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Clinical genomics and metagenomics: when to go for one or the other and what to expect

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Rossenlab
Personalised Microbiology

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Solidness
Surveillance Of mobiLome meDiated aNtibiotic rEsiStance Spread

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Disclosure of speaker's interests

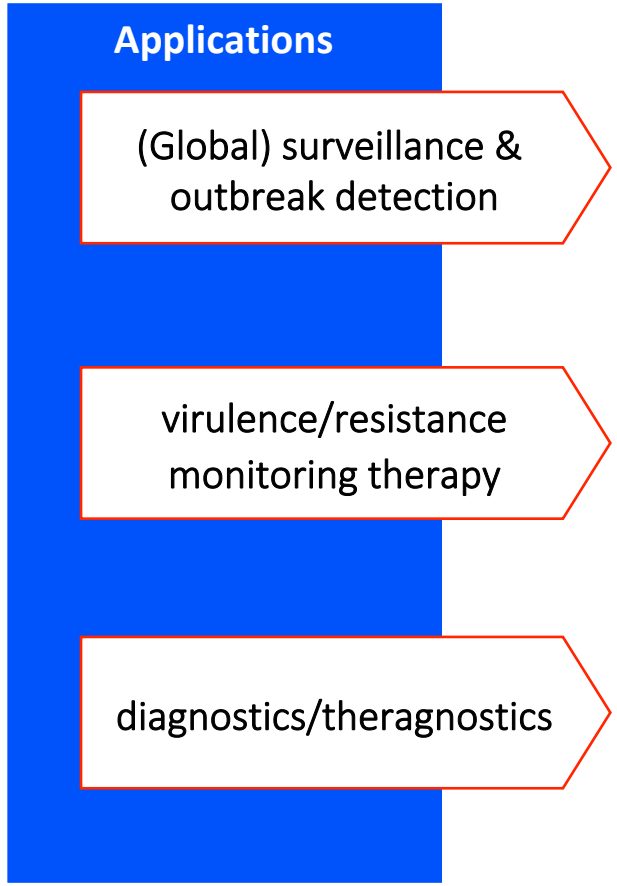
(Potential) conflict of interest	None
Potentially relevant company relationships in connection with event	Consulting for IDbyDNA
Sponsorship or research funding	National and EU-grants (H2020, InterregVA)

Applications of (meta)genomics in clinical microbiology

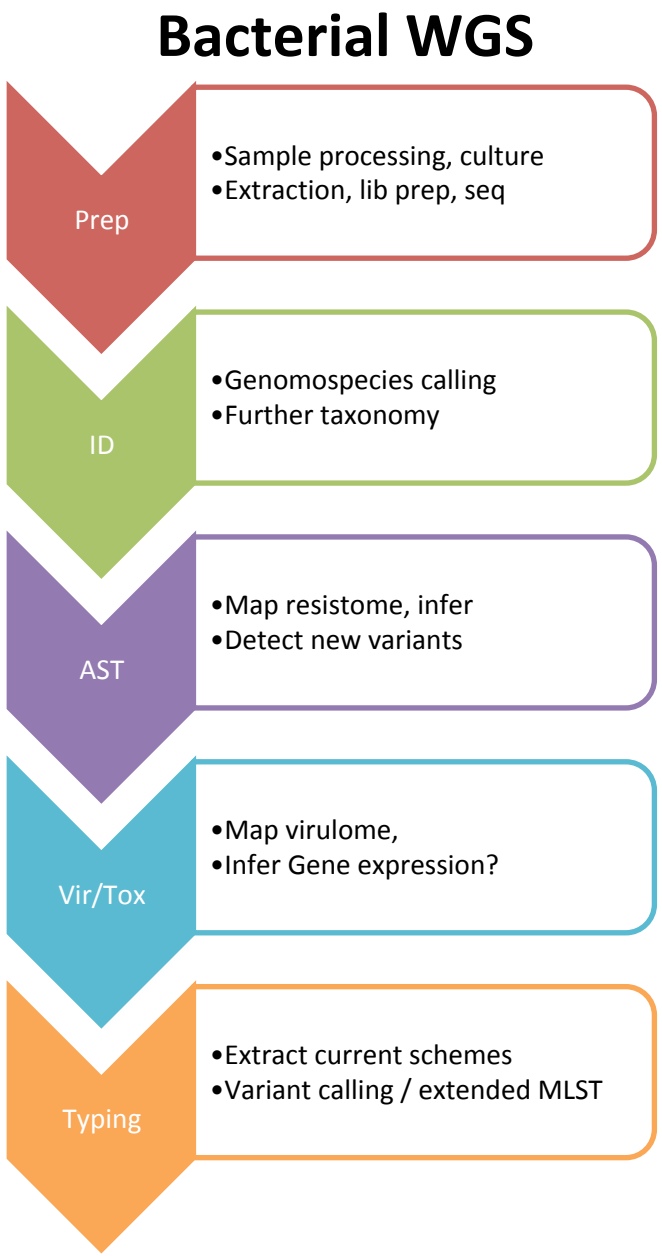
- Tracking outbreaks and identifying sources of recurrent infections
- Development of tailor-made molecular diagnostic screening tests
- Predicting resistance or virulence phenotypes from genome sequencing for optimal therapy
- Unbiased and culture free identification of pathogens

(Advanced)

- Understand host-pathogen and interactions
- Understand pathogen – microbiome/virome interactions
- Drug/vaccine development



Genomics (WGS) vs Shotgun Metagenomics

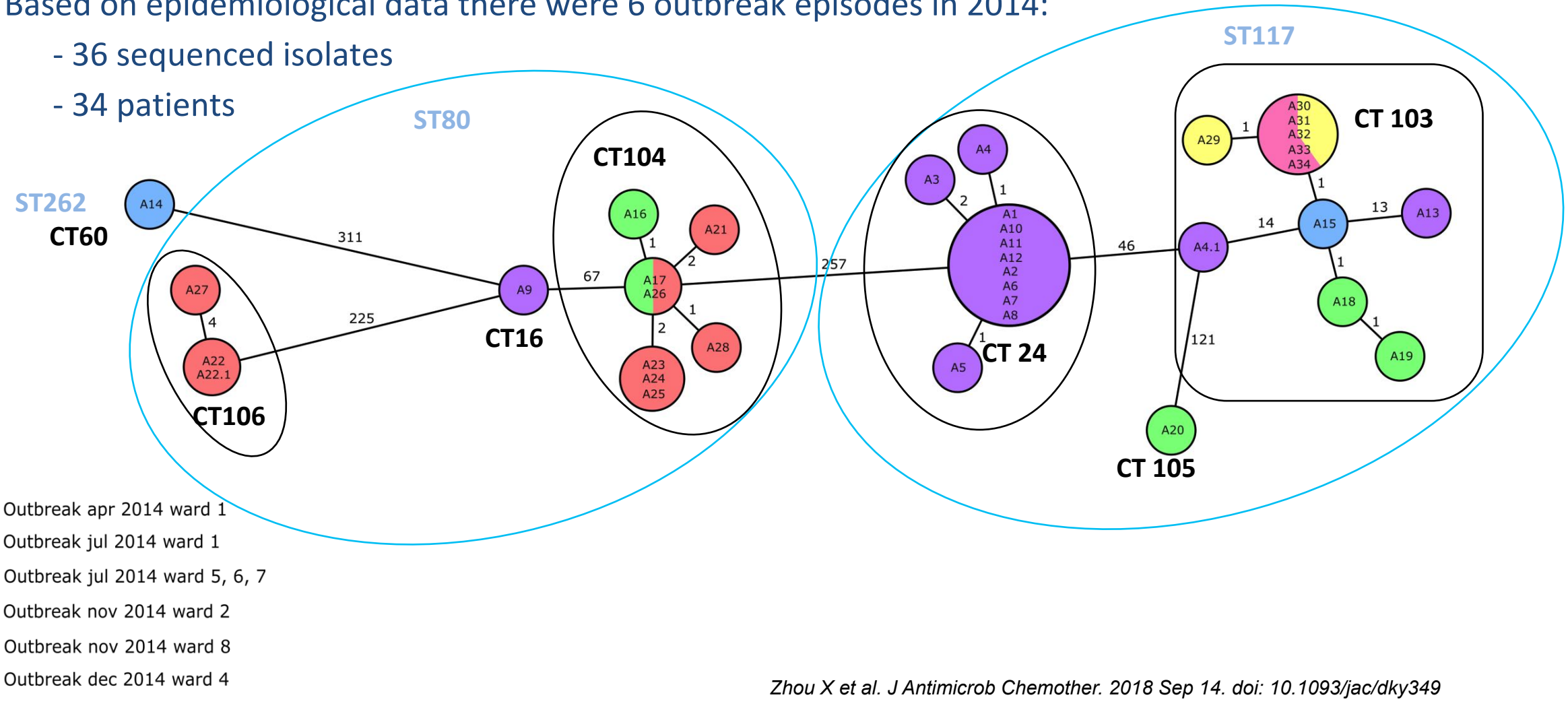


By courtesy of Prof. dr. Jacob Moran-Gilad

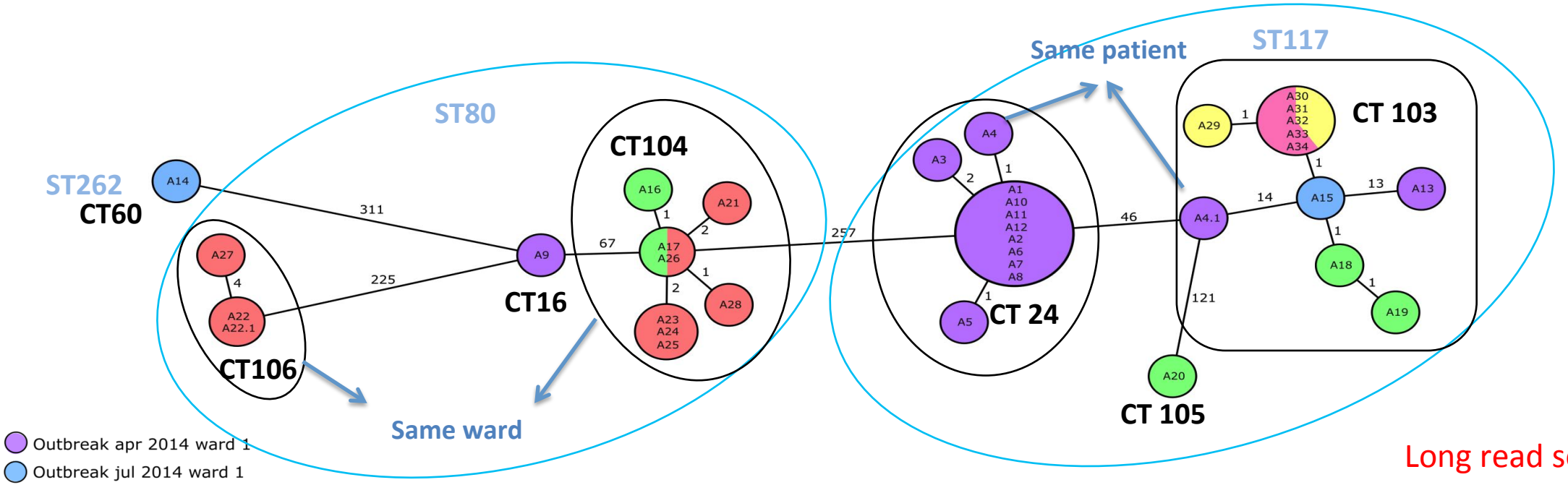
WGS to map VRE Outbreaks

Based on epidemiological data there were 6 outbreak episodes in 2014:

