The clinical microbiology laboratory at the year 2015
Thought
Prof Nathan Keller
Sheba Medical center

The role of clinical microbiology

• What is a lab test?
  – A lab test is an aid to the clinician in the path to the diagnosis of the patient
**A laboratory test**

1. What is required from the lab?
   1. A fast specimen reception and handling
   2. A prompt and fast turnaround time
   3. A clinical relevancy

2. How should we do it?
   1. An efficient transport
   2. Fast performance
   3. Fast and efficient response

**Antibiotics and the Laboratory**

- If there was no antibiotic resistance, there would be no need for a microbiology lab!
- Infections could be treated “syndromically”

**A laboratory test**

1. Report only relevant results.
2. Report only clear result
3. Interact with the client
<table>
<thead>
<tr>
<th>Deciding on whether to use an antibiotic:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Day 1 Clinical assessment</td>
</tr>
<tr>
<td>• Day 2 Positive microbiology</td>
</tr>
<tr>
<td>• Day 3 Antibiotic sensitivity tests</td>
</tr>
<tr>
<td>• Day 1: guess the disease, guess the bug, guess the sensitivity pattern</td>
</tr>
<tr>
<td>• Day 2: guess the sensitivity pattern</td>
</tr>
<tr>
<td>Day 3: know the full picture: <strong>BUT TOO LATE?</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Why do we test antimicrobial susceptibility?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• To direct &amp; predict antimicrobial chemotherapy.</td>
</tr>
<tr>
<td>• To review &amp; monitor epidemiological trends.</td>
</tr>
<tr>
<td>• To set national &amp; local antibiotic policies.</td>
</tr>
<tr>
<td>• To test the activity of a new antimicrobial agent.</td>
</tr>
<tr>
<td>• To presumptively identify isolates.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>But remember...</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Other factors are very important when we choose an antibiotic</td>
</tr>
<tr>
<td>• Will it get to where the infection is?</td>
</tr>
<tr>
<td>• Bioavailability</td>
</tr>
<tr>
<td>• Cost</td>
</tr>
<tr>
<td>• Toxicity</td>
</tr>
<tr>
<td>• Likelihood of development of resistance</td>
</tr>
<tr>
<td>• Etc...</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>How do we perform antimicrobial susceptibility tests?</th>
</tr>
</thead>
<tbody>
<tr>
<td>• We can use a number of methods including:-</td>
</tr>
<tr>
<td>• Disc susceptibility tests - Kirby-Bauer</td>
</tr>
<tr>
<td>• Agar Breakpoint method.</td>
</tr>
<tr>
<td>• Minimum Inhibitory Concentration (MIC) – Tube MIC or E-tests.</td>
</tr>
<tr>
<td>• Automated methods – Vitek, Phoenix, Microscan</td>
</tr>
<tr>
<td>• Molecular methods – PCR.</td>
</tr>
<tr>
<td>• Spectrophotometry</td>
</tr>
<tr>
<td>• Others</td>
</tr>
</tbody>
</table>
## Parting of the ways 1970 - 90

- **Microbiology & Infection Control**
  - New antibiotics
  - New societies
  - New journals
  - New guidelines
  - New diseases
- **Infection control** was the province of the IC specialists
- **Modern medicine**
  - Increased life expectancy
  - Cancer treatment
    - Immunosuppression
  - Complex surgery
    - Cardiac
    - Neurosurgery
    - Orthopaedic
  - Chronic illnesses
- **Infection** – a nuisance

## 1990s – the backroom days

<table>
<thead>
<tr>
<th>Increasing</th>
<th>Decreasing</th>
</tr>
</thead>
<tbody>
<tr>
<td>- HCAI</td>
<td>- Training numbers</td>
</tr>
<tr>
<td>- MRSA</td>
<td>- Academic input</td>
</tr>
<tr>
<td>- <em>Clostridium difficile</em></td>
<td>- Reduced medical student impact</td>
</tr>
<tr>
<td>- <em>Acinetobacter</em></td>
<td>- Effect of RAE</td>
</tr>
<tr>
<td>- <em>Norovirus</em></td>
<td>- Dislocation of academic/service interface</td>
</tr>
<tr>
<td>- Antimicrobial resistance</td>
<td>- Training in asepsis</td>
</tr>
<tr>
<td>- ESBL</td>
<td>- Supply of microbiologists!!</td>
</tr>
<tr>
<td>- Pandemic threats</td>
<td></td>
</tr>
<tr>
<td>- <em>Need for microbiology!!</em></td>
<td></td>
</tr>
</tbody>
</table>

