Antimicrobial Resistance Detection in Illumina and Nanopore Sequencing

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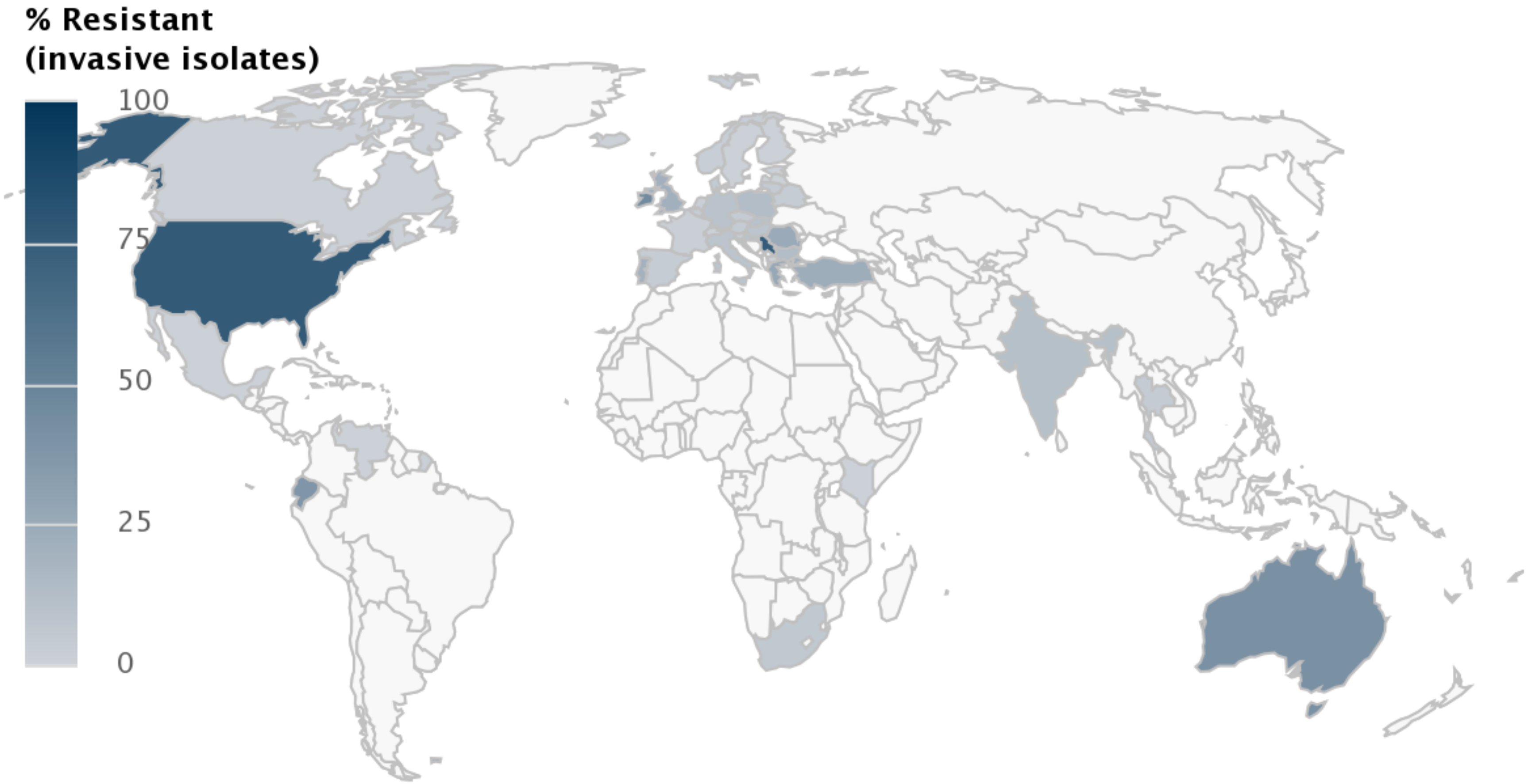
AGBT 2017

Resistance of *Enterococcus faecium* to Vancomycin



Center for Disease Dynamics, Economics & Policy (cddep.org) © Natural Earth

Vancomycin Resistance Enterococci

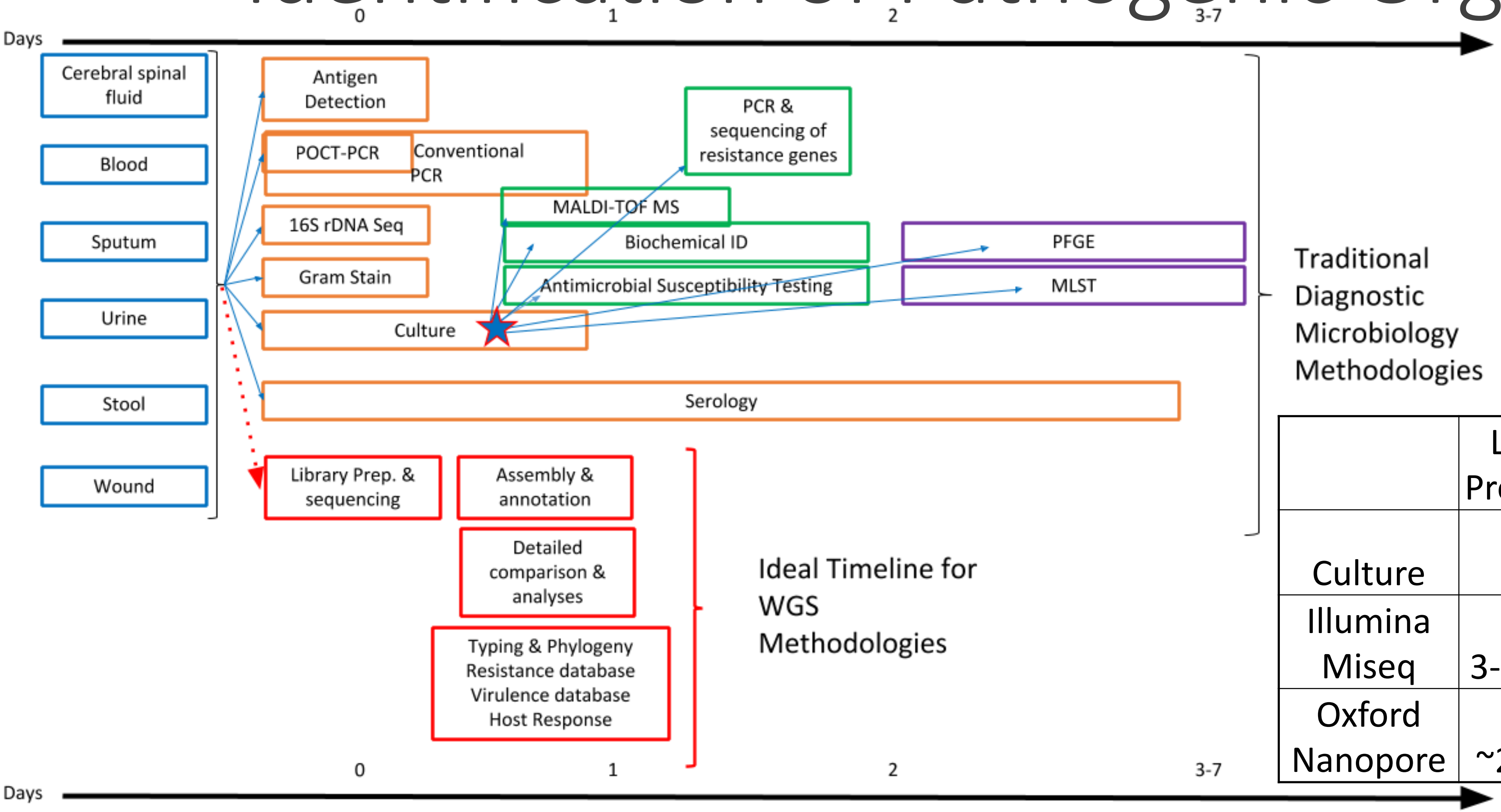
- In the United States, VRE is commonly acquired in a healthcare setting
- 20000 rectal swabs tested for VRE per year at JHHS
- Major Organisms of Interest:
  - *E. faecalis* (vanB resistance)
  - *E. faecium* (vanA resistance)

