Combating Emerging and Re-Emerging Infectious Diseases in Clinical Trials by Harnessing Innovative Technologies

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“If we are not careful, we will soon be in a post antibiotic era.....and for some patients and some microbes, we are already there!”

Tom Frieden (Director CDC)
Introduction: The Rise of the Super-Bug

- A superbug is a term used in the lay-media to describe a strain of bacteria that is resistant to most prescribed antibiotics
  - MRSA & VRSA
  - Drug-resistant tuberculosis (MDR- and XDR-TB).
  - Drug-resistant Enterococcus.
  - Drug-resistant Streptococcus pneumoniae
  - Salmonella kentucky
  - Resistant gonorrhoea
  - Malaria
Emerging Disease

CDC Definition:

- Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include
  - New infections resulting from changes or evolution of existing organisms
  - Known infections spreading to new geographic areas or populations
  - Previously unrecognized infections appearing in areas undergoing ecologic transformation
  - Old infections re-emerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures
Emerging and Other Communicable Diseases

Antimicrobial resistance

• Resistant pathogens are emerging and spreading more rapidly than in previous decades.

• Resistance is a world problem, affecting developed and developing countries, and rapidly spreading through international travel (see next slide)

• Treatment of infections caused by resistant microbes is increasingly hampered either by the prohibitive cost of existing “new generation” agents or by a total lack of effective antimicrobial agents;

• Resistance should be viewed in the larger public health context.
Spread of Antibiotic-Resistance Bacteria (ARB)

Europe
- EU: ARB costs society ~ €1.5 bn/yr & 600 million days of lost productivity.\(^{59}\)
- Russia: ARB a major concern\(^{50}\) with 83.6% of families imprudently use antibiotics at home.\(^{61}\)

Asia
- Thailand: >140,000 ARB infections/yr and >30,000/yr patients die; 2 bn in productivity losses/yr.\(^{49}\)
- Japan: Extensive levels of ARB found in Tokyo’s urban watershed.\(^{50}\)
- China: Extreme over-prescription of antibiotics\(^{51}\) and rapid growth rate of ARB.\(^{52}\)
- India: Within 4 years (02-06) ARB went from being resistant to 7, to 21 drugs.\(^{53}\)
- Vietnam: Farming practices contributing to spread of ARB through environmental contamination.\(^{54}\)
- Pakistan: 71% of infections in newborns are from ARB.\(^{55}\)

North America
- USA: ARB causes majority of 99,000 deaths/yr from infections acquired in hospitals.\(^{56}\)
- USA: Health care costs of ARB are US$21-34 bn/yr.\(^{56}\)

Middle East & North Africa
- Egypt: 38% of blood infections contracted by young cancer patients are from ARB.\(^{55}\)
- Israel: ARB found fatal in ~ 50% cases when resistant to our strongest antibiotics.\(^{63}\)

South America
- Peru, Bolivia: >51% of hospital infections caused by ARB.\(^{57}\)
- Brazil: Rates of ARB are up >60%.\(^{56}\)

Sub-Saharan Africa
- Tanzania: Death rate of ARB infected children are double that of malaria.\(^{55}\)
- Nigeria: Rapid spread of ARB that came to Africa from Asia.\(^{62}\)

Antarctica
- ARB found in Antarctic animals & water samples.\(^{64}\)
Environmental Challenges
Misuse of Antibiotics

• In many developing countries, antibiotics are readily available from hospitals, pharmacies, patent medicine stalls, roadside stalls and hawkers.

