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Applications of Nanopore Sequencing for Infectious Disease Detection

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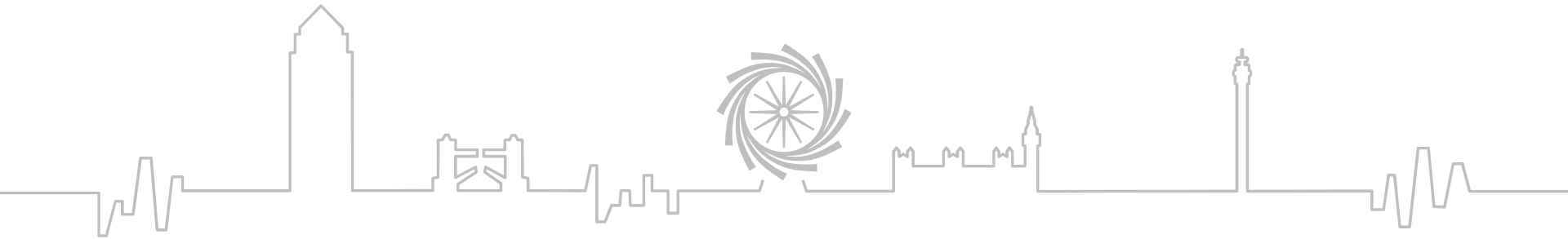
May 26, 2016

Overview

Identification of Pathogenic Organisms in the Clinical Setting

- Vancomycin resistance surveillance in rectal swabs
- Clinical case study of *K. pneumoniae*
- Flu virus



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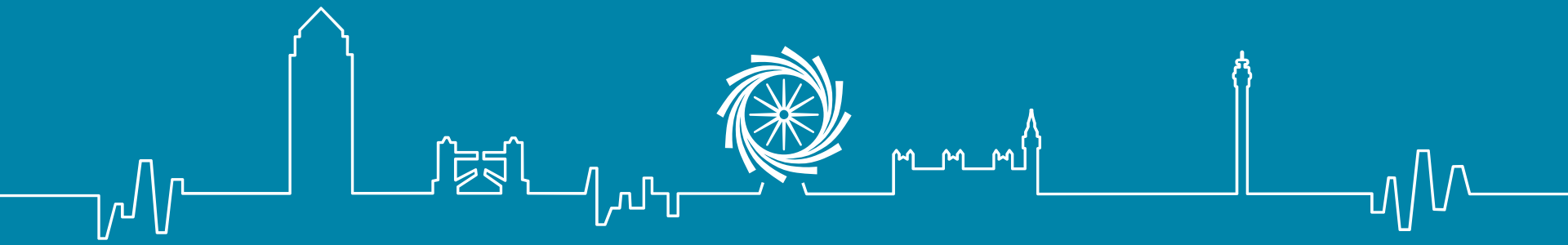
Identification of Pathogenic Organisms in the Clinical Setting



Identification of Pathogenic Organisms

Clinical Setting

- **Majority of testing is culture based**
  - **Standard is ~48 hours for identity and antimicrobial susceptibility**
    - Broadly grow organisms over wide range of medias
    - Identify organisms
    - Test for antimicrobial susceptibility
  - **Other organisms more fastidious**
    - Fungi, mycobacteria can take weeks to grow in lab

Identification of Pathogenic Organisms
Metagenomic Sequencing

Single test modality

- **No a priori knowledge necessary**
- **Only requires genomic material found in sample**
- **Unbiased – broad and amplifies whatever is present**

*courtesy of Oxford Nanopore

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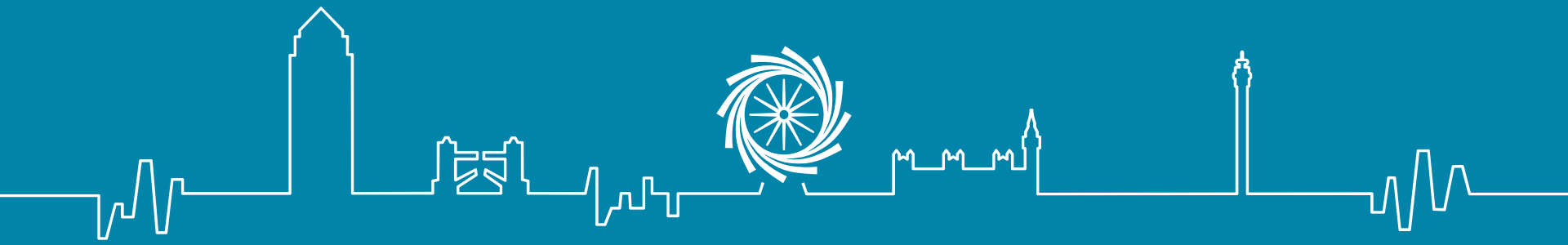
Vancomycin Resistance Detection



Vancomycin Resistance Enterococci

- **In the United States, VRE is commonly acquired in a healthcare setting**
- **VRE testing done for every admission at Johns Hopkins**
  - 20000 samples per year
- **Major Organisms of Interest:**
  - *E. faecalis* (vanB resistance)
  - *E. faecium* (vanA resistance)
  - *K. pneumonia* (KPC resistance)