## MICROBIOLOGY TESTING IN NOSOCOMIAL INFECTION (10)

**Resistance Patterns Testing**

- extended-spectrum β-lactamases (ESBL)
- stably derepressed Bush-Jacoby-Medeiros group 1 cephalosporinases among *Enterobacteriaceae*
- glycopeptide resistance among enterococci and staphylococci
- penicillin resistance among *S. pneumoniae* and viridans-group streptococci

### BUT........

**....we did not help ourselves**
Perceived dogmas

• Laboratory focused
  – What about patient focus?
• Individual clinical pathology disciplines
• A lab in every main hospital/trust
• Transport too difficult – delays
• Lab headed by medical microbiologist
  – Medical microbiologists based in labs
  – Unwilling to have cover from a distance
• Daytime (8.30 – 17.30) service
  – On-call out of hours

Results

• Traditional methods
• Lack of investment in
  – new technology, IT
• Lack of specialisation
• Isolated services

Where do we go from here?

Clinical and Laboratory Model
Technology developments

- Automation
  - Conventional tests
  - Molecular, post-genomic
- Which technologies
  - Next generation sequencing
  - Micro-arrays
  - MALDI-TOF
  - ??????

...we need a strategy for their use
Previ Isola

- 180 Plates / Hour
- Agar side up
- Full & Biplates
- No Cross contamination
- Traceability

SIS - Smart Incubator System

SMART INCUBATION SYSTEM

High imaging quality
**New BacT/Alert**

- Load & Go
- Load & Go
- Load & Go
- MYLA

**Hospital System**
- Electronic Requesting Support
- Image capture & retrieval
- Lab to Lab communication
- Electronic Requesting Support

**Infection Control**
- Epidemiological Reporting
- Result Publication Support
- Electronic Requesting Support
- Statistical Reporting

**Microscopy Expert System**
- Blood Tracking
- SMS capability
- Lab to Lab communication

**Primary Care System**
- Voice Technology
- Electronic Requesting Support
- E-mail capability
So where are we?

MICROBIOLOGY TESTING IN NOSOCOMIAL INFECTION (9)

Antimicrobial Susceptibility Testing
- automated commercial systems
  - short (3- to 5-hour) incubation periods
  - significant AST errors
  - (ESBL-producing Enterobacteriaceae, MRS, VRE, VRSA, false resistance)
- Supplement with additional methods
- keep up with current literature regarding the ability of automated systems

MICROBIOLOGY TESTING IN NOSOCOMIAL INFECTION (5)

Antimicrobial Susceptibility Testing
- macro- and microbroth dilution method

MICROBIOLOGY TESTING IN NOSOCOMIAL INFECTION (6)

Antimicrobial Susceptibility Testing
- agar dilution method
Antimicrobial Susceptibility Testing

- **disk diffusion method**

POC testing

- Healthcare settings
  - ICU
  - MRSA screening in A&E etc
  - *need to be integrated with laboratory service*

- “High Street” settings
  - Increase accessibility for patients/clients
  - Professional oversight? Interpretation? Advice?
  - Links to patient record?
  - Risks loss of surveillance data for public health
  - *Microbiologists need to be involved in pilot projects*

POC Evolution...

- Early 1990’s
  - Data Collection

- Mid-Late 1990’s
  - Data Management

- Early 2000’s and Beyond
  - Information Management

- Open IT
- Wireless
- Web
- EMR

- New Entrants
- Partnerships

- POC Testing
- Nursing Influence

- Regulatory Focus
- Dawn of Connectivity

- Reimbursement
- Patient Care
Challenges for the future

• Keep infection as a patient safety priority in healthcare
• It will never go away!
• Use modern technology to deliver a patient-focused service
• Do not repeat the mistakes of 1970 - 2000

FUTURE POSSIBILITIES

• Various models – same aims
  – Consolidation, centralisation
  – Enable technology development
  • Automation - conventional tests; new technology
  • Molecular, post-genomic – sequencing, micro-array, MALDI-TOF
  – Concentrate staff expertise
  – Extended (24/7) working
  – Cost effectiveness

...........maintain the quality of patient care and public health support

How to evaluate

• QA
• QC
• Validation with clinical relevancy

"Insanity is doing the same thing over and over again and expecting a different result”
  Albert Einstein
A small example from Sheba

**Method: rRNA Gene PCR**

- Extraction of DNA
- Amplifying the rRNA genes (using proprietary primers) by PCR
- Sequencing the PCR products
- Identification by comparing the sequence to GenBank database (public/in-house).