• Common issues with OTC drugs that contribute to ARB:
  – Substandard quality
  – Sub-therapeutic doses
  – Improper self-medication

• Internet/mail-order pharmacies present a new, easy way to obtain OTC antibiotics
  – Few regulations monitor internet drug sales
  – Difficult to impose restrictions
Hope for the Future

• New Anti-Infectives:
  – Avian Antibodies (IgY)
  – New antibiotics from different sources
  – Ethnopharmacology

• Whole Genome Sequencing and Next Generation Sequencing of bacteria
  – DNA and RNA Analysis:
  – Structural Analysis:
    • Efflux Pump Blockers
  – Auxiliary Targets:
    • Inhibit waxy coat protein of *M. tuberculosis*
    • Incorrect RNA helicase proteins
  – Virulence Factors:
    • Eliminate Lipid A component of Gram-negative organisms
The ‘Omics’ Revolution

- Genomics
- Proteomics
- Pharmacogenomics
- Functional Genomics
- Current Applications
  - Pathogen detection
  - Genotyping
  - Virulence markers
  - Antibiotic resistance
  - Identification of Tumor-specific biomarkers for treatment options
  - Outbreak investigation
  - Antibiotic development
NIAID Emerging Disease List 2015

• **Cat A**: Priority Pathogens
  - *Bacillus anthracis* (anthrax)
  - *Clostridium botulinum* toxin (botulism)
  - *Yersinia pestis* (plague)
  - Variola major (smallpox) and other related pox viruses
  - *Francisella tularensis* (tularemia)
  - Viral haemorrhagic fevers
    – Flaviviruses: Dengue
    – Filoviruses: Ebola

• **Cat B**: Priority Pathogens
  - Ricin toxin (*Ricinus communis*)
  - Typhus fever (*Rickettsia prowazekii*)
  - Food- and waterborne pathogens
  - Mosquito-borne encephalitis viruses

• **Cat C**: Priority Pathogens
  - Tuberculosis
  - SARS
  - Antimicrobial resistance: STI’s

*Source: [https://www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/Pages/CatA.aspx](https://www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/Pages/CatA.aspx)*
**Q² Solutions Capabilities**

- **Extensive Laboratory Network**
  - Europe (UK), North America (USA), South America (Brazil, Argentina), Asia-Pac (Singapore), China and South Africa

- **Microbiology**
  - Isolation and Identification using manual or automated methods
  - Susceptibility testing (disc diffusion, E-test or broth dilution)

- **Mycobacteriology**
  - Isolation and Identification using manual or automated methods
  - Genotyping and drug resistance

- **Serology and Immunology**

- **NAAT**
  - PCR and RT-PCR

- **DNA and RNA extraction**
  - Genomic studies
CASE STUDY – COMMUNITY ACQUIRED MRSA IN PAKISTAN*

*Source: Mortlock S. ‘Suspected Community Acquired MRSA at a Cancer Hospital in Pakistan.’ Scholars Journal of Applied Medical Sciences (2014); 2: 3293-3296
Case-Study MRSA
Increase of Community acquired MRSA

- Increasing worldwide
- Young, otherwise healthy patients
- No recent hospitalisations
- Predominantly skin and soft tissue infections
- Risk factors
  - People who are frequently in crowded places
  - People with weak immune systems
  - People staying or working in a health care facility for an extended period of time
  - Food handlers, veterinarians and pet owners
  - Prior antibiotic therapy

Source: Mortlock S. ‘Suspected Community Acquired MRSA at a Cancer Hospital in Pakistan.’ Scholars Journal of Applied Medical Sciences (2014); 2: 3293-3296
**Case-Study MRSA**

*Increase of Community acquired -MRSA*

<table>
<thead>
<tr>
<th></th>
<th>MSSA</th>
<th></th>
<th>MRSA</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>120</td>
<td>74.1</td>
<td>42</td>
<td>25.1</td>
<td>162</td>
</tr>
<tr>
<td>Community</td>
<td>113</td>
<td>89.7</td>
<td>13*</td>
<td>10.3</td>
<td>126</td>
</tr>
<tr>
<td>Total</td>
<td>233</td>
<td>80.9</td>
<td>55</td>
<td>19.1</td>
<td>288</td>
</tr>
</tbody>
</table>

The Fisher exact test statistic value is 0.000827. The result is significant at p < 0.05.