% Enterococci that are Vancomycin Resistant

Species	Europe	US	Canada	Asia-Pacific	Latin-America
E. faecium	8.8	79.4	22.4	14.1	48.1
E. faecalis	1.0	8.5	0.1	0.01	3.1
All enterococci	4.0	35.5	6.0	11.9	12.9

O'Driscoll, Crank, 2015. Infection and Drug Resistance

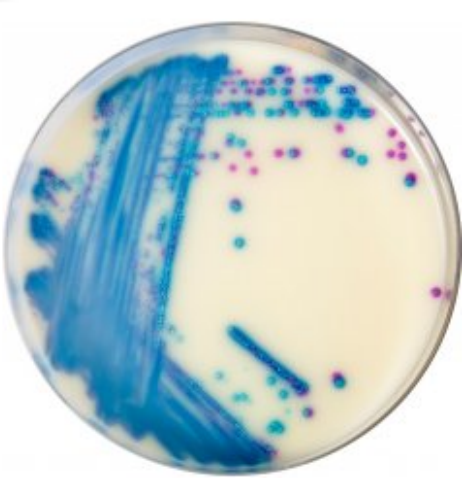
Vancomycin Resistance in Rectal Swabs
Procedure

- Remnant rectal swab samples
  - All confirmed positive for VRE by clinical testing
- Extract DNA (Zymo MiniPrep)
- Low Input Prep for Sequencing
  - Nextera XT for Illumina Miseq
  - Low Input PCR for Oxford Nanopore
- Analysis
  - Kraken
  - Comprehensive Antibiotic Resistance Database (CARD)

Samples

Results via Culture

Sample	Chromagenic results*	Carbapenem resistance?	Fermenter in sample?
7	*E. faecium*	Yes	Yes, Lactose
10	*E. faecalis*	No	Yes, Non-lactose



*On chromogenic agar, *E. faecium* isolates turn pink, *E. faecalis* isolates turn blue

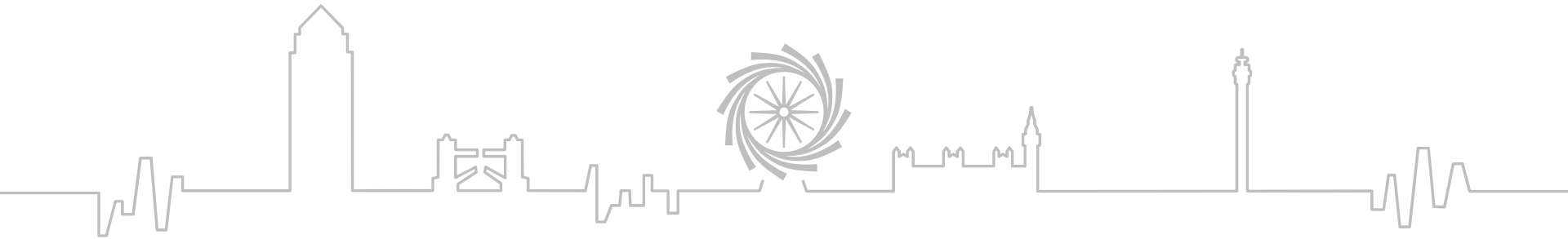
Image courtesy of CHROMAgar

Samples

Results via Culture vs NGS

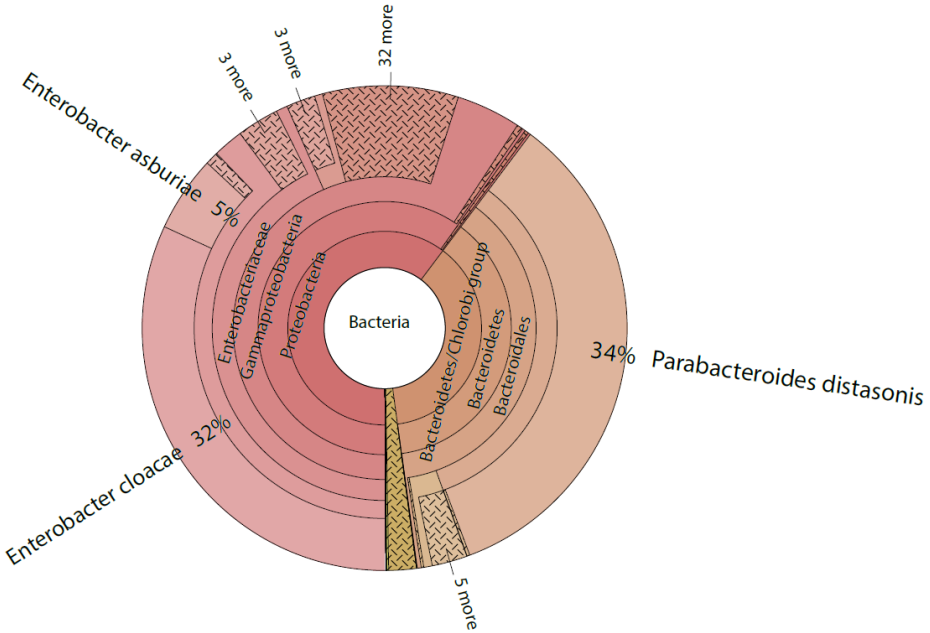
Sample	Chromagenic results	Detected via NGS	Carbapenem resistance?	KPC gene detected?	Dominant Organism
7	*E. faecium*	Yes	Yes	Yes	*K. pneumoniae*
10	*E. faecalis*	Yes	No	No	*Enterobacter, Parabacteroides*

NGS can detect what we see with culture!

