Otago Spotlight Series

Infectious Disease Research

New Sequencing Technologies for Infectious Diseases

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&

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NEW ZEALAND

'Antibiotic apocalypse': doctors sound alarm over drug resistance

The terrifying prospect that even routine operations will be impossible to perform has been raised by experts alarmed by the rise of drug-resistant genes

Resistance to the Antibiotic of Last Resort Is Silently Spreading

Just over a year after they were discovered in China, bacteria that can fend off colistin are being found all across the world.

SARAH ZHANG | JAN 12, 2017 | HEALTH

Antibiotics will soon stop working causing 'terrible human and economic cost'

It will soon be too dangerous to perform caesareans, joint replacements and chemotherapy if antibiotics become ineffective, major report warns

NEWS / EBOLA

WHO: DRC Ebola outbreak on a 'knife's edge' as urban cases rise

Efforts under way in Democratic Republic of Congo in bid to prevent Ebola outbreak spreading across borders.

NEW ZEALAND / HEALTH

Drug-resistant bug infects burns patients

6:43 am on 7 February 2018

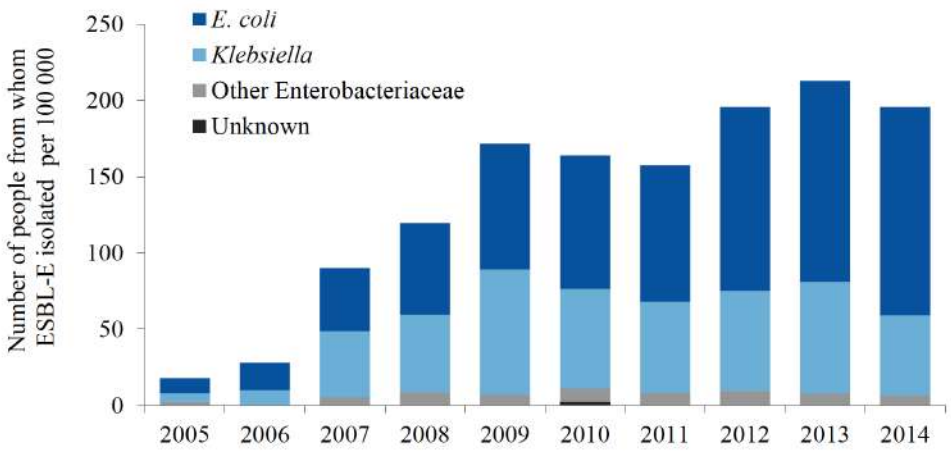
Share this

An outbreak of a multi-drug resistant organism at Middlemore Hospital's National Burns Centre has infected three already very sick patients.

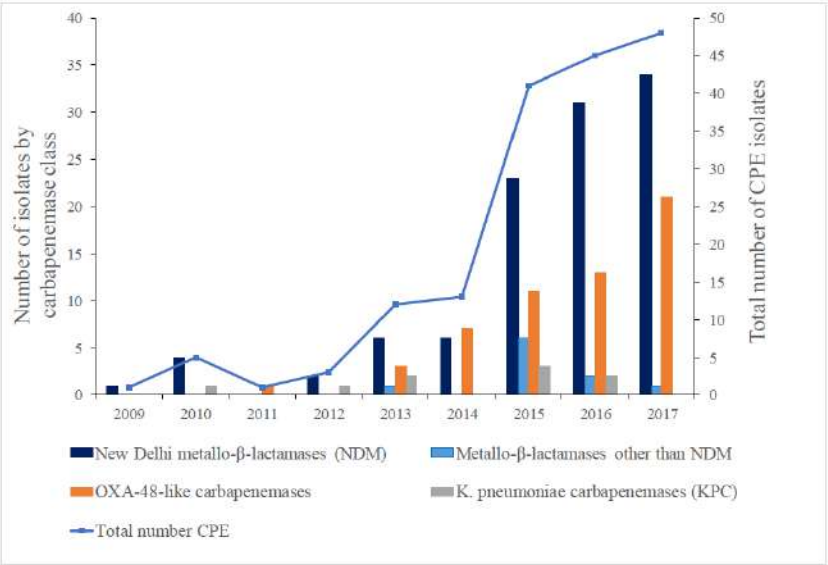
Canterbury superbug patients a 'frightening sign'

Antibiotic resistance: a 21st century nightmare

ESBL *Enterobacteriaceae*



Carbapenem-resistant *Enterobacteriaceae*



Relative risk of death with ESBL bacteraemia: 1.85

J Antimicrob Chemo 2007

Attributable mortality: 50%
Mortality risk ratio: 3.3

Infect Control Hosp Epidemiol 2009

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

CrossMark click for updates

Detection of the *mcr-1* Colistin Resistance Gene in Carbapenem-Resistant *Enterobacteriaceae* from Different Hospitals in China

Hua Yu,[a] Fen Qu,[b] Bin Shan,[c] Bin Huang,[d] Wei Jia,[e] Cha Chen,[f] Aiqing Li,[g] Minhui Miao,[g] Xin Zhang,[a] Chunmei Bao,[b] Yunmin Xu,[c] Kalyan D. Chavda,[h] Yi-Wei Tang,[i] Barry N. Kreiswirth,[h] Hong Du,[g] Liang Chen[h]

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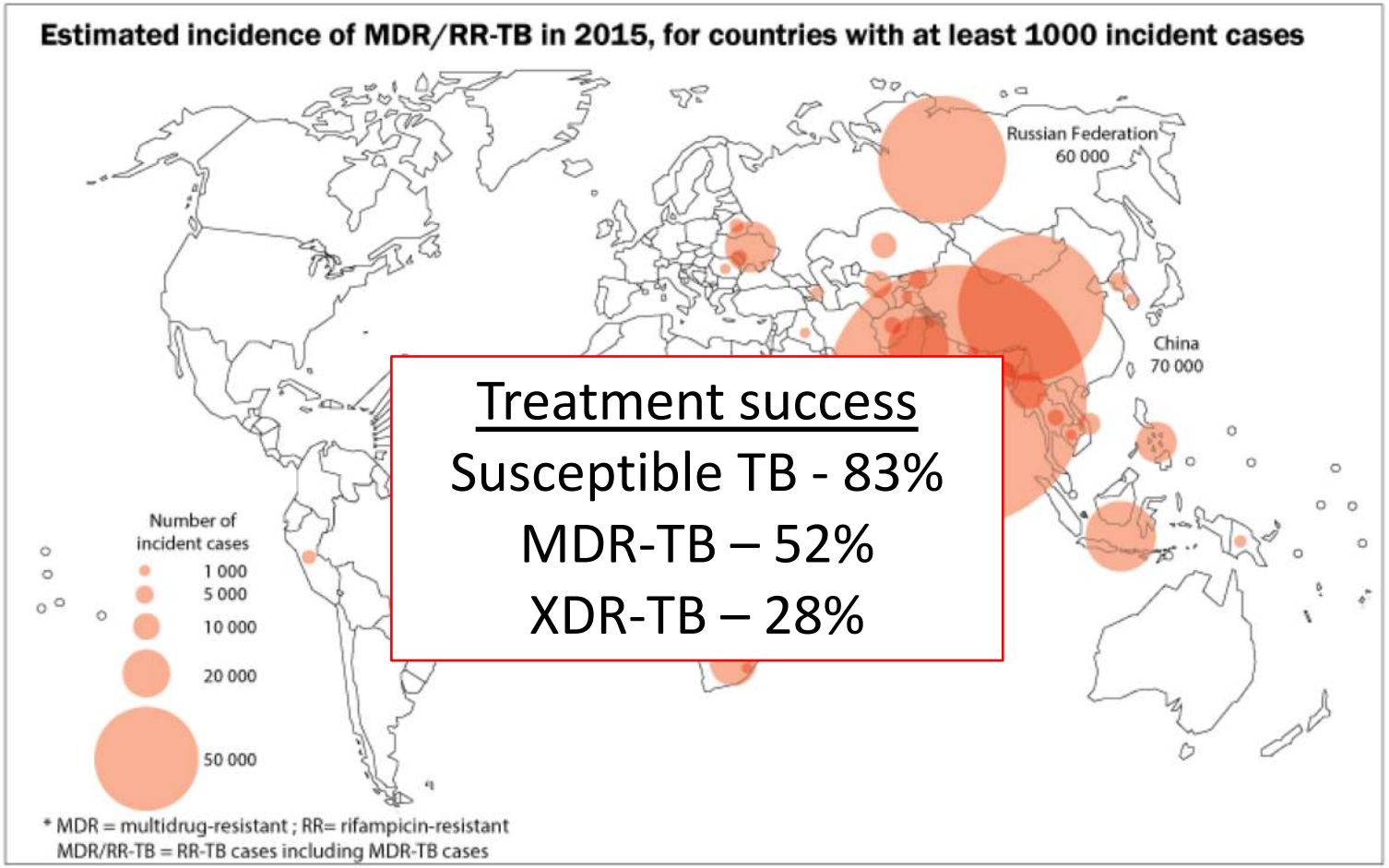
OBSERVATION

CrossMark

Colistin- and Carbapenem-Resistant *Escherichia coli* Harboring *mcr-1* and bla_{NDM-5}, Causing a Complicated Urinary Tract Infection in a Patient from the United States

José R. Mediavilla,[a] Amee Patrawalla,[b] Liang Chen,[a] Kalyan D. Chavda,[a] Barun Mathema,[c] Christopher Vinnard,[a] Lisa L. Dever,[d] Barry N. Kreiswirth[a]

Public Health Research Institute Tuberculosis Center, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA[a]; Division of Pulmonary and Critical Care Medicine, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA[b]; Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA[c]; Division of Infectious Diseases, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA[d]



Treatment success

Susceptible TB - 83%

MDR-TB – 52%

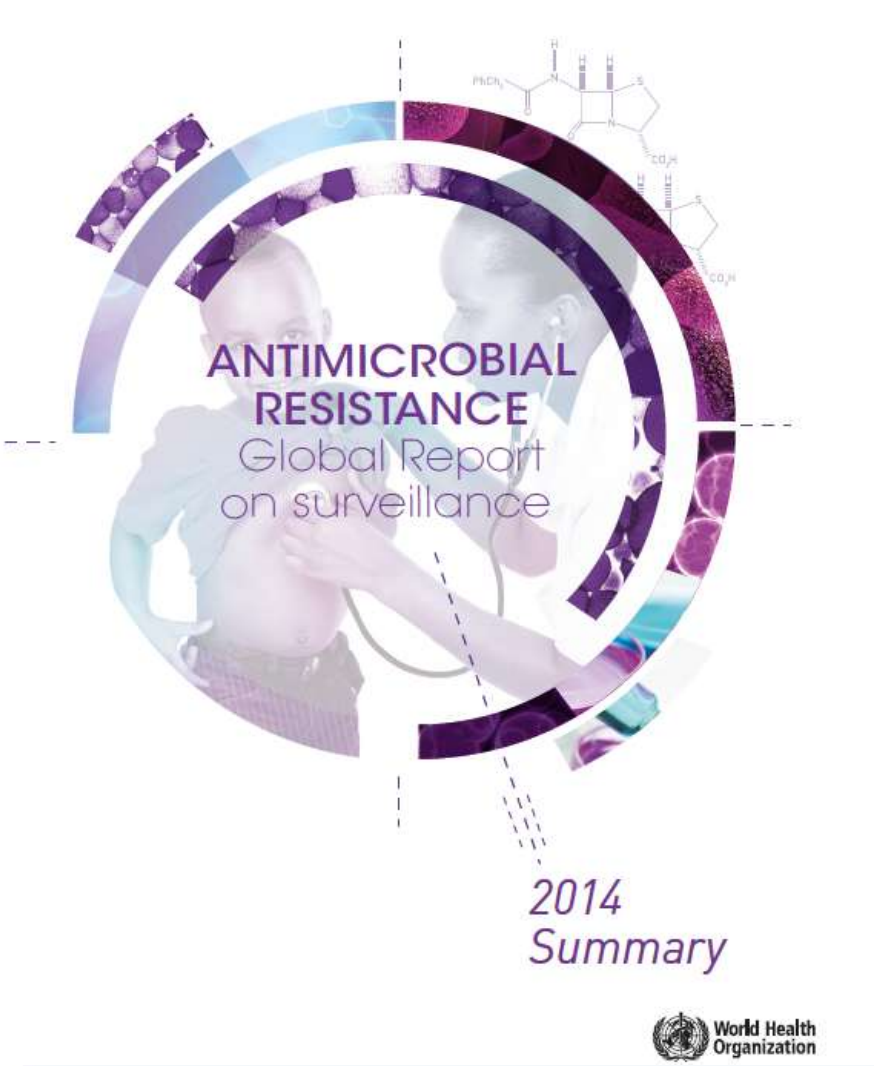
XDR-TB – 28%

10.4 million new cases of tuberculosis and 1.4 million deaths in 2015

Multidrug resistant (MDR): ~480,000 cases + 250,000 deaths

Extensively drug resistant (XDR): ~9.5% of MDR cases

WHO: Global tuberculosis report 2016



"The problem is so serious that it threatens the achievements of modern medicine. A post-antibiotic era— in which common infections and minor injuries can kill—is a very real possibility for the 21st century."