* 6 (46%) had evidence of non-prescribed antibiotic therapy

**Source:** Mortlock S. ‘Suspected Community Acquired MRSA at a Cancer Hospital in Pakistan.’ *Scholars Journal of Applied Medical Sciences* (2014); 2: 3293-3296
## Case-Study MRSA:

**PFGE Results of Isolates**

<table>
<thead>
<tr>
<th></th>
<th>USA100</th>
<th>USA200</th>
<th>USA300</th>
<th>USA400</th>
<th>USA600</th>
<th>USA800</th>
<th>Unique</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-MRSA</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td>21</td>
<td>3</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>3</td>
<td>21</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

The Chi-square test statistic value is 13.489.

p-value = 0.03589541

Yates Correction: p-value = 0.13146538

The result is not significant at p < 0.05.

*Source:* Mortlock S. ‘Suspected Community Acquired MRSA at a Cancer Hospital in Pakistan.’ *Scholars Journal of Applied Medical Sciences* (2014); 2: 3293-3296
Summary

• Emerging epidemic with Community acquired MRSA increasing worldwide
  – USA300 is still the major source of community-acquired infections
  – Always carries the Panton-Valentine leukocidin gene

• Improved Hygiene
  – Wash hands often, and clean body regularly.
  – Keep cuts, scrapes, and wounds clean and covered until healed.
  – Avoid sharing personal items such as towels and razors.

• Education
  – Correct use of antibiotics

• Screening at clinics
  – Increase recognition of MRSA infection through prospective surveillance, and reporting procedures, with coordination of referral services.
CASE STUDY – FOCUS ON TUBERCULOSIS STUDIES IN THE SOUTH AFRICAN LAB
Challenges for TB Studies from a Lab Perspective

• Ensuring the standardization and quality control of highly technical and time-consuming assays

• Navigating the constant challenges associated with
  – Trial logistics
  – Data management
  – Proposal development
  – Study set up

• State-of-the-art **Biosafety requirements.**

• Contending with the rising prevalence of **multidrug-resistant strains** (requiring more advanced assays with shorter TAT) and strategies involving **TB-HIV co-infection.**
The $Q^2$ Solutions Focus in TB Testing

• All TB related testing in a single facility

• State of the art Biosafety measure with absolute redundancy

• Extensive validation and quality control of Research based client specific assays. Ex – Quantitative CFU TB culture (Plate based)

Quantitative CFU in 2 différent Media
When a Local Lab is Required
Complicates Clinical Trial Management and Sponsor Oversight

Clinical Trial Laboratory Services

Trial Requires Same Day Lab Results, or Uses Remote Sites

yes

Local Lab
Local Lab
Local Lab
Local Lab
Local Lab
Local Lab
Local Lab
Local Lab

no

Central Lab

Sponsor Manages Data

Sponsor Oversight

Central Lab Manages Data
Local Lab Data Management
Significant burden on CRAs, Sites and Sponsors
Challenges Amplified by Trial Size
Sponsor burden, cost grows with trial complexity, size

Sponsors and CROs spend disproportionately on local lab data – contracting entire teams

Cost multiplies as complexity grows:
- x Number of regions
- x Number of local labs
- x Number of patients
- x Number of analytes
## Local Labs Issues

*Multiple vendors, additional site burden, consistency issues*

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Situation</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vendor Management</strong></td>
<td>› Each investigator site uses a different lab (and possibly more than one)› Large global studies may use several hundred local labs› Reference ranges and units are gathered separately from results</td>
<td>› Requires additional staff to oversee 100s of labs in addition to sites› Translation of local lab results and reference ranges is difficult› Testing methodologies are inconsistent globally</td>
</tr>
<tr>
<td><strong>Site Personnel and CRA Time</strong></td>
<td>› Site personnel must enter lab results into the database or CRF› CRAs required to follow up on ranges &amp; unit updates</td>
<td>› Increased burden on sites and CRAs.</td>
</tr>
<tr>
<td><strong>Data Consistency</strong></td>
<td>› Site personnel may not have lab/scientific knowledge; not trained to catch errors</td>
<td>› Results inaccurate› Additional queries, delays in data lock</td>
</tr>
</tbody>
</table>
Q² Solutions Local Lab Data Solution
Simplifies transmission for site, reduces variances and hassles
## Simplifying Complexities