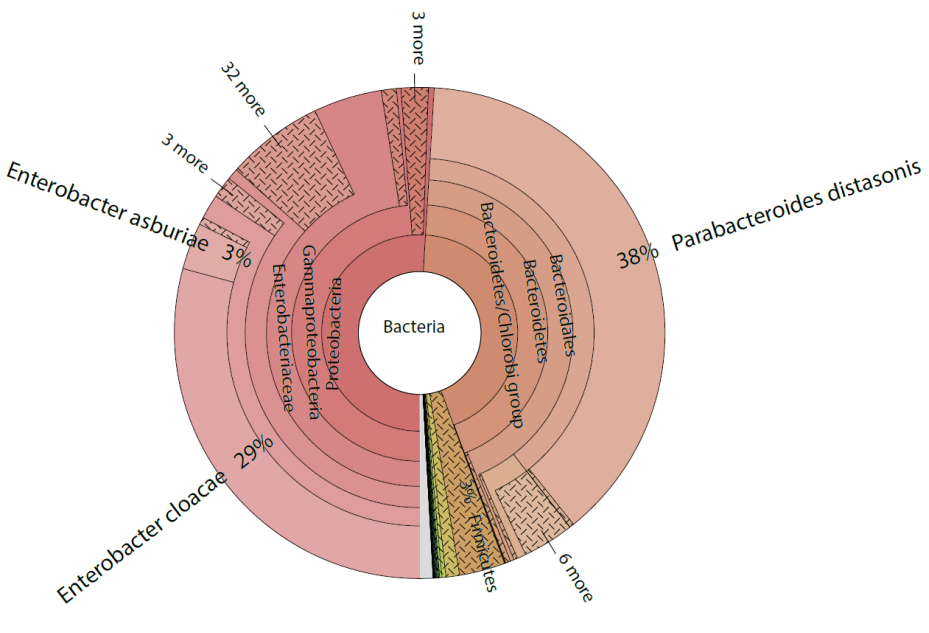


Kraken: Illumina vs. Nanopore

Illumina MiSeq (12M reads)



MinION (57k reads)



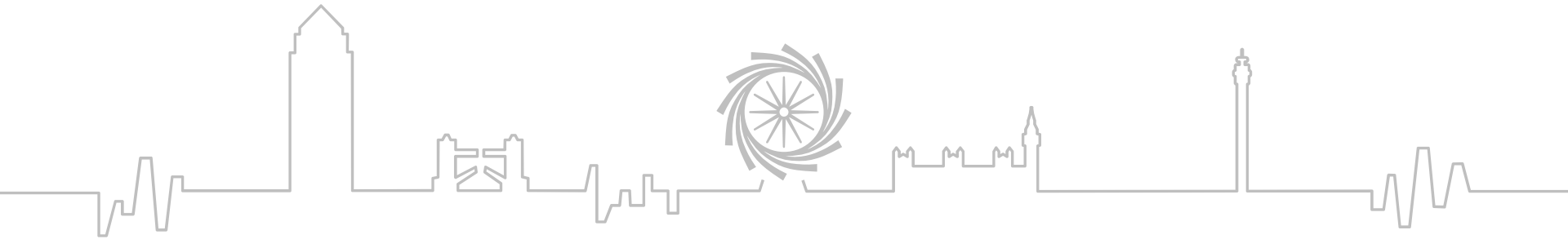
MinION shows largely the same classification as MiSeq via Kraken

Comparison of Illumina to Nanopore (Kraken)

Sample	Sequencer	Total Reads	# *E. faecium*	% *E. faecium*	# *E. faecalis*	% *E. faecalis*	# *K. pneumoniae*	% *K pneumoniae*
7	Illumina	15052147	35343	0.235	142	9.43E-4	5773568	38.36
7	Nanopore	105381	236	0.224	11	1.04E-2	39634	37.61
10	Illumina	7344961	4921	0.067	13056	0.178	29966	0.408
10	Nanopore	57225	40	0.0699	129	0.225	259	0.453

Comparison of Illumina to Nanopore: Antibiotic Resistance (BLAST)

Sample	Sequencer	Total Reads	# vanA	% vanA	# vanB	% vanB	# KPC	% KPC
7	Illumina	15052147	14	9.3E-5	0	0	7114	4.73E-2
7	Nanopore	105381	1	9.49E-4	0	0	227	2.15E-1
10	Illumina	7344961	6	8.17E-05	115	1.57E-03	3	4.08E-5
10	Nanopore	57225	0	0	4	6.99E-3	0	0