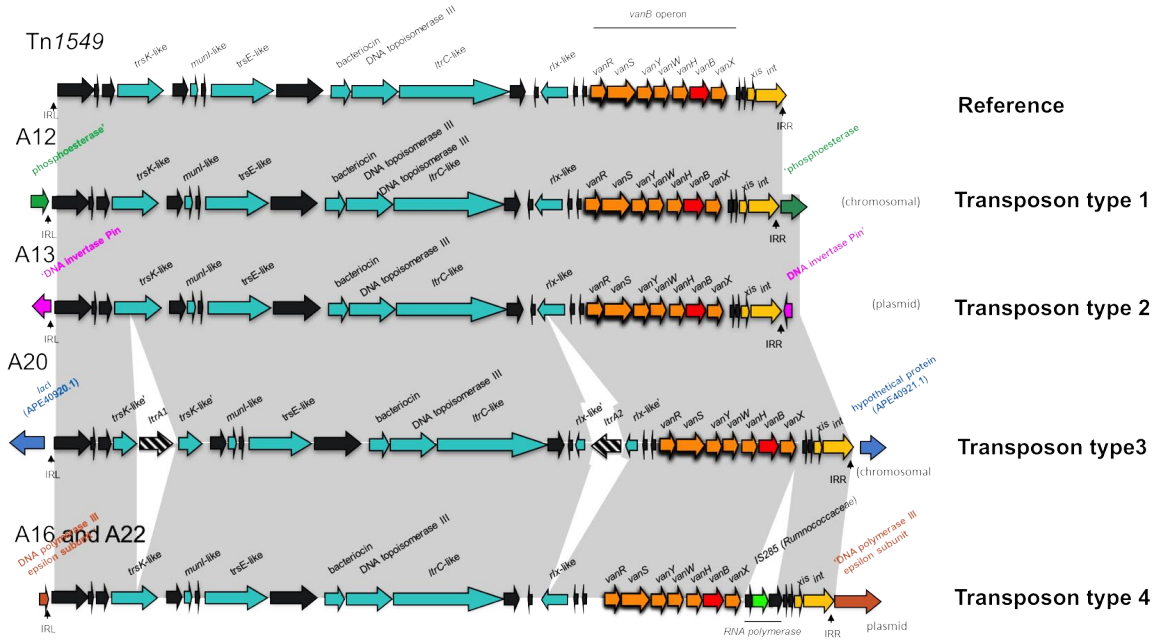
- 36 sequenced isolates
- 34 patients



Zhou X et al. J Antimicrob Chemother. 2018 Sep 14. doi: 10.1093/jac/dky349



Long read sequencing

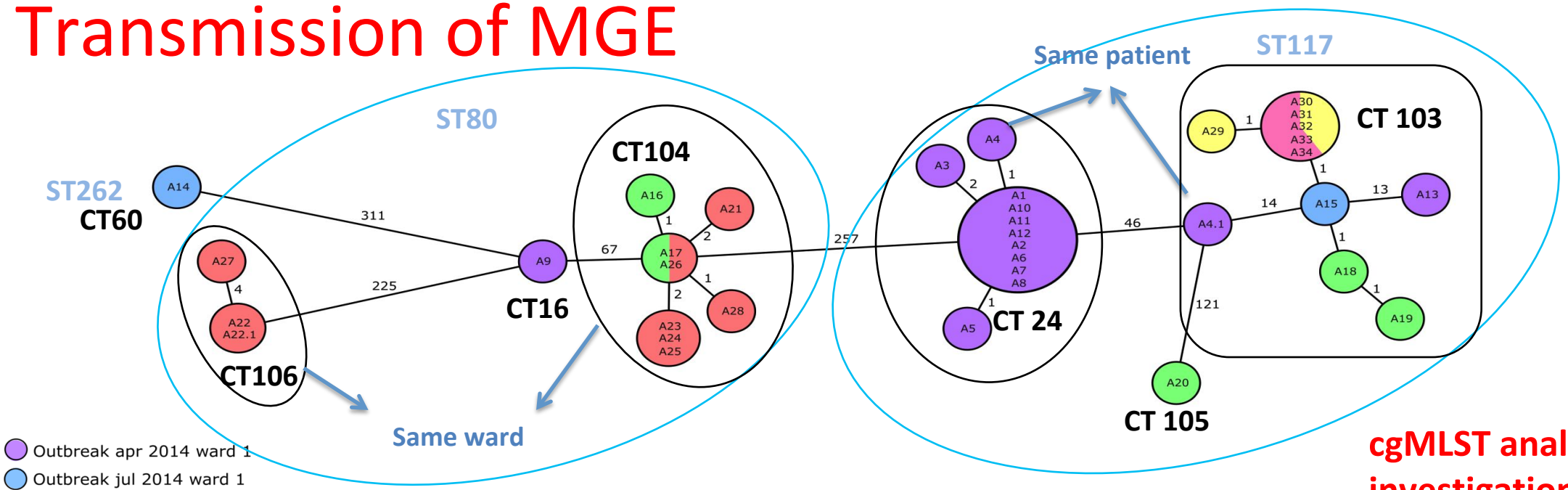


Xuewei Zhou

Monika Chlebowicz

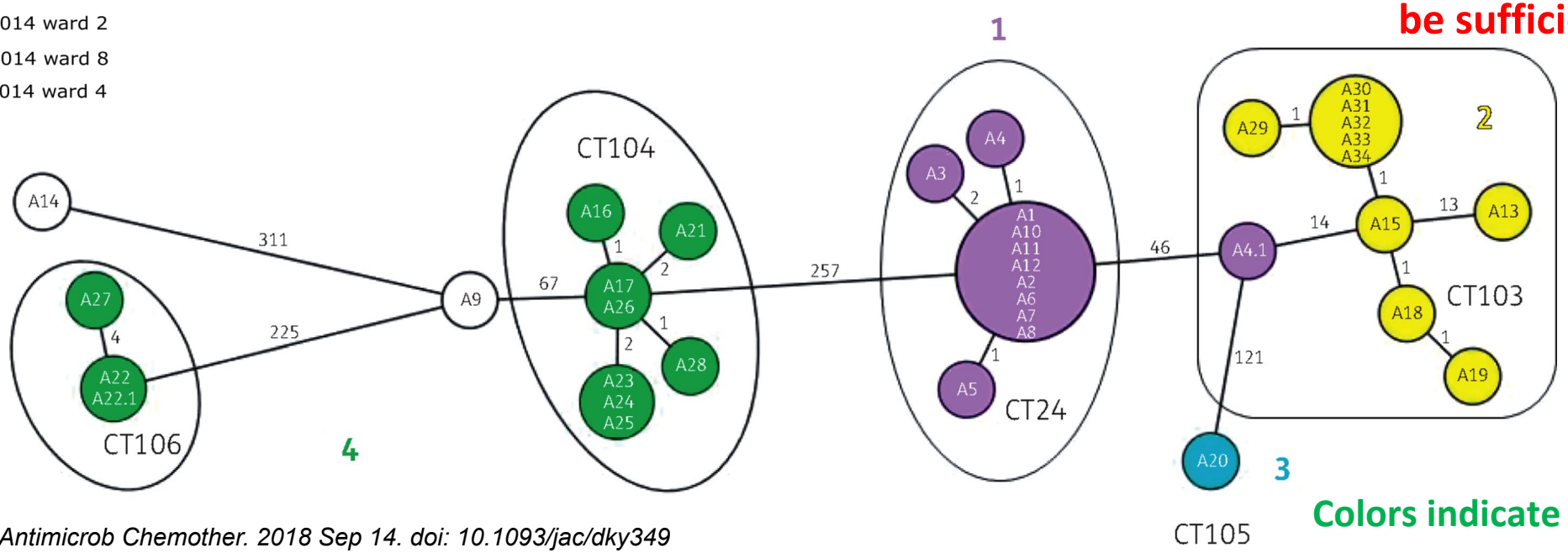
Zhou X et al. J Antimicrob Chemother. 2018 Sep 14. doi: 10.1093/jac/dky349

Transmission of MGE



- Outbreak apr 2014 ward 1
- Outbreak jul 2014 ward 1
- Outbreak jul 2014 ward 5, 6, 7
- Outbreak nov 2014 ward 2
- Outbreak nov 2014 ward 8
- Outbreak dec 2014 ward 4

cgMLST analysis in outbreak investigation may not always be sufficient



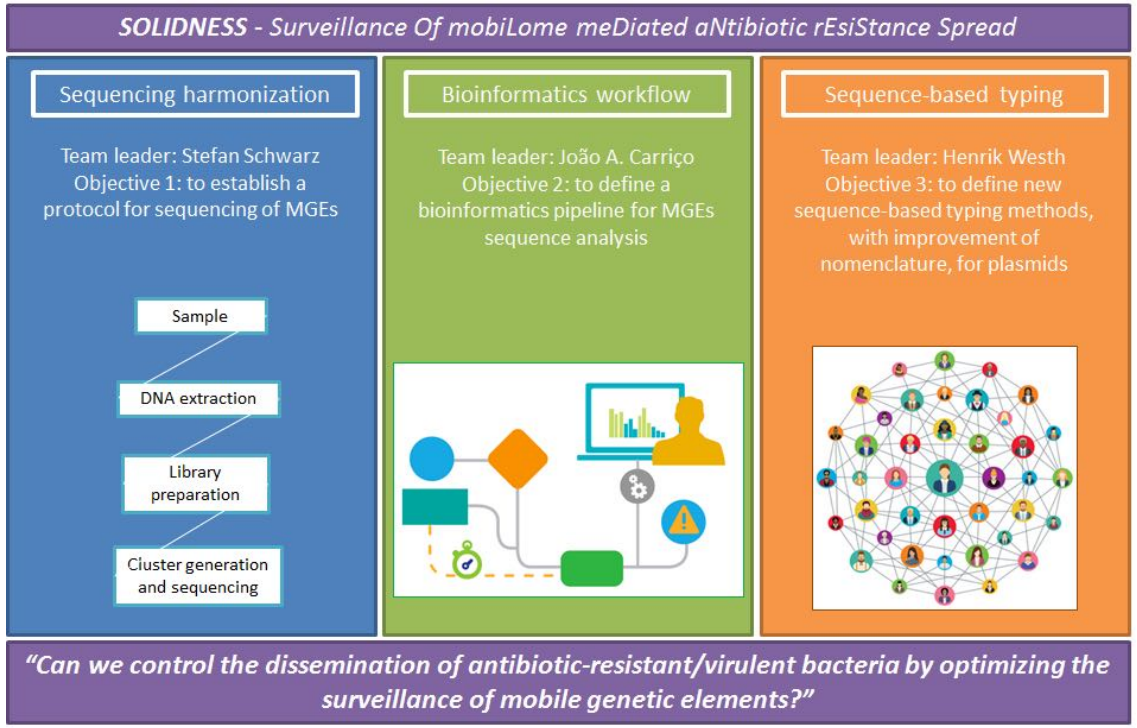
Colors indicate different transposons

Zhou X et al. J Antimicrob Chemother. 2018 Sep 14. doi: 10.1093/jac/dky349

SOLIDNESS - Surveillance Of mobiLome meDiated aNtibiotic rEsiStance Spread

The main goal of SOLIDNESS is to establish a network of excellence for surveillance of MGE- mediated antibiotic resistance and virulence spread

Natacha Couto,
scientific coordinator



solidness.eu

JPI-AMR 7th call project – supported by ZonMw

John Rossen, University of Groningen Netherlands **– Lead partner**
Stefan Schwarz Freie Universität Berlin Germany
Silke Peter University of Tübingen Germany
Alban Ramette University of Bern Switzerland
Adam Roberts Liverpool School of Tropical Medicine
Spyros Pournaras University of Athens Greece
Martin Sundqvist Örebro University Hospital Sweden
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João A. Carriço Universidade de Lisboa Portugal
Annamari Heikinheimo University of Helsinki Finland
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Hajo Grundmann University of Freiburg Germany
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Pieter-Jan Ceyssens, Sciensano, Belgium
Joana Azeredo, University of Minho, Portugal

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ZonMw jpiamr umcg

WGS in cases of unknown resistance mechanisms

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Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother
doi:10.1093/jac/dkv002

OXY-2-15, a novel variant showing increased ceftazidime hydrolytic activity

R. H. T. Nijhuis[1]*, S. Oueslati[2], K. Zhou[3], R. W. Bosboom[1], J. W. A. Rossen[3] and T. Naas[2]

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Objectives: *Klebsiella oxytoca* is a member of the family of Enterobacteriaceae and often contains the β-lactamase bla_{OXY} gene. Although this β-lactamase does not naturally hydrolyse ceftazidime, this study describes possible *in vivo* selection of a clinical *K. oxytoca* isolate showing increased MICs of ceftazidime.

RAPID COMMUNICATIONS

Isolation of an NDM-5-producing ST16 *Klebsiella pneumoniae* from a Dutch patient without travel history abroad, August 2015

E Bathoorn [1], JW Rossen [1], M Lokate [1], AW Friedrich [1], AM Hammerum [2]
1. Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
2. Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

Correspondence: Erik Bathoorn (d.bathoorn@umcg.nl)

Citation style for this article:
Bathoorn E, Rossen JW, Lokate M, Friedrich AW, Hammerum AM. Isolation of an NDM-5-producing ST16 Klebsiella pneumoniae from a Dutch patient without travel history abroad, August 2015. Euro Surveill. 2015;20(41):pii=30040. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.41.30040

Article submitted on 02 October 2015 / accepted on 14 October 2015 / published on 15 October 2015

A New Delhi Metallo-beta-lactamase-5 (NDM-5)-producing ST16 *Klebsiella pneumoniae* strain was isolated from a Dutch patient in a long-term care facility

of patients with NDM-producing *Enterobacteriaceae* is still rare [7].

ypes of NDMs have been detected, of he most prevalent type [8]. NDM-5 has

RAPID COMMUNICATIONS

Latent introduction to the Netherlands of multiple antibiotic resistance including NDM-1 after hospitalisation in Egypt, August 2013

E Bathoorn (d.bathoorn@umcg.nl)[1], A W Friedrich[1], K Zhou[1], J P Arends[1], D M Borst[1], H Grundmann[1], J W Rossen[1]
1. Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Citation style for this article:
Bathoorn E, Friedrich AW, Zhou K, Arends JP, Borst DM, Grundmann H, Rossen JW. Latent introduction to the Netherlands of multiple antibiotic resistance including NDM-1 after hospitalisation in Egypt, August 2013. Euro Surveill. 2013;18(42):pii=20610. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20610

Article submitted on 11 October 2013 / published on 17 October 2013

We describe the introduction of various multi-drug

from holidays in Egypt with his spouse and two chil-

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WGS for discovering new resistance genes/mechanisms

- Three metronidazole resistant *P. bivia* strains
  - UMCG-3721 gluteal infiltrate of a 75-year-old patient resistant to amoxicillin, clindamycin and metronidazole, susceptible for amoxicillin-clavulanic acid and meropenem
  - UMCG-93105 abdominal infection of a 68-year-old patient resistant for amoxicillin, clindamycin and metronidazole, susceptible for amoxicillin-clavulanic acid, piperacillin-tazobactam and meropenem
  - UMCG-8631 from a previously healthy 27-year-old patient treated with cefotaxime, metronidazole, and teicoplanin (later vancomycin) → finally antibiotic treatment was switched to piperacillin/tazobactam and vancomycin

Veloo et al., J Antimicrob Chemother. 2018 Oct 1;73(10):2687-2690. doi: 10.1093/jac/

New nim gene (nimK)

