Emerging Pathogens
How Do We Detect and Track Them?

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Disclosures

I have received research supplies from TechLab, Remel, Meridian, ChromAgar and Cepheid.

My wife is independent contractor for a number of pharmaceutical companies

Chance favors the prepared mind.

Pasteur

What students need to learn from this lecture

1. Infectious agents are either very well adapted to the human host (think *Streptococcus pneumoniae*) or are opportunist (environmental mycobacterium)
2. Various factors drive the emergence of pathogens
3. The specific “process” in detecting and classifying an emerging pathogen
4. Molecular methods “fingerprint” organisms and used to track them globally

Emerging Infectious Diseases in the Past 30 Years

- novel H1N1 and H3N2 influenza A
- *Clostridium difficile**#
- HIV*#
- SARS*
- *Cryptosporidium**
- Enterohaemorrhagic *E. coli* *#
- Nipah virus
- nv Creutzfeldt-Jakob disease
- Sin Nombre Virus
- West Nile Virus
- *Vibrio vulnificus**
- *Cyclospora*
- *Bacillus anthracis* #(BT agent)
- CA-ORSA*#
- TSST-1 *S. aureus**#
- MDR-, XDR- and TDR-TB*
- MDR- pneumococcus*#
- MDR-Acinetobacter*
- Rapidly growing mycobacterium*#
- *Campylobacter**#
- Rotavirus*
- Norovirus*
- BK virus*
- *Chlamydophila pneumoniae*
- *Penicillium marneffei*
- *Legionella**
- *Burkholderia cepacia* complex*#
- *Burkholderia gladioli**#
- VRE*#/VRSA
- *Helicobacter pylori**
- HHV-6*
- HPV*
- HCV*
- Avian influenza (H5N1)
- *Ehrlichia chaffenesis**
- *Borrelia burgdorferi** (Lyme disease)
- Enterotoxigenic *E. coli*#
- Enteroadherent *E. coli**
- *Bordetella avium*
- Microsporidium*

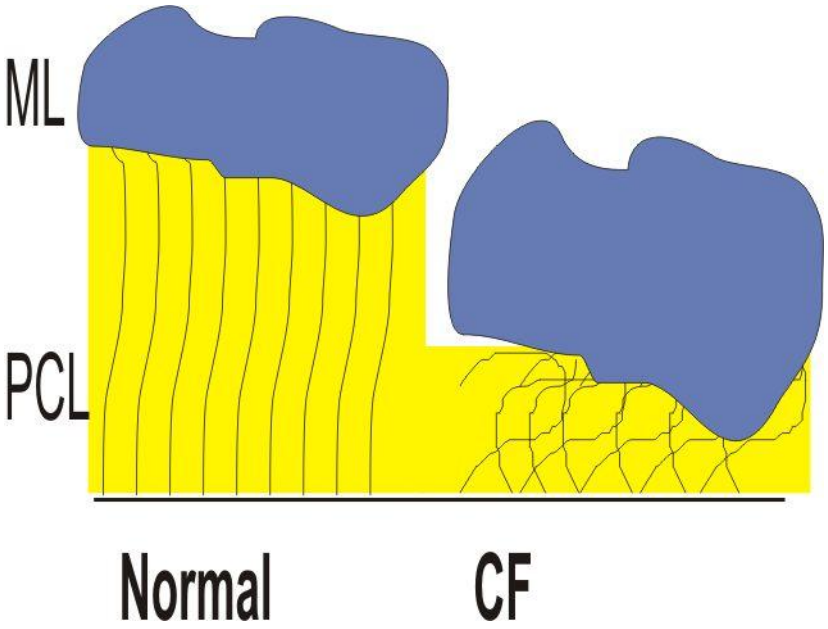
How do new pathogens emerge

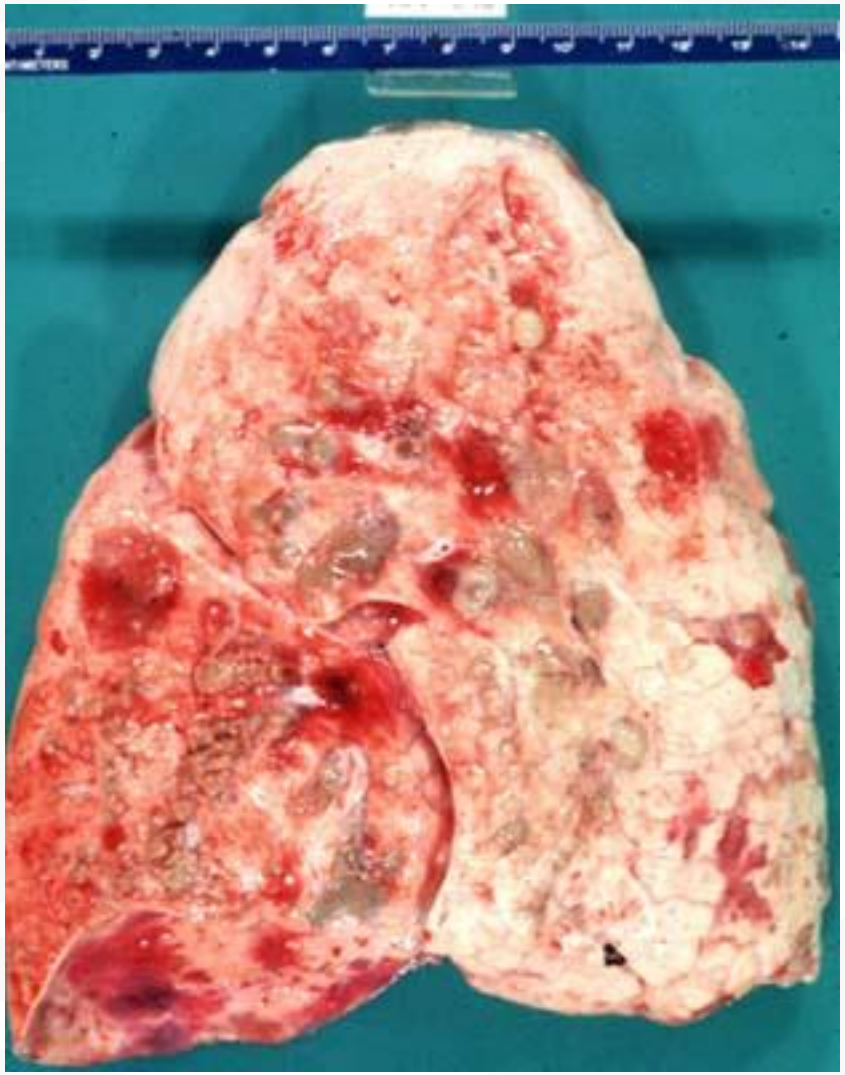
- Changing ecosystems
- Changes in food production techniques
- Evolution of medical devices and care
  - Long term survival of immunosuppressed
- Pathogens that are detected because of new technology
- Misuse of micro-organisms
  - Biocrime/bioterrorism
- Organism evolution as a result of human intervention
  - Antibiotic pressure
- Organisms that jump species barriers