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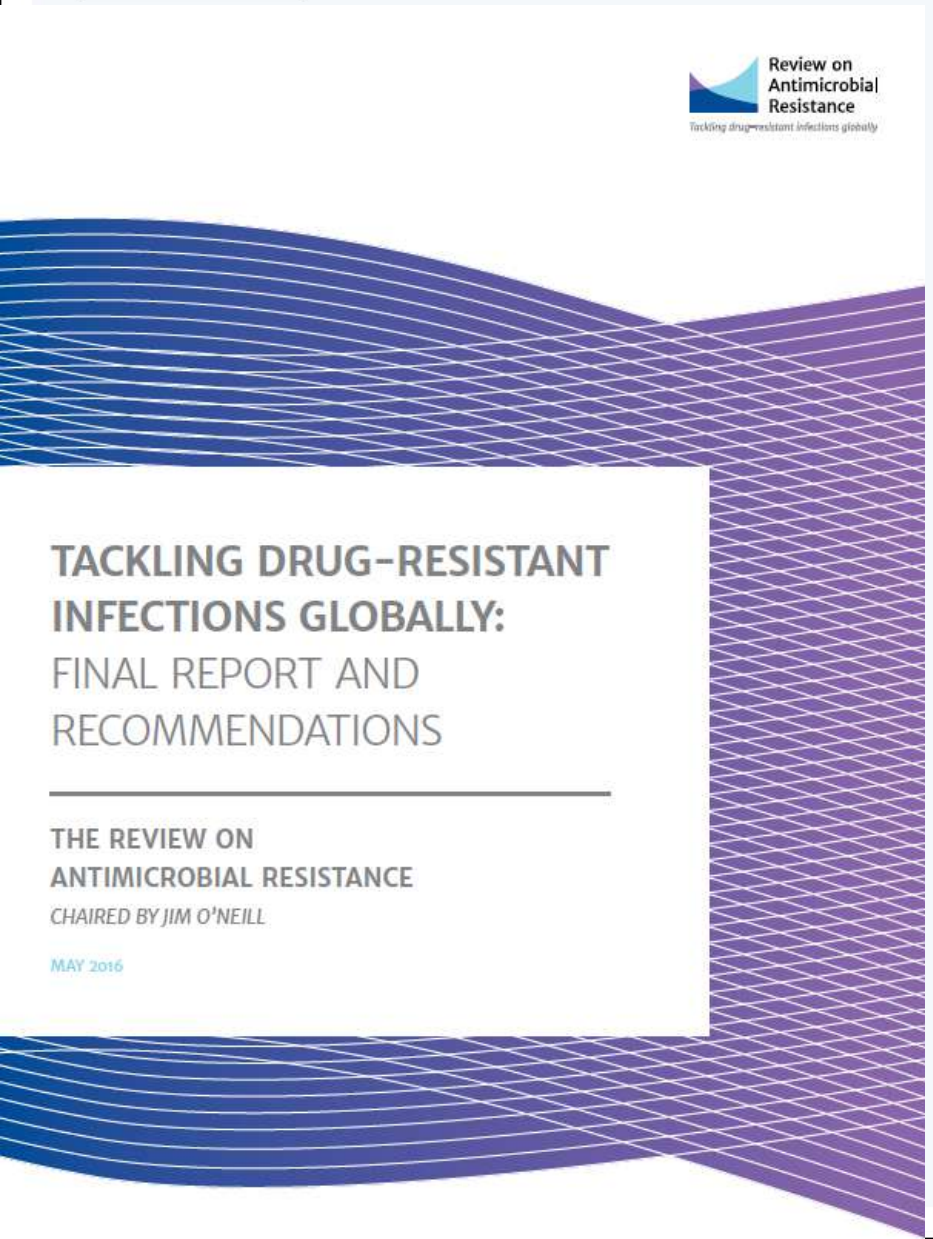

"We estimate that by 2050, 10 million lives a year and a cumulative 100 trillion USD of economic output are at risk due to the rise of drug-resistant infections if we do not find proactive solutions now to slow down the rise of drug resistance. Even today, 700,000 people die of resistant infections every year."

TACKLING ANTIMICROBIAL RESISTANCE ON TEN FRONTS

- Public awareness
- Sanitation and hygiene
- Antibiotics in agriculture and the environment
- Vaccines and alternatives
- Surveillance
- Rapid diagnostics
- Human capital
- Drugs
- Global Innovation Fund
- International coalition for action

Review on Antimicrobial Resistance

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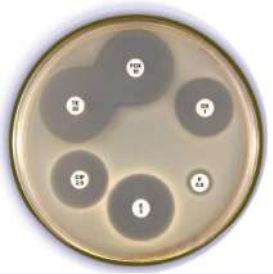
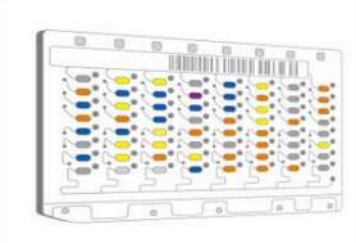
Diagnostic Laboratory

Identification



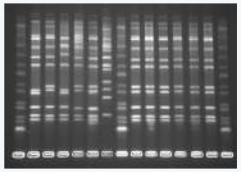
Susceptibility testing

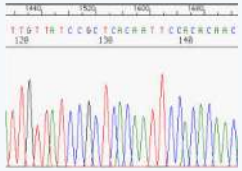


Identification and susceptibility testing

Individual patient management

Public Health Laboratory





Typing

Public health surveillance

Infection control

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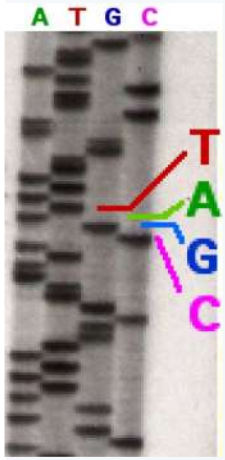
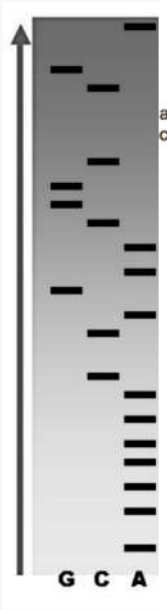
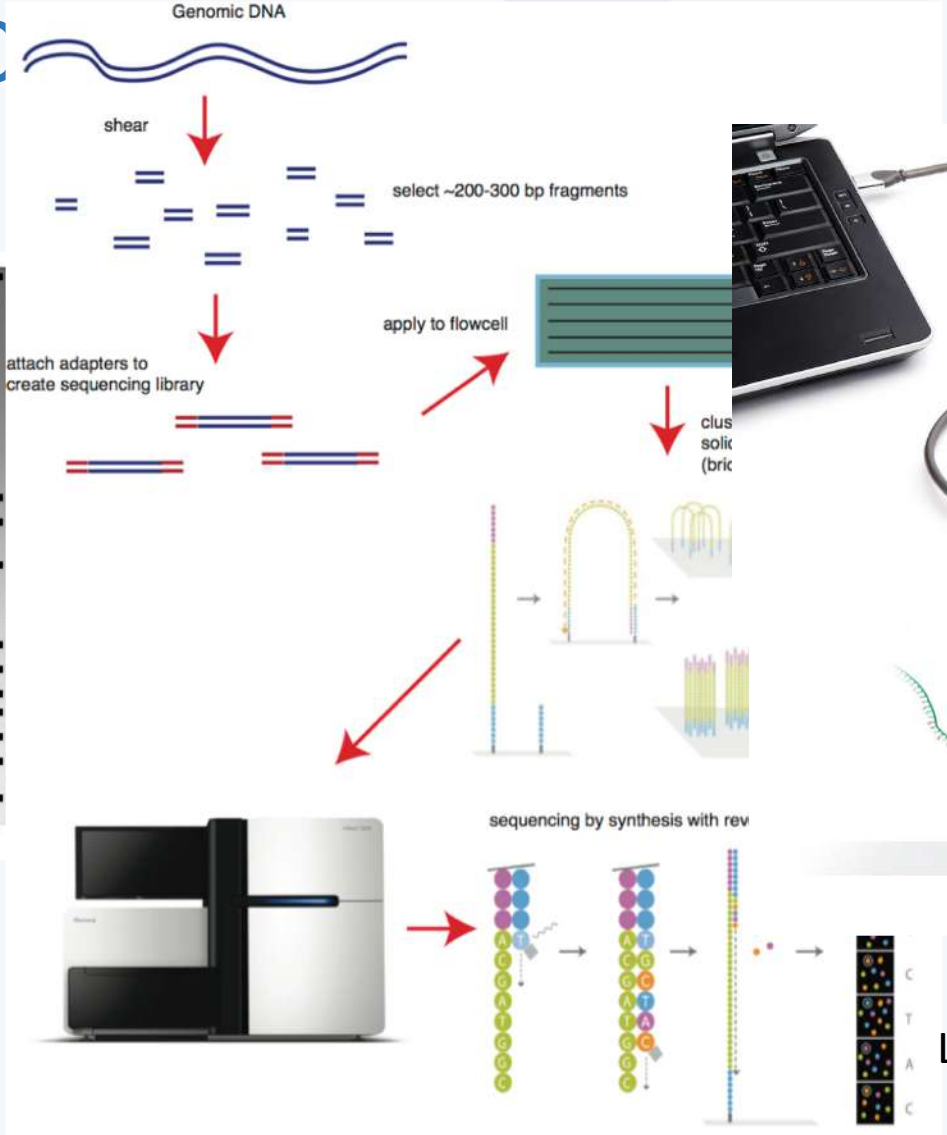
C chnology





1970s

Sanger sequencing

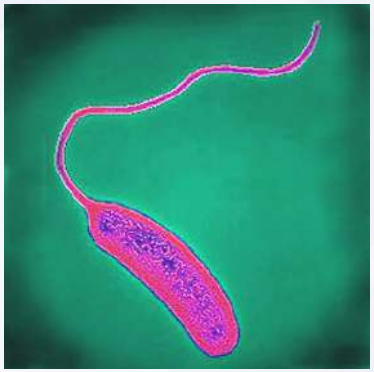


2007
Short read, massively parallel sequencing
(Illumina)

2014/5
Long read sequencing
(Oxford Nanopore)

Sanger F et al. PNAS 1977;74:5463-7
Strauss et al, Anal Biochem. 1986;154:353-60

Pace of change in sequencing technology



Size	3 billion base-pairs	4 million base-pairs
Cost (2001)	$4,100,000,000	$1,000,000
Time (2001)	10 years	18 months
Cost (2018)	$1,500	$150
Time (2018)	1-10 days	1-2 days

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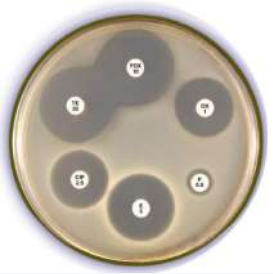
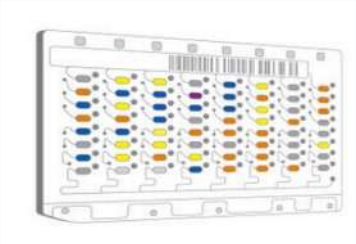
Diagnostic Laboratory

Identification



Susceptibility testing



Identification and susceptibility testing

Individual patient management

Public Health Laboratory

<u>Whole genome sequencing:</u>
typing

Public health surveillance

Infection control

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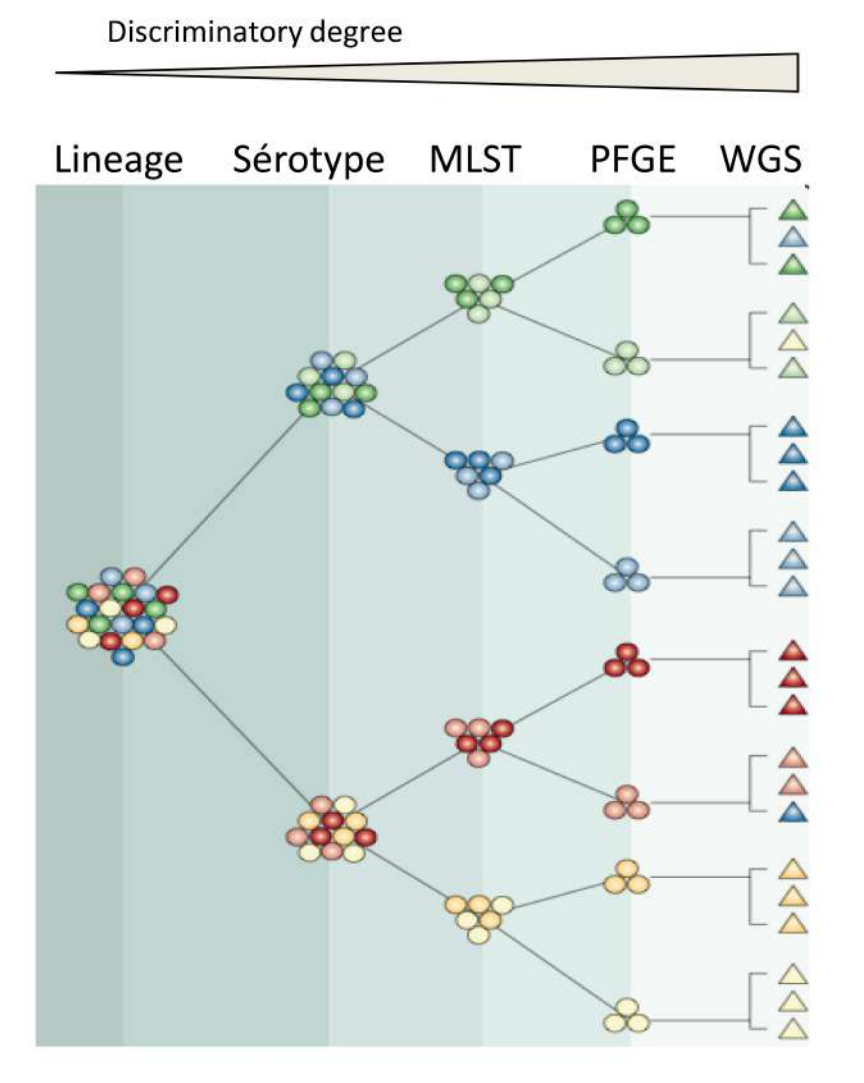
Molecular typing