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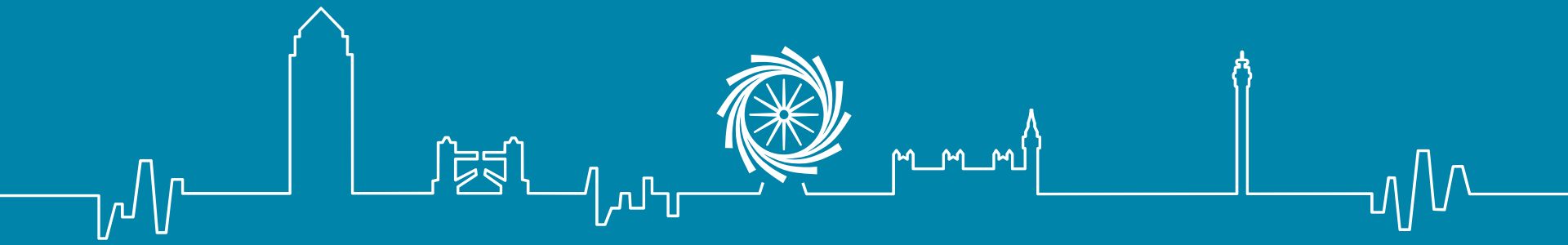
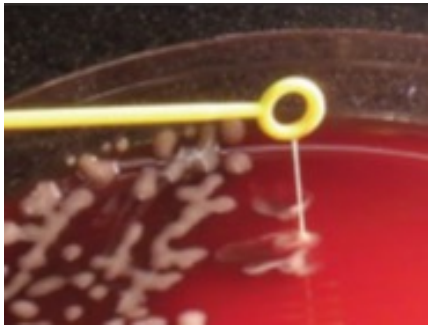
Clinical Case Study



Hypervirulent (hypermucoviscous) *K. pneumoniae*

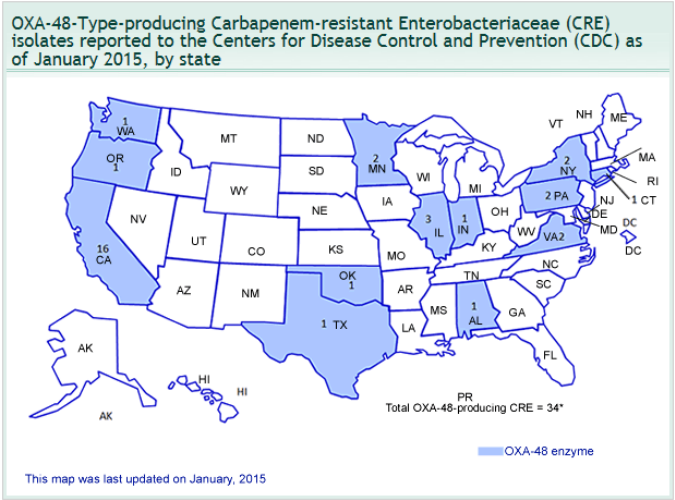


A new variant of *Klebsiella pneumoniae*

First described in the Asian Pacific Rim 1980s

Now increasingly recognized in other countries

- Defining clinical features:
  - Serious, life-threatening community-acquired infection in younger healthy hosts
  - Liver abscess, pneumonia, meningitis and endophthalmitis
  - Metastatic spread