General overview of cystic fibrosis

- Most common autosomal recessive genetic disease in Caucasian populations but can be seen in other racial groups as well
  - In US median life expectancy is approximately 37 years
- Seen primarily in North America, Northern Europe, and Australia/New Zealand but foci also seen in Brazil and Argentina
- Mutation in CFTR results in abnormal electrolyte transport causing thick, dry, sticky mucus
- This abnormal mucus adversely effects mucociliary clearance providing an ideal niche for chronic lung infection
- Over 85% of premature deaths in CF are due to cardiopulmonary failure 2° to chronic lung infection

CF Pathology





Case

- patient is an 18 y.o male with CF
- chronically infected with mucoid *Pseudomonas aeruginosa (Pa)*
- excellent physical condition-cross country runner
- presents with fever to 102 F; WBC of 22,000
- sputum culture grows mucoid *Pa*

Case

- Treated with carbenicillin and gentamicin to which *Pa* suceptible
- Has rapidly declining lung function with increasing pulmonary infiltrates, persistent fevers and elevated white count
- dies of pulmonary arrest 10 days post admission
- Autopsy is performed with frank pus present in airways
- heart blood grows *Burkholderia (Pseudomonas) cepacia* as does a lung aspirate

Burkholderia cepacia

- What we knew in 1982
  - **Called *Pseudomonas cepacia***
  - **Environmental organism- cause of onion rot**
  - **Problem in the cosmetic industry**
  - **Occasional contaminant of disinfectants causing iatrogenic infections**
  - **Very versatile metabolically could use penicillin as sole carbon source**
  - **Highly drug resistant**

Mortality associated with *B. cepacia*

- Reviewed 45 autopsy lung cultures
  - 11/45(24%) grew *B. cepacia*
  - 3/45 (7%) grew *B. cepacia* and other organisms
  - 5/45 (11%) grew *B. cepacia* and *P. aeruginosa*
  - 20/45 (44%) grew *P. aeruginosa* alone or with another organism
  - Summary: 55% grew *P. aeruginosa;* 42% *B. cepacia*

My question to Dan Schidlow was, "Tell me about *Pseudomonas cepacia* in CF patients?"

Dan's reply, "What the heck is *Pseudomonas cepacia?"*

CDC study

- 61/339 (18%) of CF patients were positive for *B. cepacia;* 5/1425 (.3%) of non-CF patients positive (p<.0001)
- 20% died in the first 90 days following infection with something that will be known as the “cepacia syndrome”
- No environmental source found (J. Ped. 1985 107:382-387)
- Two other CF centers reporting similar findings at approximately the same time

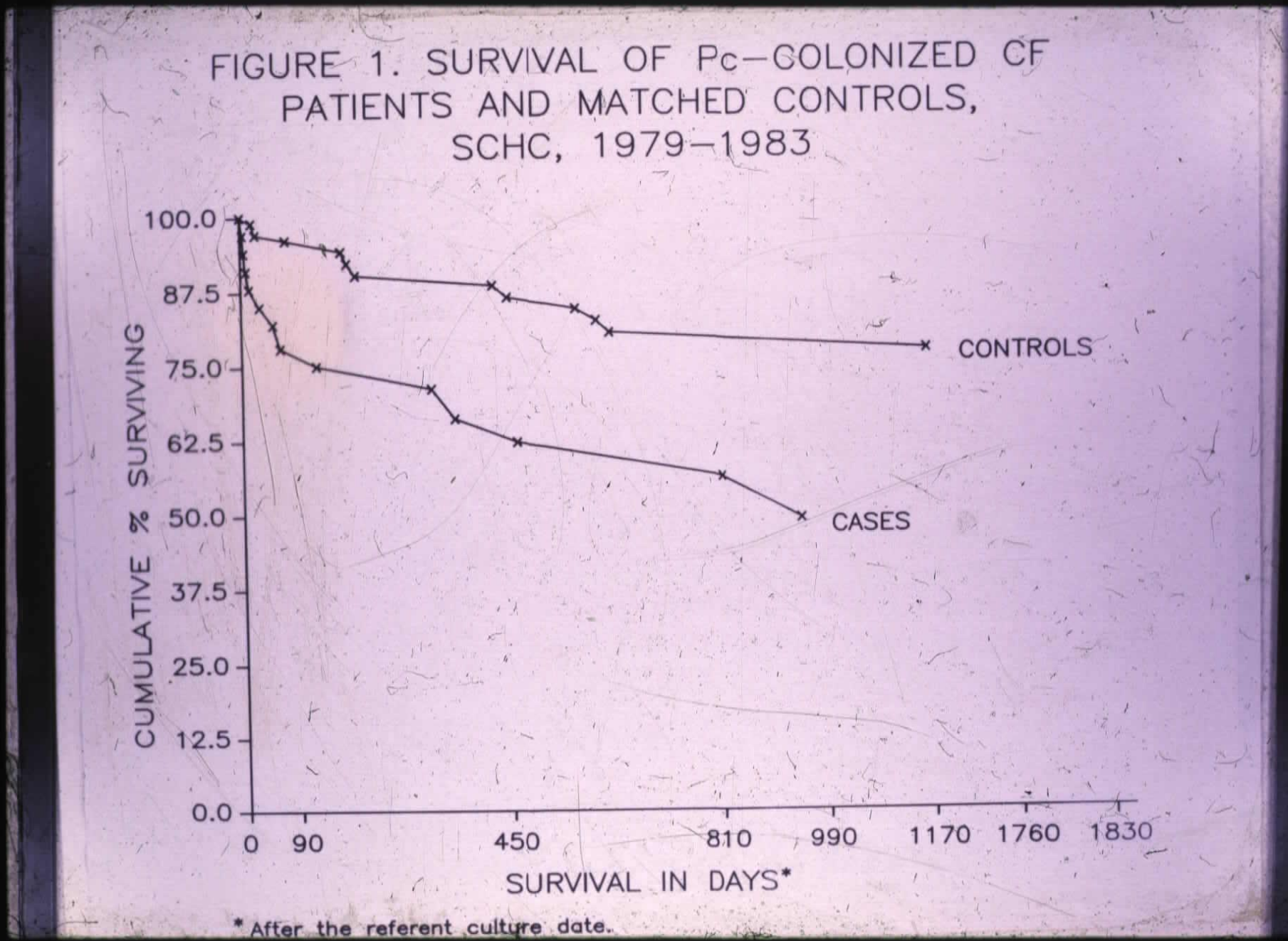
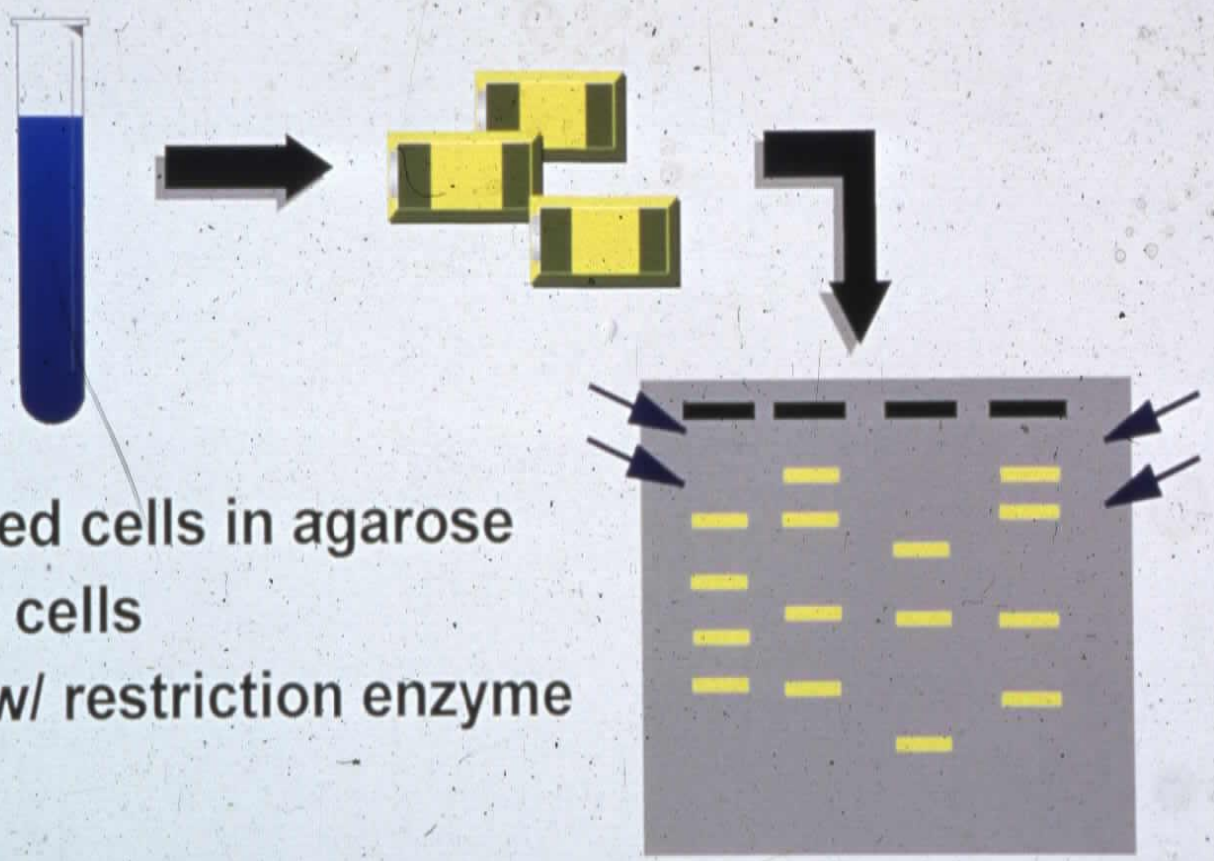