**Sample**

- Diagnostic specimens (biopsies and fluids) for detection of pathogen
- Isolated colony (agar or broth) for verifying of the identification at the species level

Applicable on a single or dominant strain only.
**Advantages**

- Identification up to species level
- No need of culturing
- No need of prolonged incubation
- No need of alive bacteria
- **PAN-PCR** (bacterial and fungal ID)
- Rare or unexpected pathogens can be found
- Turn around time of two working days

In patients receiving empiric antimicrobial treatment, molecular methods might be superior to bacterial culture.

**How does it work in the Sheba Medical Center?**

- The sample received by the laboratory.

- A test portion is removed from the sample for the shipping to molecular lab.

- Shipping.

- Checking of results via the internet / phone

**Summary of the samples:**

20.12.10 – 27.1.10

21 samples

- **11** Diagnostic specimens
- **10** Isolated microorganisms

**Validation of species identification**
מקרה ראשוני

גב רב 73, ברקע:

- פרפור פרוזדורים התחפפו
- לאחור אבליצית של נחיתת קוצב ב- 1999 AVN

מחלקת לט מסתמיות: לאחור תינוק ובמה של הצלה

וניקוסידילית קשה, ירי לוח דמ ריאתי.

-ishpa 1, 8/2009:

- חום, חלשה,ftar בד חוריות צמיחה של אנטיווקוק
- תורбин ממאטרים אק saldır שלילית, ייווש מוקר- שילית
- TEEX2 במלה + קונצ- ללא עדות לוגיציות
- באבחנה עבורה של דובים אנד-אסכולרים
- בקטארמייה ראשוית של חידק אופיני בבחינת מסתמי
- תותח קוצב ב. ל
- לא יזא הוכזב, בשילם 6 שבועות טיפול אנטיביוט.

-ishpa 2, 12/2009:

השנה אחר נפילת ובצלת ראשים 12 לא מוצא חירום

למעט המטמה הת עליר
- לוזאר היטטורייה הרפואית, לקחה תורבין דם...
- תומט תרבות לד שולחה במשר שלשה יימי ריפוי
- דוב
- מוחית של...

מגניב גרמנ חיווימ

מעבדה בקטריוולוגיה "שבראה"

Gram stain
**Culture:**

- **Erysipelothrix**
  - Esculin variable
  - H₂S positive
  - Can be identified by Phoenix
  - Can not be identified by API coryne

- **Lactobacillus**
  - Esculin negative
  - H₂S negative
  - Can not be identified by Phoenix
  - Can be identified by API coryne

**Lactobacillus sp.**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≤8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤0.5</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤4</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤4</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Infection

- 180 cases of lactobacillemia during the last 30 years
- 69 cases of IE
- 0.05%–0.4% of cases of infective endocarditis and bacteremia


Molecular Biology

Lactobacillus sp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nocardiacyriacigeorgica 99%</td>
<td>Nocardia sp &amp; AST*</td>
</tr>
<tr>
<td>Nocardiafarcinica 99%</td>
<td>Nocardia sp &amp; AST</td>
</tr>
<tr>
<td>Lactobacillusrhamnosus 99%</td>
<td>Lactobacillus sp &amp; AST</td>
</tr>
<tr>
<td>Legionella pneumophila 99%</td>
<td>Most probably Legionella sp.</td>
</tr>
<tr>
<td>Legionella pneumophila 99%</td>
<td>Most probably Legionella sp.</td>
</tr>
<tr>
<td>Ralstonia sp 99% / B. pickettii 97%</td>
<td>B. cepacia / R. pickettii &amp; AST</td>
</tr>
<tr>
<td>S. oralis 99%</td>
<td>S. oralis / S. pneumoniae &amp; AST</td>
</tr>
<tr>
<td>Staph. epidermidis / caprea 99%</td>
<td>Staph. capitis / ureolyticus / epidermidis / chromogenes &amp; AST</td>
</tr>
<tr>
<td>Propionibacterium acnes 99%</td>
<td>Propionibacterium acnes &amp; AST</td>
</tr>
<tr>
<td>Abiotrophia defectiva 99%</td>
<td>Abiotrophia sp.</td>
</tr>
</tbody>
</table>