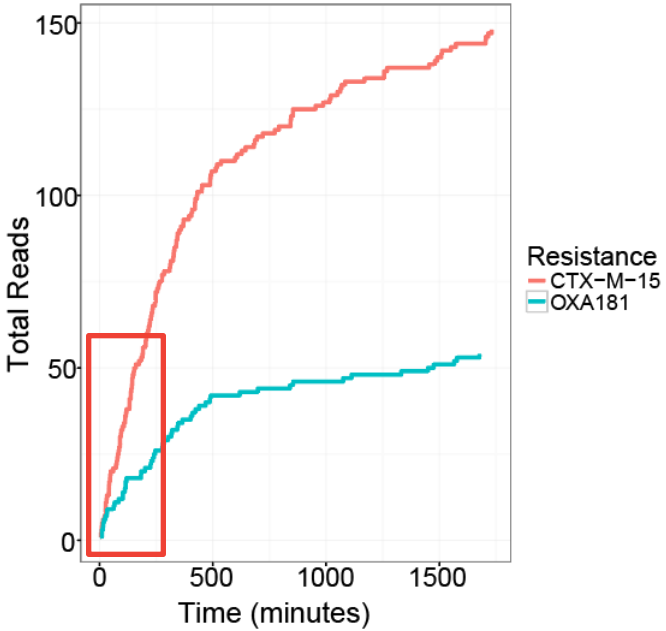


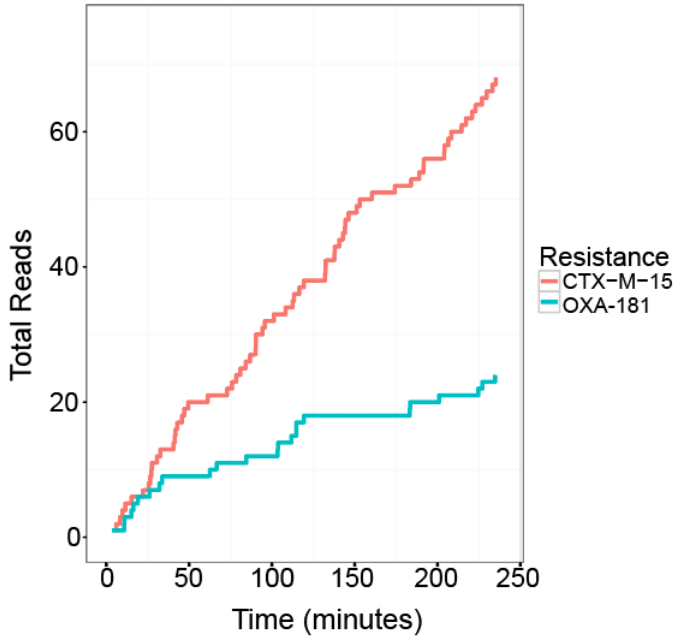
Source: Shon, Rajinda, Russo 2013
CDC
http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html#CREmapOXA

Isolates Extracted

Isolate	Date	Source	Organism	Resistance
1	July 29, 2015	Endo/Nasal	*K. pneumoniae*	no
2	August 20, 2015	Endo/Nasal	*K. pneumoniae*	yes
3	September 4, 2015	Abscess, Kidney	*K. pneumoniae*	no
4	September 10, 2015	Stool	*K. pneumoniae*	yes
5	September 10, 2015	Stool	*E. coli*	yes

Antibiotic Resistance Detection





Sample	OXA-181 detection time	CTX-M-15 detection time
2	3.5 min	5 min
4	10.3 min	3.6 min
5	10.73 min	9.37 min

Resistance identified within 11 minutes!

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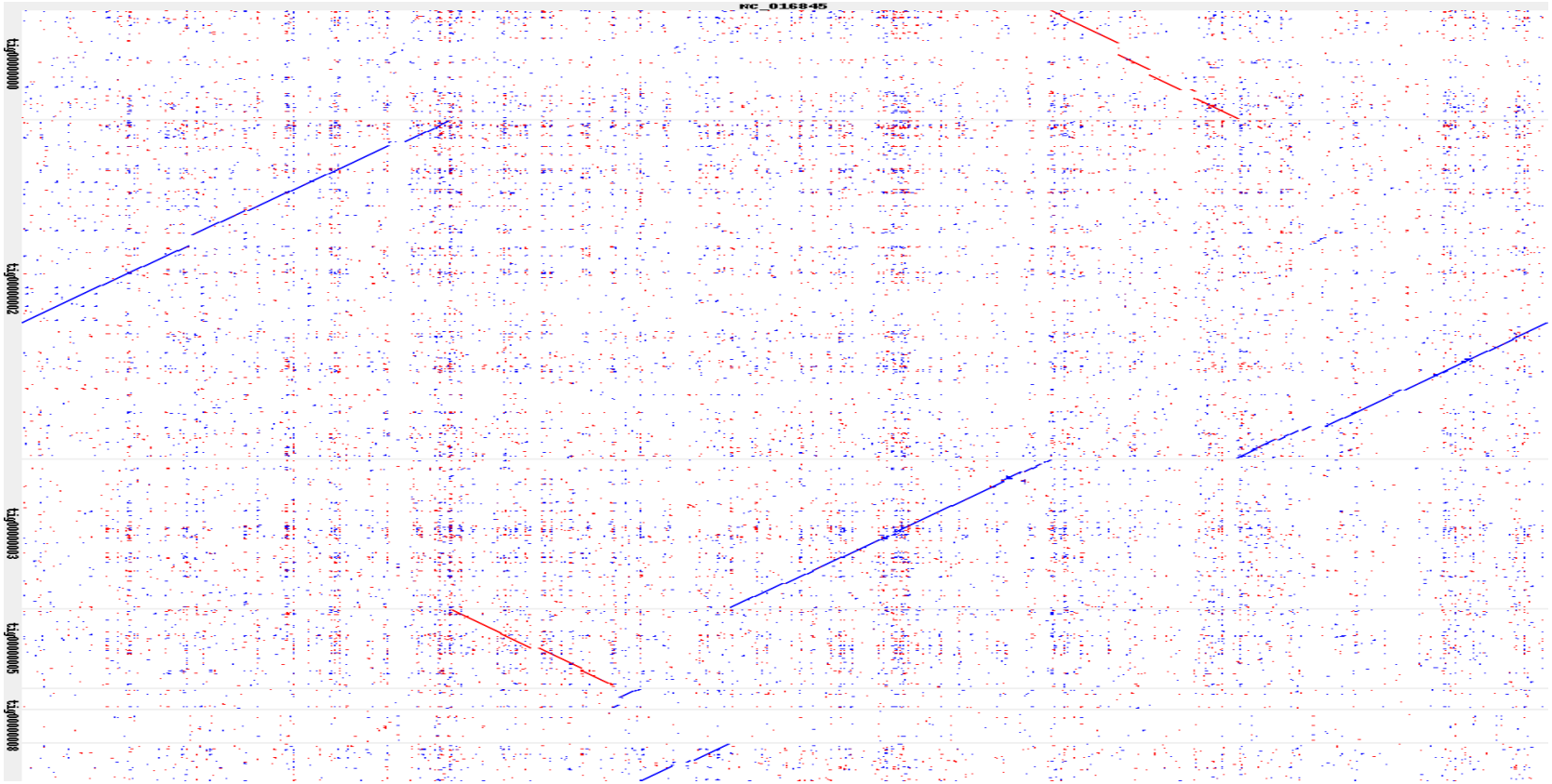
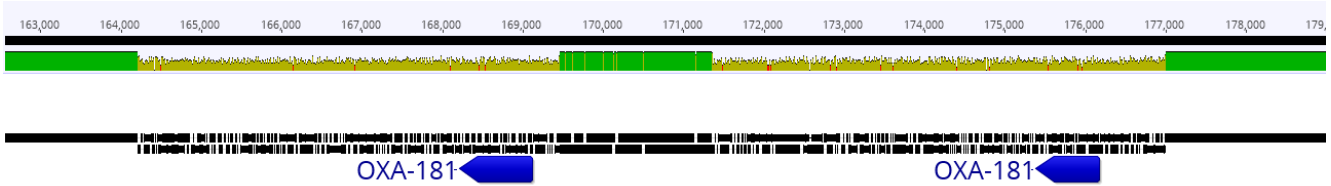
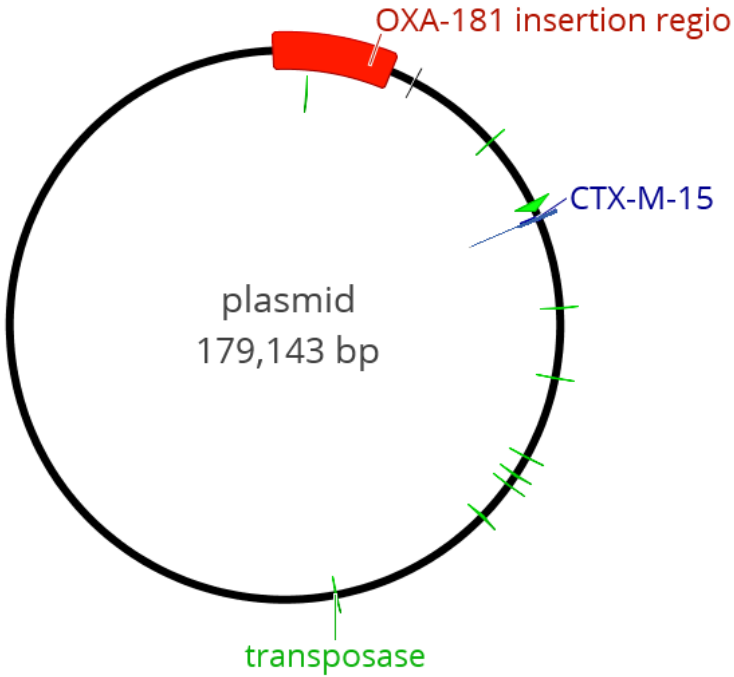
De Novo Genome Assembly