FIGURE 1. SURVIVAL OF Pc–COLONIZED CF PATIENTS AND MATCHED CONTROLS, SCHC, 1979–1983
CUMULATIVE % SURVIVING
100.0
87.5
75.0
62.5
50.0
37.5
25.0
12.5
0.0
CONTROLS
CASES
0
90
450
810
990
1170
1760
1830
SURVIVAL IN DAYS*
* After the referent culture date.

Application of molecular methods

- Pulsed field gel electrophoresis
- PCR and sequence analysis
  - » Used for speciation of *Burkholderia cepacia* complex and closely related organisms
    - 16s rRNA sequencing to get to complex and *recA* sequencing to get to species
  - » Why does it matter?

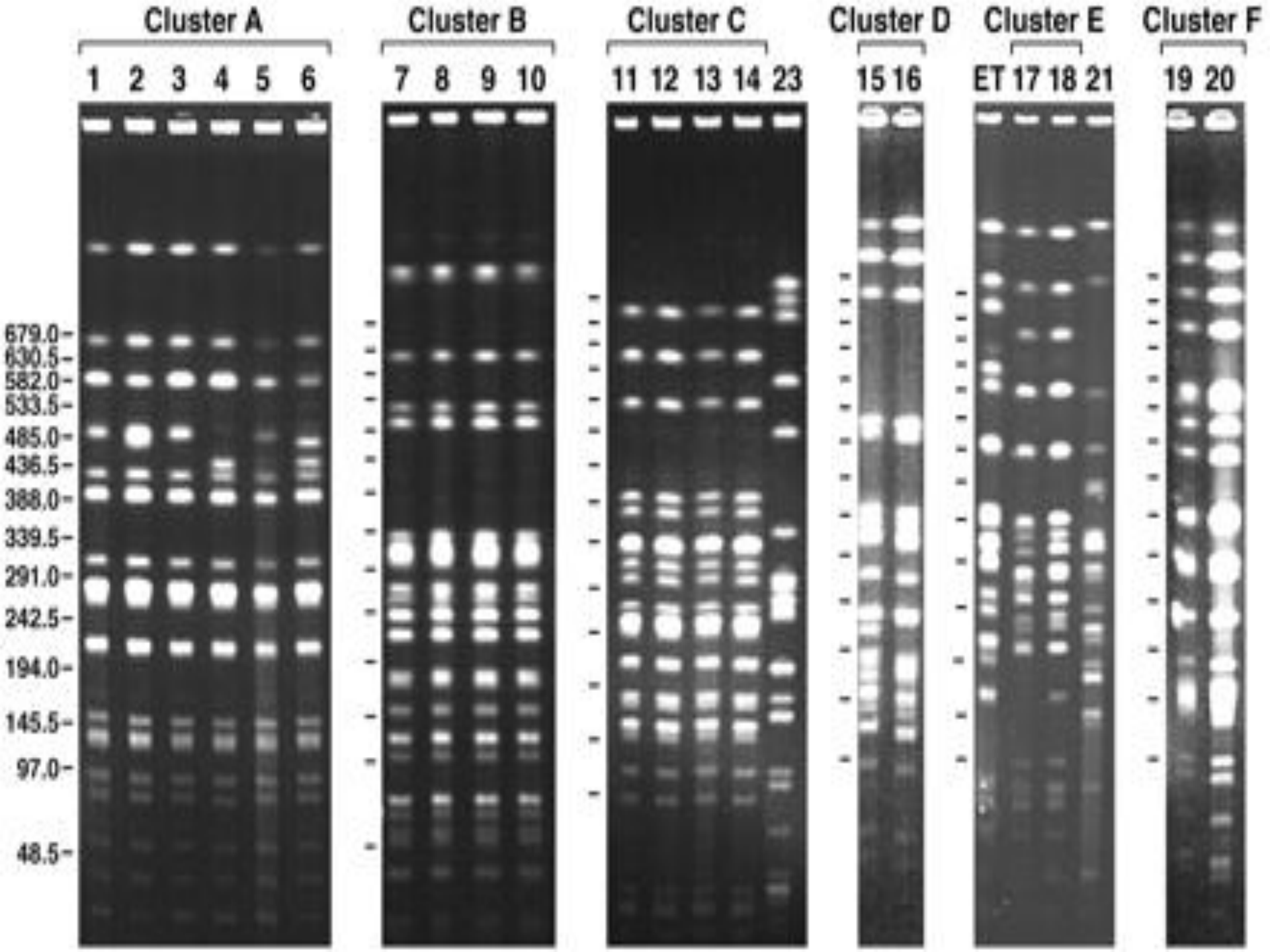
Pulsed field gel electrophoresis



*Embed cells in agarose
*Lyse cells
*Cut w/ restriction enzyme
*Gel

Molecular epidemiology

- PFGE interpretation
  - Same- 0 band difference
  - Clonally related- 1 to 3 band difference
  - Clonally possibly related- 4 to 6 band difference
  - Clonally unrelated->6 band difference


Cluster A
Cluster B
Cluster C
Cluster D
Cluster E
Cluster F
1 2 3 4 5 6
7 8 9 10
11 12 13 14 23
15 16
ET 17 18 21
19 20
679.0
630.5
582.0
533.5
485.0
436.5
388.0
339.5
291.0
242.5
194.0
145.5
97.0
48.5

Molecular epidemiology of *B. cepacia*

- The development of pulsed field gel electrophoresis for finger printing bacteria to allow us to determine if organisms are clonally related has greatly enhance our understanding of the epidemiology of *B. cepacia*
  - » Even though this technology is 20+ years old; it is still used

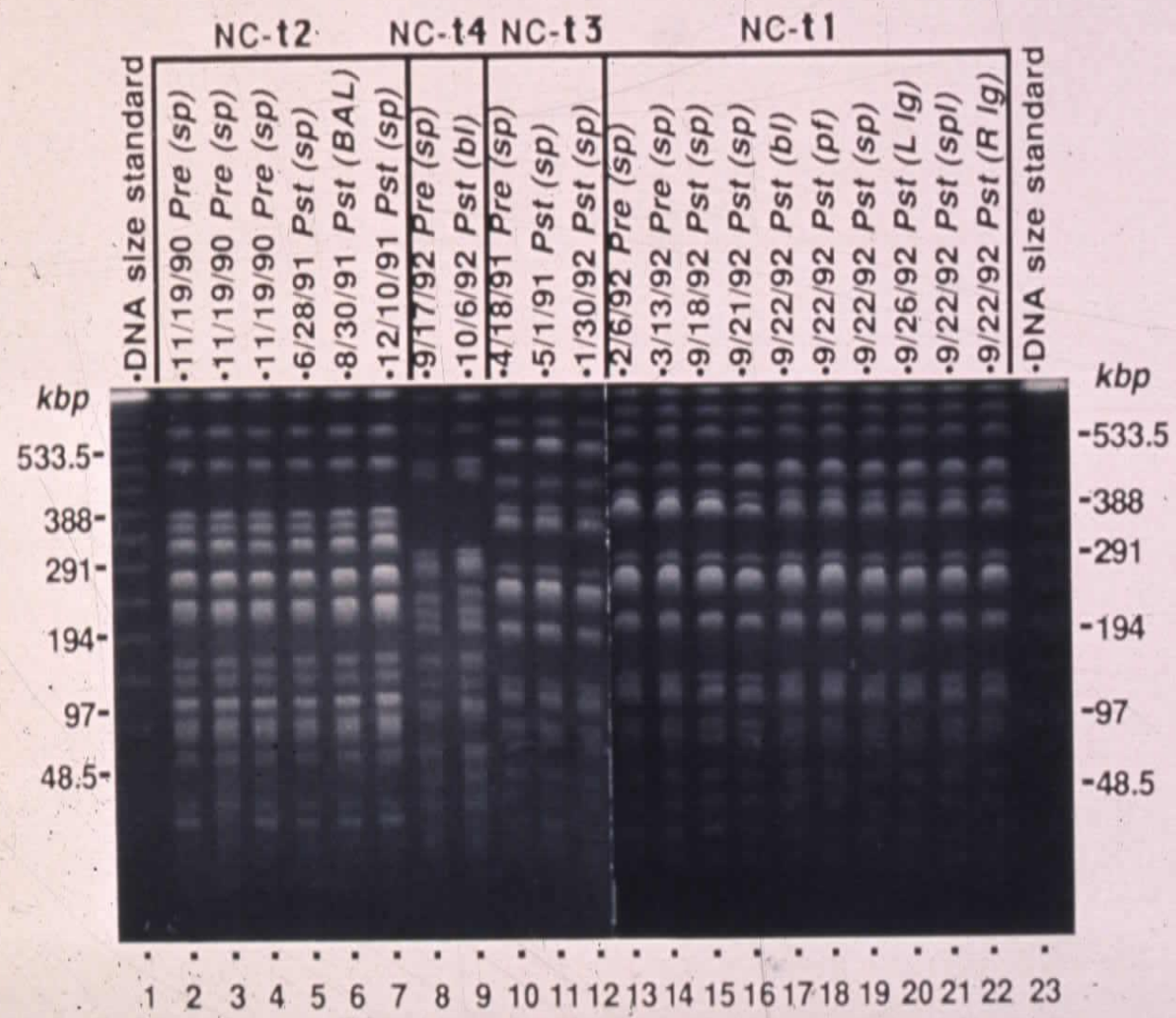
Molecular epidemiology of *B. cepacia*

- UNC one of the few centers that has done large number (n>20) of double lung transplants on *B. cepacia* positive patients
- We have studied the molecular epidemiology of *B. cepacia* in our transplant population since 1993