*AST - Antimicrobial Susceptibility Testing
סיכום המקרה הרשון

• תמיכה בשינויים סיטוריים
• תמיכה בדרכי החלפת סביבת העבודה
• תמיכה במגש החכם
• תמיכה בחברון,جزيرة אורה
• תמיכה בפעילות חברתית
• תמיכה בפעילות נוער

האגכמה הגלילית שונלולה
למעבדה המклילית לאימונים

フォト

מקרה שני

• בן 29, מעצב גורף
• ברקע: אוסטריה, פסואידוס
• מ.ג. במחנה טיל בברצלונה מגדיר一经 העני
• גבר מתון מימין של נחל האירוד

נשף י. בית: הנשרyny מטיעים את תҀר עובר. 애 المباراة 1.2 km.
Differential Diagnosis

- Lymphocutaneous sporotrichosis
- Nocardiosis caused by *N. brasiiliensis*
- Cutaneous leishmaniasis
- Mycobacterial infection caused by
  - *M. tuberculosis* (*tuberculosis cutis verrucosa*)
  - *M. marinum*
  - *M. chelonae*
  - *M. kansasii*
  - *M. fortuitum*
  - *M. leprae*

Papulonodular lesions with or without central ulceration and with one or more nodules in the proximal skin along paths of presumed lymphatic spread
After 10 days:
- Cellulomonas testoteronii
  (environmental bacteria)

Day 1: AF stain (+)

6: Genotyping Day
- M. marinum
<table>
<thead>
<tr>
<th>Organism/Malolactohytes</th>
<th>Definitive Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achromobacter xylosoxidans</em> 96%</td>
<td>(F-, A-)</td>
</tr>
<tr>
<td><em>Aureobasidium sp./ A. pullulans</em> 91%</td>
<td>(F-, A-)</td>
</tr>
<tr>
<td>Bacteria and Fungi not detected</td>
<td><em>P. acnes</em></td>
</tr>
<tr>
<td>Bacteria not detected</td>
<td><em>A. acnes</em></td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em> 90% (double seq.)</td>
<td>(F-, A-, M-, N-)</td>
</tr>
<tr>
<td>Bacteria was detected but could not be ID’d due to low amounts obtained by PCR</td>
<td>MTD+</td>
</tr>
<tr>
<td><em>M. marinum</em></td>
<td></td>
</tr>
<tr>
<td>Bacteria and Fungi not detected</td>
<td><em>M. restricta</em> (non-specific contamination)</td>
</tr>
<tr>
<td>Bacteria and Fungi not detected</td>
<td><em>P. aeruginosa</em> 99%</td>
</tr>
<tr>
<td>Bacteria and Fungi not detected</td>
<td><em>M. acnes</em></td>
</tr>
<tr>
<td>Bacteria and Fungi not detected</td>
<td><em>M. acnes</em></td>
</tr>
<tr>
<td><em>Cellulomonas testoteronii</em></td>
<td></td>
</tr>
</tbody>
</table>

Cultures: A - aerobic, F – Fungi, N – Anaerobic, M - mycobacterial

---

**Conclusion:**

- 16S rRNA PCR and sequencing is an excellent method for definitively diagnosing rare microorganisms grown in the laboratory.

- All the biological specimens were sent after manipulation in the microbiology laboratory which may account for the relatively high incidence of environmental organisms being detected.

- In addition, taking into account the low number of specimens sent we are unable to give reliable comment on the poor results received using this technique.

---

**Thanks**

- The audience for listening to my thoughts
- To my fellow in Microbiology and infectious diseases that created the data

- A comparison to culture results revealed difficulties in detecting gram-positive species and mycobacteria by PCR (breaking the cell walls of these organisms during sample preparation)

- The risk of contamination is still higher for broad-range bacterial PCR than for specific assays, since traces of environmental bacterial fragments from reagents, vessels, etc., will be amplified.
Sequence identification is only as good as the sequence database

- *Database search is part of the assay*
- A perfect reference database contains:
  - All clinically relevant organisms
  - Only sequences of high quality
  - Only sequences from well-characterized strains
  - Entries that are fully compliant with current nomenclature