.9% major error (ME)
- 4.8% minor error (mE)

---

**Gram-Positive Identification**

<table>
<thead>
<tr>
<th>Sensitivity (TP/TP+FN)</th>
<th>Specificity (TN/TN+FP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96.7%</td>
<td>99.0%</td>
</tr>
</tbody>
</table>

**C. albicans & glabrata Identification**

<table>
<thead>
<tr>
<th>Sensitivity (TP/TP+FN)</th>
<th>Specificity (TN/TN+FP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.9%</td>
<td>99.6%</td>
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</table>

**Gram-Negative Identification**

<table>
<thead>
<tr>
<th>Sensitivity (TP/TP+FN)</th>
<th>Specificity (TN/TN+FP)</th>
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<tbody>
<tr>
<td>98.5%</td>
<td>99.8%</td>
</tr>
</tbody>
</table>

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A significant portion of this data set was used to support FDA regulatory submission - FDA Clearance February 23rd 2017

Study comparators: ID = Vitek®2   AST = triplicate broth microdilution

---

(VME) S result, R by comparator. (ME) R result, S by comparator. (mE) I v R or I v S
Do resistance marker tests get results?

ONLY one randomized controlled trial: N= 361 patients

YES 😊
- Reduce treatment of contaminants
- Reduce use of broad spectrum antimicrobials
- Earlier escalation of therapy
- Earlier de-escalation (with stewardship team)

NO 😞
- no impact on management of Gram-negative infections

Banerjee et al CID 2015 61: 1071-80
<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (μg/mL)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;32</td>
<td>R</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>&gt;32</td>
<td>R</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>&gt;8</td>
<td>R</td>
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<tr>
<td>Ceftazidime-avibactam</td>
<td>32</td>
<td>R</td>
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<td>Fluoroquinolones</td>
<td>&gt;2</td>
<td>R</td>
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<td>Colistin</td>
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<tr>
<td>Piperacillin-Tazobactam</td>
<td>&gt;128</td>
<td>R</td>
</tr>
<tr>
<td>Trimethoprim-Sulfa</td>
<td>&gt;4</td>
<td>R</td>
</tr>
</tbody>
</table>

How this could be a problem...

“Oh No!!! It’s a plasmid AmpC + porin mutation by WGS

Carbapenemase-negative *K. pneumoniae* (KPC, NDM, IMP, VIM, Oxas)
What if I had **ALL** the genome information?

- For most bacteria available evidence for using WGS to infer antibiotic susceptibility is poor or non-existent
- Assessing genotypic data vs. clinical breakpoints is a tough challenge
- Published evidence does not currently support use of WGS-inferred susceptibility to guide clinical decision making

Consultation on Report from the EUCAST Subcommittee on the Role of Whole Genome Sequencing (WGS) in Antimicrobial Susceptibility Testing of Bacteria
Next Generation of BSI Diagnostics: Direct from blood

Blood drawn, sent to lab → Cultured on automated system → Flagged as positive → Gram stain performed and called → Subcultured to solid media → ID & AST

Examples:
- Genotype ID: T2 BioSystems
- Genotype ID & R genes: DNAe, Qvella
Impact of Antimicrobial Therapy on Mortality

Mortality (%)

1996 vs 2017

Conclusions

• On the verge of a new era in rapid microbial diagnostics
• MALDI-TOF is now standard in most academic and large community diagnostic microbiology laboratories
• Newer integrated rapid microbial diagnostics with time to ID and genomic and one phenotypic AST < 12 hrs are beginning to be available
• Even more advanced direct from blood ID-AST analysis within 1 hr are on the horizon
• Advent of new diagnostic technologies has the potential to challenge and demand revision of current treatment paradigms
## Genotype ID Direct from Blood: T2

![Flowchart Image]

### Flowchart Details:

- **4mL vacutainer** minutes
- **2mL blood sample** seconds
- **Blood cell lysis & concentration** minutes
- **Lysis of bacteria cells** minutes
- **Amplification 50 uL lysate** ~2 hours
- **Hybridize with particles** minutes
- **T2 Detection** seconds

### Table: CFU/mL, N, %Detected, Mean T2, %CV

<table>
<thead>
<tr>
<th></th>
<th>CFU/mL</th>
<th>N</th>
<th>%Detected</th>
<th>Mean T2</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. baumannii</strong></td>
<td>2</td>
<td>20</td>
<td>95%</td>
<td>737</td>
<td>16%</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>3</td>
<td>20</td>
<td>100%</td>
<td>806</td>
<td>21%</td>
</tr>
<tr>
<td><strong>E. faecium</strong></td>
<td>5</td>
<td>20</td>
<td>100%</td>
<td>322</td>
<td>9%</td>
</tr>
<tr>
<td><strong>K. pneumoniae</strong></td>
<td>2</td>
<td>20</td>
<td>100%</td>
<td>883</td>
<td>12%</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>1</td>
<td>20</td>
<td>95%</td>
<td>1078</td>
<td>13%</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>4</td>
<td>20</td>
<td>100%</td>
<td>531</td>
<td>12%</td>
</tr>
</tbody>
</table>
Genotype Direct from Blood: DNAe
DNAe: how it works

Hydrogen ions are released as a natural by-product of nucleotide incorporation during sequencing or during real-time PCR. These hydrogen ions are then detected by the chip using Ion-Sensitive Field Effect Transistor (ISFET) sensors.
Forthcoming tests: Qvella™ FAST ID BSI Panel

How does it work?
Bacteria isolated and concentrated from whole blood
E-lysis™ of bacteria in tailored electric field Denatures/inactivates proteins
FAST™ amplification of targets & detection on microarray

Performance?
15 species/genus level pathogens
Feasibility study (vs blood culture)
>96% sensitive
>99% specific

*Under development. Not for sale or use.
Genotype Direct from Blood: Biofire

- Childhood systemic infections (CSI) panel (early development)

<table>
<thead>
<tr>
<th>Viral Targets</th>
<th>Bacterial Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>E. coli</td>
</tr>
<tr>
<td>CMV</td>
<td>H. influenzae</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>EBV</td>
<td>N. meningitidis</td>
</tr>
<tr>
<td>HHV-6</td>
<td>S. aureus</td>
</tr>
<tr>
<td>HHV-7</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>Parechovirus</td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td>S. agalactiae</td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td></td>
</tr>
</tbody>
</table>

**Viruses, plasma or whole blood**

**Bacteria for children < 90 days of age, whole blood**
I’ve heard a lot about metagenomics – is that coming for BSI diagnostics?

• Deep sequencing technologies allow detection & characterization of bacteria even at $10^{-8}$ the concentration of human DNA

One study of 9 patients neutropenic fever$^1$

Also: *E. coli* is everywhere

1 Gyarmati et al Nature Scientific Reprots 23532 20166
Next generation dx = next generation lab?

Current laboratory workflow (and clinician workflow!) based on ~12 hours “off” time