Molecular epidemiology of *B. cepacia*

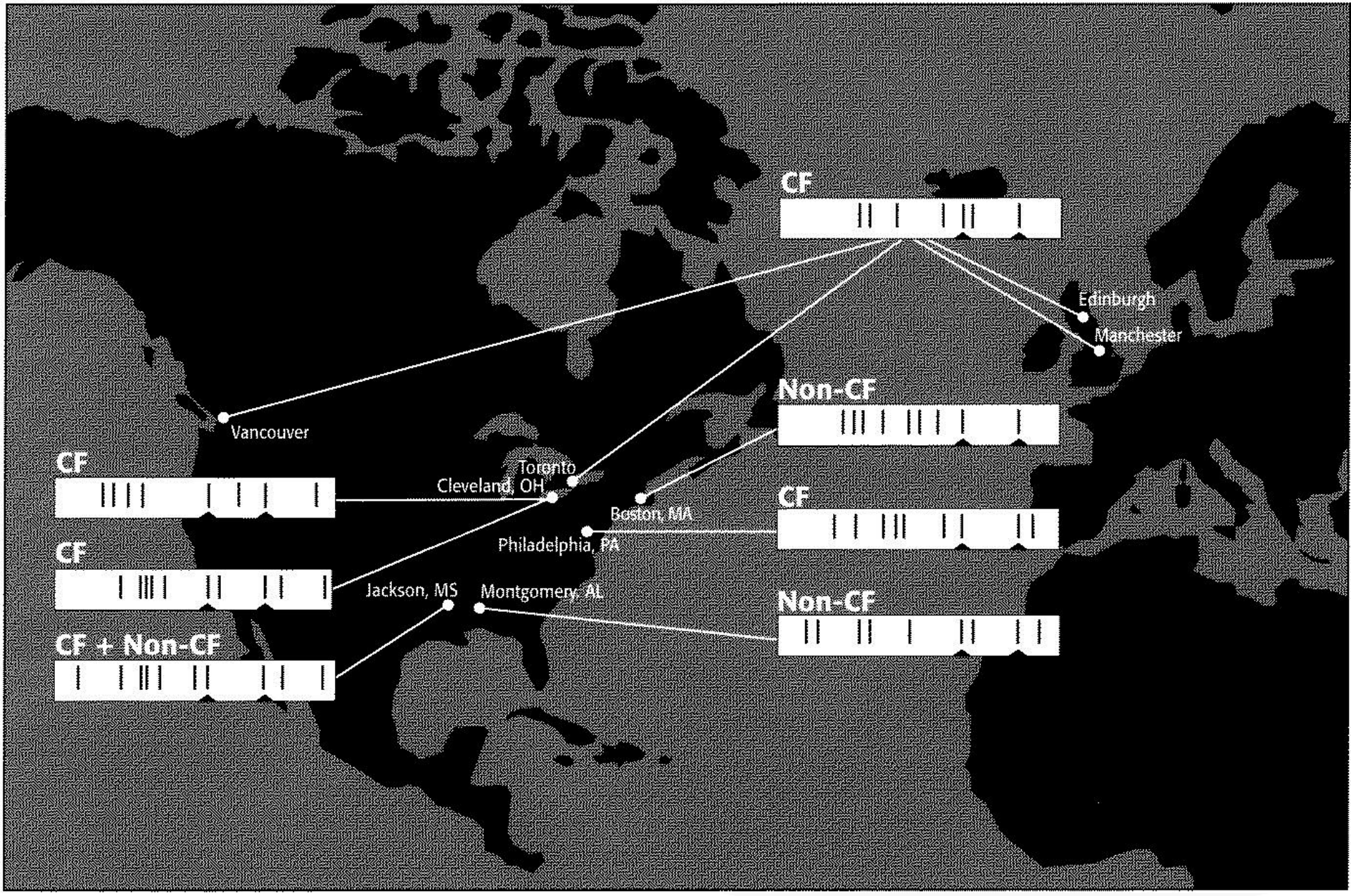
- Initial studies showed
  - **Patients had the same clone pre- and post-transplant**
  - **That these isolates, like *P. aeruginosa* persist over time**
  - **That there was not any person-to-person spread in our very small transplant population (NEJM. 1994 331:981-987)**

PFGE-resolved chromosomal RFLP profiles of *P. cepacia* isolates indicate 'persistent' clonal infection of CF transplant patients



Molecular epidemiology of *B. cepacia*

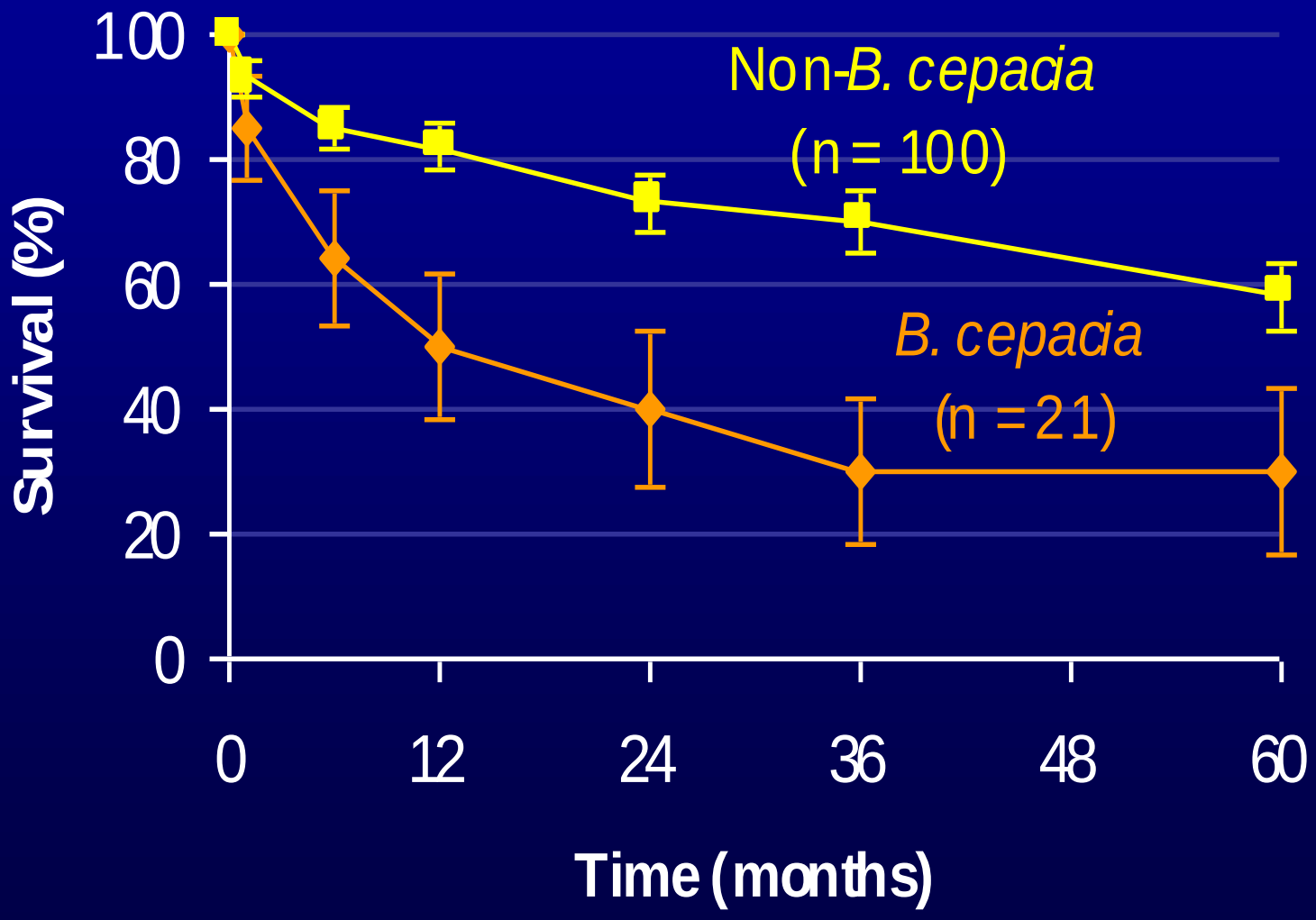
- Subsequent findings showed that a *B. cenocepacia* clone referred to as the “Toronto strain” had been spread intercontinentally
- The organism had a unusual pilus called the “cable pilus” which was thought to enhance its transmissibility including from CF to non-CF patients
- Subsequently two other major clones of *B. cenocepacia* have been recognized, the PHDC clone and what we call the “Midwestern” clone