Fig 1. An aligment of the amino acids of the new NimK and other Nim proteins.

```
          10        20        30        40        50
nimK  -------MFREMRRKRQQLSTEECIAILEKMTSGVLALNEEGGYPYAVPLS
nimJ  MN------EFREMRRKRQQLSEEESIAILQKATAGTLALLGDNDYPYAVPIS
nimI  MNRLDNMEFREMRRKRQQLSDAECVGILENATSGTLALQGDGGYPYAVPIS
nimH  --------FREMRRKRQLLPTEESITILEKMTNGTLALHGDNGYPYAVPVS
nimF  -------MFREMRRKRQQLSAEESIAILERMTNGVLALHGDEGYPYAVPVS
nimE  -------MFREMRRKRQLLPQEESVAILEKMTNGTLALHGDNGYPYAVPLS
nimD  -------MFREMPRKRQLLPTEESVAILERMTNGTLALHGDDGYPYAVPVS
nimC  -------MFRAMRPKRHELPTDESVGILKRMTNGTLALHGDGDYPYAVPVS
nimB  -------MFREMRRKRQLLPTEESVAILERMTNGTLALHGDDGYPYAVPIS
nim   -------MFKEMRRKRQQLSTEECVAVLEKMTSGVLALNEENGYPYAVPLS
nimA  -------MFREMRRKRQLLPPEESLAILERMTGGTLALHGDNGYPYAVPVS

          60        70        80        90       100
nimK  YVYSDNKIYFHSAIKGHKIELLEKNENVSFCVIFQDHIVPEEFTTYFQSV
nimJ  YVYADGRLYFHSALSGHKVDAIRKCDKASFCVIEQDEVHPEKYTTFFRSV
nimI  YVHADGKLYFHSALKGHKVDAIRGCDKASFCVIEQDEVHGEEYTPYFRSV
nimH  YVYADGKIYFHSAVKGHKVDAILRNNKVSFCVVEQDDVKPAEFTTYFRSV
nimF  YVYADGKIYFHSAMKGHKVDAIMRNERVSFCVVERDDVCPGEFTTYFRSV
nimE  YVYADGKIYFHCAKIGHKVDAIMQNNKVSFCVVEQDNIKPAEFTTYFRSV
nimD  YVYADGKIYFHSAMQGPKVDAILRNDKVSFCVVEQDEVKPAEFTTYFRSV
nimC  YVYSDGRIYFHTATQGHKVDALMRNDKVSFCVVEQDDVKSAEFTTYFRSV
nimB  YVYADGKIYFHSAMKGHKVDAILQNDKVSFCVVEQDDIRPSEFTTYFRSV
nim   YVYADNKIYFHSAVKGHKIDLLKENCNVSFCVIGQDCIVPEEFTTYFRSV
nimA  YVYADGKIYFHGAVQGHKMDAIRQHPEVSFCVVEQDRIVPAEFTTYFRSV

          110       120       130       140       150
nimK  IVFGKARILLDEKEKMSALWKLGEKYSSGNAEALSAEISKGKNHLLIIEI
nimJ  IAFGRIHIIEDETEKLETARMLVNRYNPNQEEALQKELENGLSRMLMIRF
nimI  IAFGRIRILEDEAERMAAARLLGDRYHPHHEEALGRELAKSFSHMLVICL
nimH  IAFGKARILADEGEKQLAFRLLADKYSHGEV-GMEAEIAKGFNHLLMVEI
nimF  VLFGKTRILTEEAEKLAALSLLADKYSPGEP-GKDAEIAKGFN-LLMVEI
nimE  IVFGKAYILTDETEKRMAMTLLVNKYSFGEP-GLSDEIAKSFNHLLMVKI
nimD  IVFGKARILTDENEKRNALNLLADKYSHGEA-GMEAEMAKGFNHLLMIEI
nimC  IPFGRARILTDETENGAALQLLADKYSSGMP-GLEAVIAKGFRHLLMVEI
nimB  IVFGKAHILTDELEKRVALGLLADKYSYGEA-GMEAEIAKGFNHLLIVKI
nim   IVFGKARILTDENEKMSALRKLGEKYSSDNPEGLSEEIHKGFNHLLMVEI
nimA  IVFGKARILTDEVEKRAALLRLAEKYSSGES-GMQDEIDKGFDHLVMVEI

          160       170       180
nimK  AIEHMTGKESIELVRAKK---------------
nimJ  DIEHLTGKEAIELVRRHQK--------------
nimI  DIEHMTGKEAIELVRMKRQRA-------------
nimH  MVEHMTGKEA-----------------------
nimF  AVEHMTGKEA-----------------------
nimE  DIEHMTGKEAIELVREKEKFCTSEKTS------
nimD  TVEQMTGKEAIELTRGRNGCS-------------
nimC  DIEHLTGKESIELVREKNDM--------------
nimB  AIEHITGKEAIELTKNRNDRP-------------
nim   TIEHMTGKESIELVRAKE---------------
nimA  TVEHMTGKEAIQLVRRKGNNRWDAFPSKDVFIR
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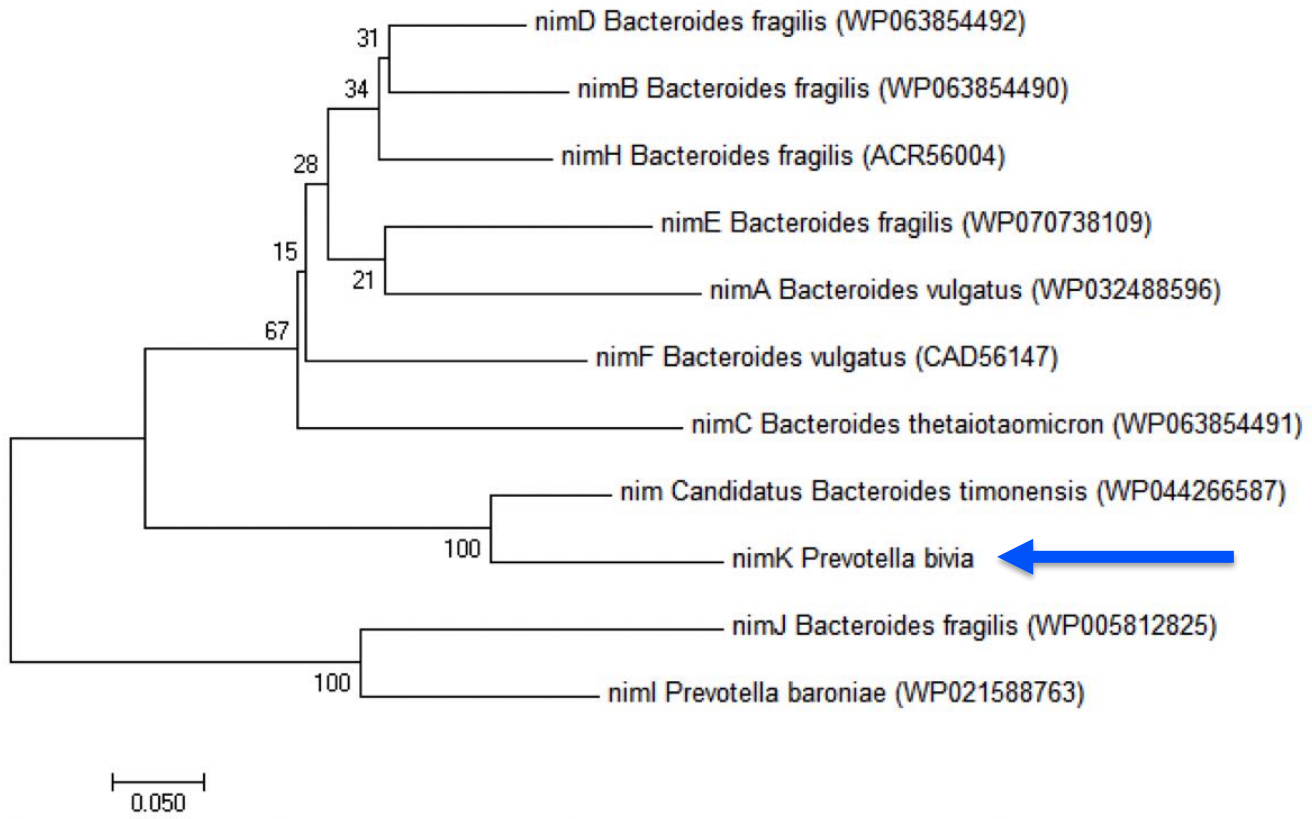

Fig 2. A phylogenetic analysis of the *nimK* gene was performed using the maximum likelihood method. Amino acid sequences were aligned using the MUSCLE method in MEGA7. A consensus tree was calculated from 500 bootstraps. The final dataset consisted out of 151 positions.

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Veloo et al., J Antimicrob Chemother. 2018 Oct 1;73(10):2687-2690. doi: 10.1093/jac/

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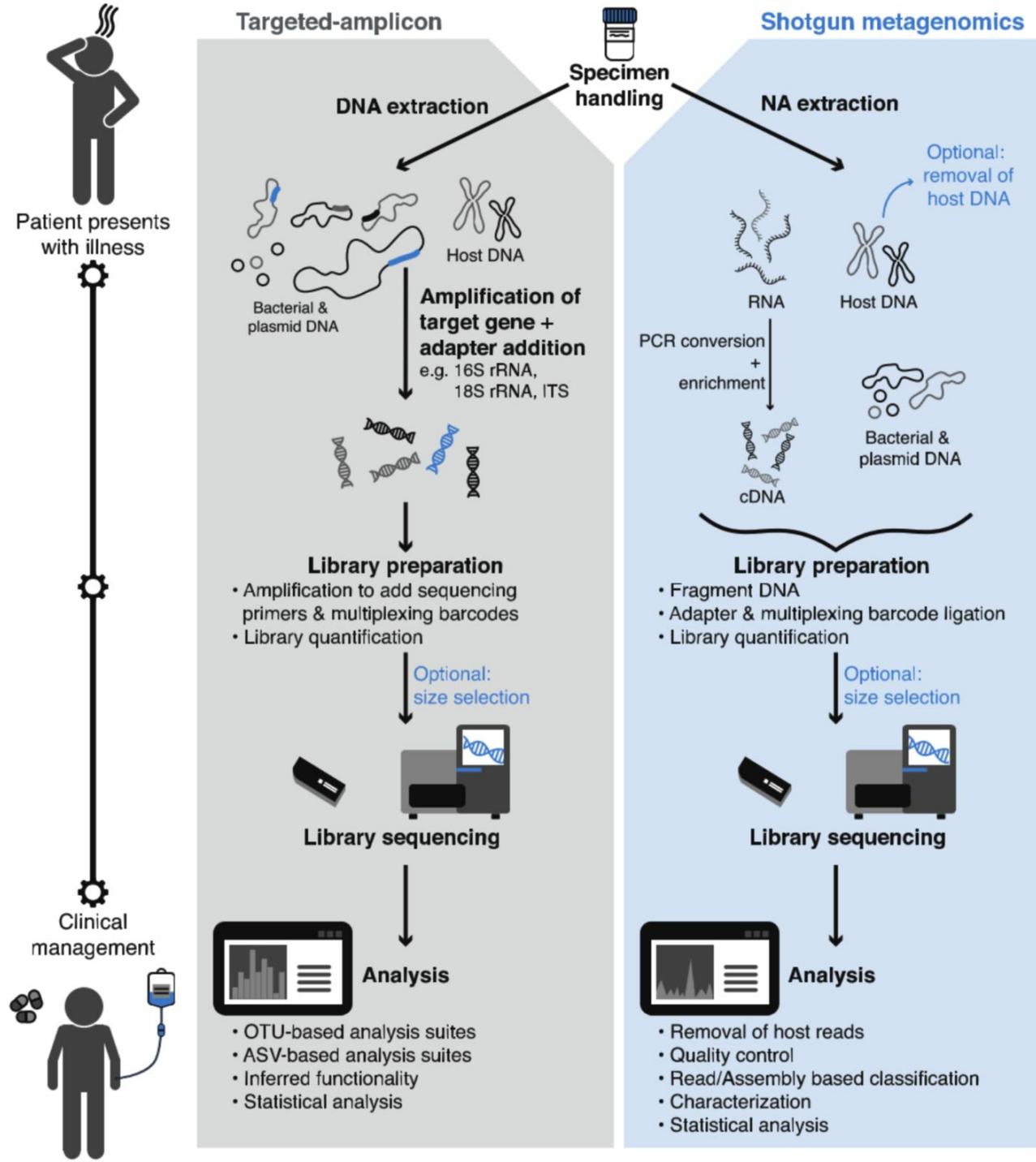
Metagenomics

Targets of amplicon sequencing

- Eukaryotes:
  - 18S rRNA gene
- Fungi:
  - ITS: internal transcribed spacer
- Bacteria and Archaea
  - 16S rRNA
  - *rpoB*
  - *cpn60*
  - 5S rRNA
  - 23S rRNA



Forbes 2018
Comp Struc Biotech J

Amplicon based metagenomics: species ID using 16S-23S NGS

- 1
- 1
- 1

Material	Routine Diagnostic culture	16S rDNA load (Ct)	16S rDNA Sanger result	16S-23S rDNA result	ID score (%)	Relative abundance (%)	Eukaryote DNA (%)	Background (%)
bronchial fluid	Haemophilus influenzae	22,2	Meng sequentie	Streptococcus parasanguinis	99,39	48	0	6
				Veillonella spp	98,10	13		
				Prevotella melaninogenica	99,15	8		
				Haemophilus influenzae	99,66	5		
				Actinomyces spp	94,99	3		
				Streptococcus spp	95,34	3		
				Streptococcus pneumoniae	98,89	3		
				Selenomonas spp	97,19	2		
				Streptococcus mitis	99,17	2		
				Prevotella spp	97,47	1		
				Solobacterium moorei	99,84	1		
				Selenomonas sputigena	100,00	1		
				Gemella spp	96,00	1		
				Enterococcus spp	90,81	1		
				Leptotrichia genomosp	99,65	1		
				Leptotrichia spp	93,62	1		
				Fusobacterium nucleatum	99,41	0		
				Prevotella spp	92,80	0		
				Megasphaera spp	92,68	0		
				Eubacterium spp	96,35	0		