% 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

Identification of Pathogenic Organisms



Days: 0, 1, 2, 3-7

Cerebral spinal fluid

Blood

Sputum

Urine

Stool

Wound

Antigen Detection

POCT-PCR

Conventional PCR

16S rDNA Seq

Gram Stain

Culture

PCR & sequencing of resistance genes

MALDI-TOF MS

Biochemical ID

Antimicrobial Susceptibility Testing

PFGE

MLST

Serology

Traditional Diagnostic Microbiology Methodologies

Library Prep. & sequencing

Assembly & annotation

Detailed comparison & analyses

Typing & Phylogeny
Resistance database
Virulence database
Host Response

Ideal Timeline for WGS Methodologies

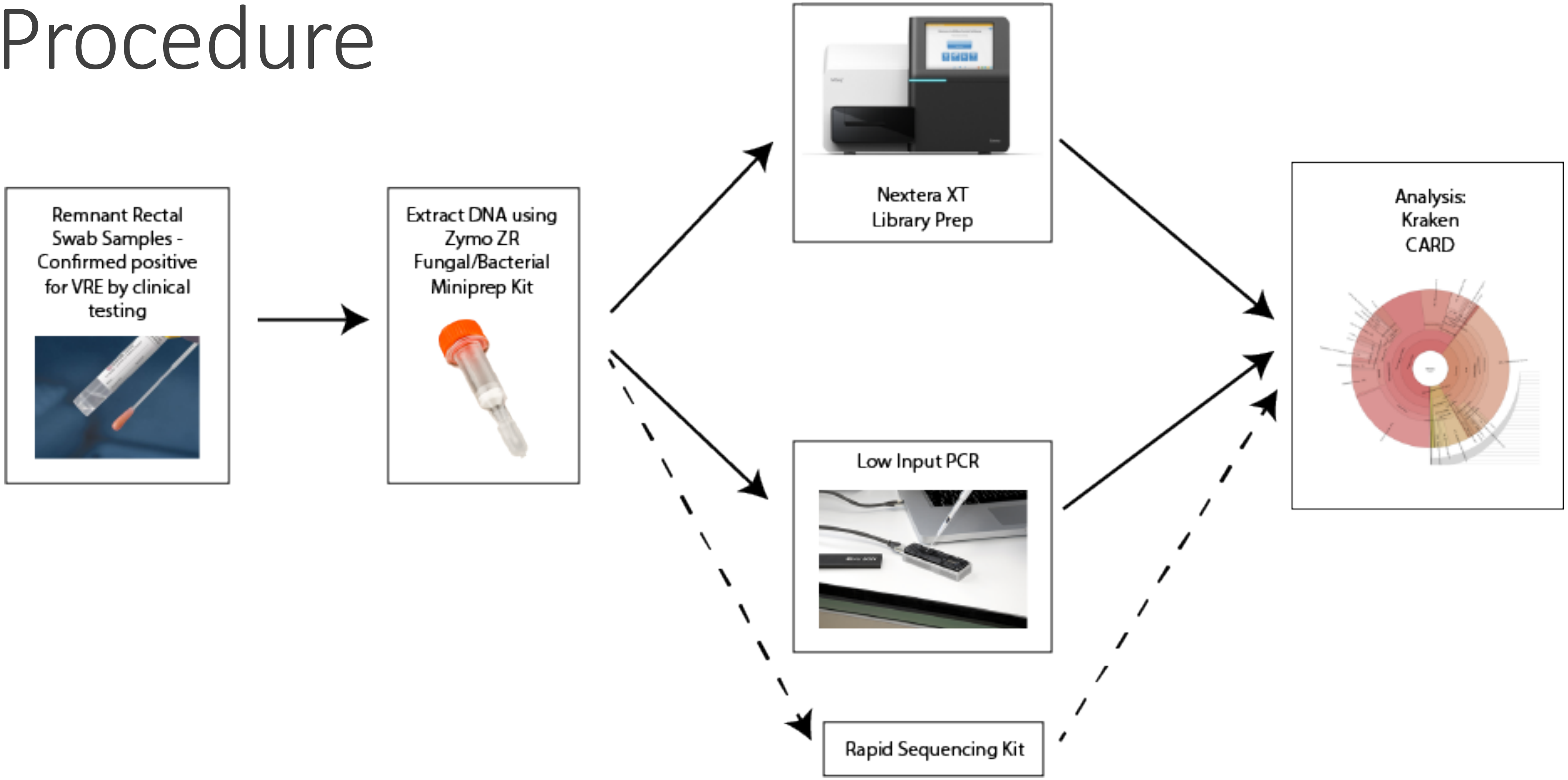
Days: 0, 1, 2, 3-7

	Library Prep Time	Sequencing/ Growth Time	Cost per Sample
Culture	N/A	~24-48 hours	$45*
Illumina Miseq	3-4 hours	~14-16 hours	$400
Oxford Nanopore	~2 hours	~ 8 hours	$650

*Cost is for ChromAgar plate + PCR testing for VRE

TIMELINE COURTESY OF SIMNER,, VRE COSTS FROM HTTP://DX.DOI.ORG/10.1016/J.AJIC.2015.11.008

Procedure



Remnant Rectal Swab Samples - Confirmed positive for VRE by clinical testing

Extract DNA using Zymo ZR Fungal/Bacterial Miniprep Kit

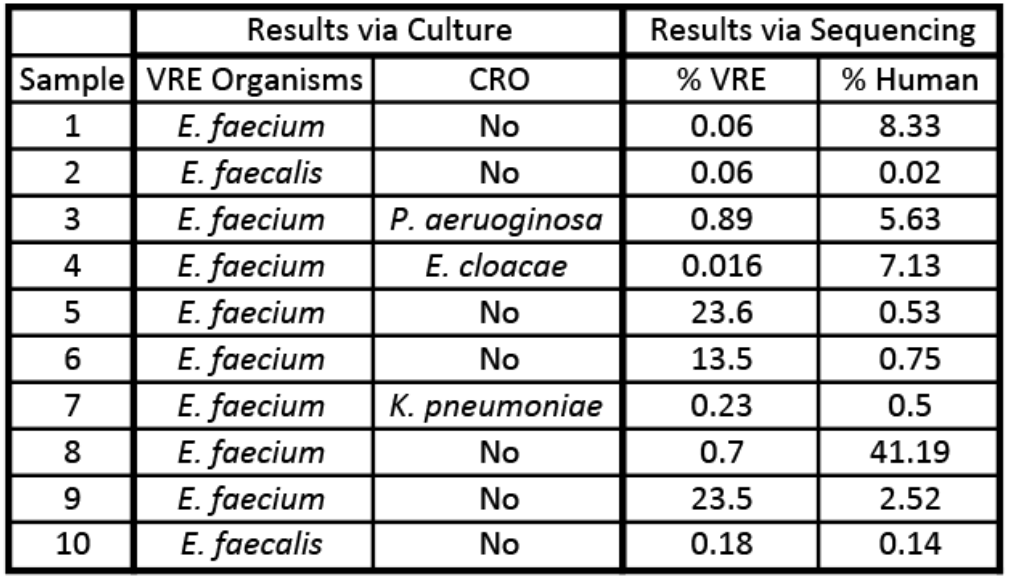
Nextera XT Library Prep

Low Input PCR

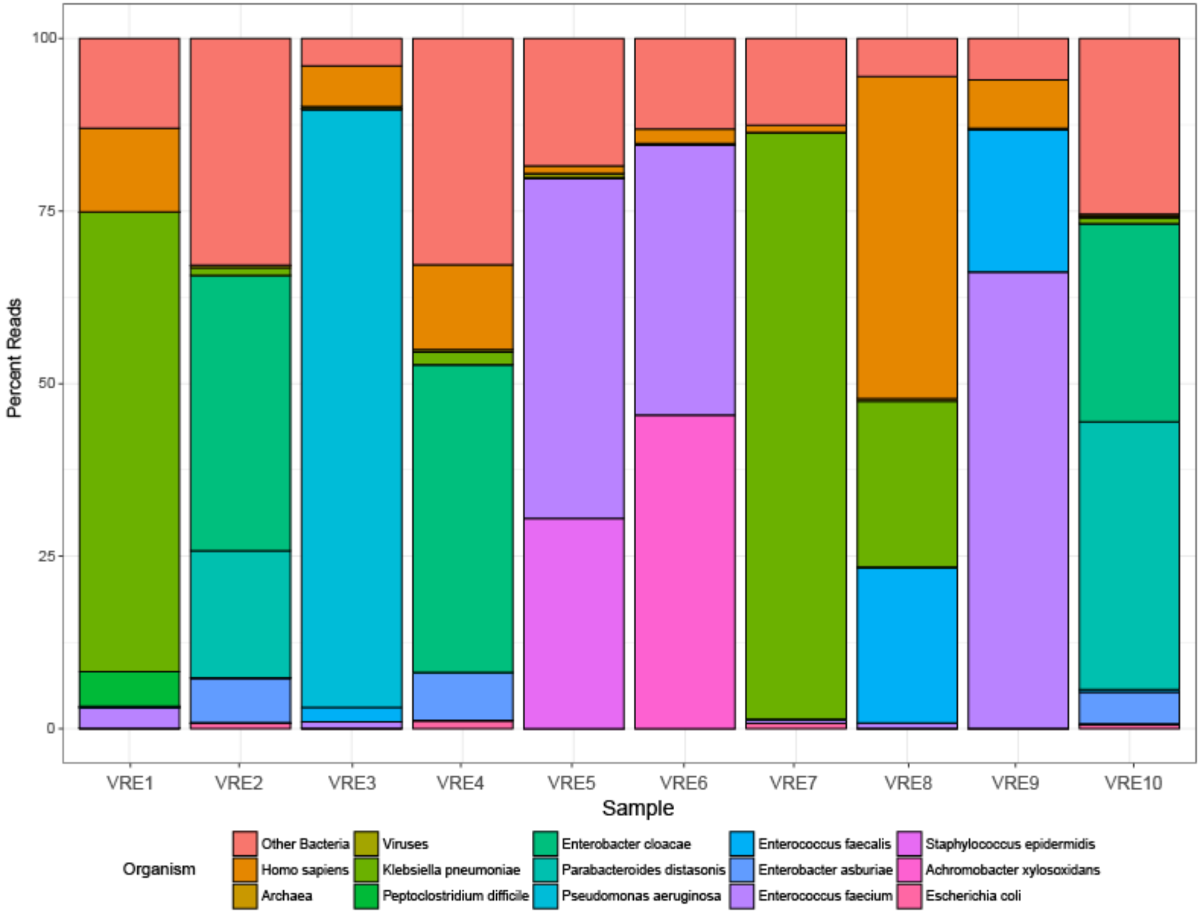
Rapid Sequencing Kit

Analysis:
Kraken
CARD

Organism Distribution



	Results via Culture		Results via Sequencing	
Sample	VRE Organisms	CRO	% VRE	% Human
1	*E. faecium*	No	0.06	8.33
2	*E. faecalis*	No	0.06	0.02
3	*E. faecium*	*P. aeruoginosa*	0.89	5.63
4	*E. faecium*	*E. cloacae*	0.016	7.13
5	*E. faecium*	No	23.6	0.53
6	*E. faecium*	No	13.5	0.75
7	*E. faecium*	*K. pneumoniae*	0.23	0.5
8	*E. faecium*	No	0.7	41.19
9	*E. faecium*	No	23.5	2.52
10	*E. faecalis*	No	0.18	0.14