CF
Edinburgh
Manchester
Non-CF
Vancouver
CF
Toronto
Cleveland, OH
Boston, MA
CF
Philadelphia, PA
CF
Jackson, MS
Montgomery, AL
Non-CF
CF + Non-CF

Molecular epidemiology of *B. cepacia*

- Based on the new taxonomic findings, the description of cable pilus and our finding of several deaths post transplant to a *B. cepacia* sepsis syndrome, we re-examined the issue of *B. cepacia* in our transplant population

CF Survival Comparisons



Lung transplantation in CF patients infected with *B. cepacia*

- What we have learned about *B. cepacia* complex in CF patients who undergo transplant (Am J Respir Crit Care Med 2001164:2102-2106)
  - Infected with the same organism pre- and post-transplant
  - Have a much poorer outcome than all other transplanted CF patients (both UNC and Toronto's experience)
  - UNC has different *B. cenocepacia* clones than Toronto but we have similar poor outcomes
  - Most of the early deaths (in first 12 months) are associated with *B. cenocepacia*
  - Outcomes with *B. multivorans* may not be as poor as *B. cenocepacia* but numbers are too small to say for sure

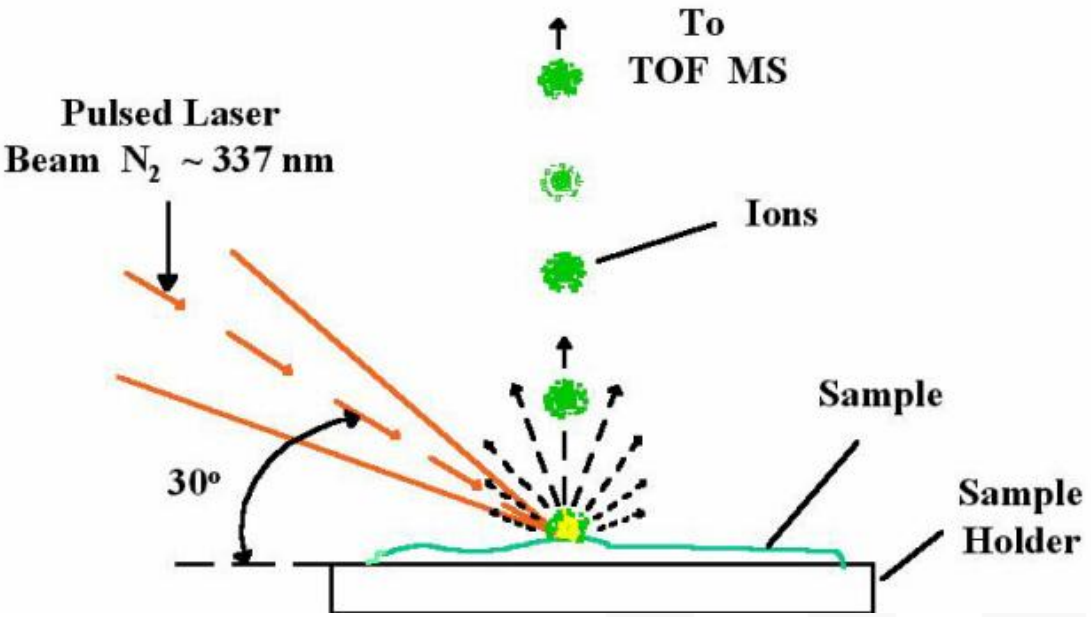
Lung transplantation in CF patients infected with *B. cepacia*

- There has been a general reluctance to transplant CF patients infected with *B. cepacia* complex
- In the US, only Duke and UNC have transplanted more than a handful of CF patients with *B. cepacia* infection
  - Duke discontinued their *B. cepacia* transplant program following two outbreak of bacteremia in non-CF patients due to two different clones of *B. cenocepacia.* (not the ET 12 strain) Mortality in the 2 outbreaks was 67%.
  - We have not seen any outbreaks in non-CF patients in our institution and patient to patient spread of *B. cepacia* strains is unusual in our center (J. Clin. Microbiol. 2002, 40:1188-1193.)

Applying molecular techniques to understanding new CF pathogens

- What have these molecular techniques allowed us to do?
  - Recognize that there are 17 species within the *B. cepacia* complex.
  - Importantly recognize that two species *B. cenocepacia* and *B. multivorans* are most common among CF patients
  - For *B. cenocepacia,* allowed us to recognize distinct subtype and also clones. Molecular tools have allowed us to better understand the molecular epidemiology of this organism and has resulted in changes in specific care protocols especially transplant ones
  - Recognize that a third member of the complex, *B. dolosa* can cause outbreaks among CF patients and that this organism can cause accelerated decline in lung function and decreased survival.

Applying molecular techniques to understanding new CF pathogens



MALDI-TOF- replace PCR and sequence analysis organism identification for *B. cepacia* and phenotypically closely related organisms

Changes in food production techniques

- Increased use of factory farming
- Feedlots bring together large numbers of animals who produce large amounts of waste
  - » Waste can lead to run-off of STEC that can contaminant adjacent fields as was seen in recent spinach outbreaks
- Large meat packing operations can result in 50 ton lots of ground meat containing 100s of animals
  - » Meat can be distributed throughout the US
  - » Contaminated lots can then lead to large scale outbreaks