				Campylobacter concisus	99,82	0		

gene

) score (%)	Relative abundance (%)	Eukaryote DNA (%)	Background (%)
97,16	85	0	0
99,25	6		
99,73	5		
95,18	2		
90,53	1		
93,63	1		
99,78	1		
99,2	99	1	0
99,27	38	2	2

				Mycobacterium abscessus	100	23		
	Mycobacterium abscessus (Molecular detection)			Corynebacterium accolens	99,8	17		
				Corynebacterium propinquum	100	10		
				Enterococcus faecalis	99,95	5		
				Staphylococcus spp	92,95	1		
				Klebsiella oxytoca	99,83	0		
				Staphylococcus capitis	99,91	0		
Plural fluid	Fusobacterium nucleatum	21,8	Mixed sequence patern	Prevotella pleuritidis	99.59	77	0	0
				Fusobacterium nucleatum	99.80	21		
				Actinomyces meyeri	100.00	1		

Dr. Mirjam Kooistra-Smid, Certe

Sabat et al, Sci Rep. 2017 Jun 13;7(1): 3434. doi: 10.1038/ s41598-017-03458-6.

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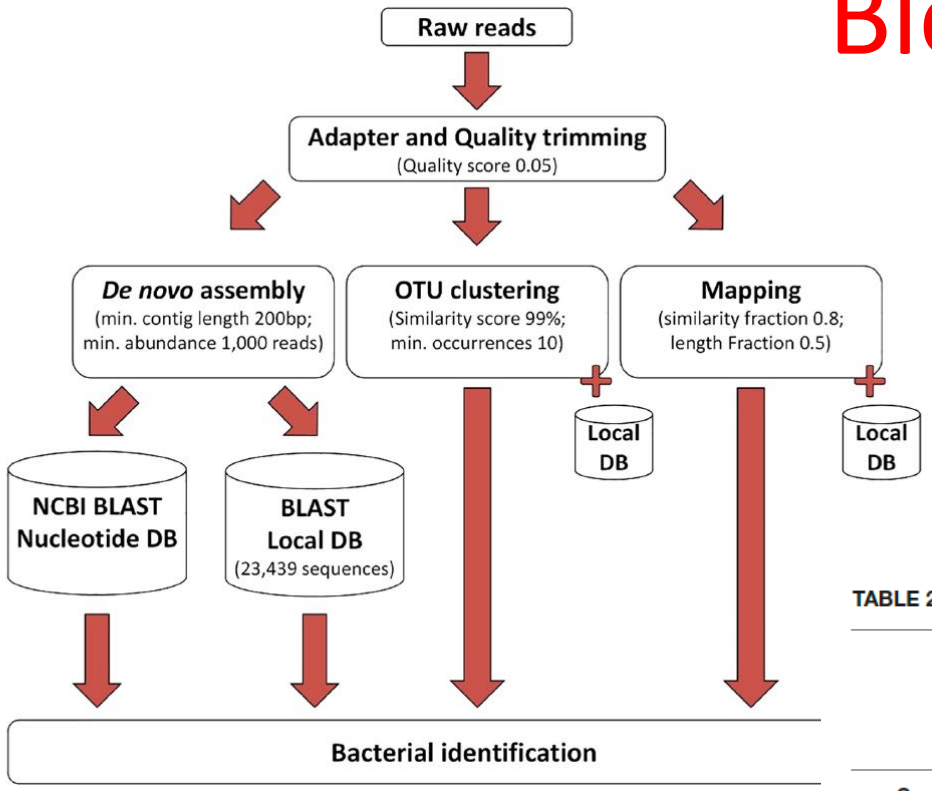
Bioinformatics



- *De novo* assembly and subsequent BLASTN analysis using an in-house developed database most accurate and fastest
- OTU clustering considered as a second approach if no pathogen species are identified
- Database needs to be continuously updated

TABLE 2 | Continued

		NGS of 16S–23S rRNA encoding region			Conventional methods	
		***De novo* assembly+BLAST (cut-off: 0.3%)**	**OTU clustering (cut-off: 0.2%)**	**Mapping (cut-off: 0.4%)**	**16S rRNA gene Sanger sequencing**	**Culturing**
Sample number		**Bacteria (relative abundance, %)**	**Bacteria (relative abundance, %)**	**Bacteria (relative abundance, %)**	**Bacteria**	**Bacteria**
27##		*Actinotignum schaalii* (1.4%) *Actinotignum* sp. (10.0%) *Aerococcus urinae* (6.2%) *Cutibacterium acnes* (1.0%)	*Actinotignum schaalii* (17.5%) *Aerococcus urinae* (13.9%)	*Actinotignum schaalii* (14.2%) *Aerococcus urinae* (7.4%)	*Actinotignum schaalii*	Negative
Time*	CLC analysis	~1 h 20 min	~3 h	~2 h 30 min		
	Hands on	~45 min	~1 h	~4 h		
	Total	~2 h 5 min	~4 h	~6 h 30 min		

*Analysis time is for all 30 samples (including positive and negative control) using a i7-6700 CPU @ 3.40 GHz, 32 GB RAM, 64-bit operating system computer. ¥In later analysis, Cutibacterium acnes was identified. #Tissue sample (heart valve); ##Fluid sample; Cutibacterium acnes had been formerly referred to as Propionibacterium acnes.

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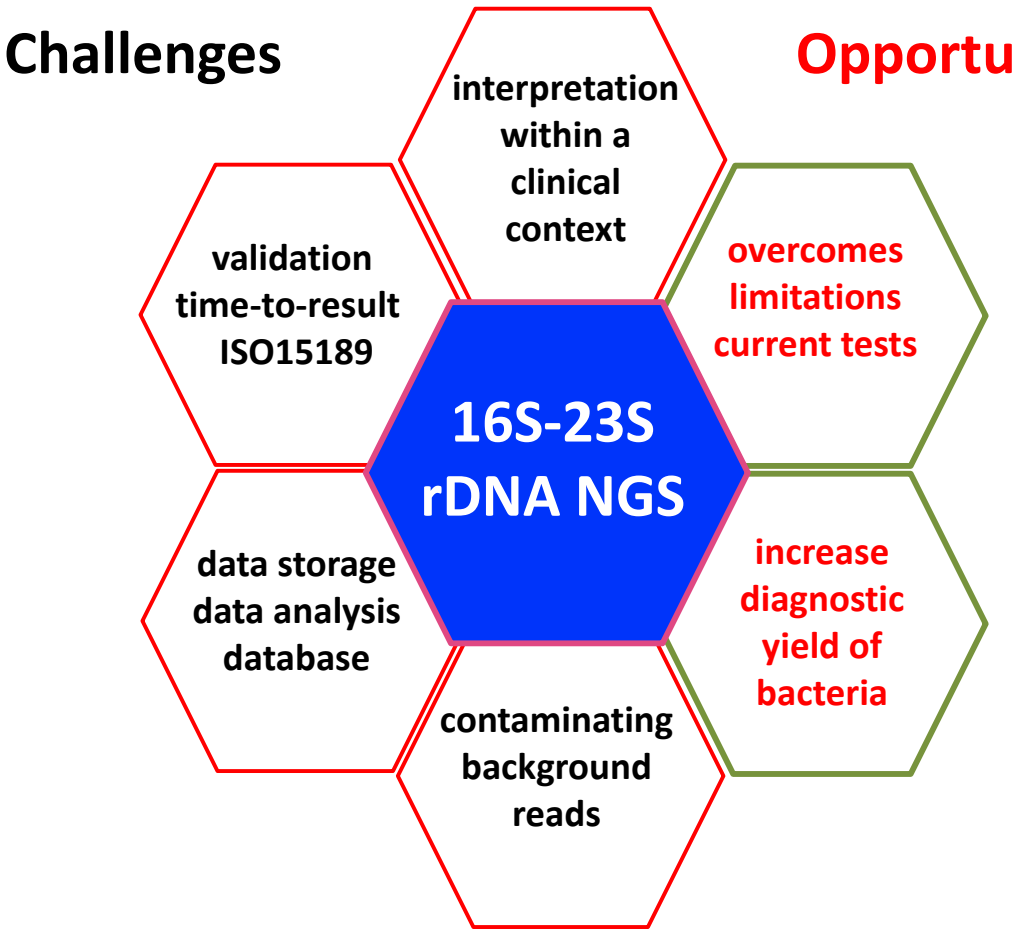
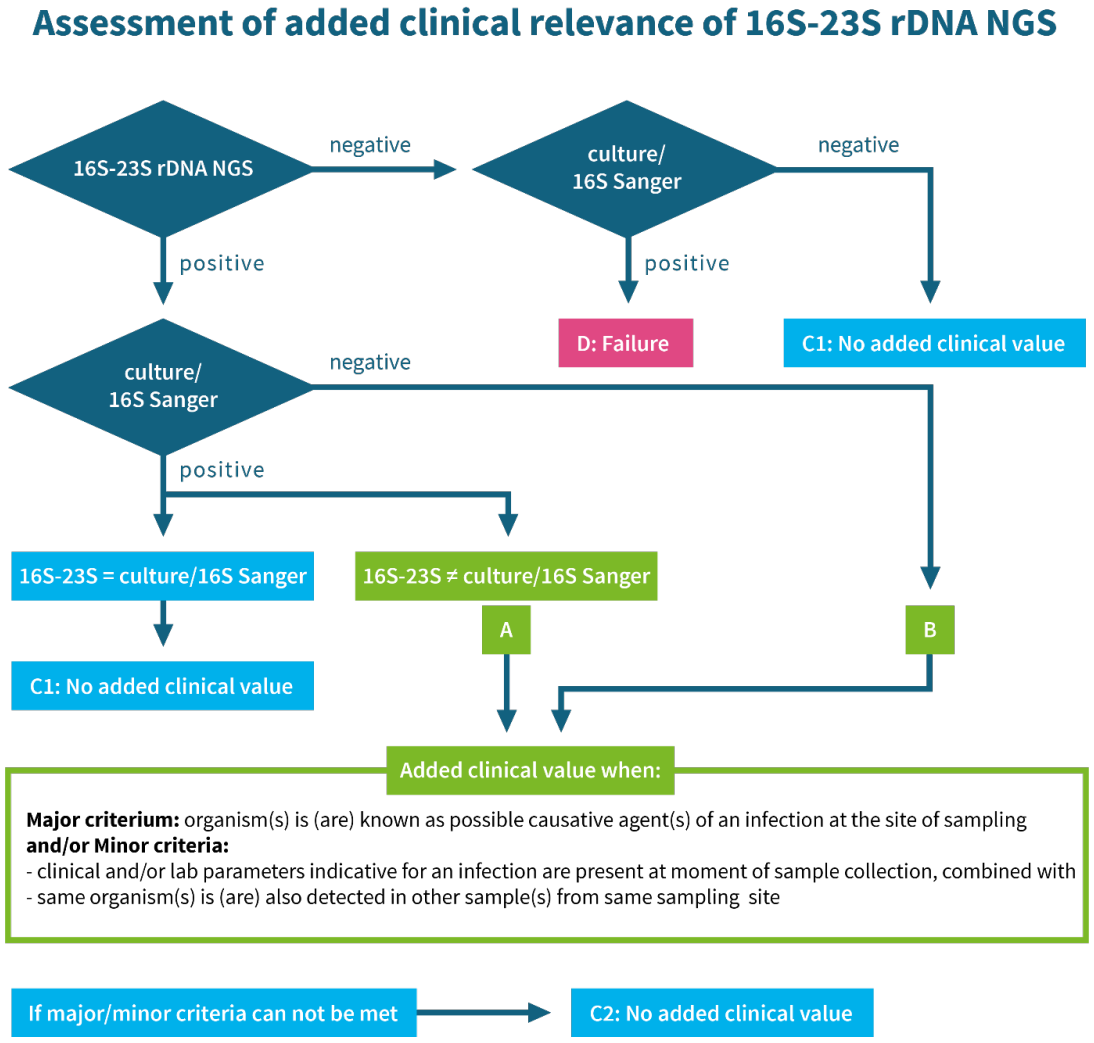
Peker et al., Frontiers in Microbiology, doi: 10.3389/fmicb.2019.00620

Assessment of the added clinical value of 16S-23S rDNA NGS in a clinical setting





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By courtesy of Dr. Mirjam Kooistra-Smid, CERTE

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Metagenomics

Targeted-amplicon sequencing

- Taxonomical assignment
- Relative quantification
- Change over time

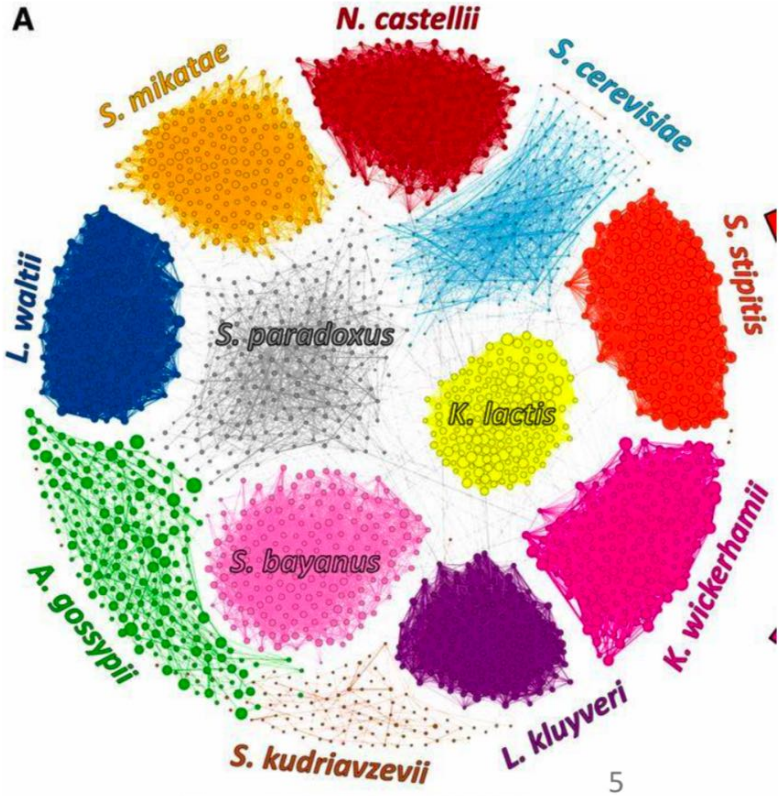
- Known Genes

No Human DNA
More sensitive
Less data per sample
$

Shotgun metagenomics

- Taxonomical assignment
- Relative quantification
- Change over time
- Genomes
- Functions & pathways
- All Genes

Human DNA
Less sensitive
More data
$$$$$



Burton 2014 G3

By courtesy of Claire Bertelli

First pathogen detections

Nakamura 2008 Emerging infect Dis

Metagenomic Diagnosis of Bacterial Infections

Shota Nakamura, Norihiro Maeda, Ionut Mihai Miron, Myonsun Yoh, Kaori Izutsu, Chidoh Kataoka, Takeshi Honda, Teruo Yasunaga, Takaaki Nakaya, Jun Kawai, Yoshihide Hayashizaki, Toshihiro Horii, and Tetsuya Iida

Author affiliations: Osaka University, Suita, Japan (S. Nakamura, I.M. Miron, M. Yoh, K. Izutsu, C. Kataoka, T. Honda, T. Yasunaga, T. Nakaya, T. Horii, T. Iida); RIKEN Yokohama Institute, Yokohama, Japan (N. Maeda, J. Kawai, Y. Hayashizaki);

Cite This Article

Abstract

To test the ability of high-throughput DNA sequencing to detect bacterial pathogens, we used it on DNA from a patient's feces during and after diarrheal illness. Sequences showing best matches for *Campylobacter jejuni* were detected only in the illness sample. Various bacteria may be detectable with this metagenomic approach.

34 year old male

Negative culture for enteric pathogens

Neg Norovirus PCR

Metagenomics: *C. jejuni*

Pathogen discovery

A Novel Cause of Chronic Viral Meningoencephalitis: Cache Valley Virus

Michael R. Wilson, MD, MAS,[1,2] Dan Suan, MBBS, PhD,[3] Andrew Duggins, MBBS, PhD,[4] Ryan D. Schubert, MD,[1,2] Lillian M. Khan, BS,[5] Hannah A. Sample, BS,[5] Kelsey C. Zorn, MHS,[5] Aline Rodrigues Hoffman, DVM, PhD,[6] Anna Blick, BS,[6] Meena Shingde, FRCPA,[7] and Joseph L. DeRisi, PhD[5,8]