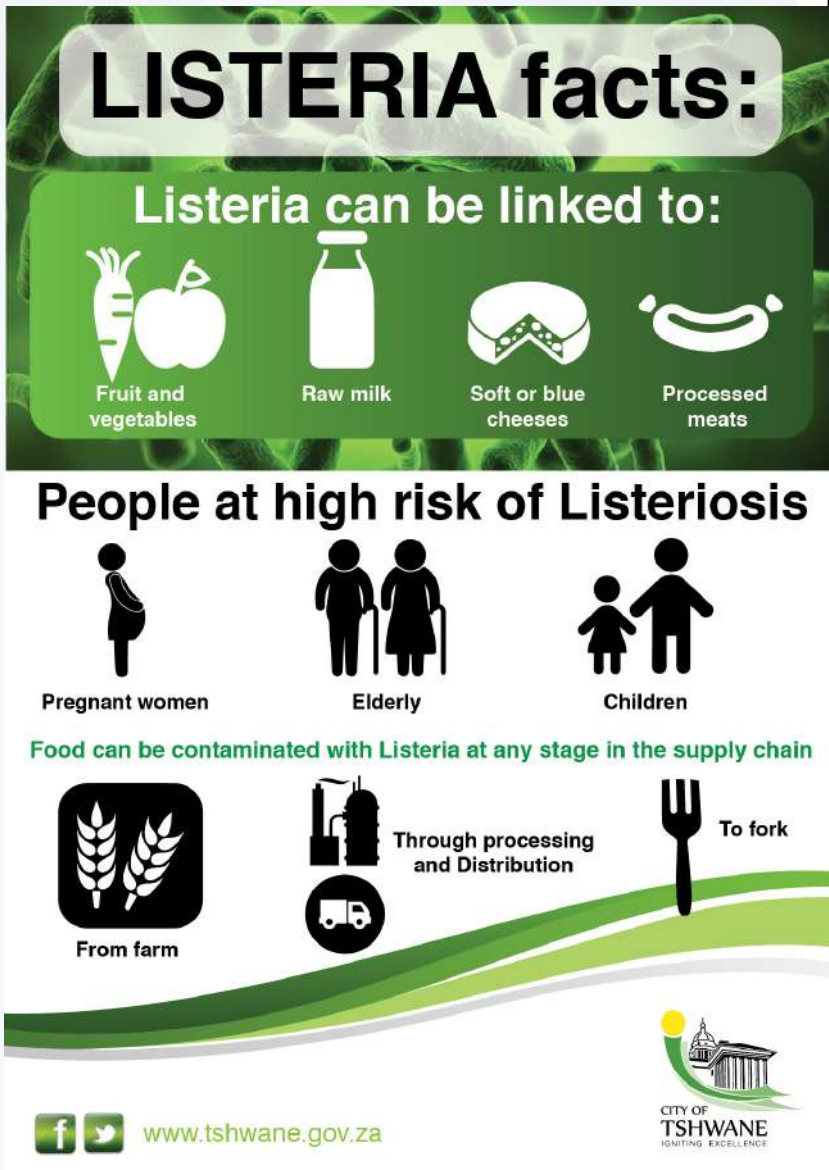
- Identify phylogenetic relationships to identify clusters/outbreaks
  - Hospital
  - Community
- Traditional methods lack resolution and accuracy

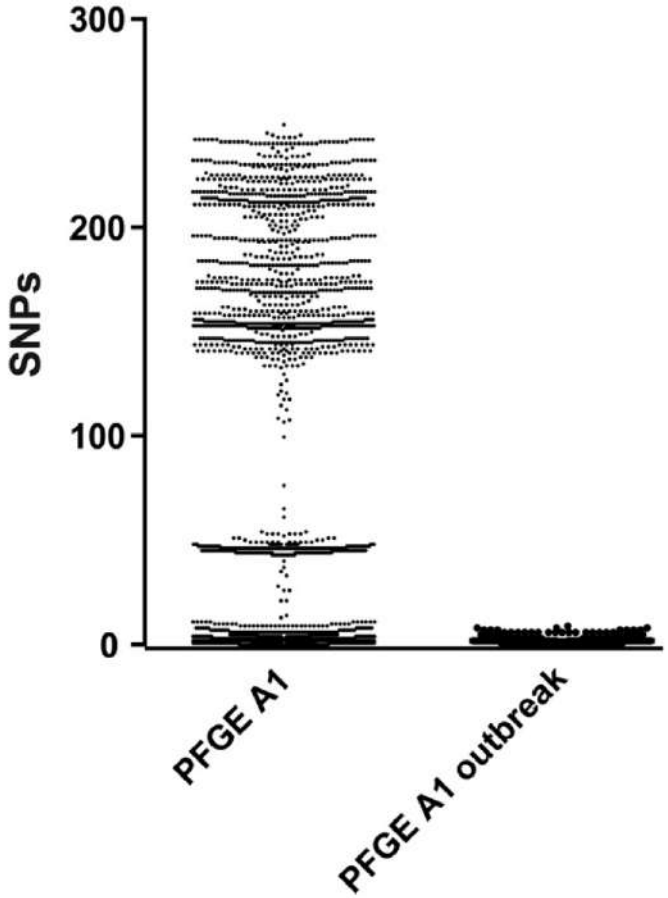


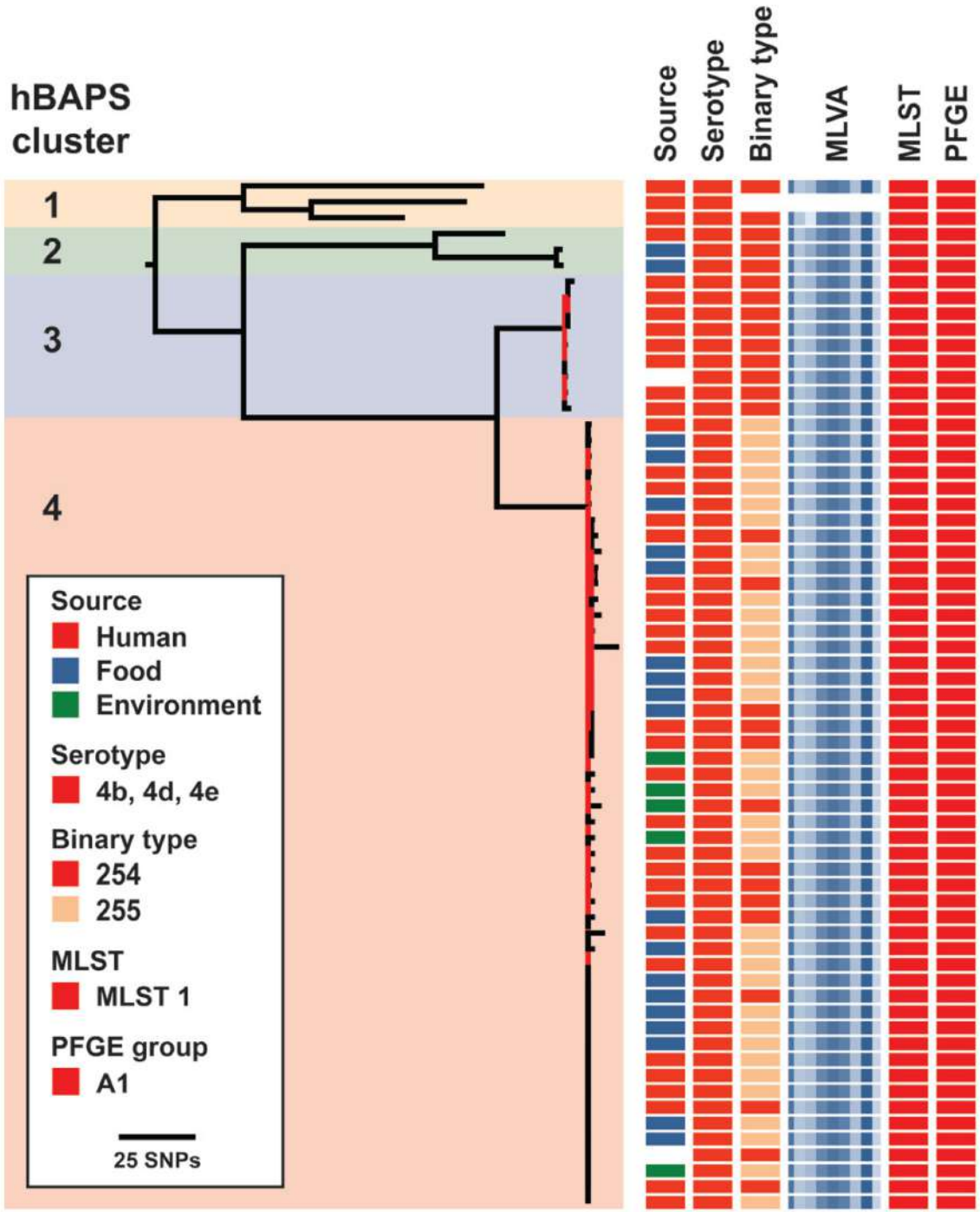
Sophie Roussel, Anses, Maisons- Alfort; France

Listeriosis

- *Listeria monocytogenes*
- Foodborne-illness
  - US: 19% of deaths from foodborne-illness
- Outbreaks
  - Difficult to solve:
    - Often small
    - Long incubation period
    - High mortality
- Only a small proportion of cases linked to recognised outbreaks
- Molecular subtyping allows identification of clusters
  - PFGE lacks discriminatory ability







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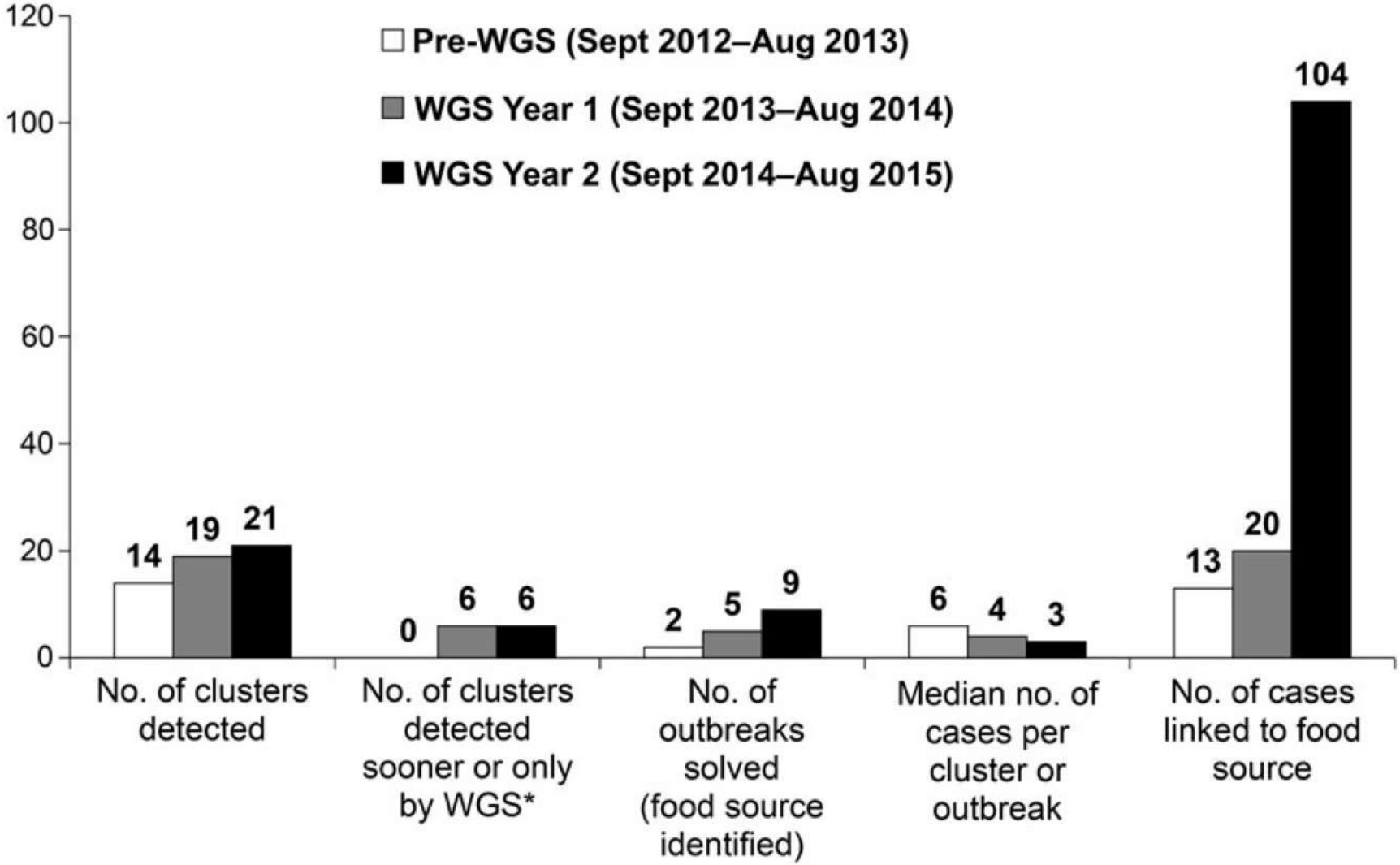
Kwong et al. J Clin Microbiol 2016;54:333–342.