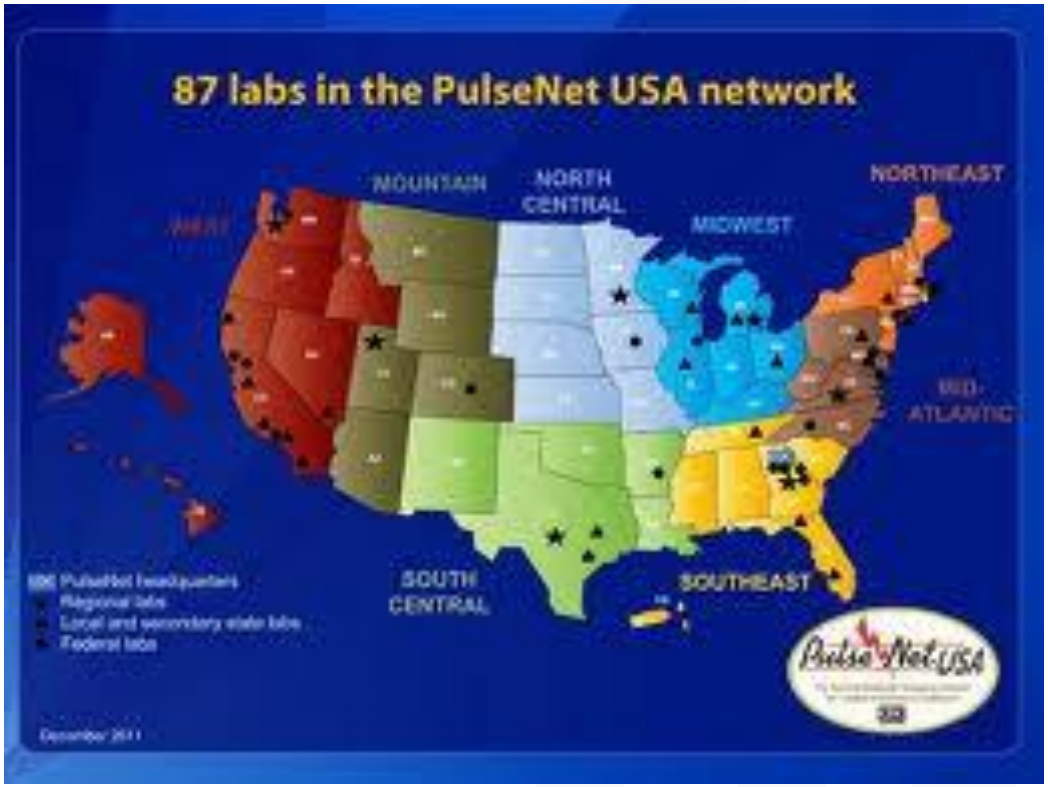
STEC

- Causes a spectrum of disease from mild diarrhea to haemorrhagic colitis
- Major complication-Hemolytic uremic syndrome
  - » More common in children
  - » Has a mortality of 5 to 10%
  - » Morbidity includes the need for dialysis in some children

FoodNet

Population based surveillance for food borne illnesses
Including STEC

PulseNet



Type STEC organisms using standardized PFGE

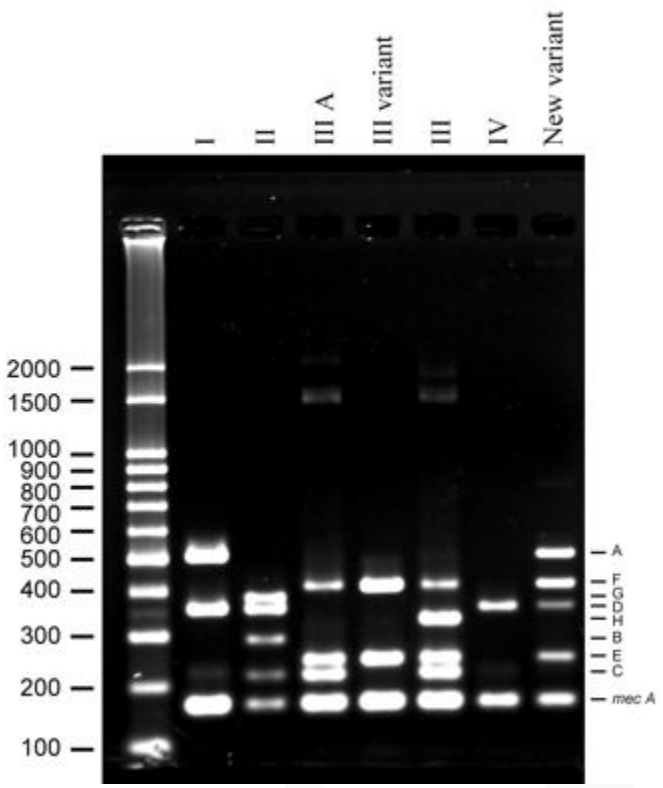
Have means to electronically track specific "fingerprints"- outbreak strains

Sequence of events

- Child first seen on 9-13 during a nationwide outbreak of STEC due to contaminated spinach
- Child had consumed spinach from an unknown source prior to onset of diarrhea
- During hospitalization, child had bloody diarrhea
- *E. coli* O157:H7 was recovered and confirmed on 9-21 only after she developed HUS on 9-19
- PFGE was done revealing a common pulsotype different from the outbreak strain: 9-29