Annals of Neurology, 2017;82:105-114

Pathogen discovery

Doan *et al. Genome Medicine* (2016) 8:90
DOI 10.1186/s13073-016-0344-6

Genome Medicine

RESEARCH Open Access

Illuminating uveitis: metagenomic deep sequencing identifies common and rare pathogens

Thuy Doan[1,2†], Michael R. Wilson[3,4†], Emily D. Crawford[3,5], Eric D. Chow[3], Lillian M. Khan[3], Kristeene A. Knopp[3], Brian D. O'Donovan[3], Dongxiang Xia[6], Jill K. Hacker[6], Jay M. Stewart[2], John A. Gonzales[1,2], Nisha R. Acharya[1,2] and Joseph L. DeRisi[3*]

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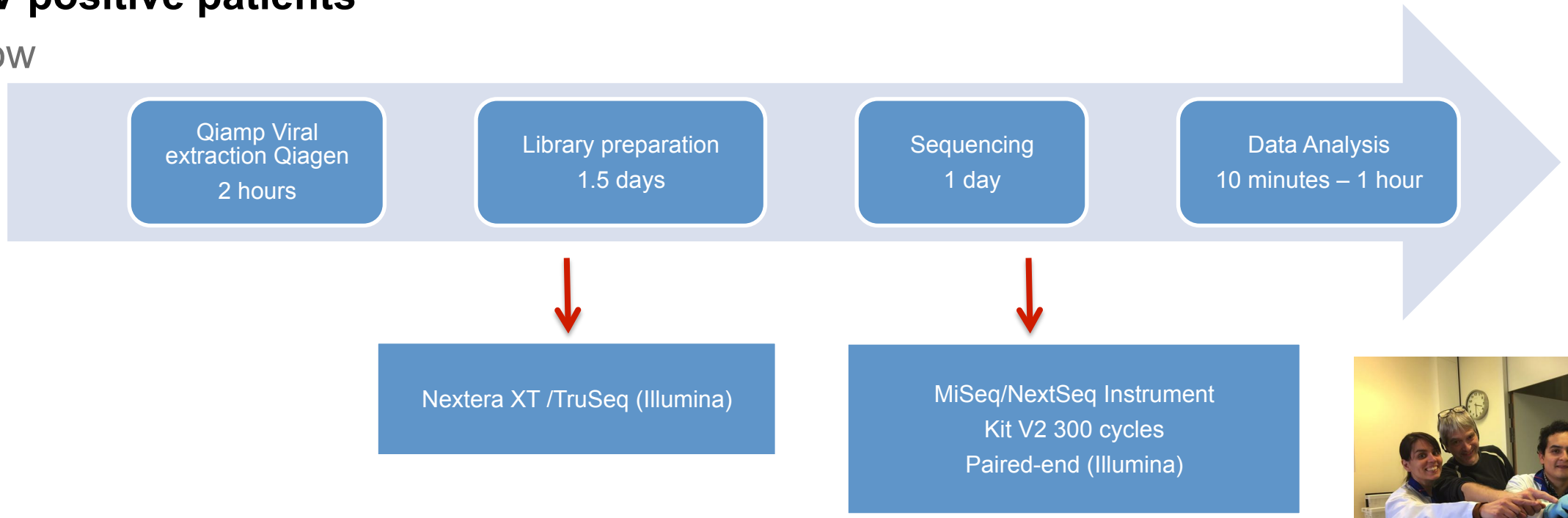
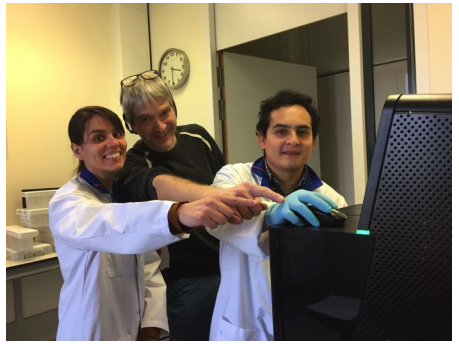
Dengue virus detection and typing from blood samples

17 DENV positive patients

Workflow





Classic approach: Sanger sequencing

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Lizarazo/Couto 2019, In : Journal of Biotechnology: X.2, 10 p., 100009.

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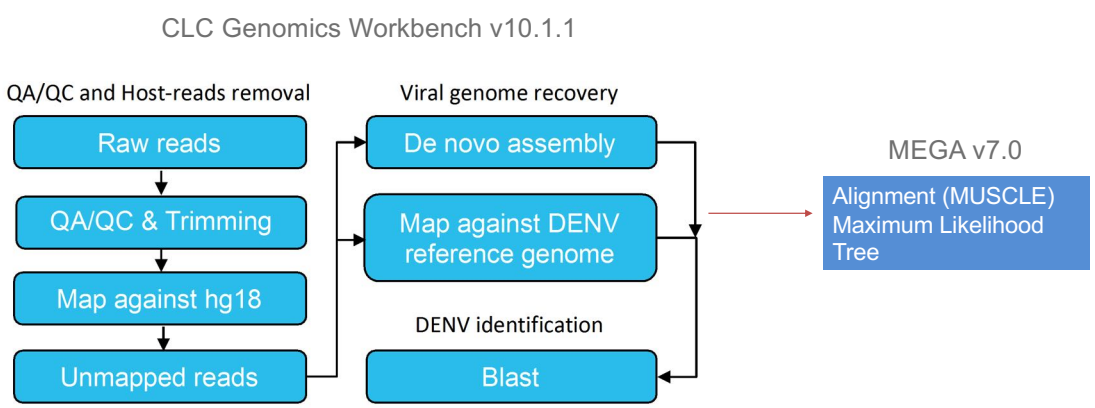
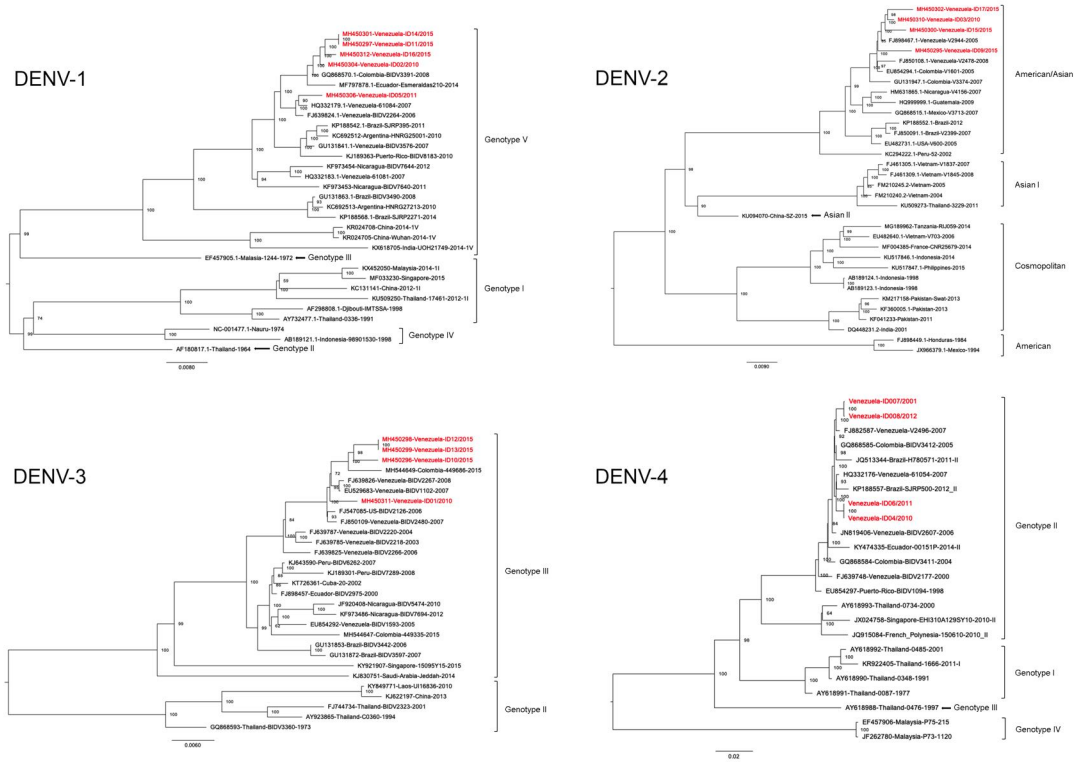
Bioinformatics analysis

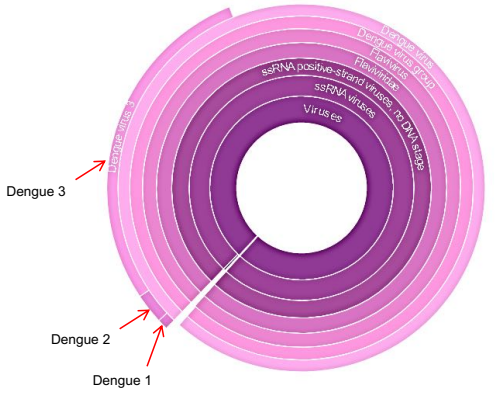


Sample	Total number of reads	Mapped reads against hg18	Unmapped reads against hg 18	Dengue-2 mapped reads	Average coverage	Longest contig
91-0109a	1,243,122	834,830 (67.2%)	408,292 (32.8%)	238,112 (19.2%)	3,301.7	10,599
91-0105b	1,427,648	1,215,507 (85.1%)	212,141 (14.9%)	51,914 (4.0%)	668.4	10,691
91-0121	2,802,530	2,220,772 (79.2%)	581,758 (20.8%)	288,694 (10.3%)	3,747.2	10,763
91-0131a	9,743,154	8,313,085 (85.3%)	1,430,069 (14.7%)	721,536 (7,4%)	10,053.8	10,529
91-0135b	562,914	302,336 (53.7%)	260,578 (46.3%)	87,679 (16.0%)	1,114.3	10,691
92-1095	3,640,058	3,038,591 (83.5%)	601,467 (16.5%)	165,422 (4.5%)	2,146.9	10,711
92-1096	3,918,662	3,671,259 (93.7%)	247,403 (6.3%)	110,770 (2.8%)	1,480.6	10,694
92-1099	2,810,772	2,251,377 (80.1%)	559,395 (19.9%)	53,517 (1.9%)	704.6	10,619
cc0007	2,654,296	2,144,891 (80.8%)	509,405 (19.2%)	60,185 (2.3%)	604.3	10,716

hg18: human genome



Detection and visualization Taxonomer IDbyDNA)



Spiked sample

CLC Genomics Workbench v10.1.1

Virus	Mapped reads	Coverage	Consensus (bp)	De Novo Assembly
DENV1	1180	15.45	10614	2796
DENV2	2514	32.4	10675	6736
DENV3	55952	733.66	10675	10555

Lizarazo/Couto 2019, In : Journal of Biotechnology: X.2, 10 p., 100009

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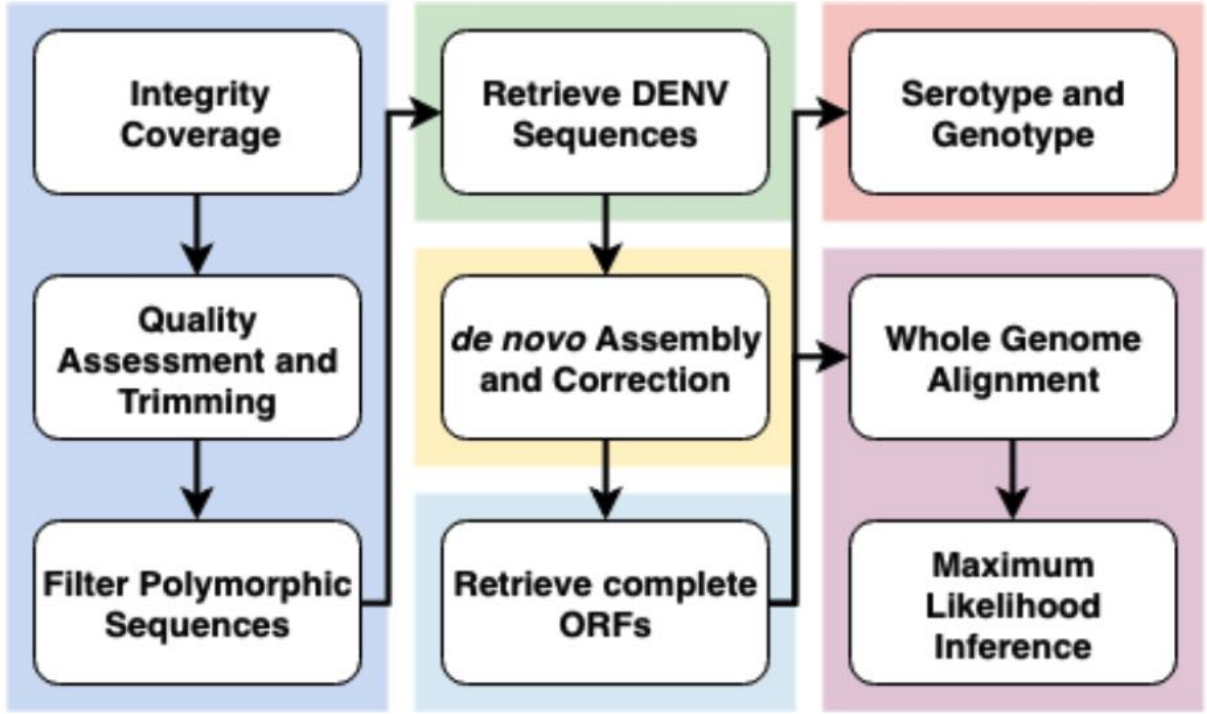
DEN-IM: Dengue Virus Identification from Metagenomic and Targeted Sequencing Data (Open source)

An automated workflow for identification, serotyping, genotyping, and phylogenetic analysis of DENV

Implemented in Nextflow alongside Docker containers to facilitate installation

Inês Mendes

João Carriço



https://github.com/B-UMMI/DEN-IM

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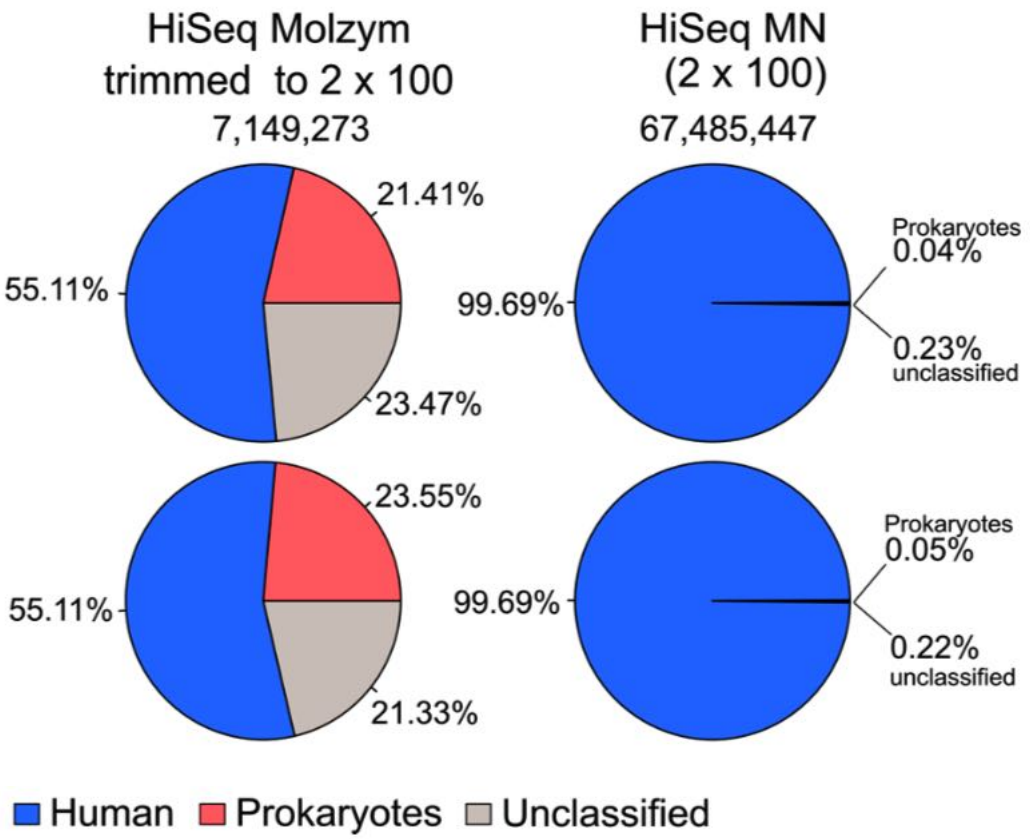
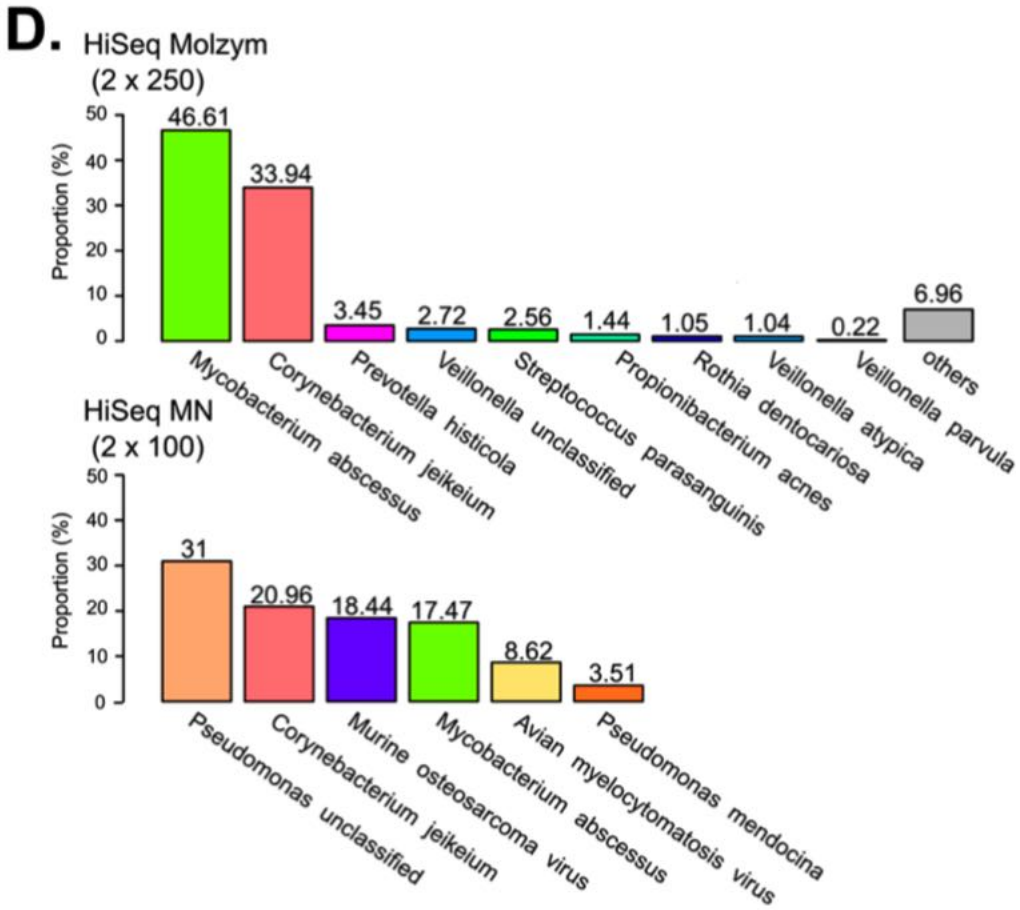
Mendes et al, biorxiv.org, https://doi.org/10.1101/628073

Human DNA depletion and DNA extraction

Depletion

No Depletion





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Leo et al., Int. J. Mol. Sci. **2017**, *18*, 2011

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Getting rid of the human reads

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Negative control
Sample type	Peritoneal fluid	Pus (abscess)	Synovial fluid	Synovial fluid	Pus (abscess)	Pus (empyema)	Pus (empyema)	Bone biopsy	Pus (abscess)	Sputum	Water
DNA extraction method	Ultra-Deep Microbiome Prep (Molzym)	Ultra-Deep Microbiome Prep (Molzym)	Ultra-Deep Microbiome Prep (Molzym)	Ultra-Deep Microbiome Prep (Molzym)	Ultra-Deep Microbiome Prep (Molzym)	QIAamp DNA Microbiome Kit (Qiagen)	QIAamp DNA Microbiome Kit (Qiagen)	Micro-DX™ (Molzym)	Micro-DX™ (Molzym)	Micro-DX™ (Molzym)	QIAamp DNA Microbiome Kit (Qiagen)
Total number of reads	5,892,978	9,603,346	8,615,810	6,078,166	8,368,930	2,912,802	1,486,700	6,534,866	6,173,132	7,596,836	1,730,738
Mapped reads against hg19	5,249,063 (89.2%)	7,828.746 (81.6%)	8,254,594 (95.9%)	6,015,945 (99.0%)	309,588 (3.7%)	2,877,066 (98.8%)	922,932 (62.2%)	229,149 (3.5%)	6,081,612 (98.5%)	7,337,832 (96.7%)	1,706,861 (98.9%)
Unmapped reads	632,951 (10.8%)	1,770,558 (18.4%)	355,200 (4.1%)	61,099 (1.0%)	8,052,272 (96.3%)	34,506 (1.1%)	561,772 (37.8%)	6,303,803 (96.5%)	89,922 (1.5%)	235,520 (3.3%)	19,805 (1.2%)