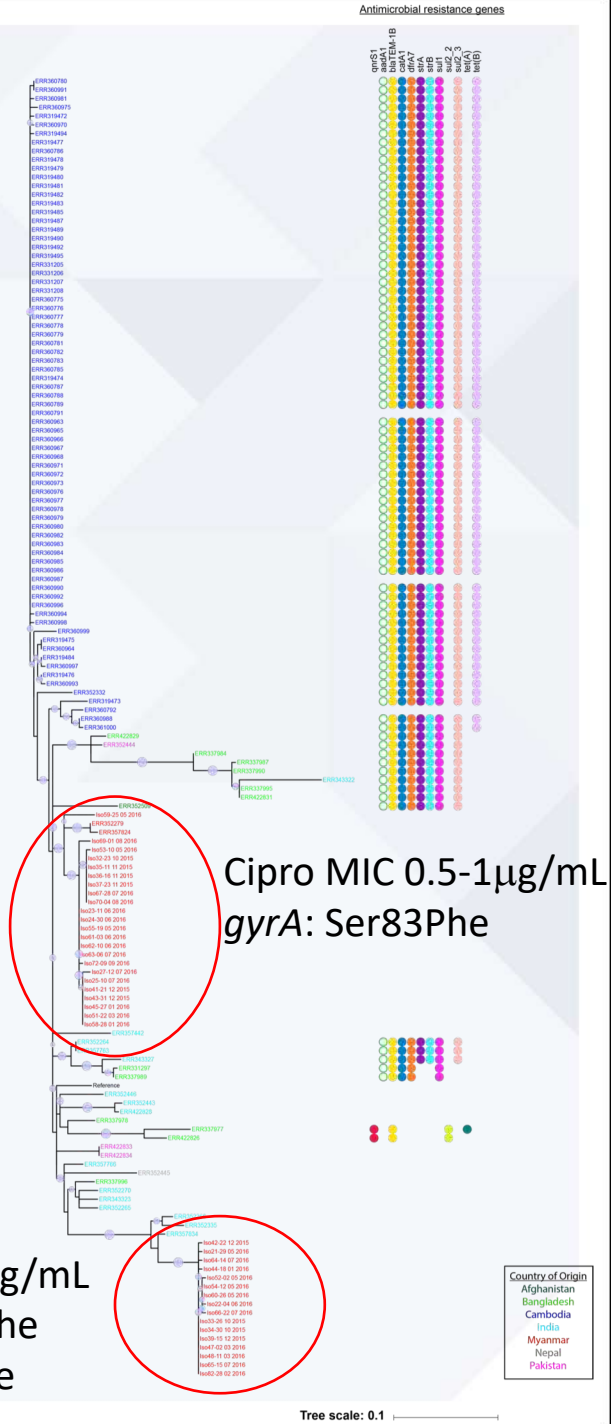
WGS detects more clusters sooner and solves more outbreaks



Jackson et al. Clin Infect Dis 2016:63(3):380-6

Enteric fever in Myanmar

- *Salmonella enterica* serovar Typhi and Paratyphi A-C
  - 53% serovar Typhi
  - 47% serovar Paratyphi A
- Common in Myanmar
  - 498 per 100,000 persons
- Universally ciprofloxacin resistant
- Genetic basis of resistance?
- Phylogenetic relationship?
  - 4.3.1 (H58) clade



Core genome SNP alignment
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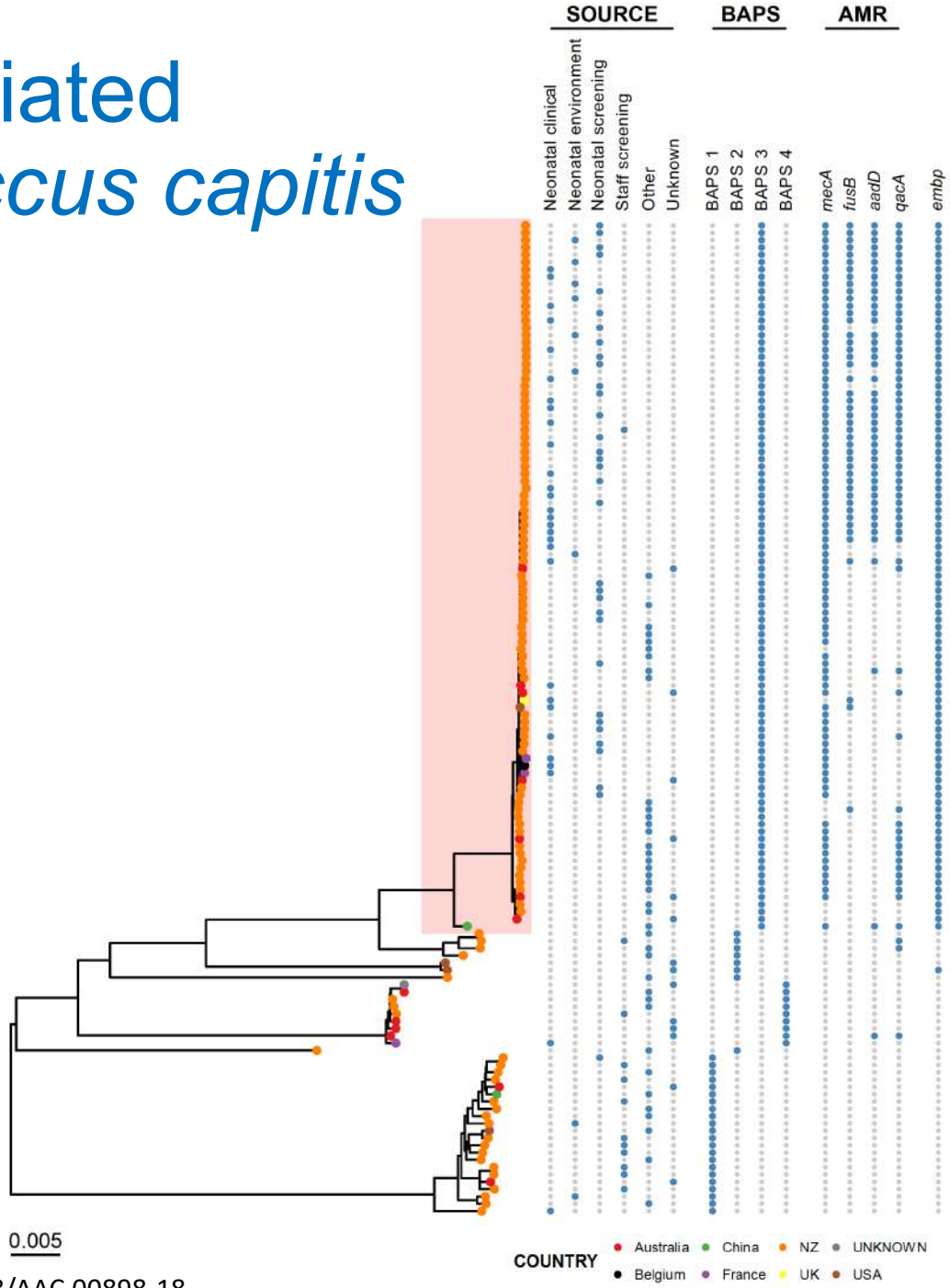
Unpublished data

NICU-associated *Staphylococcus capitis*

- Cause of neonatal bacteraemia
- 40 of 127 (31.5%) CoNS blood culture isolates Jan 2007 - July 2016
- Colonisation: 28.5% of neonates become colonised
- Closely related PFGE pattern
- Phylogenetic relationship?

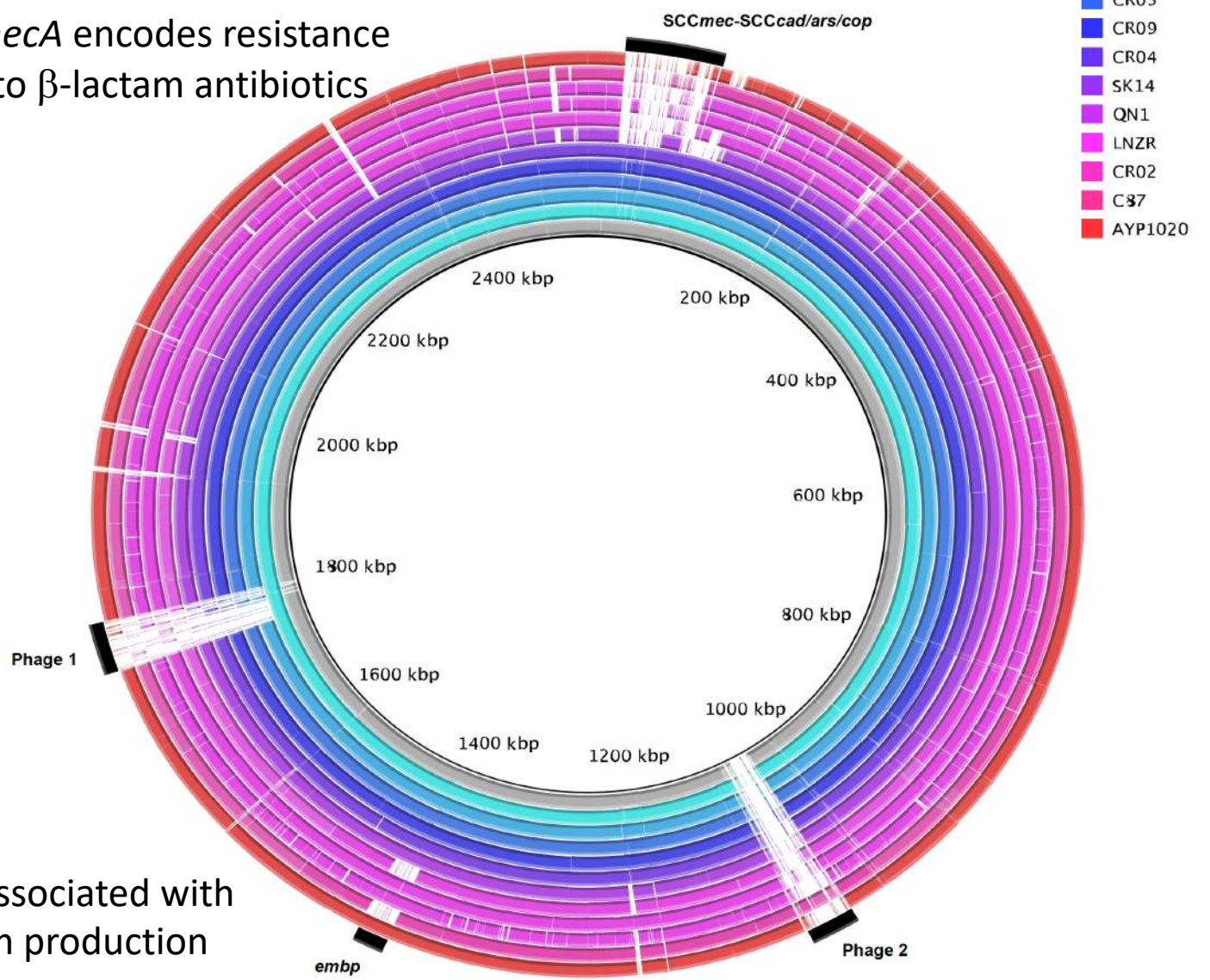


Core genome SNP alignment
otago.ac.nz/infectious-disease

Carter, Ussher et al. Antimicrob. Agents Chemother. doi:10.1128/AAC.00898-18

High level of conservation amongst the international NICU-associated *S. capitis* isolates

mecA encodes resistance to β-lactam antibiotics



embp associated with biofilm production

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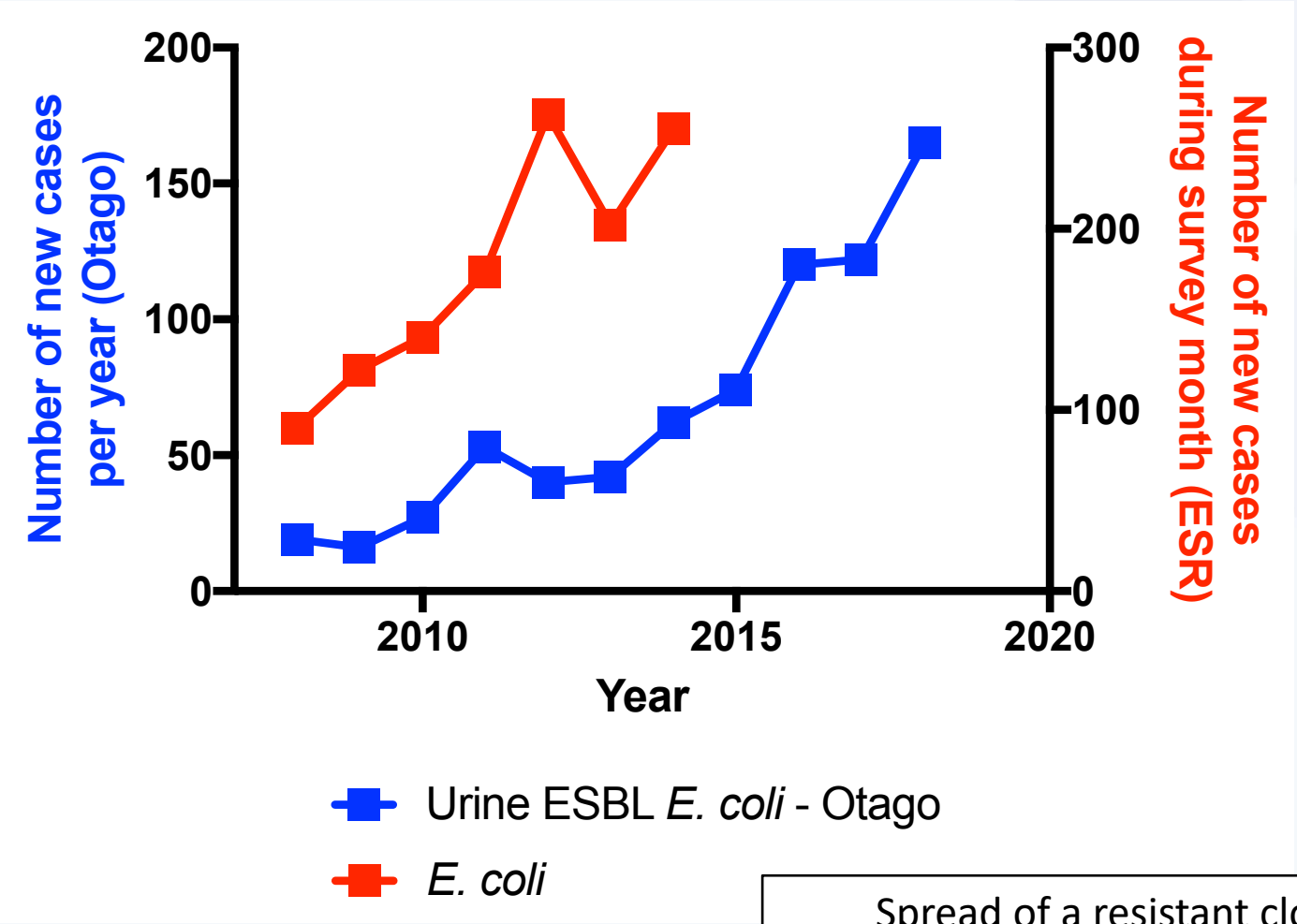
Carter, Ussher et al. Antimicrob. Agents Chemother. doi:10.1128/AAC.00898-18