Organism Distribution - VRE

	Results via Culture		Results via Sequencing	
Sample	VRE Organisms	CRO	% VRE	% Human
1	*E. faecium*	No	0.06	8.33
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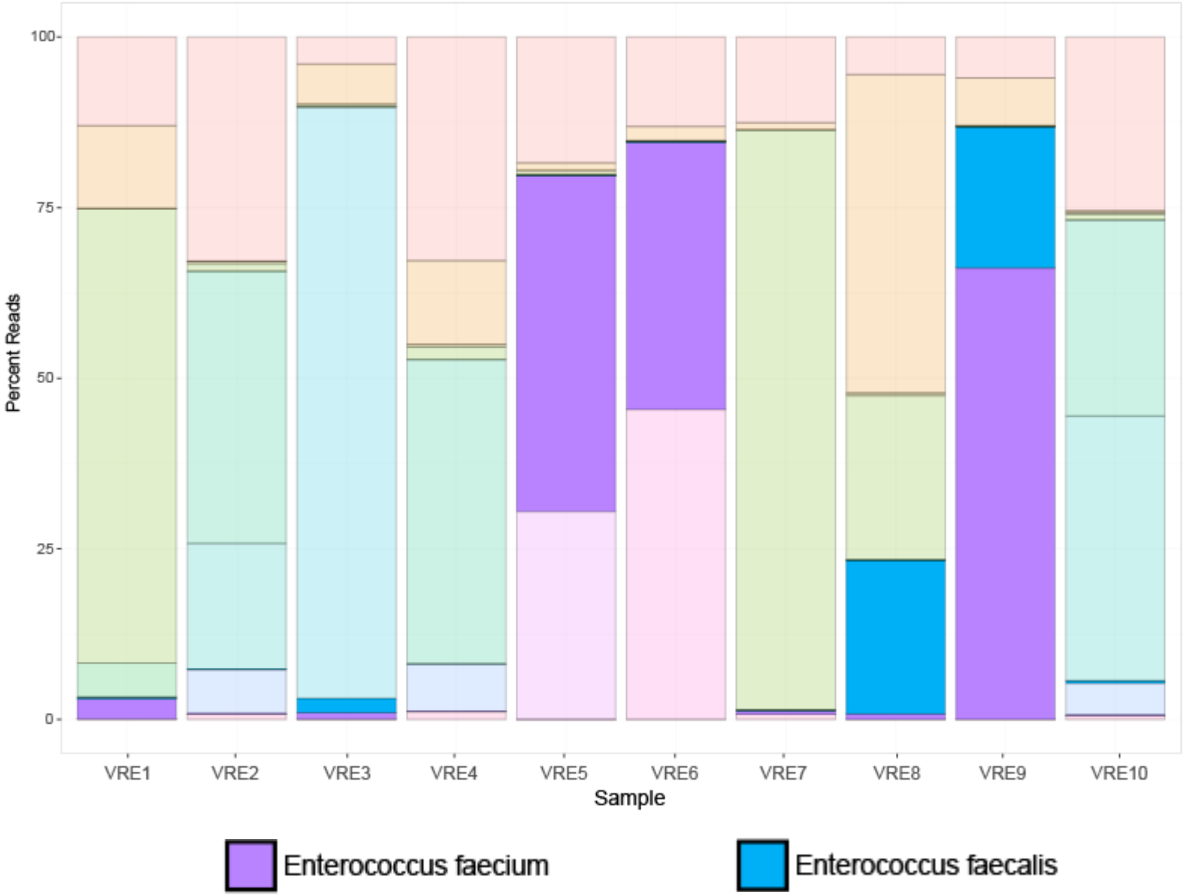
3	*E. faecium*	*P. aeruoginosa*	0.89	5.63
4	*E. faecium*	*E. cloacae*	0.016	7.13
5	*E. faecium*	No	23.6	0.53
6	*E. faecium*	No	13.5	0.75
7	*E. faecium*	*K. pneumoniae*	0.23	0.5
8	*E. faecium*	No	0.7	41.19
9	*E. faecium*	No	23.5	2.52
10	*E. faecalis*	No	0.18	0.14

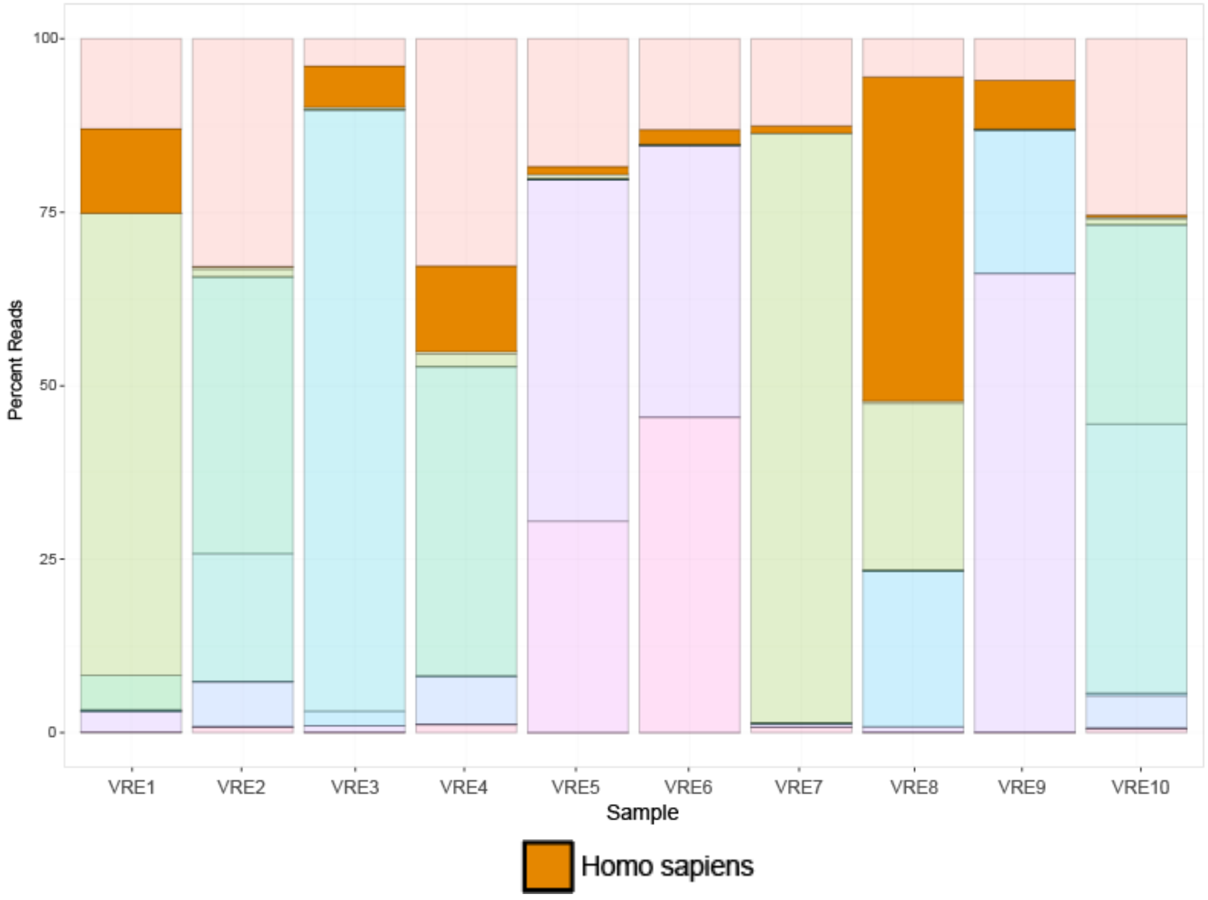
VRE do not generally make up a large part of the microbiome



Organism Distribution - Human

	Results via Culture		Results via Sequencing	
Sample	VRE Organisms	CRO	% VRE	% Human
1	*E. faecium*	No	0.06	8.33
2	*E. faecalis*	No	0.06	0.02
3	*E. faecium*	*P. aeruginosa*	0.89	5.63
4	*E. faecium*	*E. cloacae*	0.016	7.13
5	*E. faecium*	No	23.6	0.53
6	*E. faecium*	No	13.5	0.75
7	*E. faecium*	*K. pneumoniae*	0.23	0.5
8	*E. faecium*	No	0.7	41.19
9	*E. faecium*	No	23.5	2.52
10	*E. faecalis*	No	0.18	0.14

