Historical overview of molecular biology and strain typing

Dr. George Broukhanski
Assistant Professor,
Laboratory Medicine and Pathobiology,
University of Toronto
Molecular Specialist
Public Health Ontario Laboratories

Whole Genome Sequencing Conference
Austin, TX
July 26-27, 2017
OBJECTIVES

• Goals for bacteria typing
• Traditional methods of typing
• Molecular typing of isolates
• Investigation of outbreaks using molecular typing
Goals for typing bacteria

- Identification if isolated bacteria are novel, may be causing severe disease or are not preventable by vaccine
- Investigation of epidemiology of an outbreak – routes of transmission, persistence, efficiency of infection control measures
Traditional typing methods

- Serotyping – first study published by Rebecca C. Lancefield in 1933 on hemolytic streptococci
- Phage typing (Identification of *S. aureus* strains by means of bacteriophage, Fisk, 1942)
- Biotyping (biochemistry, antibiotic resistance, growth characteristics etc.)
Significance of serotyping

- *V. cholera* – 200 serotypes, only 2 are significant (Inaba and Ogawa)
- *E. coli* – hemorragic O157:H7 and verocytotoxin-producing O104:H4
- *N. meningitidis* group B – account for >50% of cases but no broadly effective vaccine is available
Polysaccharide capsule is essential for survival in the blood, and thus for virulence. Immunochemically distinct capsular polysaccharides define serotypes. Remarkably, the total size of alternative coding DNA at this one locus exceeds 1.8 Mbp, almost equivalent to the entire S. pneumoniae chromosomal complement.
Phage typing - characterization of bacteria, extending to strain differences, by demonstration of susceptibility to one or more bacteriophage *

*http://medical-dictionary.thefreedictionary.com/phage+typing
Strain typing by detection of phages

FIG 2 PFGE analysis of the genomic DNA of isolated phages. Undigested purified phage DNA was separated by PFGE and stained with ethidium bromide. The sizes of the mass marker fragments are indicated on the left.

“Lysogenic conversion,” now well known as the mechanism responsible for the acquisition of important traits in microbes such as Vibrio cholerae, Staphylococcus aureus, enterohemorrhagic Escherichia coli, and many others*

Upon introduction of CD38-2 into a NAP1/027 representative isolate, up to 1.6- and 2.1-fold more TcdA and TcdB were detected**

Phage therapy as a realistic alternative to antibiotics depends on our capacity to overcome the hurdles faced by this therapeutic option***

*** Nobrega et al. Revisiting phage therapy: new applications for old resources.Trends in Microbiology, April 2015, Vol. 23, No. 4
Molecular typing targets

- Detection of specific biomarkers (toxin and antibiotic resistance genes etc.): dot blot hybridization, PCR, toxinotyping
- Detection of insertion sequences: RFLP, spoligotyping
- Analysis of chromosomal repeat regions: VNTR, MLVA
- Detection of random variations of bacterial genome: MLST, PFGE, REA, AFLP, rep-PCR, ribotyping, SNP analysis
- Detection of multiple targets: WGS
Same DNA – different appearance
Dot-blot hybridization to detect presence of specific genes

- DNA extraction
- Applying of drop of DNA solution to a membrane
- Hybridizing overnight with a labelled probe
- Visualizing by exposing to X-ray film
Comparison of Seven Techniques for Typing International Epidemic Strains of *Clostridium difficile*: Restriction Endonuclease Analysis, Pulsed-Field Gel Electrophoresis, PCR-Ribotyping, Multilocus Sequence Typing, Multilocus Variable-Number Tandem-Repeat Analysis, Amplified Fragment Length Polymorphism, and Surface Layer Protein A Gene Sequence Typing

George Killgore,1 Angela Thompson,1 Stuart Johnson,2 Jon Brazier,3 Ed Kuijper,4 Jacques Pepin,5 Eric H. Frost,6 Paul Savelkoul,8 Brad Nicholson,6 Renate J. van den Berg,4 Haru Kato,7 Susan P. Sambol,2 Walter Zukowski,2 Christopher Woods,6 Brandi Limbago,1 Dale N. Gerding,2 and L. Clifford McDonald1

Centers for Disease Control and Prevention, Atlanta, Georgia1; Hines VA Hospital, Hines, Illinois2; Anarobe Reference Laboratory, National Public Health Service for Wales, Microbiology Cardiff University Hospital of Wales, Cardiff, United Kingdom3; Department of Medical Microbiology, Center for Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands4; Department of Microbiology and Infectious Diseases, University of Sherbrooke, Sherbrooke, Quebec, Canada5; Division of Infectious Diseases, Department of Medicine, Duke University School of Medicine, Durham, North Carolina6; Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo, Japan7; and Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands8

Received 24 July 2007/Returned for modification 11 September 2007/Accepted 14 November 2007
Typing of *M. tuberculosis* strains with IS6110-based RFLP (restriction fragment length polymorphism) analysis

PvuII digestion

IS6110 hybridization

Spoligotyping of *M. tuberculosis* strains

The method is called spacer oligotyping or “spoligotyping” because it is based on strain-dependent hybridization patterns of in vitro-amplified DNA with multiple spacer oligonucleotides.