Increasing prevalence of ESBL-producing *E. coli* in Otago and New Zealand

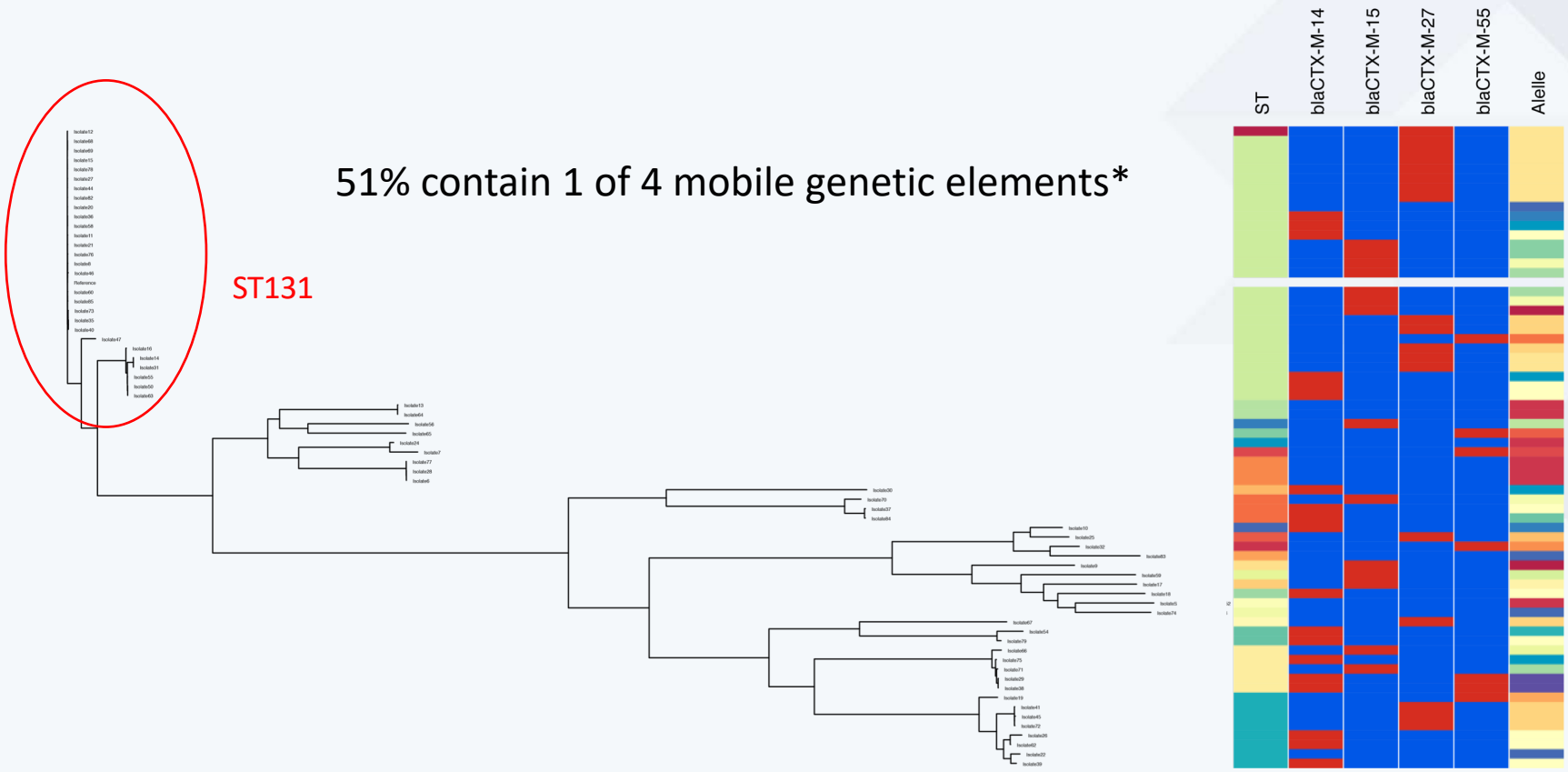


Spread of a resistant clone?
Spread of a mobile genetic element?
Multiple introductions?

Multiple different strains of *E. coli* encoding different CTX-M variants

Whole genome sequencing of 67 *E. coli* from Otago from 2/2015-2/2016

51% contain 1 of 4 mobile genetic elements*



Core genome SNP alignment

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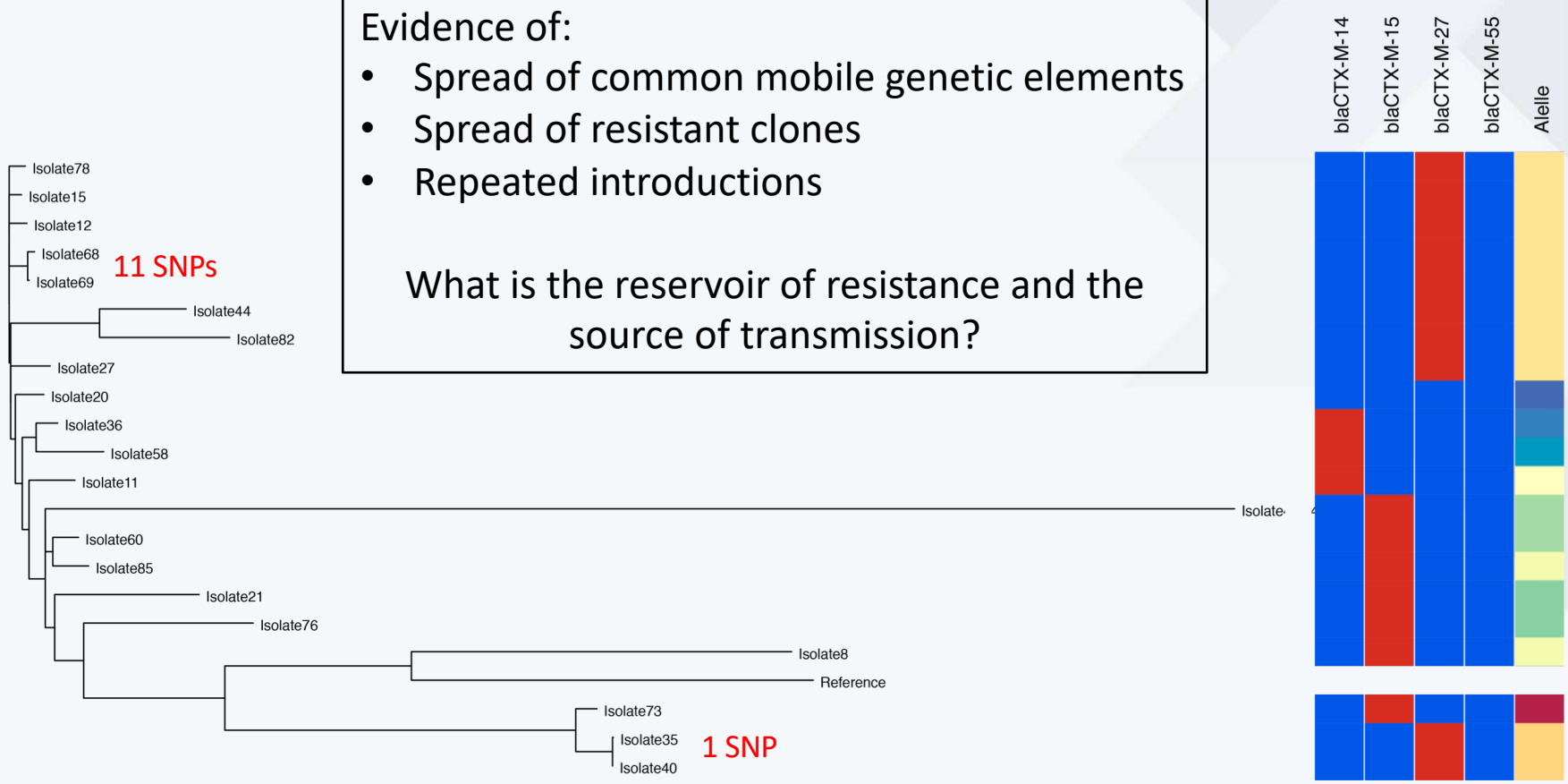
* Defined by combination of ESBL gene and insertion sequences

Phylogenetic relatedness of ST131 isolates

Evidence of:

- Spread of common mobile genetic elements
- Spread of resistant clones
- Repeated introductions

What is the reservoir of resistance and the source of transmission?



Core genome SNP alignment

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Diagnostic Laboratory

Metagenomics

Whole genome sequencing:
identification and susceptibility testing

Individual patient management

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Whole genome sequencing:
typing

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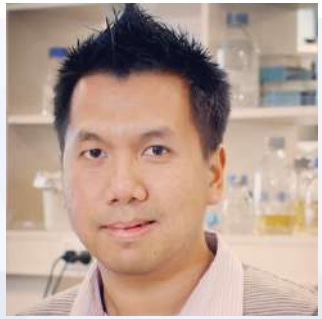
Treatment of MDR-TB in Myanmar: resistance to 2nd-line drugs associated with poor outcomes



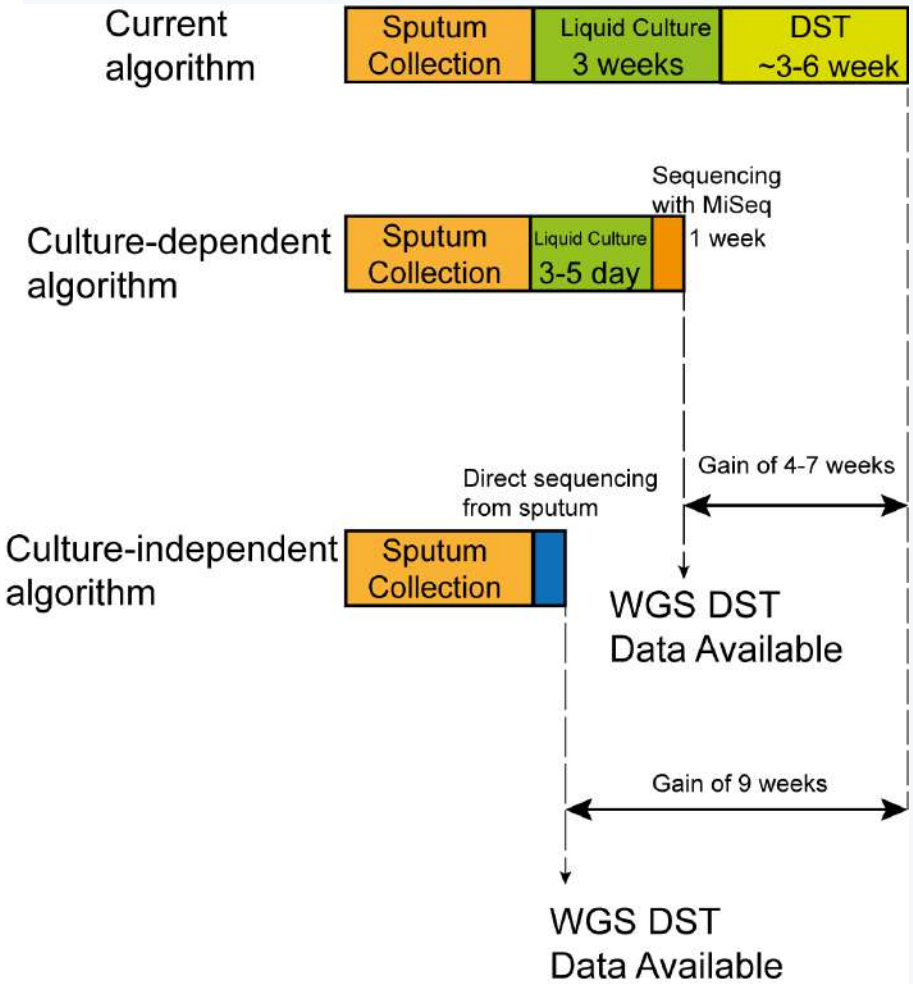
Dr Htin Lin Aung

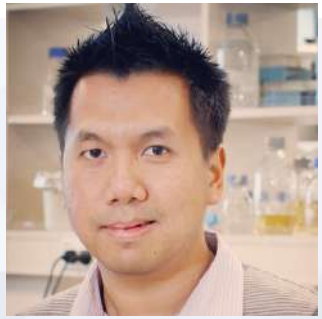
Table 1. Demographic and clinical outcome of patients with mutations in resistance genes for drugs that are commonly used in the treatment of drug-susceptible and MDR-TB in Myanmar identified by WGS.

	ID	Type of patient	Age	Sex	RIF *rpoB*	INH *katG*	STR *rpsL*	EMB *embB*	AMK	ETH *ethA*	PZA *pncA*	LFX *gyrA*	Treatment Outcome
1	M00001	Cat II failure	55	F	S450L	S315T	K43R	L402V			W119G		Cured
2	M00003	Close contact	28	M	S450L	S315T	K43R	G406D					Cured
3	M00004	Cat II failure	32	M	S450L	S315T	K43R	M306I			FS	A90V	Died
4	M00005	Cat II failure	51	F	H445Y	S315T	K43R	G406A			Q10P		Cured
5	M00008	Relapse after Cat II	75	M	D435V	S315T							Cured
6	M00010	Cat II failure	24	F	S450L	S315T	K43R						Cured
7	M00011	Cat II failure	63	M	S450L	G299C	K43R	M306V				A90V, D94A	Cured
8	M00012	Cat II failure	44	M	H445Y	S315T							Cured
9	M00013	Cat II failure	19	M	S450L	S315T	K43R	M306V		M1R	Y103*		Cured
10	M00016	Cat II failure	48	M	S450L	C-15T *inhA*				C-15T *inhA*			Cured
11	M00017	Relapse after Cat II	68	M	S450L	S315T	K43R	E504D	G1484T		Q10P		Died
12	M00018	Cat II failure	63	F	S450L	S315T							Cured
13	M00019	Cat II failure	27	M	S450L	S315T	K43R	M306V		M1R	T142A		Cured
14	M00020	Relapse after Cat II	42	M	S450L	C-15T *inhA*				C-15T *inhA*			Cured
15	M00022	Relapse after Cat II	43	M	S450L	S315T	K43R						Died
16	M00023	Cat II failure	33	F	S450L	S315T	K43R	M306V		M1R			Died
17	M00029	Relapse after Cat II	24	M	H445N	S315T	K43R	M306I		D219A*	G17C	D94Y	Died
18	M00040	Cat II failure	28	M	S450L	S315T	K43R						Cured
19	M00042	Cat II failure	33	M	S450L	S315T	K43R				Q10P		Died
20	M00071	Cat II failure	55	M	S450L	S315T	K43R	Q197L		P334A*	S32FS*	D94G	Died
21	M00087	Cat II failure	51	M	S450L	S315T	K43R	M306V		M1R	A146E*		Died
22	M00088	Relapse after Cat II	48	F	S450L	S315T	K43R		V163I* *eis*				Died
23	M00090	Close contact	24	F	S450L	S315T	K43R	M306I	G1484T *rrs*		T-11C		Died
24	M00097	Cat II failure	31	M	D435V	S315T	K43R						Cured
25	M00098	Cat II failure	33	M	S431T	S315T	K43R	M306V	G1484T *rrs*		Q10P		Died
26	M00102	Cat II failure	38	F	S450L	S315T							Died

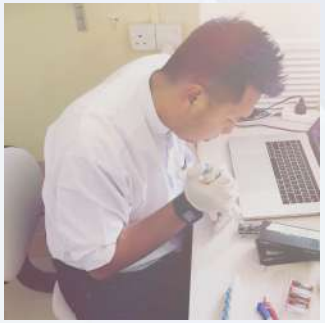
AMK: amikacin; EMB: ethambutol; ETH: ethionamide; FS: frameshift; INH: isoniazid; LFX: levofloxacin; PZA: pyrazinamide; RIF: rifampicin; STR: streptomycin.
*Novel mutation with unknown effects.