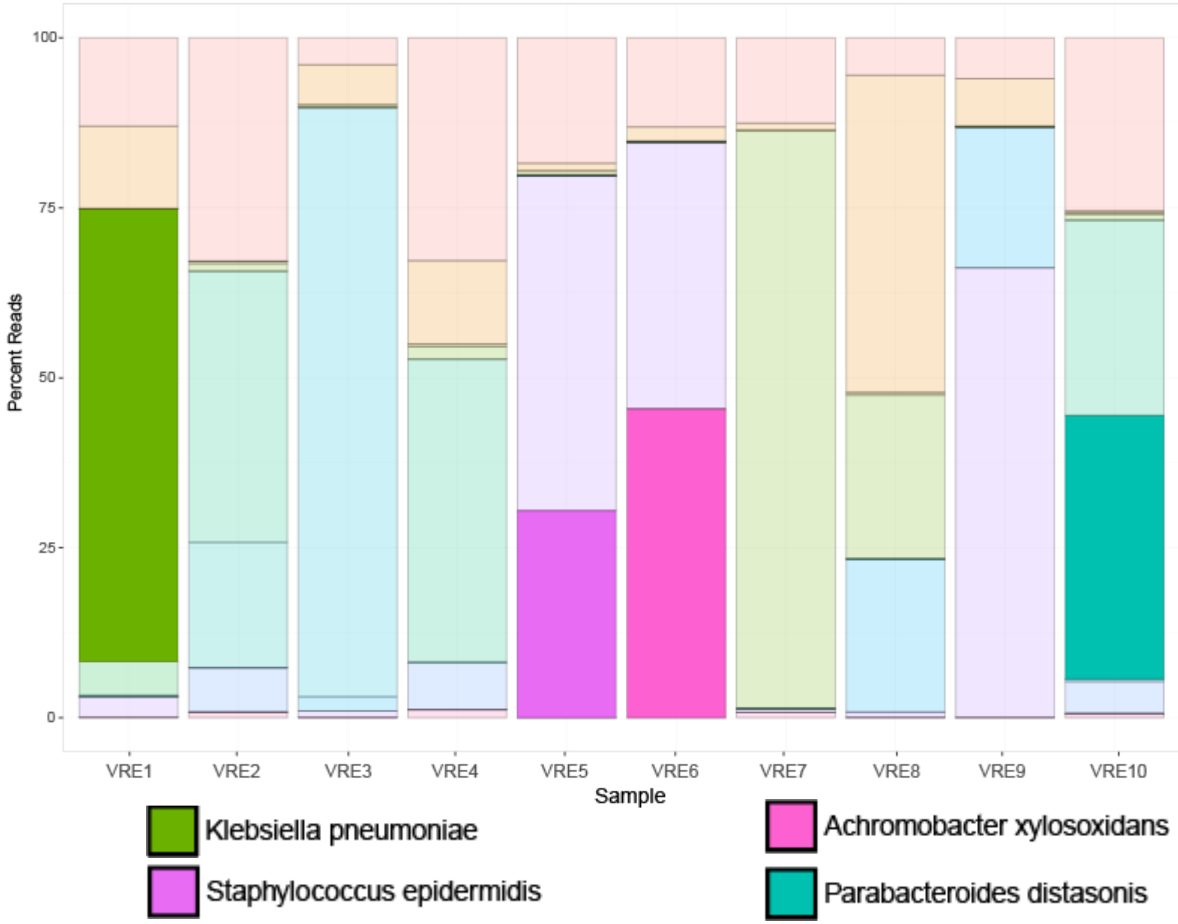
Human contamination levels vary, but are typically low.



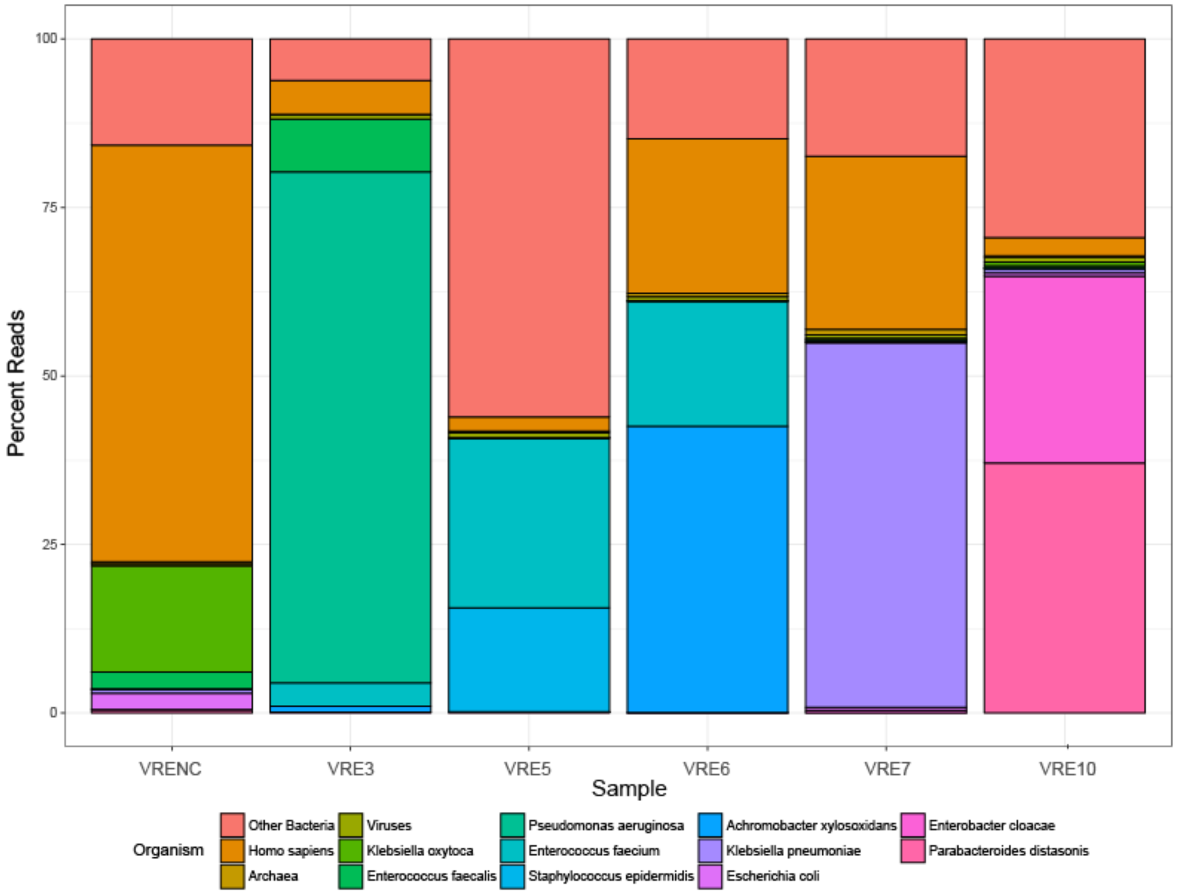
Organism Distribution – Other

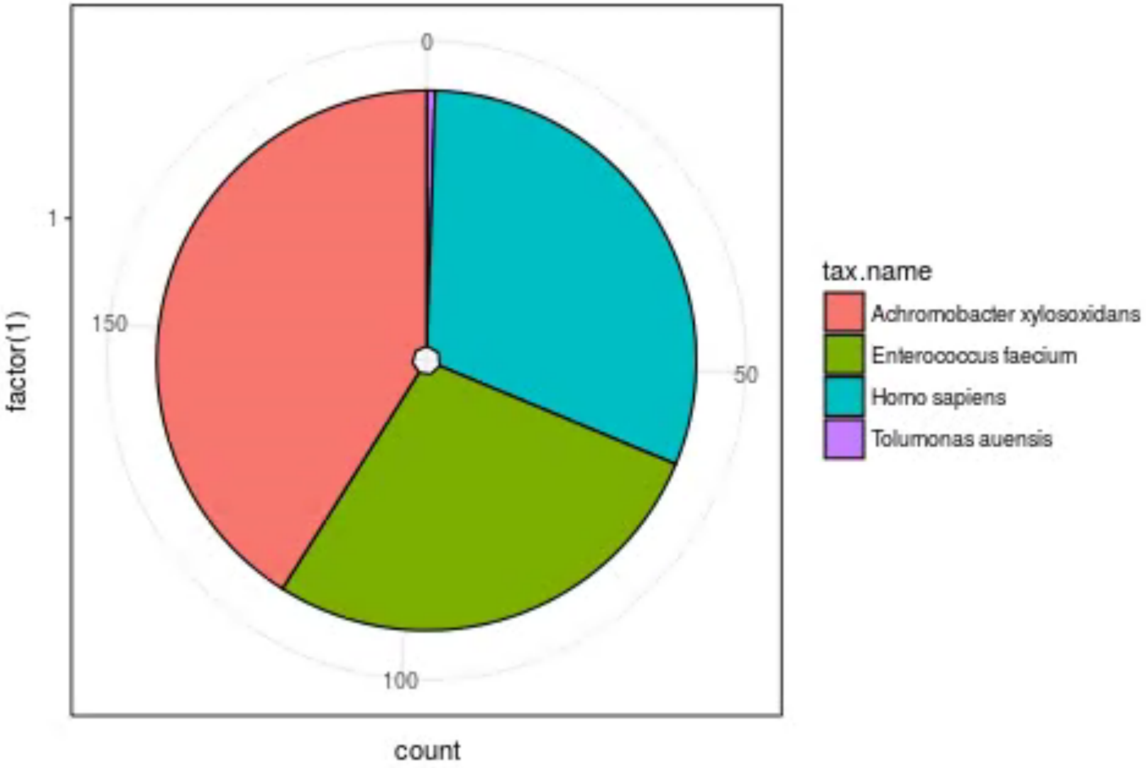
	Results via Culture		Results via Sequencing	
Sample	VRE Organisms	CRO	% VRE	% Human
1	*E. faecium*	No	0.06	8.33
2	*E. faecalis*	No	0.06	0.02
3	*E. faecium*	*P. aeruginosa*	0.89	5.63
4	*E. faecium*	*E. cloacae*	0.016	7.13
5	*E. faecium*	No	23.6	0.53
6	*E. faecium*	No	13.5	0.75
7	*E. faecium*	*K. pneumoniae*	0.23	0.5
8	*E. faecium*	No	0.7	41.19
9	*E. faecium*	No	23.5	2.52
10	*E. faecalis*	No	0.18	0.14

We can get more information using shotgun metagenomics sequencing than just from culture alone