Table 1. Characteristics of the samples and mapping of trimmed reads against a human genome hg19 (%) using CLC Genomics Workbench v10.0.1.

hg19 – human genome

Couto N et al., Sci Rep. 2018 Sep 13;8(1):13767. doi: 10.1038/s41598-018-31873-w

Bioinformatics

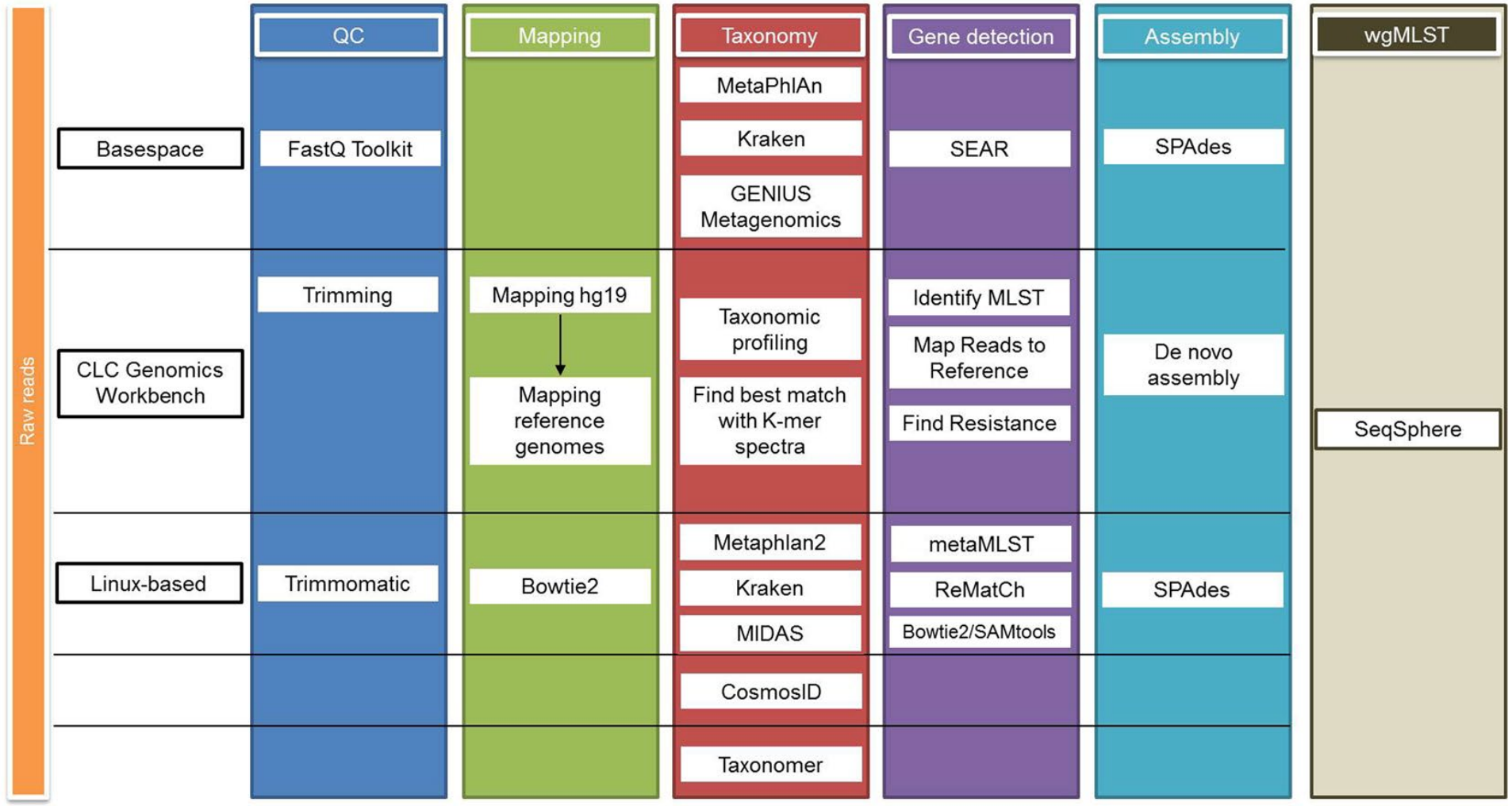

Figure 1. Scheme of the bioinformatic analysis of the metagenomics samples.

Unix-based software

Sample number	Culture result (CFU)[a]	Conventional identification (MALDI-TOF)	WGS-based identification	Shotgun metagenomics		
				Kraken[b]	MIDAS[c]	MetaPhlAn[c]
1	10^3 10^3 10	*E. faecium* *S. haemolyticus* *C. glabrata*	*E. faecium* *S. haemolyticus* —	*E. faecium* (34.6%) *S. haemolyticus* (10.1%) —	*E. faecium* (62.0%) *S. haemolyticus* (28.0%) —	*E. faecium* (66.6%) *S. haemolyticys* (27.7%) —
2	10^3 1 Not determined	*E. avium* *E. coli* Anaerobes	—# —# —#	Not identified* Not identified* Several species (29.5%)	Not identified* Not identified* Several species (100.0%)	Not identified* Not identified* Several species (100.0%)
3	1	*S. epidermidis*	—#	*S. aureus* (0.2%)	Not identified*	Not identified*
4	10^3	*S. aureus*	*S. aureus*	*S. aureus* (0.73%)	*S. aureus* (100%)	*S. aureus* (100%)
5	$\geq 10^5 \geq 10^5$ 10^3 10^3 Not determined 10	*E. coli* *K. oxytoca* *S. anginosus* *E. faecalis* Anaerobes *C. albicans*	*E. coli* *K. oxytoca* —# *E. faecalis* —# —#	*E. coli* (9.7%) *K. oxytoca* (0.5%) *S. anginosus* (0.07%) *E. faecalis* (0.3%) Several species (12.7%) —	*E. coli* (6.5%) *K. oxytoca* (0.3%) *S. anginosus* (0.01%) *E. faecalis* (0.9%) Several species (96.7%) —	*E. coli* (8.5%) *K. oxytoca* (0.3%) *Streptococcus* spp. (0.09%) *E. faecalis* (0.7%) Several species (90.4%) —
6	10^3	*E. faecium*	*E. faecium*	*E. faecium* (0.77%)	Not identified*	Not identified*
7	10^2	*S. aureus*	—#	*S. aureus* (82.9%)	*S. aureus* (100%)	*S. aureus* (100%)
8	10^3	*O. intermedium*	*O. intermedium*	*O. anthropi* (21.3%)	*O. intermedium* (99.4%)	*O. intermedium* (99.1%)
9	10^3	*S. aureus*	*S. aureus*	*S. aureus* (22.9%)	*S. aureus* (100%)	*S. aureus* (100%)
10	10^3	*S. marcescens*	—#	*S. marcescens* (64.7%)	*S. marcescens* (99.1%)	*S. marcescens* (100%)

CLC Genomics workbench

Sample number	Culture result (CFU)[a]	Conventional identification (MALDI-TOF)	WGS-based identification	Shotgun metagenomics	
				Taxonomic Profiling (CLC)[b]	Best match with K-mer spectra (CLC)[c]
1	10^3 10^3 10	*E. faecium* *S. haemolyticus* *C. glabrata*	*E. faecium* *S. haemolyticus* —	*E. faecium* (71%) *S. haemolyticus* (24%) *C. glabrata* (100%)	*E. faecium* (41.4%) *S. haemolyticus* (13.8%) *C. glabrata* (0.5%)
2	10^3 1 Not determined	*E. avium* *E. coli* Anaerobes	—[#] —[#] —[#]	Not identified* Not identified* Several species (97%)	Not identified* Not identified* Several species (13.2%)
3	1	*S. epidermidis*	—[#]	Not identified*	*S. aureus* (4%)
4	10^3	*S. aureus*	*S. aureus*	Not identified*	*S. aureus* (9.7%)
5	$\geq 10^5 \geq 10^5$ 10^3 10^3 Not determined 10	*E. coli* *K. oxytoca* *S. anginosus* *E. faecalis* Anaerobes *C. albicans*	*E. coli* *K. oxytoca* —[#] *E. faecalis* —[#] —[#]	*E. coli* (25%) *K. michiganensis* (0.3%) Not identified* *E. faecalis* (2%) Several species (70.0%) Not identified*	*E. coli* (11.5%) Not identified* Not identified* *E. faecalis* (0.6%) Not identified* *C. albicans* (<0.05%)
6	10^3	*E. faecium*	*E. faecium*	Not identified*	*E. faecium* (4.0%)
7	10^2	*S. aureus*	—[#]	*S. aureus* (100%)	*S. aureus* (95.5%)
8	10^3	*O. intermedium*	*O. intermedium*	*O. intermedium* (86.0%)	*O. intermedium* (91.2%)
9	10^3	*S. aureus*	*S. aureus*	*S. aureus* (100%)	*S. aureus* (81.2%)
10	10^3	*S. marcescens*	—[#]	*S. marscescens* (100%)	*S. marcescens* (79.7%)

Web-based tools

Sample number	Culture result (CFU)[a]	Conventional identification (MALDI-TOF)	WGS-based identification	Shotgun metagenomics				
				Genius (Basespace)[c]	Kraken (Basespace)[c,d]	MetaPhlAn (Basespace)[c]	Taxonomer (Utah)[b,e]	Cosmos ID[a]
1	10^3 10^3 10	*E. faecium* *S. haemolyticus* *C. glabrata*	*E. faecium* *S. haemolyticus* —	*E. faecium* (14.4%) *S. haemolyticus* (55.8%) —	*E. faecium* (25.0%) *S. haemolyticus* (20.1%) —	*E. faecium* (65.1%) *S. haemolyticys* (30.4%) —	*E. faecium* (22.9%) *S. haemolyticus* (20.1%) Not identified*	*E. faecium* (50.3%) *S. haemolyticus* (22.1%) *C. glabrata* (88.6%)