Aung et al Infect Dis 2017





Dr Htin Lin Aung



Aung et al 2018 unpublished data

Rachel Hannaway
Tin Ohn Myat
Isuri Hapuarachchi

John Crump

David Murdoch

Xochitl Morgan
Ambarish Biswas

Htin Lin Aung

Deborah Williamson
Glen Carter

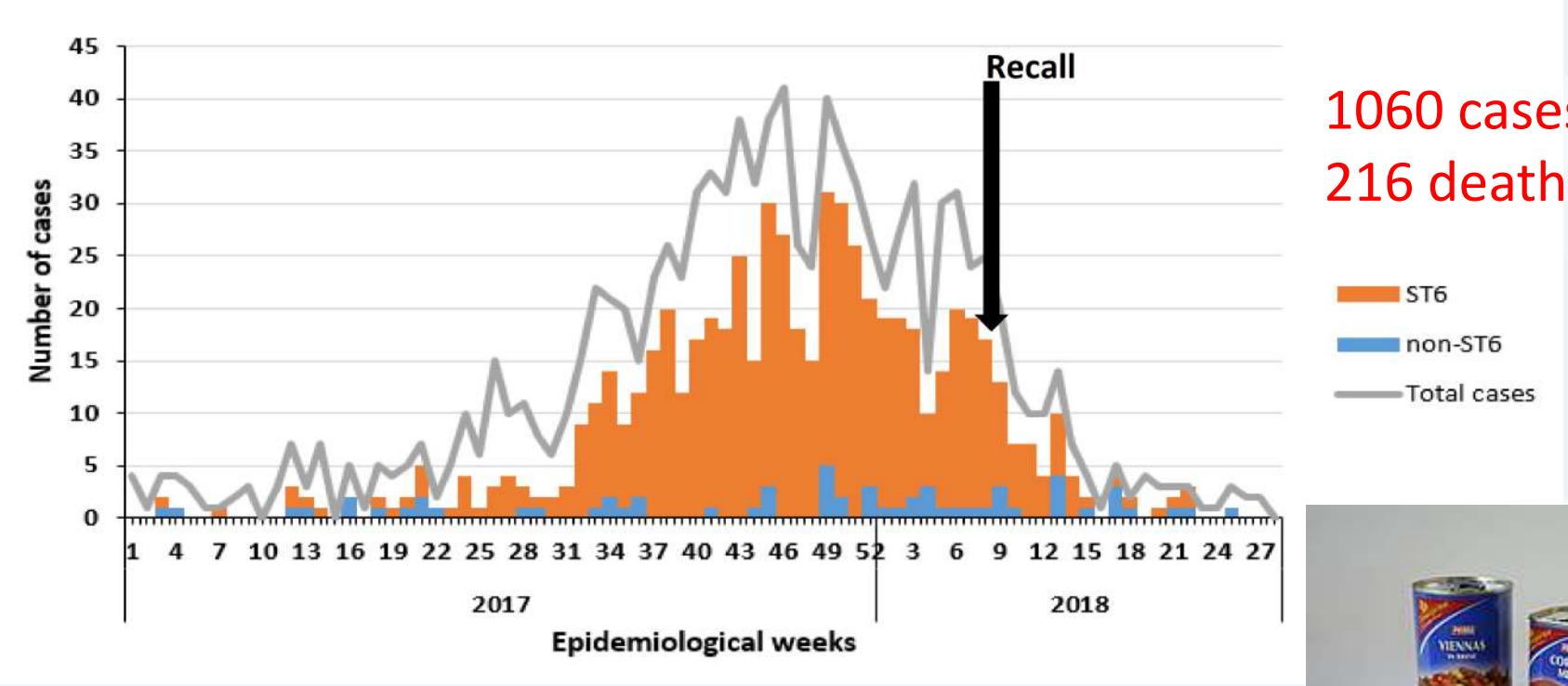
Wah Win Htike
Kyu Kyu Win
Khine Mar Oo

Khwar Nyo Zin

Funders

University of Otago Collaborative Research Grant
William Sherriff Charitable Trust
Otago Medical Research Foundation

South African listeriosis outbreak 2017/8



1060 cases
216 deaths

Ready-to-eat processed meat products manufactured at Enterprise Foods' Polokwane production facility

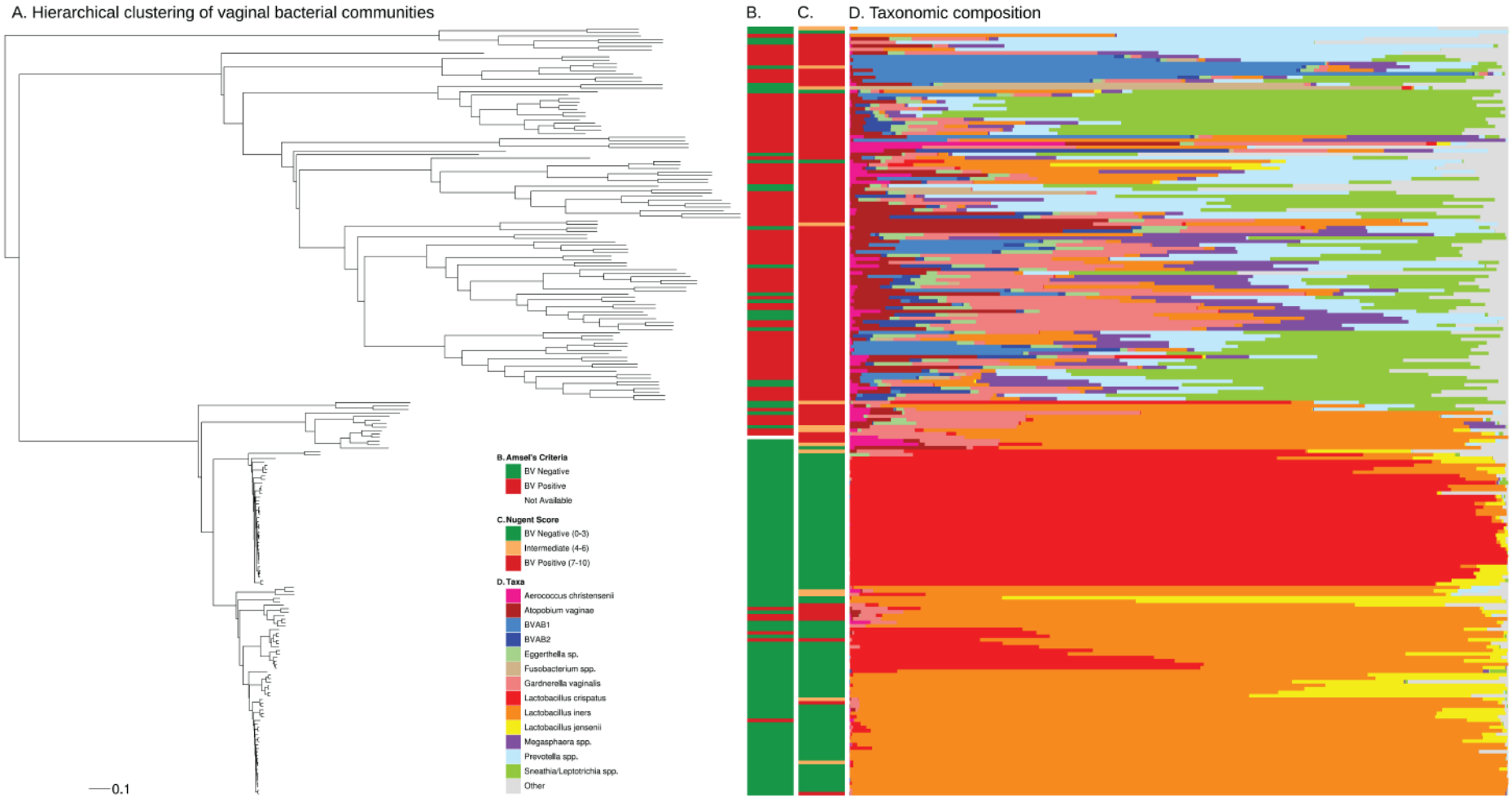
otago.ac.nz/infectious-disease

Department of Health, RSA. Listeriosis outbreak situation report – 26/07/2018

16S rRNA sequencing: Who's there?

Amsel's criteria
Nugent score

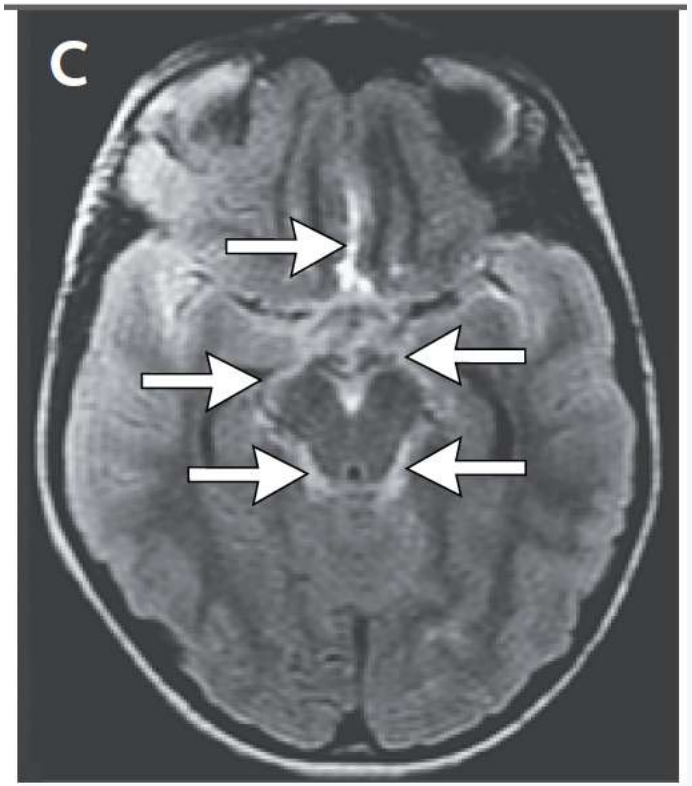
Taxonomic composition



Increased diversity in vaginal microbiome in bacterial vaginosis

PLoS ONE 2012;7(6): e37818

Shotgun metagenomics



- 14-year-old with severe combined immunodeficiency
- Presented 3 times with fever and headache
- Advanced to intractable seizures
- Routine diagnostic workup inconclusive
- MRI - encephalitis-like condition
- Shotgun metagenomics on CSF and serum
  - 48 hours
- CSF: 475 of 3,063,784 reads (0.016%) matched *Leptospira borgpetersenii*
- PCR and serology confirmed neuroleptospirosis diagnosis
- Treated with intravenous penicillin
- Improved and discharged

N Engl J Med 2014;370:2408-17.
DOI: 10.1056/NEJMoa1401268

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Same-Day Diagnostic and Surveillance Data for Tuberculosis via Whole-Genome Sequencing of Direct Respiratory Samples

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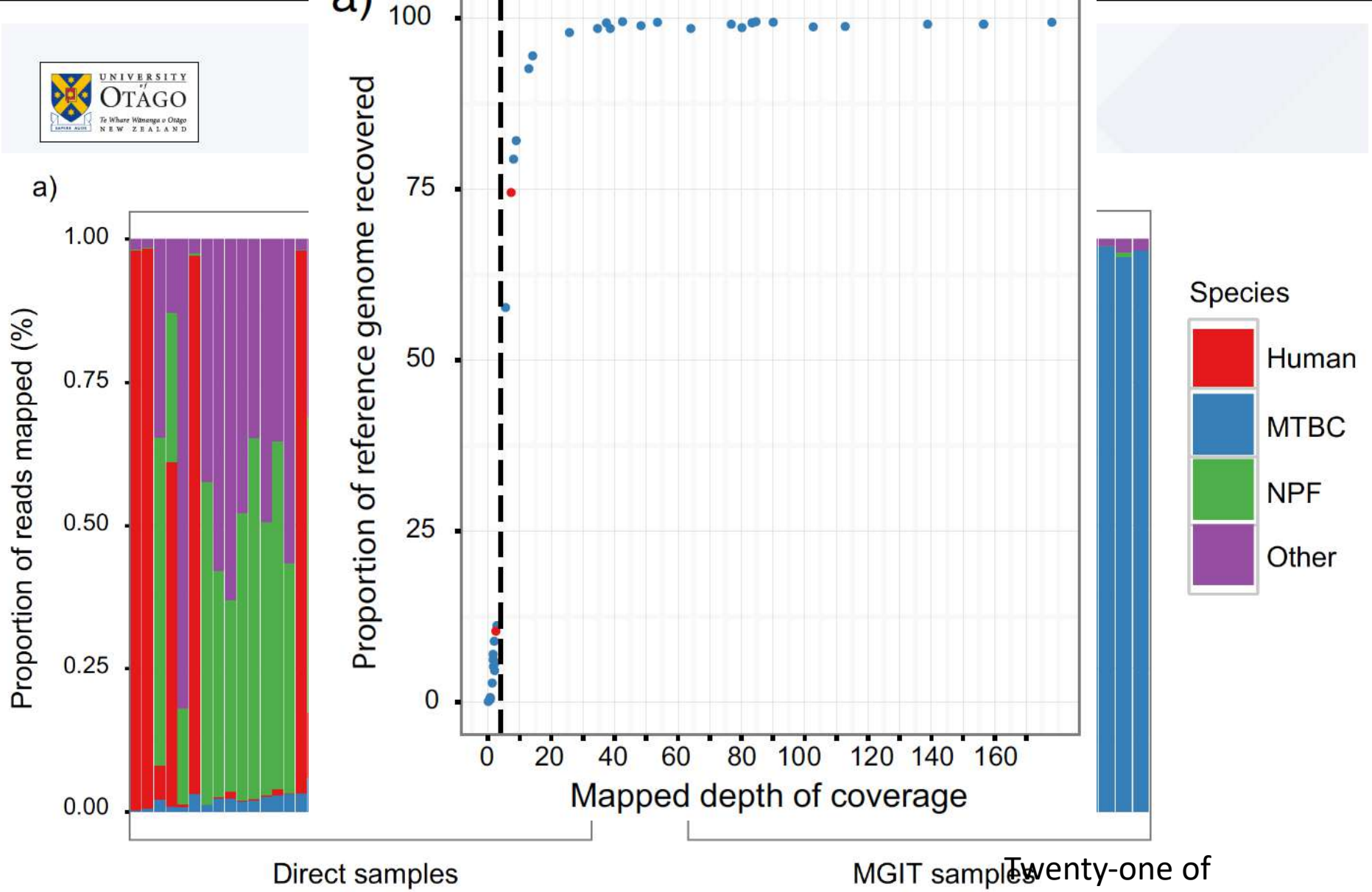


mycobacterial cells mixed with other bacterial and human cells,
with the latter accounting for