**Trained lab professionals, specialized systems and technology**

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Solution</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vendor Management</strong></td>
<td>- Quintiles works with sites and local labs so you don’t have to</td>
<td>- Reduced Sponsor Oversight Costs</td>
</tr>
<tr>
<td></td>
<td>- Our direct entry Lab Results Portal streamlines reporting to sites and transmission to CRF/database</td>
<td></td>
</tr>
<tr>
<td><strong>Site Personnel and CRA Time</strong></td>
<td>- Easy process for sites</td>
<td>- CRAs focus on site compliance and accreditation</td>
</tr>
<tr>
<td></td>
<td>- Online Infosario portal and other simple options for delivering lab reports to Quintiles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Everything is provided to make the job easy for them</td>
<td></td>
</tr>
<tr>
<td><strong>Data Consistency</strong></td>
<td>- Smart data entry</td>
<td>- Lab trained personnel deal with units and ranges</td>
</tr>
<tr>
<td></td>
<td>- Experienced team of dedicated staff with direct supervision by Medical Technologists</td>
<td>- Data ready for use</td>
</tr>
<tr>
<td></td>
<td>- Edit checks developed specifically for local lab data from years of experience</td>
<td>- Translated, reviewed, confirmed, and checked</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Online within a day of entry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Ready for data transfers using well-established systems and processes</td>
</tr>
</tbody>
</table>
Case Study: Tools to Assess Immunogenicity in the Human Ebola Vaccine Trials
Epicenter of the Ebola Outbreak
March 2014 to October 2015

Source: WHO, Ebola Response Roadmap
21 October 2015
### Cases Per CDC, October 27, 2015

<table>
<thead>
<tr>
<th>Countries</th>
<th>Total Cases (suspected, confirmed)</th>
<th>Laboratory Confirmed</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea</td>
<td>3804</td>
<td>3351</td>
<td>2536</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>14122</td>
<td>8704</td>
<td>3955</td>
</tr>
<tr>
<td>Liberia</td>
<td>10675</td>
<td>3160</td>
<td>4809</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>28,601</strong></td>
<td><strong>15,215</strong></td>
<td><strong>11,300</strong></td>
</tr>
</tbody>
</table>

## Cases Outside the Epicenter

*Per CDC, October 27, 2015*

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Cases (Suspected, Probable, and Confirmed)</th>
<th>Laboratory-Confirmed Cases</th>
<th>Total Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigeria</td>
<td>20</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Senegal</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>United States</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Mali</td>
<td>8</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Italy</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36</strong></td>
<td><strong>34</strong></td>
<td><strong>15</strong></td>
</tr>
</tbody>
</table>
Vaccines move to the forefront for Ebola prevention

• 15 VACCINE CANDIDATES IN THE FIELD
  ▪ North America
  ▪ Europe
  ▪ Russia
  ▪ China

• Funded by
  ▪ US Government, in particular the US Army (BARTA, JVAP, USAMRIID) and NIH
  ▪ EU
  ▪ Denmark
  ▪ Gates Foundation
  ▪ WHO

• Most advanced vaccines to the clinic
  ▪ Merck/New Link Genetics
  ▪ Crucell - J&J
  ▪ GSK
  ▪ Novavax
Ebola Vaccine Clinical Trials in the Field