Pulsed Field Gel Electrophoresis

- PFGE is used for genotyping or genetic fingerprinting
- It is commonly considered a gold standard in epidemiological studies of pathogenic organisms
Amplified Fragment Length Polymorphism

AFLP allows visualization of sets of restriction fragments without knowledge of nucleotide sequence. It involves three steps:

• restriction of the DNA and ligation of oligonucleotide adapters
• selective amplification of sets of restriction fragments
• gel analysis of the amplified fragments

AFLP of *Legionella pneumophila*

1. DNA ladder
2. Outbreak isolate, patient #1
3. Outbreak isolate, patient #2
4. 1215, washroom sink
5. 1216, condensing tower
6. Lp1 isolate #1, unrelated
7. Lp1 isolate #2, unrelated
8. Lp1 isolate #3, unrelated
9. *L. bellingham*
10. *L. micdadei*
11. 1280, condensing tower #3
12. 1281, condensing tower #3
13. 1296, condensing tower #3
14. 1327, condensing tower #3
15. 1279 Fr cont plate
16. Neg. control
17. DNA ladder

Ribotyping of *C. difficile*

PFGE

ribotyping
Modified Multiple-Locus Variable number of tandem repeats Analysis (MMLVA)
Clustering of 50 isolates by ribo-MMLVA
DNA-sequence-based typing

- Unambiguous
- Portable
- Reproducible
- Scalable
This report describes the emergence and rapid spread since January 2005 of reassortant H3N2 influenza A viruses among pigs and turkeys across Canada and isolation of a related virus from the nasal passages of a farm worker in Ontario and is the first to describe isolation of a human/ classical swine/avian triple reassortant H3N2 virus from a human.
Variation in DNA sequences of the *fla* gene of *C. jejuni*
Multilocus sequence typing (MLST)

- MLST was used to investigate evolutionary relationships between strains of Campylobacter, *N. meningitidis*, *S. aureus*, *S. pyogenes*, *C. albicans* etc.
- Sequencing of 450-500 bp internal fragments
- For each gene, the different sequences present are assigned as distinct alleles; For each isolate, the alleles at each of the loci define the allelic profile or sequence type (ST)


Whole Genome Sequencing of *Clostridium difficile* using Illumina platform
WGS alignment (MAUVE) – E. coli and C. difficile
SMRT sequencing was used in this study as it produces high quality genome sequences, with resolution of repeat regions (including those found in mobile elements) and can generate data to determine methylation modifications across the sequence (the methylome)*

Our results constrain the set of genomic differences possibly affecting virulence by more than half, which focuses laboratory investigation on pertinent targets and demonstrates the power of SMRT sequencing for producing high-quality reference genomes.

Hargreaves et al. Use of single molecule sequencing for comparative genomics of an environmental and a clinical isolate of Clostridium difficile ribotype 078. BMC Genomics. 2016 Dec 13;17(1):1020.*

Elghraoui A et al. SMRT genome assembly corrects reference errors, resolving the genetic basis of virulence in Mycobacterium tuberculosis, BMC Genomics201718:302**
We find evidence of horizontal transfer of carbapenemase-encoding plasmids between *K. pneumoniae*, *E. cloacae*, and *C. freundii* in the hospital environment

*K. pneumoniae* and *E. cloacae* isolated simultaneously from a single patient harbored two different carbapenemase-encoding plasmids, indicating that plasmid transfer between organisms was unlikely within this patient
Graph of possible transmission links among patients.

Putative map of *K. pneumoniae* transmission during outbreak. The transmission map was constructed with genetic and patient trace data.

AN IDEAL WGS TYPING PROTOCOL

• User-friendly sample preparation
• Inexpensive (costs less than multiplex PCR, $100)
• Follows an established and verified interpretation protocol
• Has high resolution and is scalable
• Capable of detecting pathogenicity related genes/structures
• Can detect population variations of the same pathogen (e.g. ribotypes, drug resistance etc.)
• Can differentiate chromosomal and plasmid/transposon/phage sequences
Thank you!