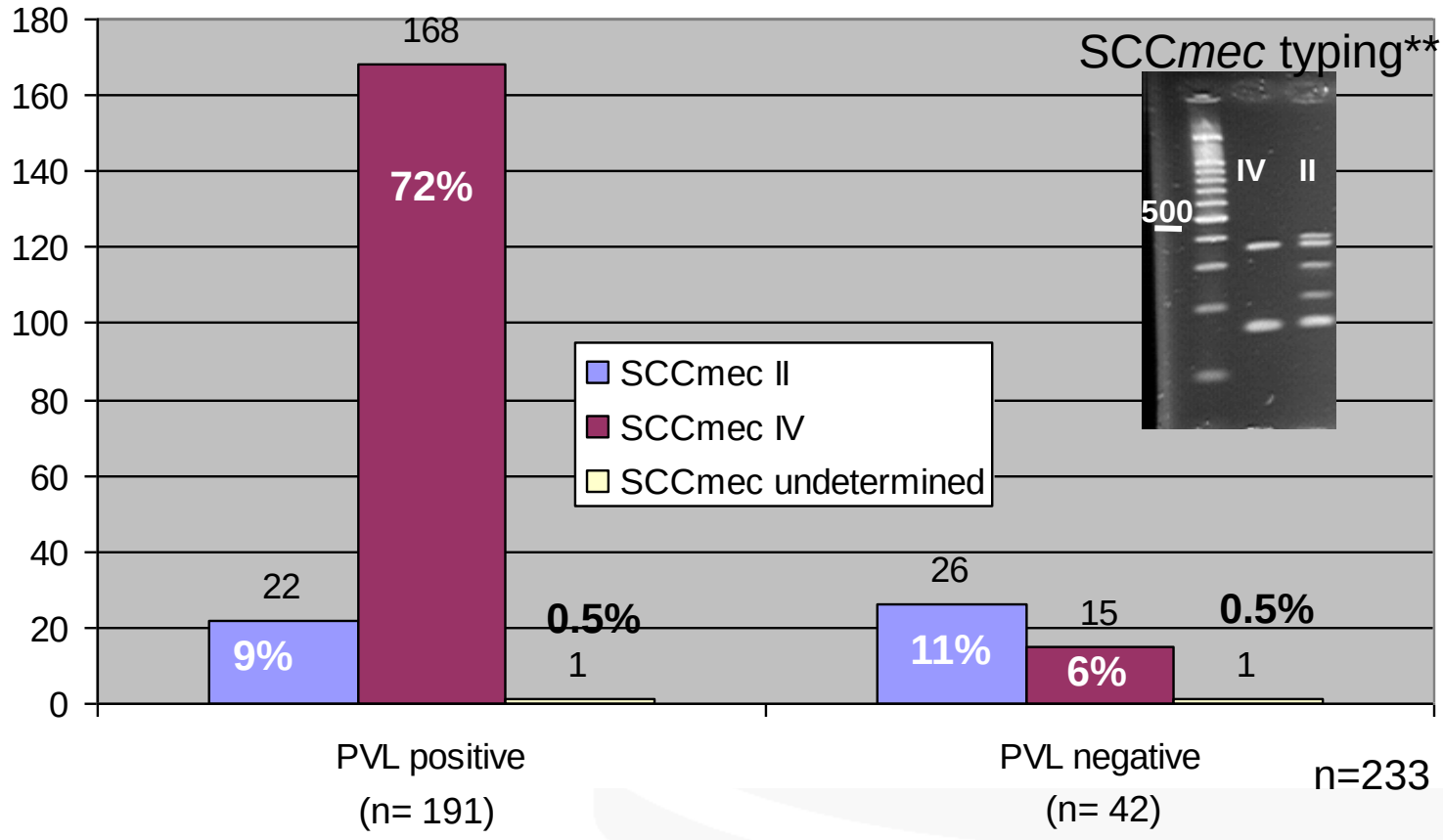
UNC-ED

- 6% of wounds from ED in 1st quarter of 2005 grew MRSA
- 45% of wounds from ED in 2nd quarter of 2005 grew MRSA
- ? Due to proliferation of CA-MRSA?
- GOAL
  - » To characterize and determine the prevalence of CA-MRSA isolates at UNC hospitals



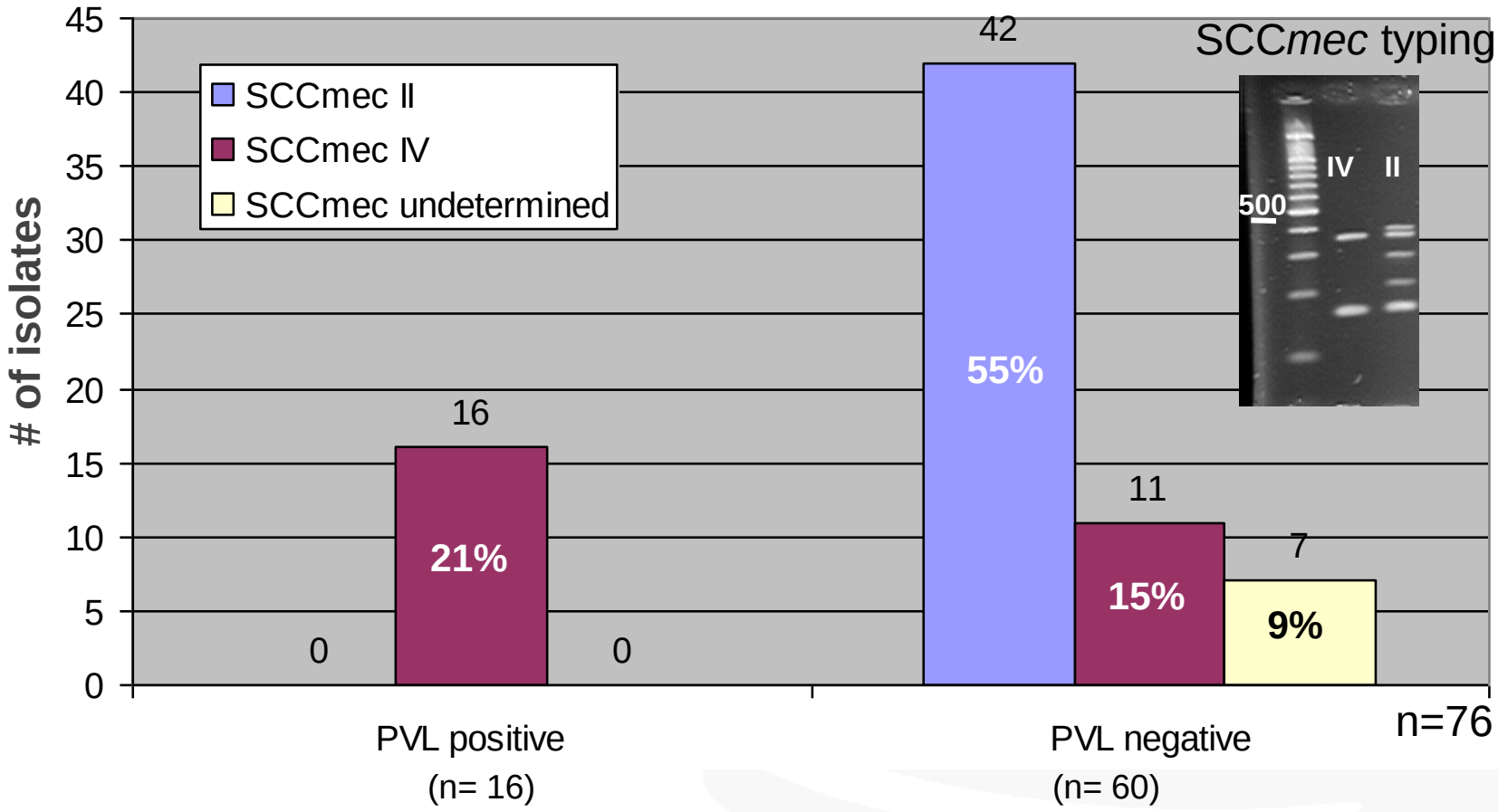
CA-MRSA= PVL pos and SCC*mec* IV

I. PVL and SCC*mec* Characterization of outpatient wound isolates



** Oliveira and Lencastre (2002) *Antimicrob Agents Chemother* **46,** 2155-61.

II. PVL and SCC*mec* Characterization nosocomial blood isolates



Typing of *S. aureus*

Two systems widely used:

PFGE or MLST/SCC*mec* typing

Cookson et al J Clin Micro 2007; 45:1830-7

Genotypic Typing Systems

Multilocus sequence typing (MLST)

- Sequence internal fragments (450-500 bp) of seven house-keeping genes
  - The 7 genes sequenced vary by organism
  - For each house-keeping gene, the different sequences present within a bacterial species are assigned as distinct alleles and, for each isolate, the alleles at each of the seven loci define the allelic profile (sequence type)
- Allelic profiles of isolates compared to those in a large database on the internet
- Results highly portable but expensive

As Brian the scientist would say, "Any Questions?"