2	10^3 1 Not determined	*E. avium* *E. coli* Anaerobes	—[#] —[#] —[#]	Not identified* Not identified* Several species (94.0%)	Not identified* Not identified* Several species (27.0%)	Not identified* Not identified* Several species (54.2%)	Not identified* Not identified* Several species (14.2%)	Not identified* Not identified* Several species (100%)
3	1	*S. epidermidis*	—[#]	*S. aureus* (100%)	*S. aureus* (0.1%)	Not identified*	*S. pseudintermedius* (3.4%)	Not identified*
4	10^3	*S. aureus*	*S. aureus*	*S. aureus* (100%)	*S. aureus* (0.3%)	*S. aureus* (100%)	*S. aureus* (8.3%)	*S. aureus* (100%)
5	$\geq 10^5 \geq 10^5$ 10^3 10^3 Not determined 10	*E. coli* *K. oxytoca* *S. anginosus* *E. faecalis* Anaerobes *C. albicans*	*E. coli* *K. oxytoca* —[#] *E. faecalis* —[#] —[#]	*E. coli* (0.4%) Not identified* *S. anginosus* (0.03%) *E. faecalis* (0.8%) Several species (45.0%) —	*E. coli* (10.2%) *K. oxytoca* (0.5%) *S. anginosus* (0.4%) *E. faecalis* (0.3%) Several species (8.0%) —	*E. coli* (7.0%) *K. pneumoniae* (0.01%) *S. anginosus* (0.3%) *E. faecalis* (0.7%) Several species (89.1%) —	*E. coli* (3.6%) *K. michiganensis* (0.1%) *S. anginosus* (0.1%) *E. faecalis* (0.1%) Several species (60.3%) —	*E. coli* (7.6%) *K. oxytoca* (1.7%) *S. anginosus* (0.09%) *E. faecalis* (3.7%) Several species (86.2%) Not identified*
6	10^3	*E. faecium*	*E. faecium*	*E. faecium* (4.2%)	*E. faecium* (14.8%)	*E. faecium* (5.5%)	*E. faecium* (1.4%)	*E. faecium* (4.1%)
7	10^2	*S. aureus*	—[#]	*S. aureus* (100%)	*S. aureus* (93.8%)	*S. aureus* (100%)	*S. aureus* (14.2%)	*S. aureus* (100%)
8	10^3	*O. intermedium*	*O. intermedium*	*O. intermedium* (100%)	*O. nthropic* (88.9%)	*O. intermedium* (99.8%)	*O. intermedium* (13.1%)	*O. intermedium* (49.5%)
9	10^3	*S. aureus*	*S. aureus*	*S. aureus* (100%)	*S. aureus* (99.5%)	*S. aureus* (100%)	*S. aureus* (12.7%)	*S. aureus* (100%)
10	10^3	*S. marcescens*	—[#]	*S. marcescens* (32.5%)	*S. marcescens* (94.8%)	*Serratia* spp. (100%)	*S. marcescens* (1.4%)	*S. marscescens* (38.4%)

Bioinformatics impact - summary

Method	Total number of bacteria identified[a]	True positives[a]	False positives	False negatives	Sensitivity (%)	PPV (%)
Culture/MALDI-TOF	9	9	0	0	100%	100%
MetaPhlAn (BaseSpace)	16	7	9	2	78%	44%
Genius (BaseSpace)	35	8	27	1	89%	23%
Kraken (BaseSpace)	959	7	952	2	78%	1%
Taxonomer (Full Analysis)	4649	8	4641	1	89%	0%
CosmosID	35	8	27	1	89%	23%
Taxonomic Profiling (CLC Genomics Workbench v10.0.1)	17	6	11	3	67%	35%
Best match K-mer spectra (CLC Genomics Workbench v10.0.1)	12	8	4	1	89%	67%
Kraken (Unix)	198	7	191	2	78%	4%
MetaPhlAn2 (Unix)	15	7	6	4	78%	54%
MIDAS (Unix)	34	7	26	2	78%	21%

Couto N et al., Sci Rep. 2018 Sep 13;8(1):13767. doi: 10.1038/s41598-018-31873-w

AMR detection in the WGS era a no go?

- published evidence for using WGS as a tool to infer antimicrobial susceptibility accurately --> poor or non-existent
- for most bacterial species major limitations are
  - current high-cost
  - limited speed
  - dependency on previous culture
- for most bacterial species there is currently insufficient evidence to support the use of WGS-inferred AST to guide clinical decision making

Ellington et al., Clinical Microbiology and Infection 23 (2017) 2e22

Antimicrobial resistance

Sample number	Conventional identification (MALDI-TOF)	Conventional susceptibility testing (VITEK 2)[b]	WGS CLC Genomics Workbench	Shotgun metagenomics	
				ReMatCh (Unix)	CLC Genomics Workbench[a]
1	*E. faecium* *S. haemolyticus*	LEV, ERY, CLI OXA, GEN, CIP, FOS, ERY, CLI	*erm(B), msr(C), ant(6')-Ia, aph(3')-III, dfrG* *blaZ, mecA, ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), erm(C), mph(C), msr(A), dfrG*	*erm(B), msr(C), ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), blaZ, mecA, erm(C), mph(C), msr(A), dfrG*	*erm(B), msr(C), ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), blaZ, mecA, erm(C), mph(C), msr(A), dfrG*
2	*E. avium* *E. coli* Anaerobes	DOX, CLI susceptible —	—[#] —[#] —[#]	Not detected Not detected *catS, lnu(D), lsa(C), cepA-44, tet(Q)*	Not detected Not detected *catS, lnu(D), lsa(C), cepA-44, tet(Q), fusA*
3	*S. epidermidis*	OXA, GEN, TEC, FUS, CIP, ERY, CLI	—[#]	Not detected	Not detected
4	*S. aureus*	PEN, ERY	*blaZ, spc, erm(A)*	Not detected	Not detected
5	*E. coli* *K. oxytoca* *S. anginosus* *E. faecalis* Anaerobes	susceptible AMX susceptible DOX, CLI —	—[#] *blaOXY-1–3* —[#] *tet(M), lsa(A)* —[#]	— Not detected — *tet(M)* *cfxA4, tet(Q)*	— Not detected — *tet(O)* *cfxA4, tet(Q)*