Twenty-one of 39 samples have 12 depth a
recover 90% of the genome
14/39 samples
have 3 depth and recover 1

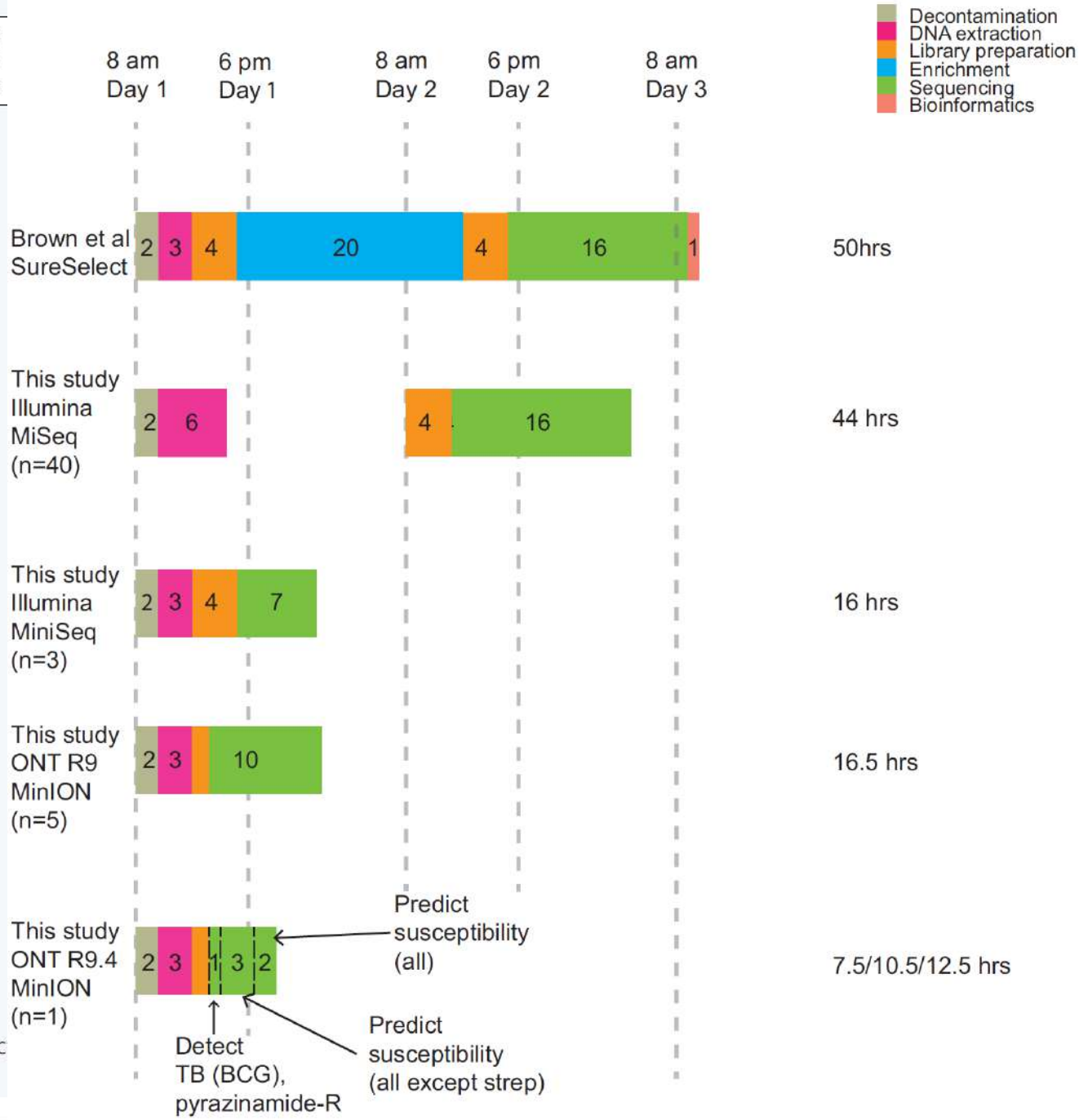
L, del Ojo Elias C, Loose M, Nilgiriwala K, Chatterjee A, Smith EG, Sanderson N, Walker TM, Morgan MR, Wyllie DH, Walker AS, Peto TEA, Crook DW, Iqbal Z. 2017. Same-day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples. J Clin Microbiol 55:1285–1298

TABLE 3 Susceptibility prediction at time stamps during R9.4 run

Hr	% of AMR mutations typed	No. of mutations ungenotyped (total, 175)	Ungenotyped mutation(s)	Drug(s) awaiting results
1	57.1	75	—[a]	All but pyrazinamide
2	88.5	20	*katG* S700, L141, V633, W191, D142, L704; *gid* L26, V41, G34, R47, G117, A205, R118, Q125; *rpoB* H445; *embB* D328, G406; *rpsL* K43; *pncA* T47, K48[b]	Isoniazid, streptomycin, rifampin, ethambutol
3	97.1	5	*embB* D328; *gid* G34, A205; *katG* W191; *pncA* T47	Ethambutol, streptomycin, isoniazid
4	98.2	3	*gid* G34, A205; *pncA* T47	Streptomycin
5	98.8	2	*gid* G34; *pncA* T47	Streptomycin
6–9	99.4	1	*pncA* T47	
9	100	0		

[a]Ungenotyped mutations omitted because 75 is too many to list.
[b]Further ungenotyped *pncA* mutations could be ignored, as H57D had already been detected at 1 h. The sample was already predicted to be pyrazinamide resistant; thus, pyrazinamide is not listed.

In conclusion, diagnostic and surveillance information can now be obtained directly from patient specimens in 16/44 h with the Illumina MiniSeq and MiSeq platforms, a considerable step forward. In addition, the


Decontamination
DNA extraction
Library preparation
Enrichment
Sequencing
Bioinformatics
8 am Day 1
6 pm Day 1
8 am Day 2
6 pm Day 2
8 am Day 3
Brown et al SureSelect
2 3 4 20 4 16 1
50hrs
This study Illumina MiSeq (n=40)
2 6 4 16
44 hrs
This study Illumina MiniSeq (n=3)
2 3 4 7
16 hrs
This study ONT R9 MinION (n=5)
2 3 10
16.5 hrs
This study ONT R9.4 MinION (n=1)
2 3 1 3 2
7.5/10.5/12.5 hrs
Predict susceptibility (all)
Predict susceptibility (all except strep)
Detect TB (BCG), pyrazinamide-R

Transmission

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A Prolonged Outbreak of KPC-3-Producing *Enterobacter cloacae* and *Klebsiella pneumoniae* Driven by Multiple Mechanisms of Resistance Transmission at a Large Academic Burn Center

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Division of Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA[a]; Hospital Epidemiology, University of North Carolina Health Care, Chapel Hill, North Carolina, USA[b]; University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA[c]; North Carolina Jaycee Burn Center, University of North Carolina Health Care, Chapel Hill, North Carolina, USA[d]

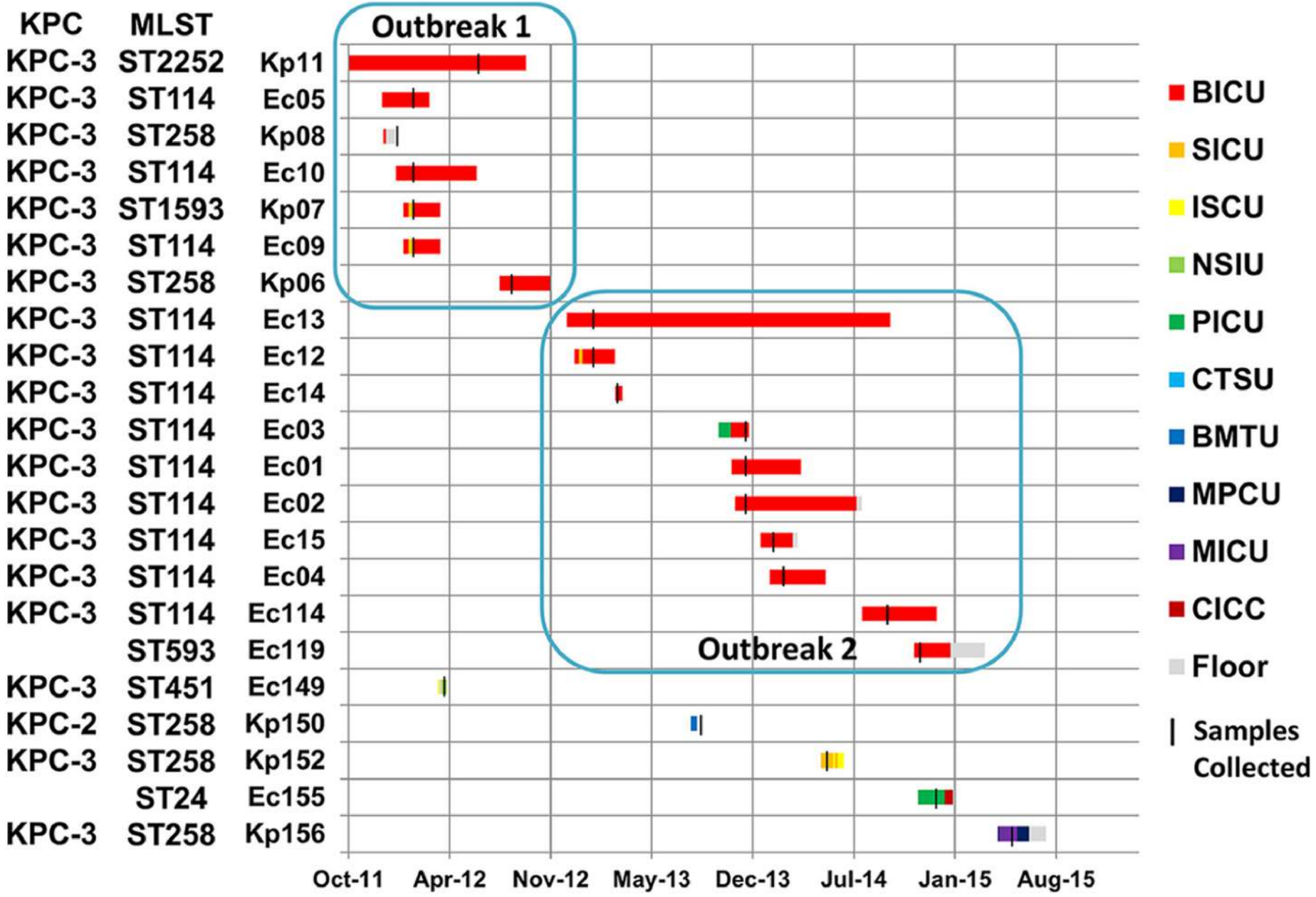
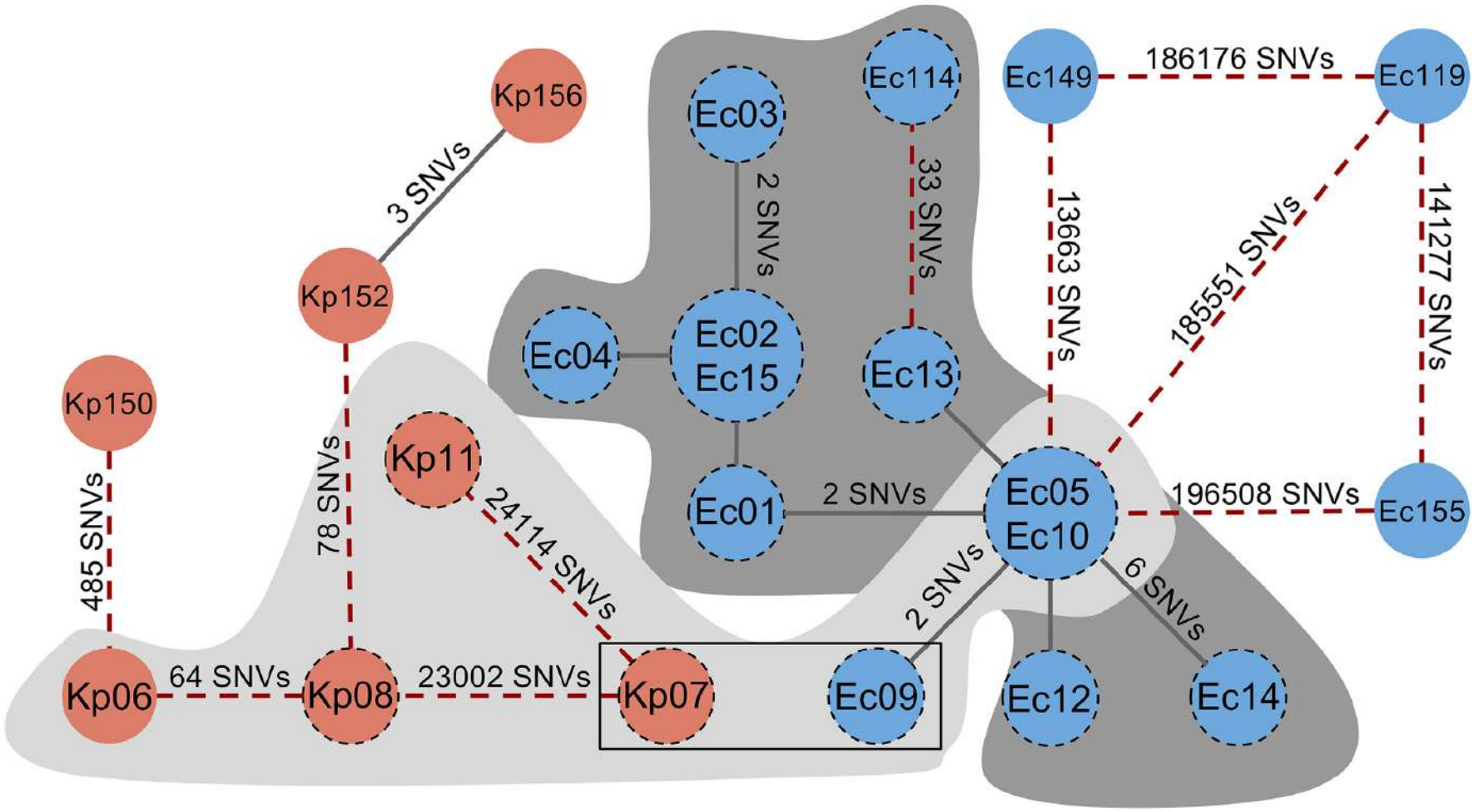
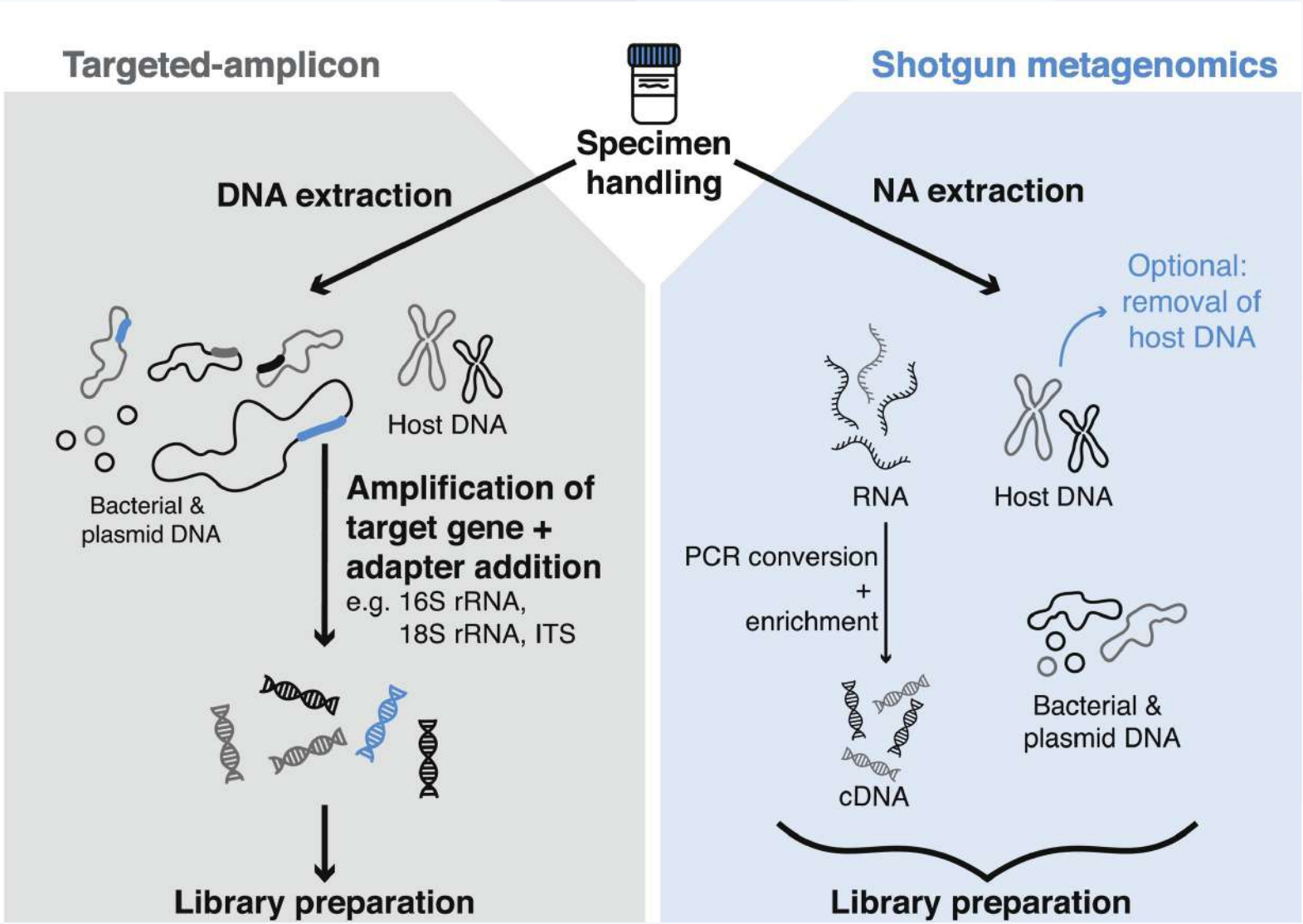