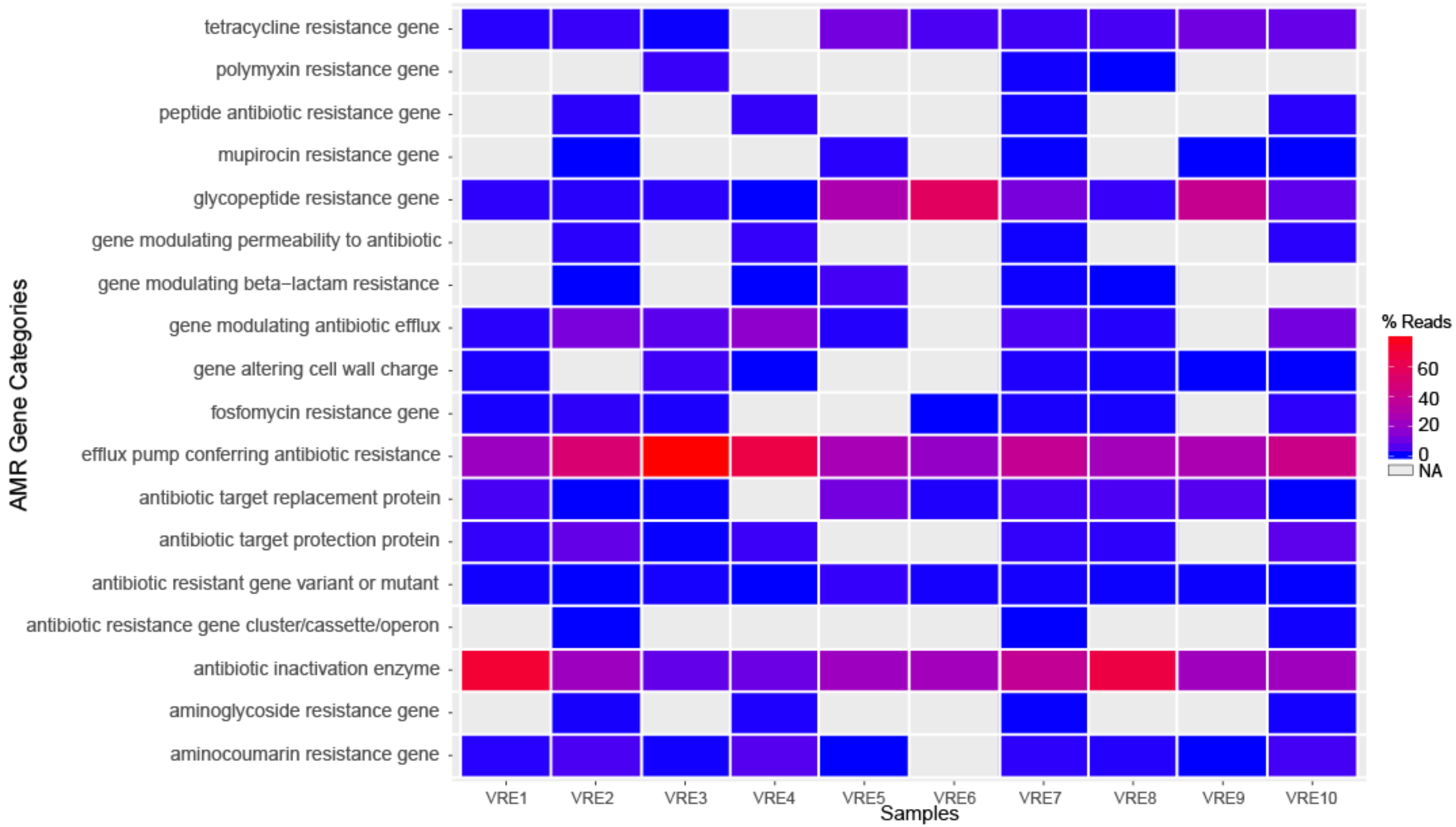


Samples – Nanopore



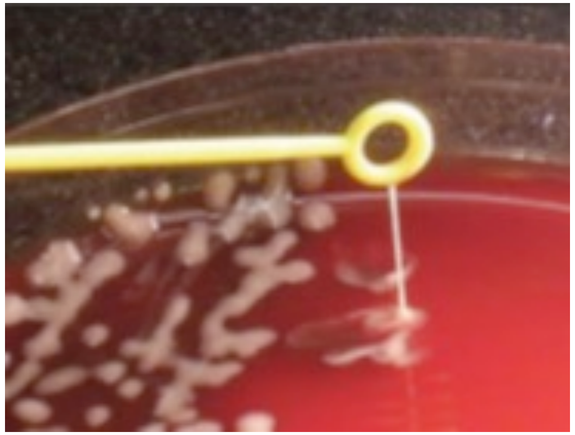


Kraken with customized CARD database



Klebsiella pneumoniae Case Study

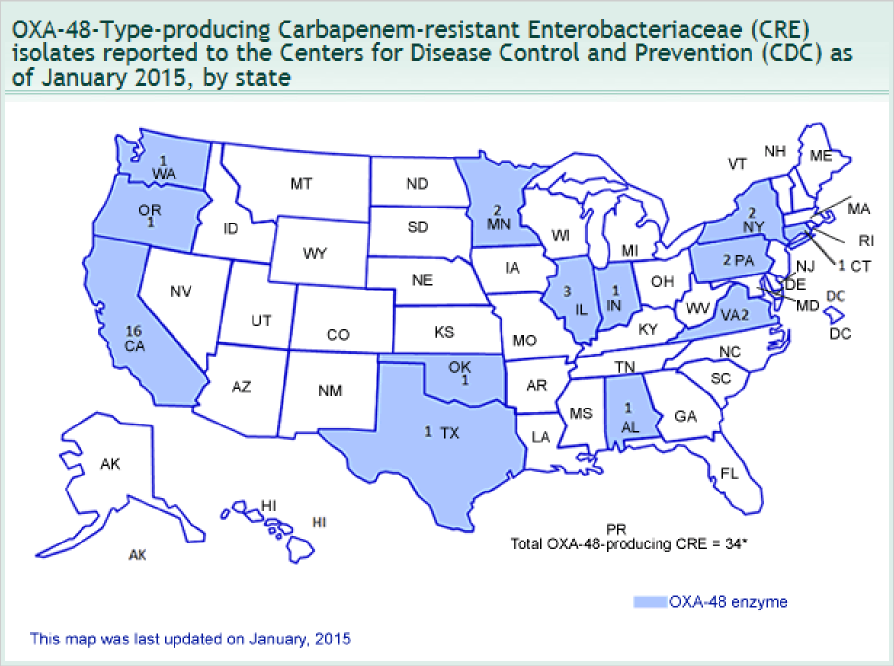
Hypervirulent (hypermucoviscous) *K. pneumoniae*



A new variant of *Klebsiella pneumoniae*

First described in the Asian Pacific Rim 1980s

Now increasingly recognized in other countries



OXA-48-Type-producing Carbapenem-resistant Enterobacteriaceae (CRE) isolates reported to the Centers for Disease Control and Prevention (CDC) as of January 2015, by state