Plasmids





Non-MDR strains have 1 plasmid

MDR strains have 4 plasmids

- ~180kb plasmid relatively similar in all 3 strains
- OXA-181 is on a separate contig, inserts itself into plasmid with CTX-M-15

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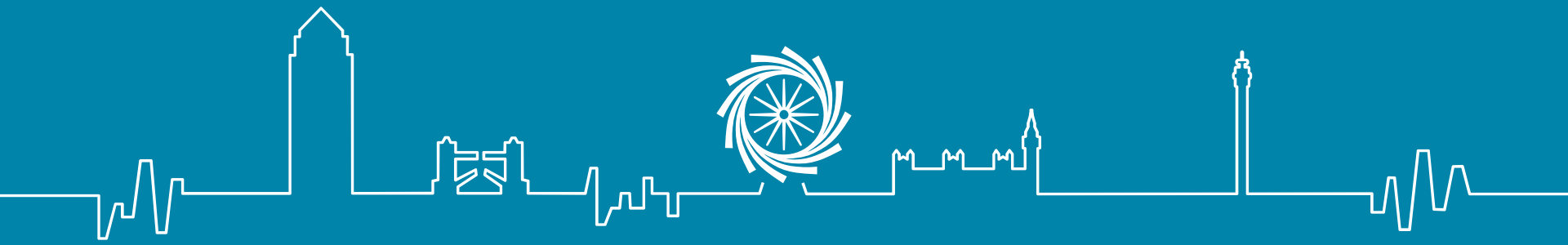
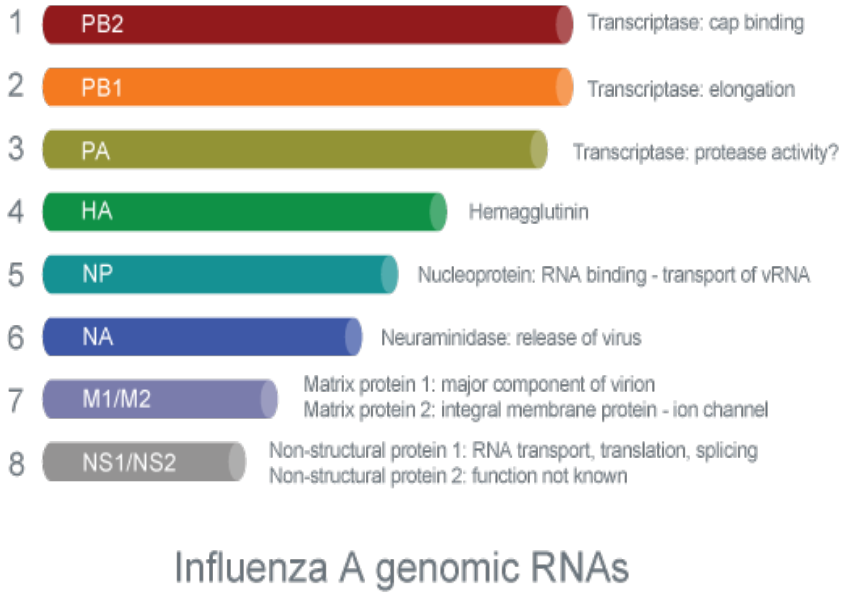
Flu Strain Analysis