KPC
MLST
KPC-3 ST2252 Kp11
KPC-3 ST114 Ec05
KPC-3 ST258 Kp08
KPC-3 ST114 Ec10
KPC-3 ST1593 Kp07
KPC-3 ST114 Ec09
KPC-3 ST258 Kp06
KPC-3 ST114 Ec13
KPC-3 ST114 Ec12
KPC-3 ST114 Ec14
KPC-3 ST114 Ec03
KPC-3 ST114 Ec01
KPC-3 ST114 Ec02
KPC-3 ST114 Ec15
KPC-3 ST114 Ec04
KPC-3 ST114 Ec114
ST593 Ec119
KPC-3 ST451 Ec149
KPC-2 ST258 Kp150
KPC-3 ST258 Kp152
ST24 Ec155
KPC-3 ST258 Kp156
Outbreak 1
Outbreak 2
Oct-11
Apr-12
Nov-12
May-13
Dec-13
Jul-14
Jan-15
Aug-15
BICU
SICU
ISCU
NSIU
PICU
CTSU
BMTU
MPCU
MICU
CICC
Floor
Samples Collected

TABLE 2 Summary of the genetic context for bla_{KPC} among KPC-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates during a prolonged outbreak

Strain ID	Organism	KPC	MLST	Strain relatedness	Plasmid variant[a]	Tn*4401* variant[b]	Flanking sequences[c]	Composite Tn*4401*/Tn2-like structure
Ec01	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec02	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec03	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec04	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec05	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Kp06	*K. pneumoniae*	bla_{KPC-3}	ST258			Tn*4401*d	GTTCT/TCTCT	
Kp07	*K. pneumoniae*	bla_{KPC-3}	ST1593		pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Kp08	*K. pneumoniae*	bla_{KPC-3}	ST258			Tn*4401*b**	GTTCT/GTTCT	Partial, identical
Ec09	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec10	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Kp11	*K. pneumoniae*	bla_{KPC-3}	ST2252		pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec12	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec13	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec14	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec15	*E. cloacae*	bla_{KPC-3}	ST114	Ec_UNC	pKPC-3_UNC	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec114	*E. cloacae*	bla_{KPC-3}	ST114		pKPC-3_UNC*	Tn*4401*b	GTTCT/GTTCT	Complete, identical
Ec149	*E. cloacae*	bla_{KPC-3}	ST451			Tn*4401* novel***	GTTCT/GTTCT	
Kp150	*K. pneumoniae*	bla_{KPC-2}	ST258			Tn*4401*a	ATTGA/ATTGA	
Kp152	*K. pneumoniae*	bla_{KPC-3}	ST258	Kp_UNC		Tn*4401*d	GTTCT/TCTCT	
Kp156	*K. pneumoniae*	bla_{KPC-3}	ST258	Kp_UNC		Tn*4401*d	GTTCT/TCTCT	

[a]Isolates with shared plasmid variants indicate possible plasmid-mediated bla_{KPC-3} transmission among isolates (pKPC-3_UNC). *, Ec114 differed from others by 1 SNV.
[b]All isolates carried Tn*4401*b (except for one, marked with two asterisks, isolate Kp08 with 1 SNV) and are identical, and all isolates carrying Tn*4401*d are identical. "Tn*4401* novel" (indicated with three asterisks, isolate Ec149) is an isoform of Tn*4401* with a 91-bp deletion downstream of bla_{KPC}.
[c]Flanking sequences of Tn*4401* are shown as 5-bp target site duplications in transposition. There was no evidence of target site duplication for Tn*4401*d.

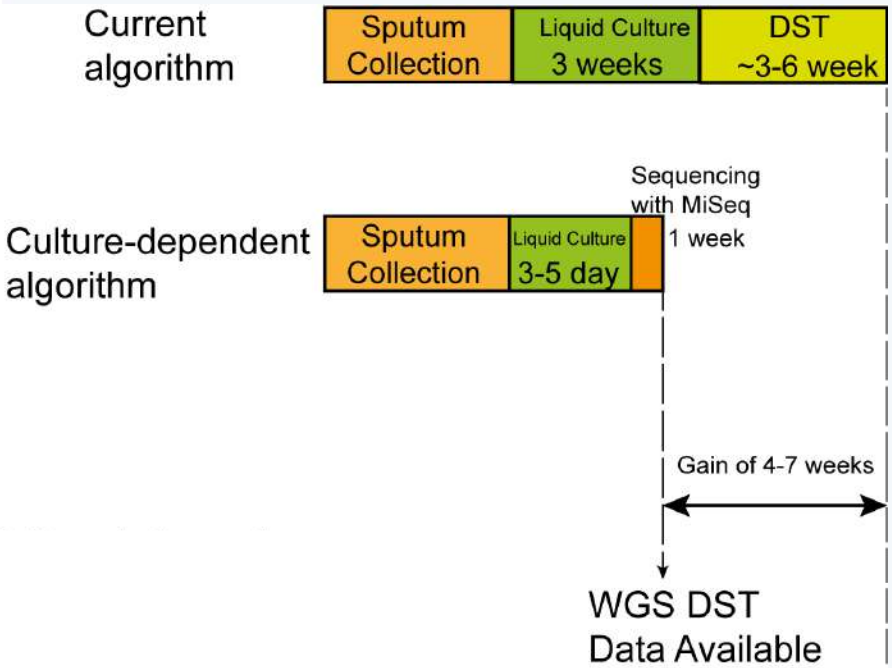

Kp156
3 SNVs
Kp152
Kp150
485 SNVs
78 SNVs
Kp11
24114 SNVs
Kp06
64 SNVs
Kp08
23002 SNVs
Kp07
Ec03
2 SNVs
Ec04
Ec02
Ec15
Ec01
2 SNVs
Ec114
33 SNVs
Ec13
Ec09
2 SNVs
Ec05
Ec10
6 SNVs
Ec12
Ec14
Ec149
186176 SNVs
Ec119
13663 SNVs
185551 SNVs
141277 SNVs
196508 SNVs
Ec155

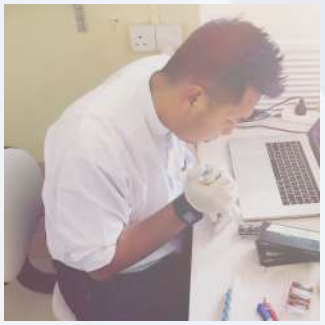


Library sequencing
Analysis

Library sequencing
Analysis

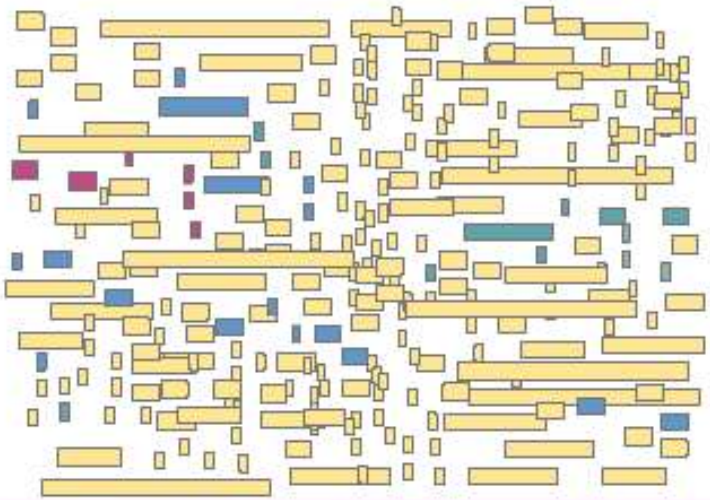
Comput Struct Biotechnol J 2018





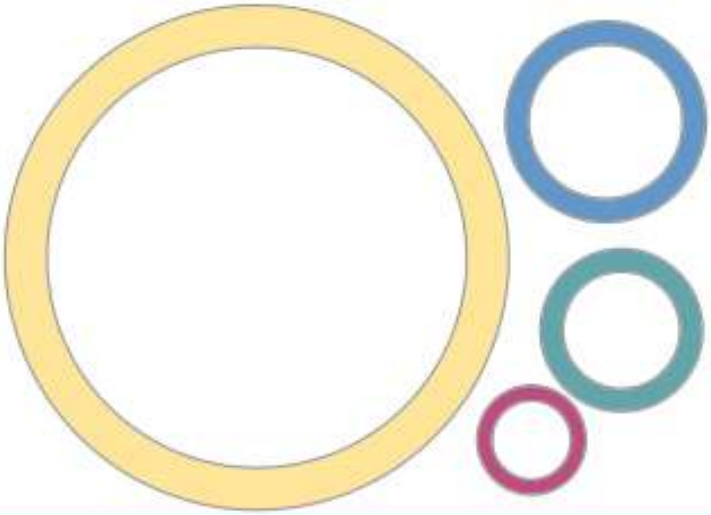
Aung et al 2018 unpublished data

Illumina assembly



311 contigs

Illumina + Nanopore assembly



1 chromosome and 3 plasmids

https://era7bioinformatics.com/en/page.cfm?id=510

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