6	*E. faecium*	PEN, AMX, CFX, IMP, GENhl, STRhl, LEV, ERY, CLI, AMP/SUL	*erm(B), msr(C), ant(6')-Ia, aph(3')-III, aac(6')-aph(2"), dfrG*	Not detected	Not detected
7	*S. aureus*	PEN	*blaZ*	*blaZ, norA*	*blaZ*
8	*O. intermedium*	AMX, PIP/TAZ, CFX, CFT, CTZ, IMP, FOX, TOB, FOS, NIT, TMP	*bla*OCH-2	*bla*OCH-5	*bla*OCH-2
9	*S. aureus*	PEN	—[#]	*blaZ*	*blaZ*
10	*S. marcescens*	AMX, AMC, CFX, FOX, NIT, POL	—[#]	*blaSST-1, tet(41), oqxB, aac(6')-Ic*	*tet(41), oqxB, aac(6')-Ic*

- 1, 7 and 9 genotypes and phenotypes correlated well
- Other samples not all AMR genes explaining phenotypic resistance identified
- 1, 5, 7 and 10 different results ReMatCh vs CLC Genomics workbench

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Couto N et al., Sci Rep. 2018 Sep 13;8(1):13767. doi: 10.1038/s41598-018-31873-w

There is hope…

- WGS-based MIC prediction allows reliable MIC prediction for five gonorrhoea antimicrobials *Eyre et al. J Antimicrob Chemother 2017; 72: 1937–1947*
- WGS can aid in the timely diagnosis of *Mycobacterium tuberculosis* drug resistance and guide clinical decision-making *Ruesen et al., scientific reports| (2018) 8:9676 | DOI:10.1038/s41598-018-27962-5*
- Whole-genome sequencing effective tool for predicting antibiotic resistance in nontyphoidal *Salmonella*, although the use of more appropriate surveillance breakpoints and increased knowledge of new resistance alleles will further improve correlations *McDermott et al. Antimicrob Agents Chemother 60:5515–5520. doi:10.1128/AAC.01030-16.*

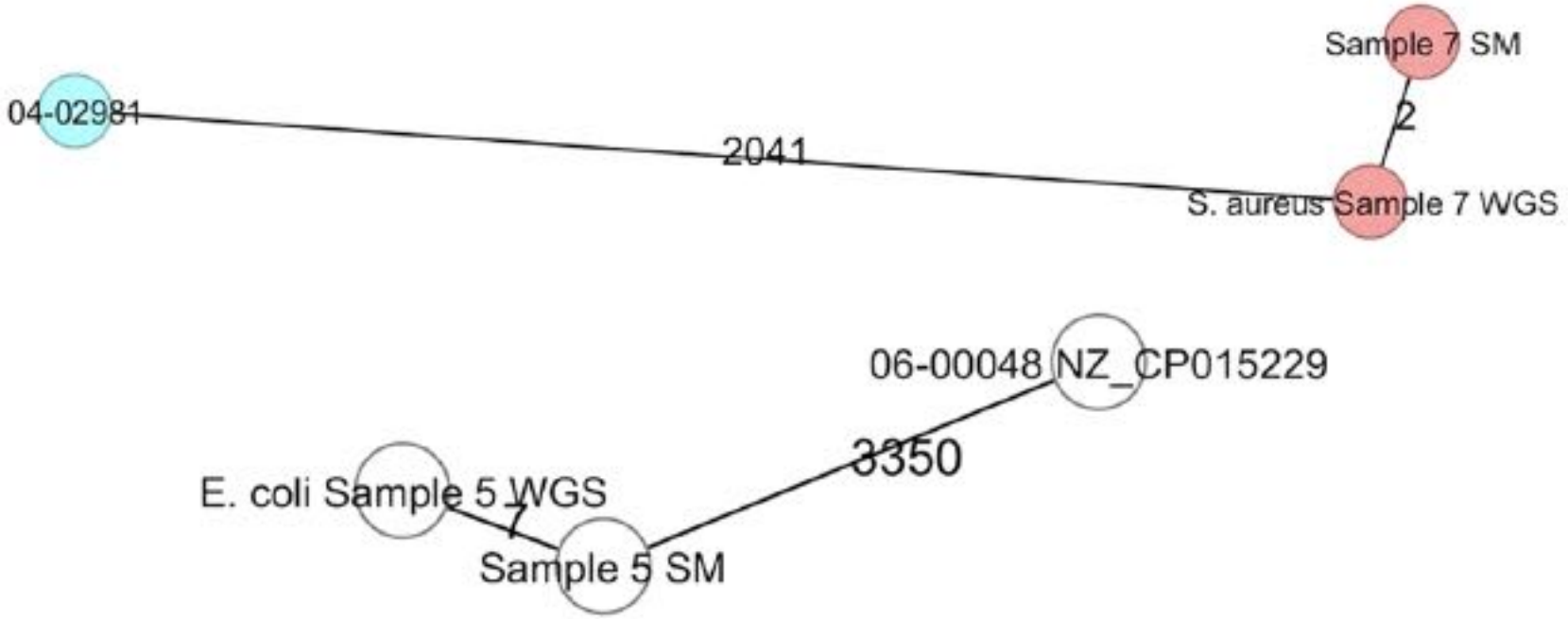
Turn-around-time

- Will depend on eventual pre-enrichment, extraction methods, sequencing technologies, computational platforms

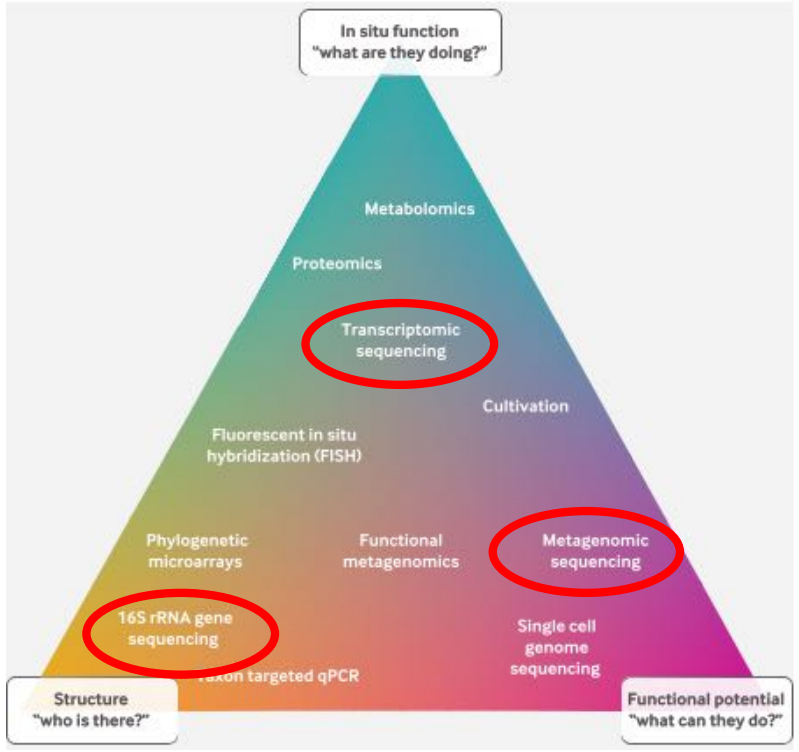
Sample type	Sequencing platform	Total reads	Matching reads (percentage)	Pathogen	TaT	Reference
CSF	MiSeq	3,063,784	475 (0.016%)	*Leptospira santarosai*	48h	Wilson 2014 N Eng J Med
Spinal cord or Brain	Illumina	8.3 – 29.1 Mio	15-9000 (<0.033%)	*M. Tuberculosis* JC polyomavirus EBV	24h	Salzberg 2016 NNN
BAL	MinIon	NA	1 x 3,217 bp 6 x 909-6,288 bp	*P. aeruginosa* *S. aureus*	9h	Pendleton 2017 Am J Crit Care Med
Stools	MinIon	NA		*K. pneumoniae*	Few h	Leggett 2017 BioRxiv
Urine	MinIon	NA	(76%-98%)	*E. coli* *K. pneumoniae* *E. cloacae*	4h	Schmidt 2017 J Antimicrob Chemother

Metagenomic Typing

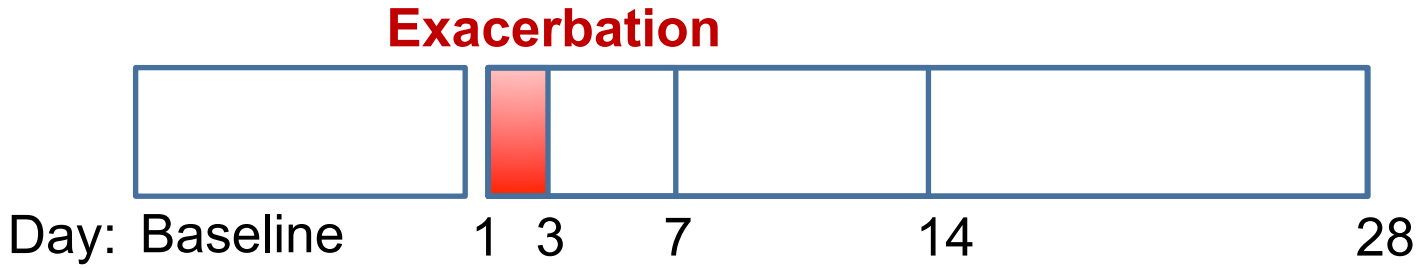
Sample number	Conventional identification (MALDI-TOF)	WGS	Shotgun metagenomics	
		CLC Genomics Workbench v10.1.1	CLC Genomics Workbench v10.1.1	metaMLST (Unix-based)
1	*E. faecium* *S. haemolyticus*	ST117 ST25	Not detected (6 alleles identified correctly) Not detected (3 alleles identified correctly)	ST117 Not detected
2	*E. avium* *E. coli* Anaerobes			
3	*S. epidermidis*			
4	*S. aureus*			
5	*E. coli* *K. oxytoca* *S. anginosus* *E. faecalis* Anaerobes			
6	*E. faecium*			
7	*S. aureus*			
8	*O. intermedium*			
9	*S. aureus*			
10	*S. marcescens*			



Study of the microbiome in Obstructive Pulmonary Diseases



44 COPD patients followed longitudinally during stability and acute exacerbations

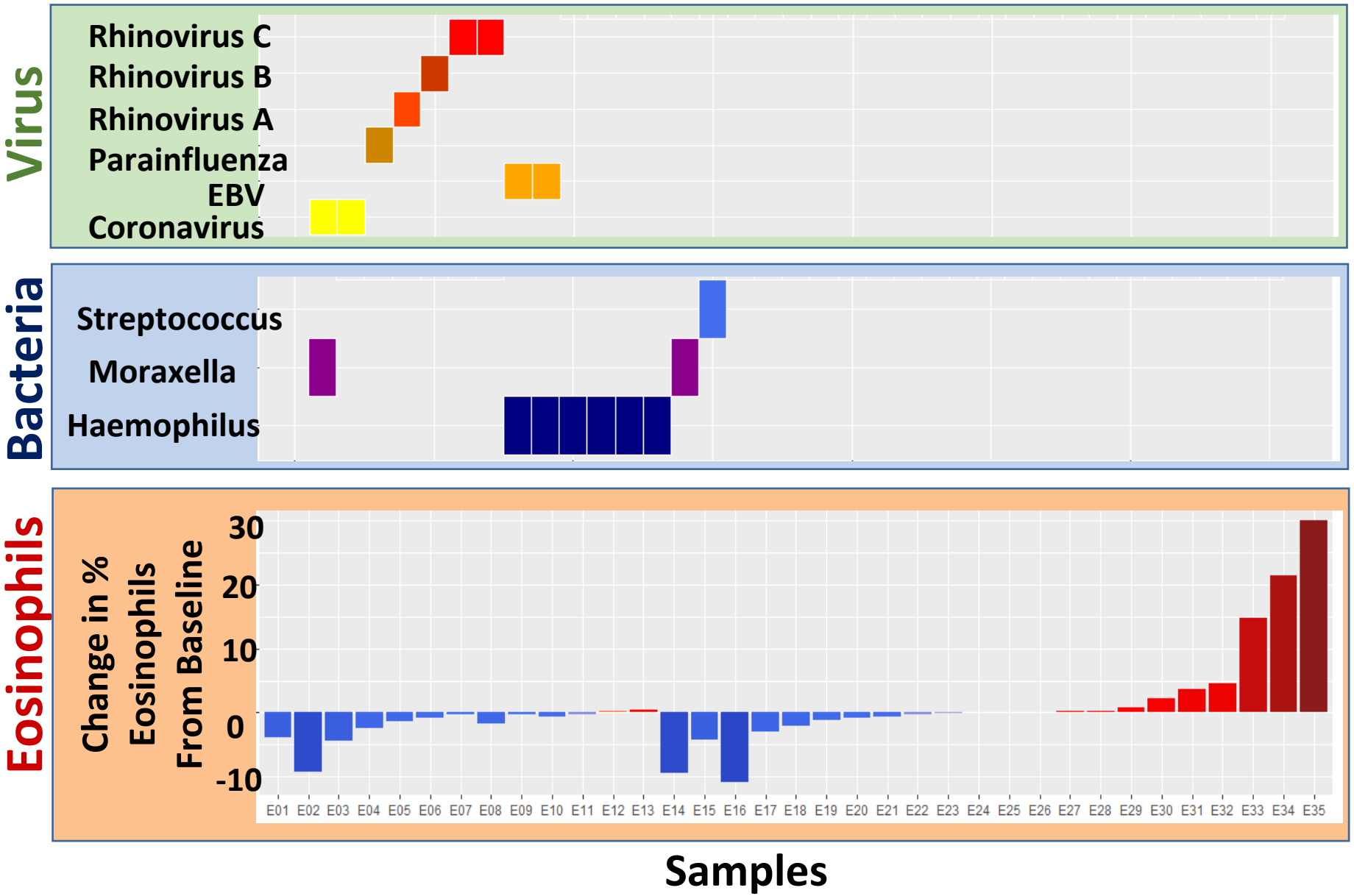


- Induced sputum at each of 6 visits
- Cell count/differential
- RNA for metagenomic sequencing
- Extensive clinical phenotyping

Treatments:

- Doxycycline
- Bronchodilators
- +/- Steroids

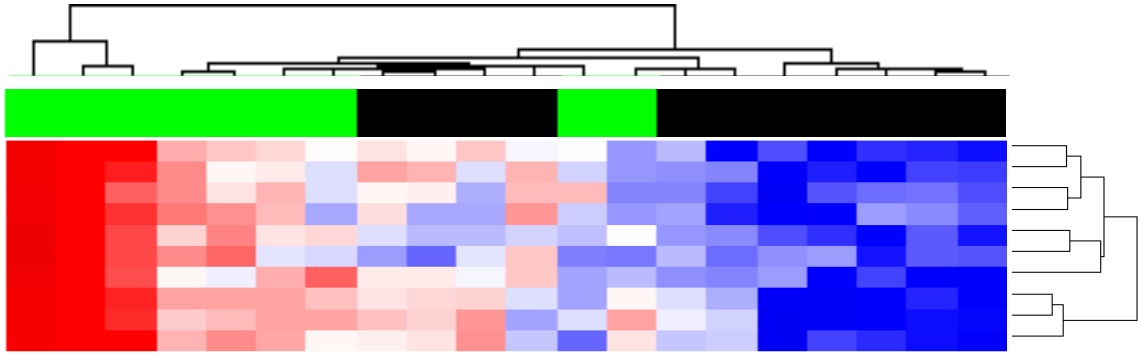
Cross sectional data at AECOPD



Cross sectional data at AECOPD: Host immune response

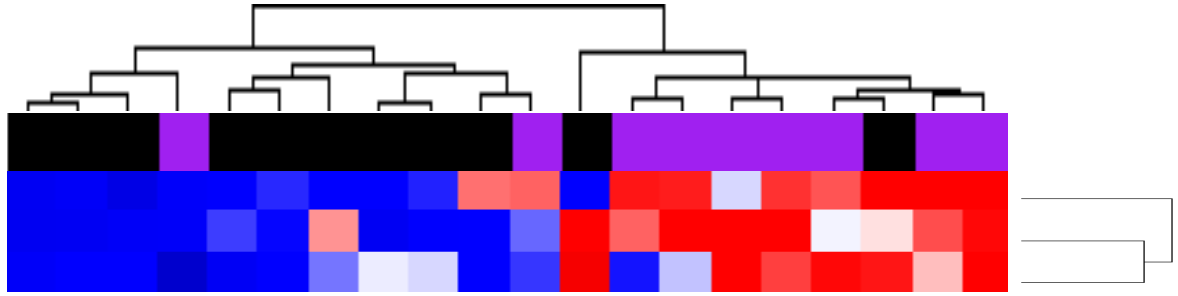
Virus

Anti-viral
(Interferon)



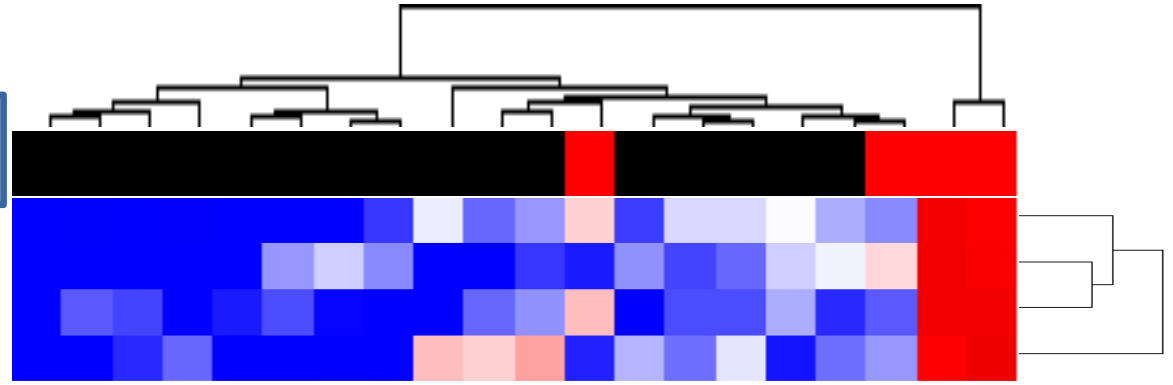
Bacterial

Anti-
Bacterial
(IL-17)

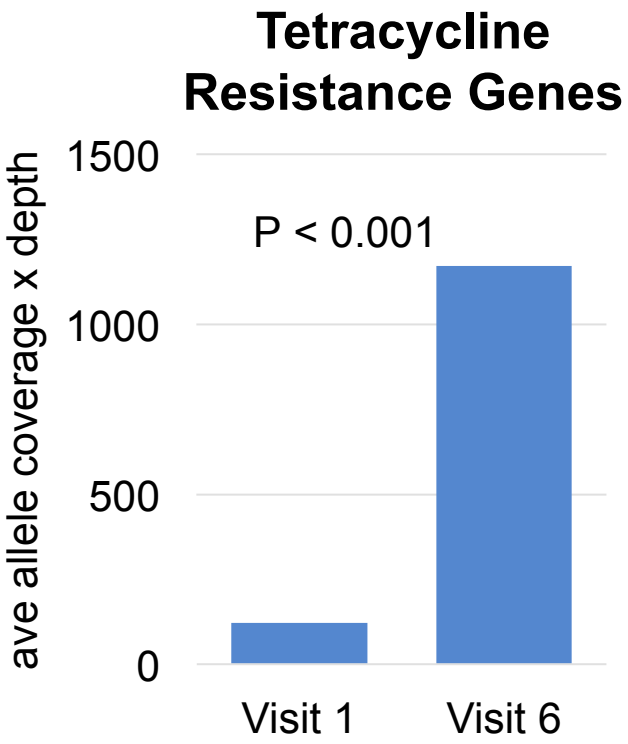


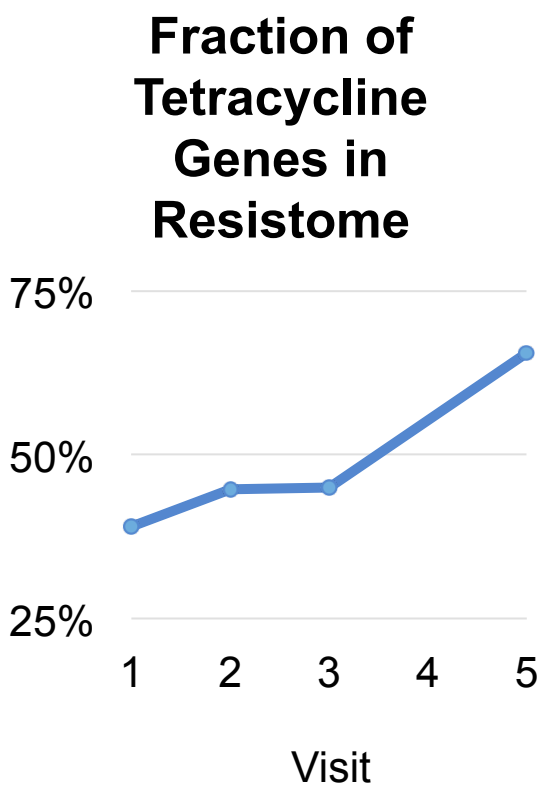
Eosinophilic

Allergic
(Type 2)



Tracking antibiotic resistance





Challenges

- Clinical sensitivity and specificity → depletion human DNA / enrichment microbial DNA/RNA
- Genotype not always phenotype (AMR) → RNAseq?
- Which genes belong to which pathogen ? → Single cell sequencing/ cross-linking plasmid and genome/CG content?
- Presence of contaminant DNA → reagents, sample taking
- Persistence of DNA from dead microbes → RNAseq
- Colonization versus infection → host response, RNAseq?

Collaborations and acknowledgements

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I apopologize in advance if I forget to mention people – please contact me afterwards if you think your name should be on this slide

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