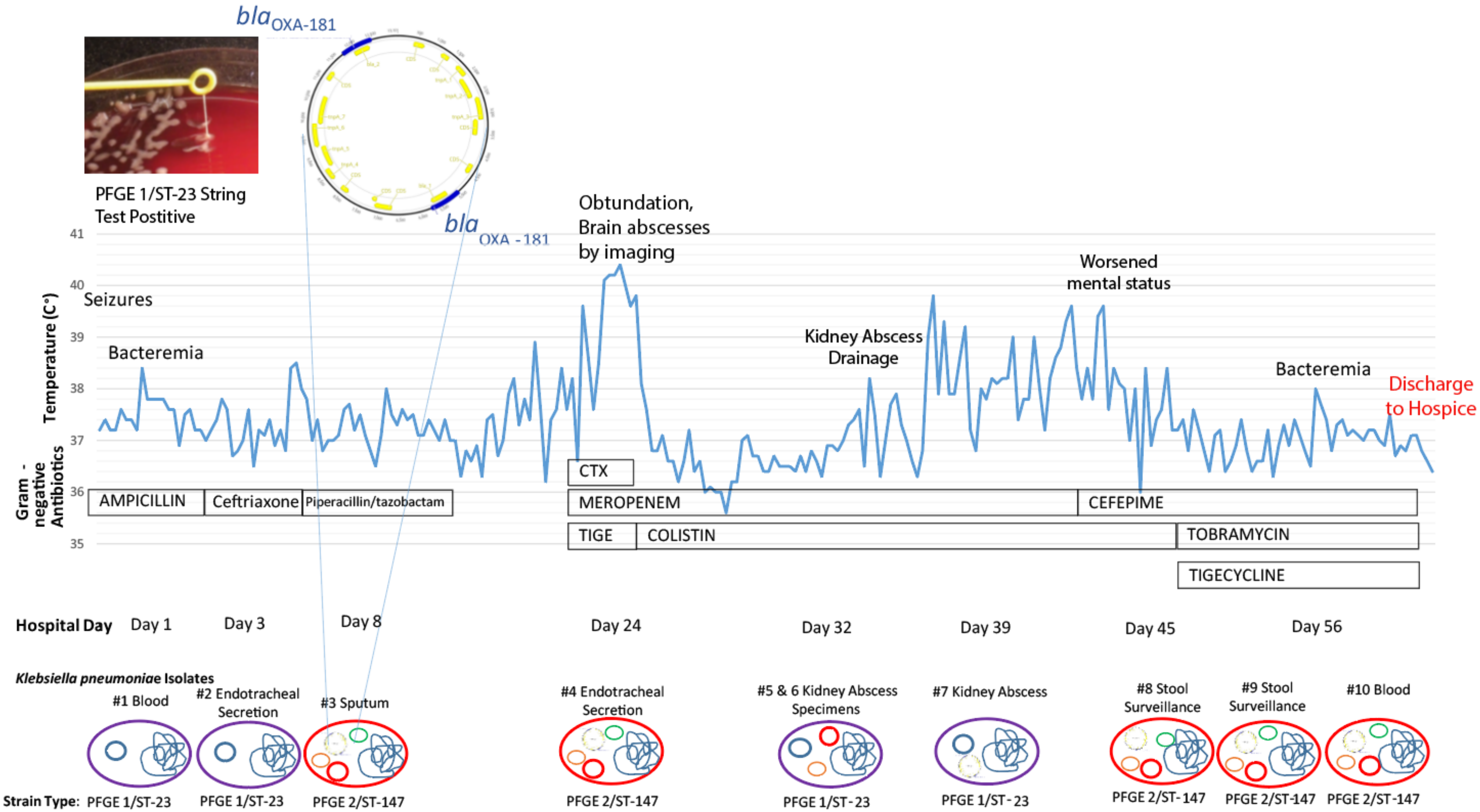
Total OXA-48-producing CRE = 34*

▨ OXA-48 enzyme

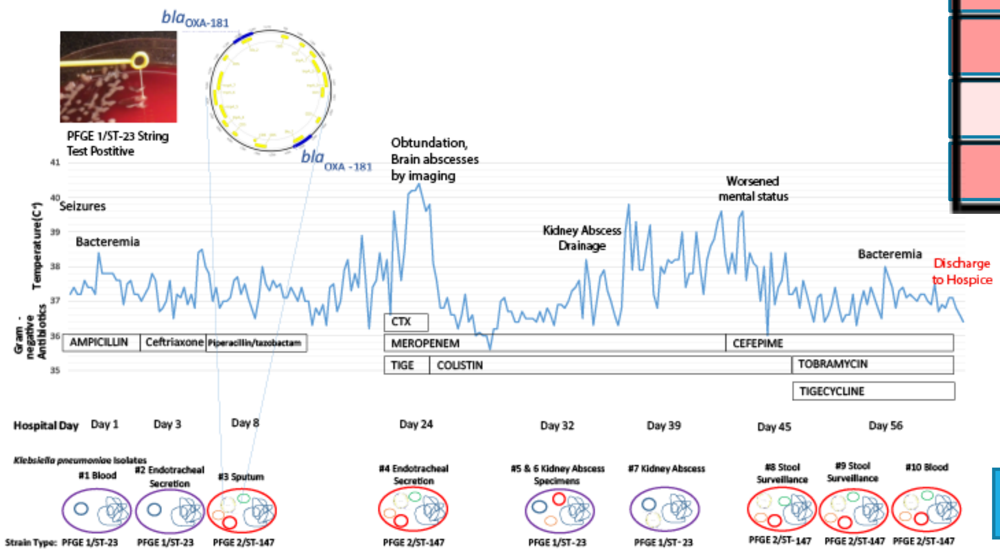
This map was last updated on January, 2015

- 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


*bla*OXA-181
PFGE 1/ST-23 String Test Postitive
bla OXA -181
Obtundation, Brain abscesses by imaging
Worsened mental status
Seizures
Bacteremia
Kidney Abscess Drainage
Bacteremia
Discharge to Hospice
Temperature (C°)
Gram - negative Antibiotics
41
40
39
38
37
36
35
AMPICILLIN
Ceftriaxone
Piperacillin/tazobactam
CTX
MEROPENEM
CEFEPIME
TIGE
COLISTIN
TOBRAMYCIN
TIGECYCLINE
Hospital Day
Day 1
Day 3
Day 8
Day 24
Day 32
Day 39
Day 45
Day 56
Klebsiella pneumoniae Isolates
#1 Blood
#2 Endotracheal Secretion
#3 Sputum
#4 Endotracheal Secretion
#5 & 6 Kidney Abscess Specimens
#7 Kidney Abscess
#8 Stool Surveillance
#9 Stool Surveillance
#10 Blood
Strain Type:
PFGE 1/ST-23
PFGE 1/ST-23
PFGE 2/ST-147
PFGE 2/ST-147
PFGE 1/ST-23
PFGE 1/ST-23
PFGE 2/ST-147
PFGE 2/ST-147
PFGE 2/ST-147

- Two strains – HMV and XDR
- 9 isolates sequenced on both platforms



Isolate	Hospital Day	Source	Resistance	Sequencer
1	1	Blood	No	Illumina and Nanopore
2	3	Endo/Nasal	No	Illumina and Nanopore
3	8	Sputum	Yes	Illumina Only
4	24	Endo/Nasal	Yes	Illumina and Nanopore
5	32	Kidney Abcess	No	Illumina Only
6	32	Kidney Abcess	No	Illumina and Nanopore
7	39	Kidney Abcess	No	Illumina and Nanopore
8	45	Stool	Yes	Illumina and Nanopore
9	45	Stool	Yes	Illumina and Nanopore
10	56	Blood	Yes	Illumina and Nanopore
11	50	Room	Yes	Illumina Only
12	50	Room	Yes	Illumina and Nanopore

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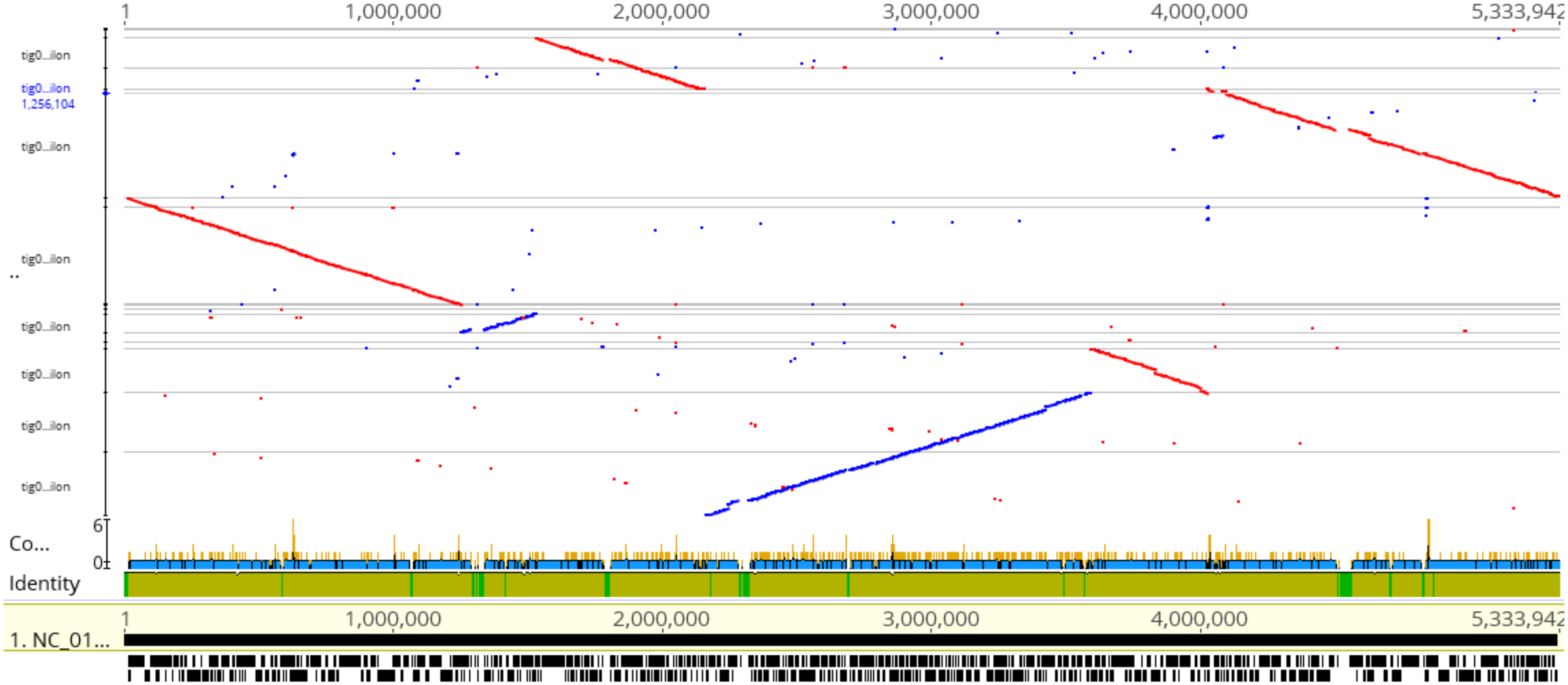
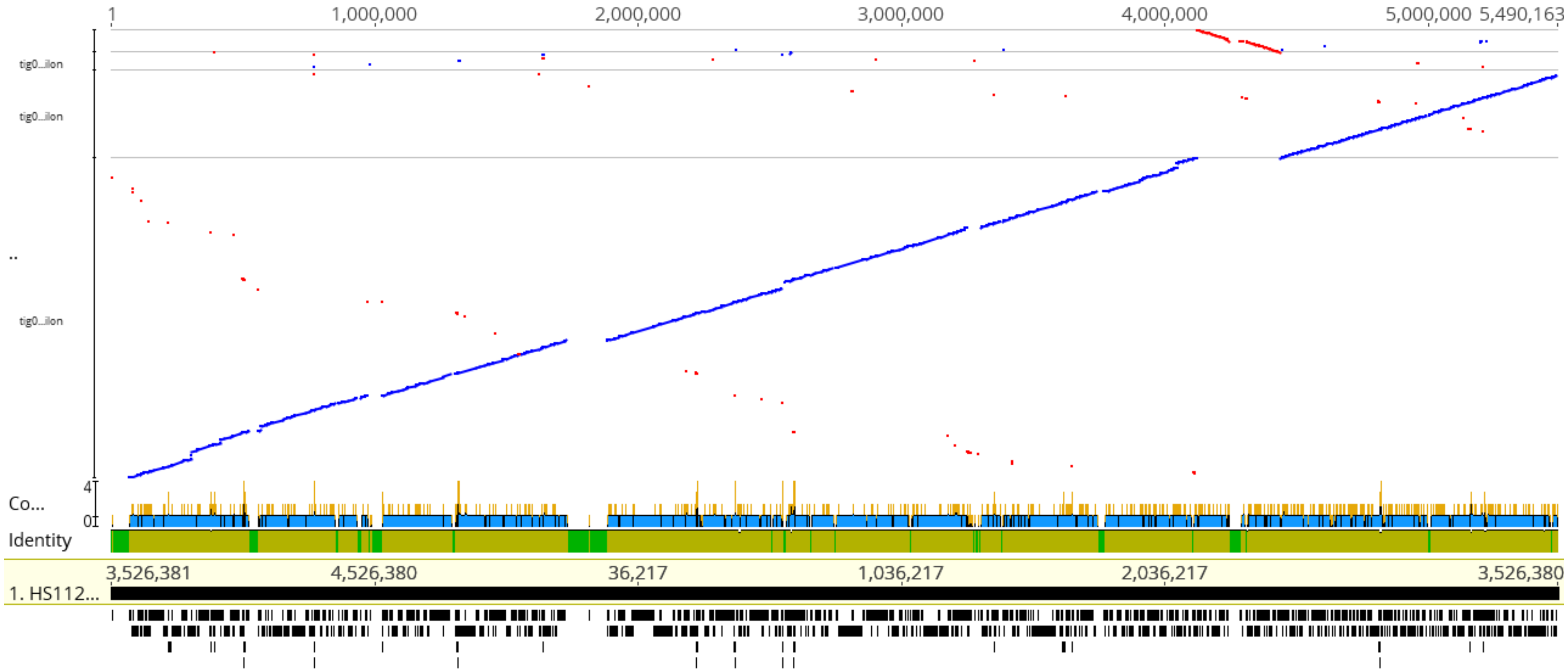
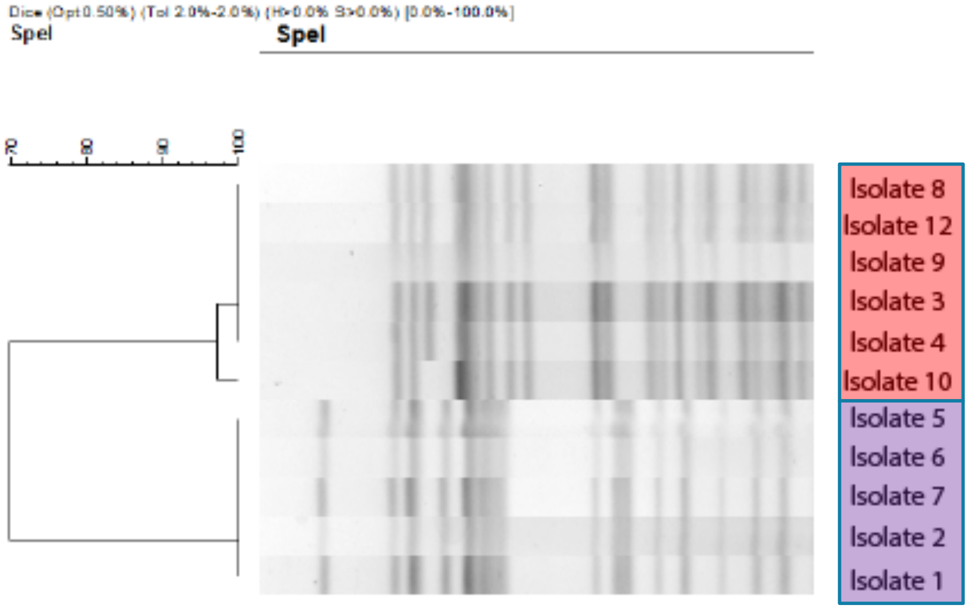
Assembly with canu