Primary Studies in the Endemic Zone

- Prevail Study: NIH/Liberia
- Ring Study: Guinea
- Strive Study: Sierre Leone

Studies Outside of the Epicenter

- >8 African sites
- Multiple USA sites (phase 1 and 2)
- Multiple European sites (phase 1 and 2)
Current Challenge: Vaccine efficacy no longer a measurable endpoint

- Vaccine efficacy was the primary endpoint for all trials in the endemic zone
- Immunogenicity of each vaccine candidate also included in all trials
- However with the outbreak subsided – the primary endpoint of efficacy no longer possible
- Left with the Animal Rule to get vaccine licensure
  - Demonstrate correlate of immunity in NHP via the immunogenicity data and viral challenge
  - Then correlate human and NHP immunogenicity
- The immunogenicity data and establishment of correlates of immunity now are primary endpoints for vaccine licensure
**Immunogenicity Tools**

- Immunoassays
  - Assess immunoglobulin titers to GP

- Viral neutralization assays
  - Assess antibody function
    - Ebola/Filoviruses are BSL-4 agents = limited, low throughput testing capacity for large volume phase 3 studies
    - Alternative, non-WT virus technology required

- Standardization of assay platforms required
  - “WHO Collaborative Study to Assess the Suitability of an Interim Standard for Antibodies to Ebola Virus” – by the WHO Expert Committee on Biological Standardization
    - Q1 2015: convalescent, post-vaccine, and TG bovine sera used
    - 17 labs participated
    - All data submitted in Q2 2015
    - Report published 16 October 2015 and interim standard (convalescent sera) available at NIBSC
### WHO Collaborative Study

#### Summary of Tools

**Immunoassays:**

1. IFA – Fixed viral infected cells
2. EIA
   - i. Indirect
   - ii. IgG/IgM capture
   - iii. Sandwich
   - iv. “Direct”
   - v. One commercial kit (ADI)
   - vi. Competitive
3. Antigen sources
   - i. GP ‘particles’
   - ii. Inactivated virus
   - iii. VSV-GP virus ‘particles’
   - iv. rZEOBV GP

**Virus Neutralization Assays:**

1. Pseudovirion lentiviral particle- EBOV GP
2. VSV pseudovirus – EBOV GP
3. BSL-4 PRNT
4. BSL-4 FRNA

**Readout formats:**

1. RLU
2. Fluorescence
3. PFU
4. FFU

**Zaire strains used:** Kikwit, Makona, Mayinga
Summary: Ebola Vaccine Studies

• The Animal Rule will be the probable path forward for Ebola vaccine licensure

• Raises the stringency of immunogenicity assessment for both human and NHP clinical studies

• Immunogenicity assessment for Ebola vaccines will include both Immunoassay (ELISA) and functional (viral neutralization) antibody assessment

• National (USAMRIID/JVAP) and International (WHO/NIBSC) efforts ongoing to standardize assays used for the multiple vaccine candidates.

• The Fang ELISA is a leading candidate for a standardized ELISA

• Viral neutralization has several non-WT options available
Genomics Case Study
Case Study: Respiratory Syncytial Virus

- Causes acute infection in young infants, elderly, and immune-compromised patients.

- Current therapies ineffective either due to low efficacy or drug delivery challenges (especially in regards to infants)

- Several inhibitors tested in cell culture, RNA-Seq performed
  - Assess viral transcript levels → provides information on where in the viral life cycle the drug acts
  - Assess cellular pathway changes, especially the interferon response.

Summary

• Emerging diseases and antibiotic resistance place a considerable burden on health care.

• Experts predict that it is not ‘if’ but ‘when’ the next pandemic occurs.

• Long term strategies need to be rationalised.
  – Strengthen Infectious Disease surveillance and response programmes
  – Integrate new technologies to create quantitative, predictive models of infectious disease.
  – Develop new anti-infective agents.
  – Education
And Finally

‘C’est les microbes qui auront le dernier mot.’

Louis Pasteur (1822-1895)

Translation: “The microbes will have the last word”
Thank You

Q&A