Influenza Genome

Influenza – single stranded, helically shaped RNA virus

- **Influenza is made of 8 different genomic RNA segments.**
- **These segments can be amplified by primers which are specific to each segment, but conserved between influenza strains**
- **Fragments range in size between 900-2.4kb; nanopore would allow for full length sequencing.**

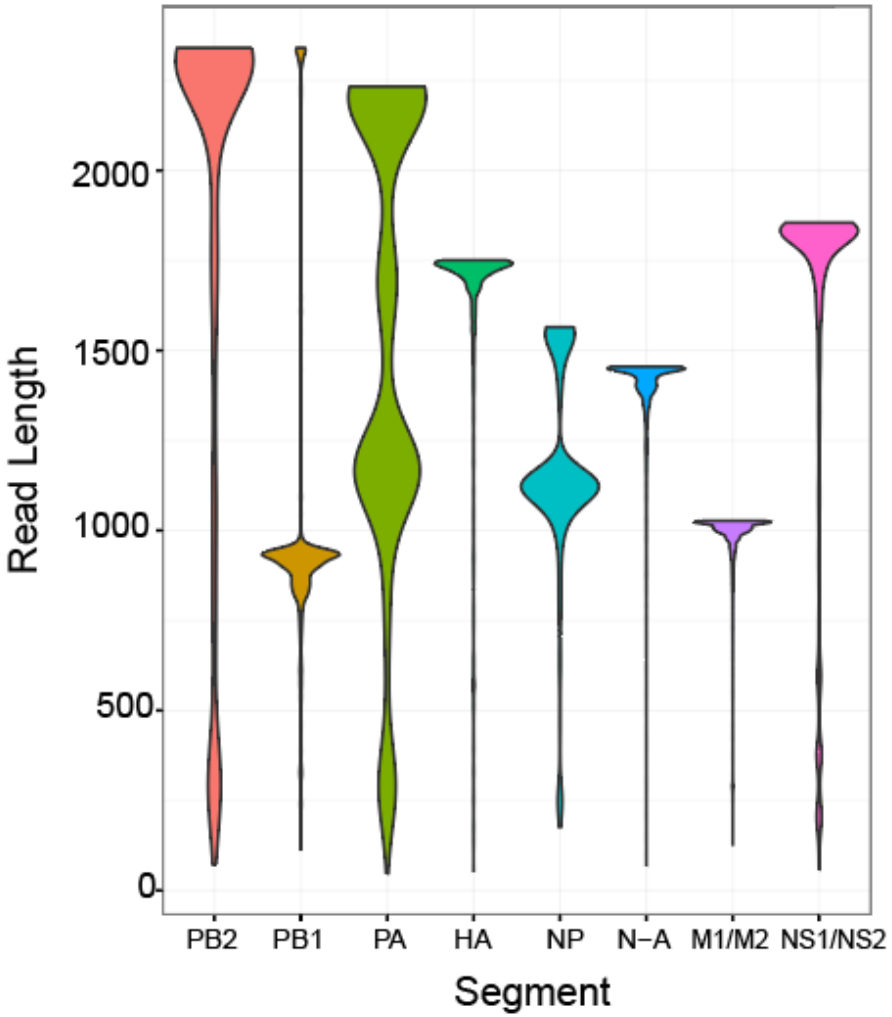


Influenza A genomic RNAs

From Thermo Fisher Scientific

Flu Sequencing

Segment	# of Reads
PB2	622
PB1	1147
PA	4267
HA	2196
NP	1291
N-A	4354
M1/M2	10905
NS1/NS2	1478



Majority of reads are full length

Full length reads don't occur in some segments due to defective interference particles

Observed mutations

Control

	miseq	oxford
Segment_1|PB2	0	1*
Segment_2|PB1	1*	NA
Segment_3|PA	0	0
Segment_4|HA	0	0
Segment_5|NP	0	0
Segment_6|NA	0	0
Segment_7|M1	0	0
Segment_8|NS1	0	0