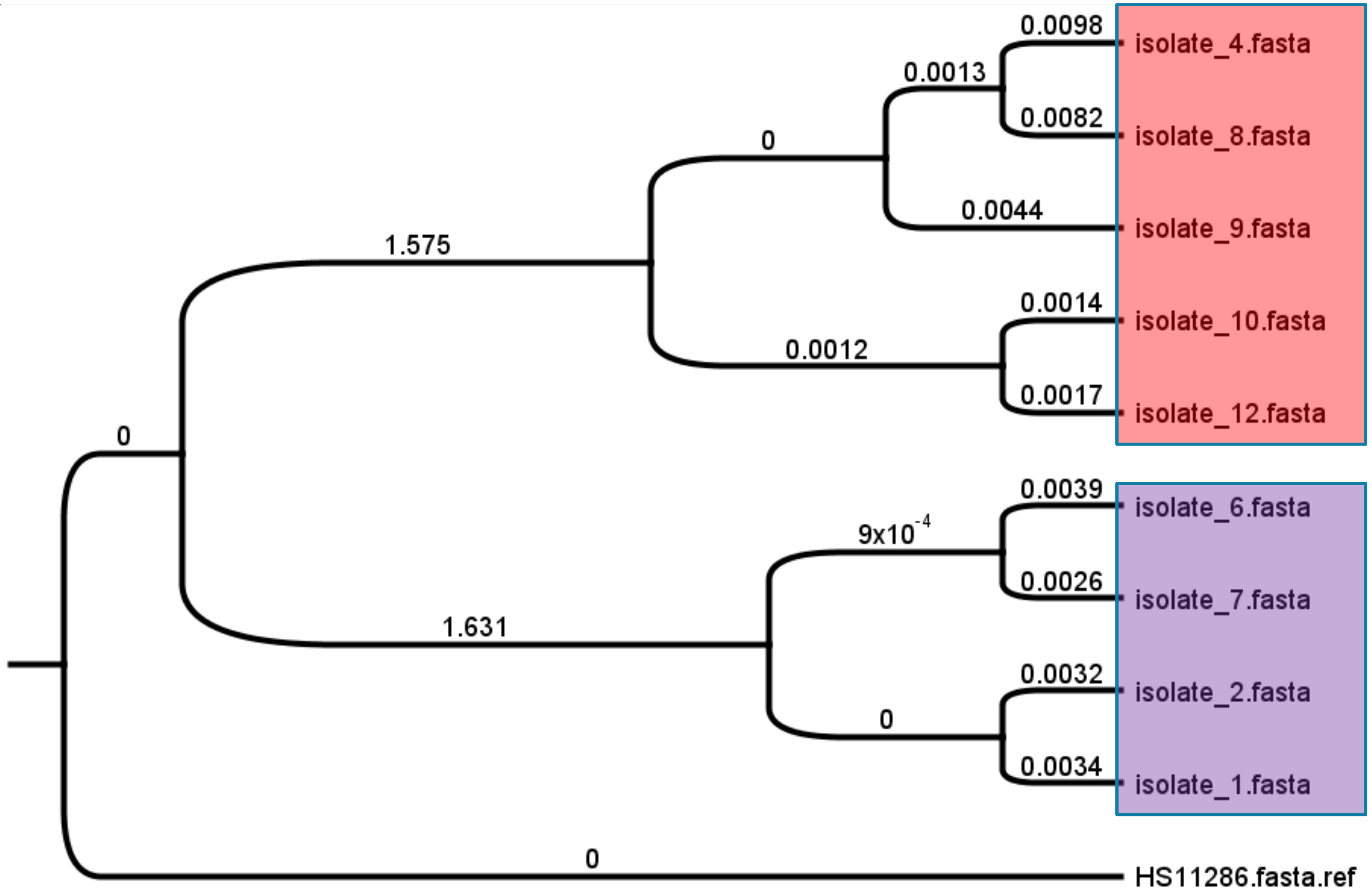


Assembly with sequencing from v9.4 flowcell

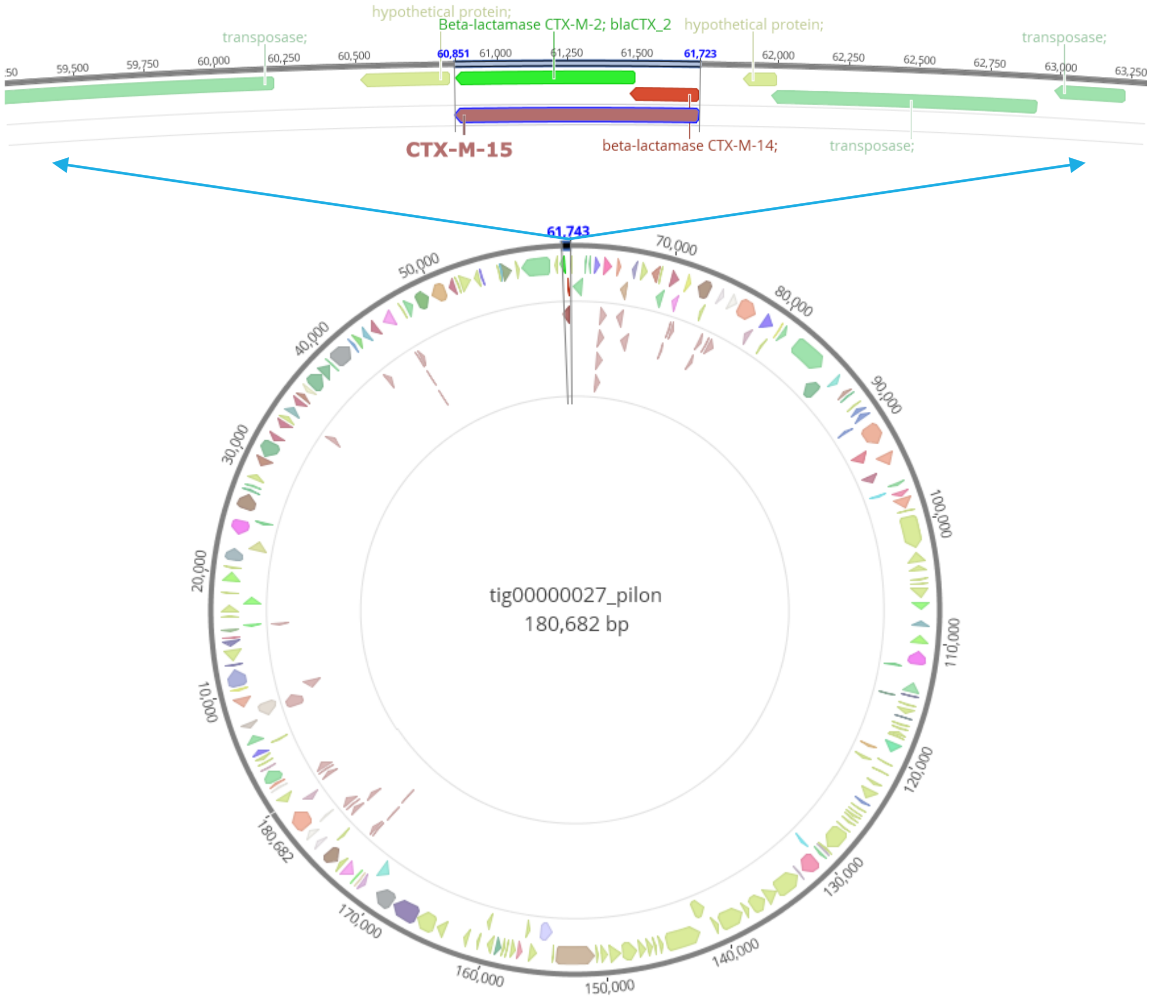


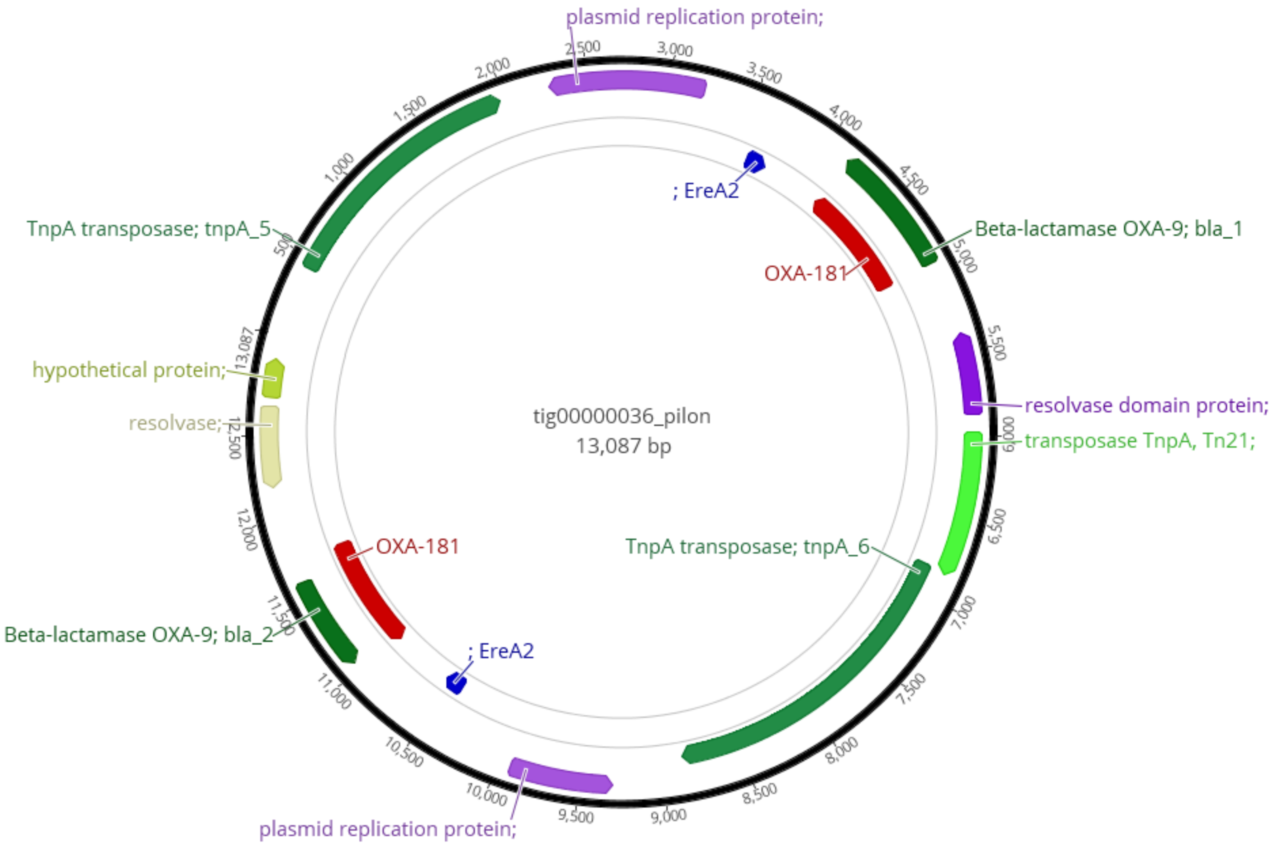
Nanopore Tree



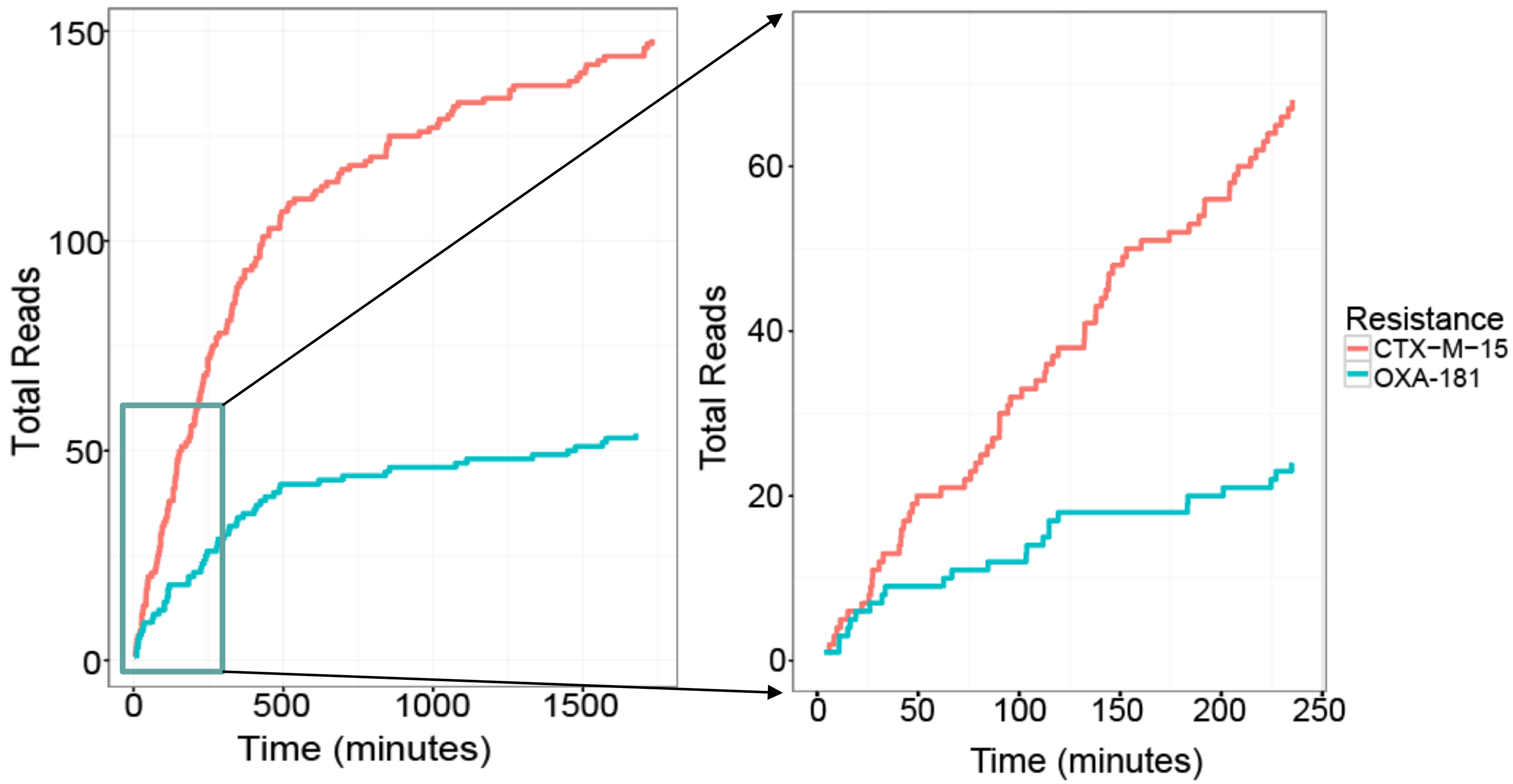


Genes of Interest





Antibiotic Resistance Detection



Sample	OXA-181 detection time	CTX-M-15 detection time
4	**4.8 min**	**2.8 min**
8	**10.3 min**	**3.6 min**
9	**10.73 min**	**9.37 min**
10	**0.99 min**	**1.00 min**
12	**13.07 min**	**6.04 min**

Resistance identified within 15 minutes!

Conclusions

Sequencing can provide more insight into environmental context of organisms than just culture alone

Nanopore sequencing could be useful as an aid in providing proper treatment for infectious diseases

- Get full coverage of pathogenic organisms with one flowcell
- Rapid time of detection of genes of interest

Acknowledgements

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- James Gurtowski

Salzberg Lab

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