*mutations appear at ends of sequence or internal abrupt changes in sequencing depth

Clinical

	miseq	oxford
Segment_1|PB2	28	14*
Segment_2|PB1	13	NA*
Segment_3|PA	25	9*
Segment_4|HA	31	NA*
Segment_5|NP	17	14**
Segment_6|NA	14	14
Segment_7|M1	2	2
Segment_8|NS1	9	9

*discrepancy due to incomplete segment coverage
**discrepancy due to stringency of consensus generation

Slide courtesy of Thomas Mehoke, JHU APL

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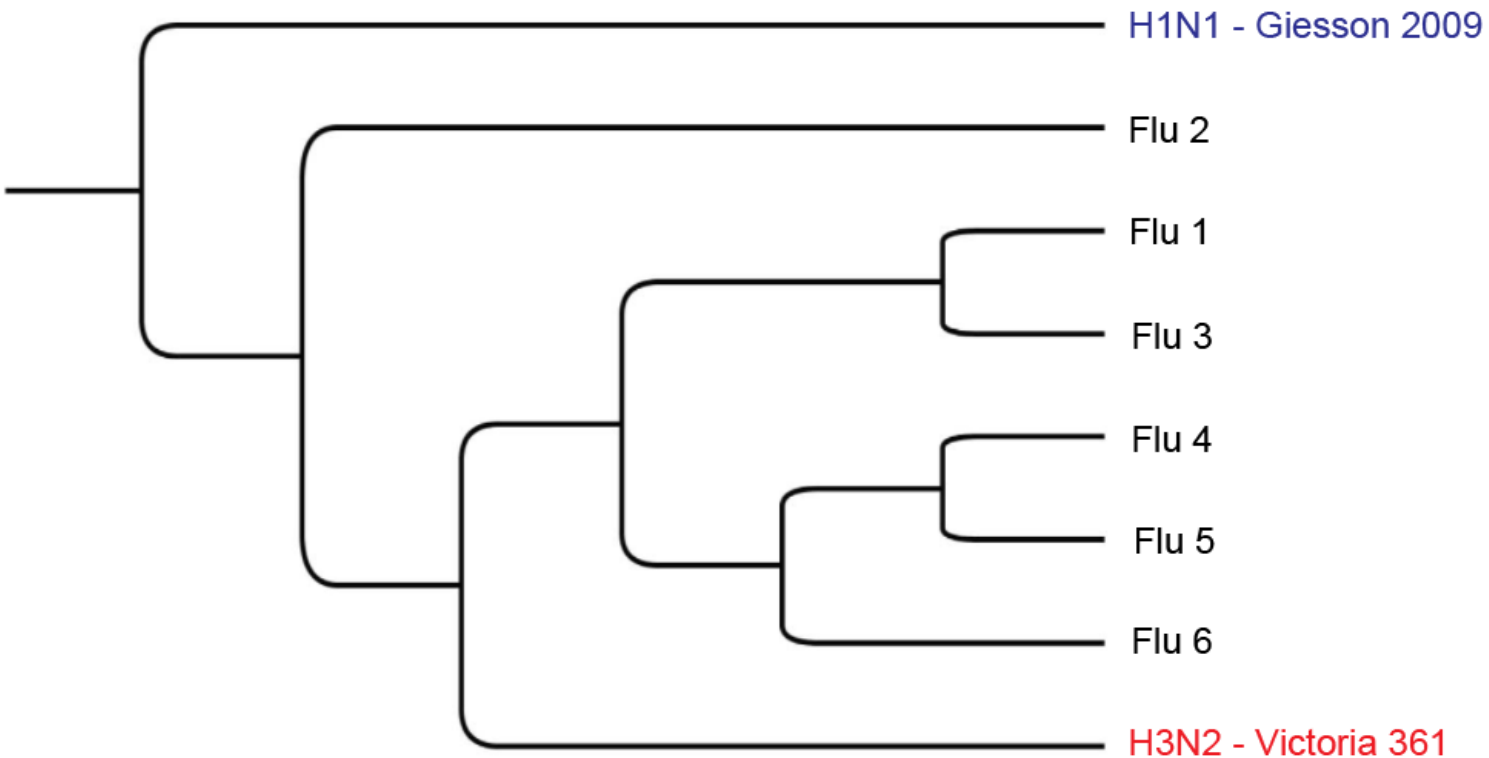
Flu Clinical Variations



Conclusions

Nanopore sequencing looks promising for detection of infection and antibiotic resistance

- Rapid results (under 15 min)
- Long/Full length reads
- Low capital investments
- Portable

Still a bit noisy in terms of detecting individual mutations and rare cases

- Increased yield and accuracy with further development

Acknowledgements

Timp Lab (JHU BME)

- Rachael Workman
- Isac Lee
- Yunfan Fan
- Winston Timp

Infectious Diseases/Medical Microbiology (JHMI)

- Annie Antar
- Patricia Simner
- Belita Opene

Schatz Lab (CSHL/JHU)

- James Gurtowski
- Michael Schatz

Applied Physics Laboratory (JHU)

- Peter Thielen
- Thomas Mehoke

Funding

- CEIRS Foundation