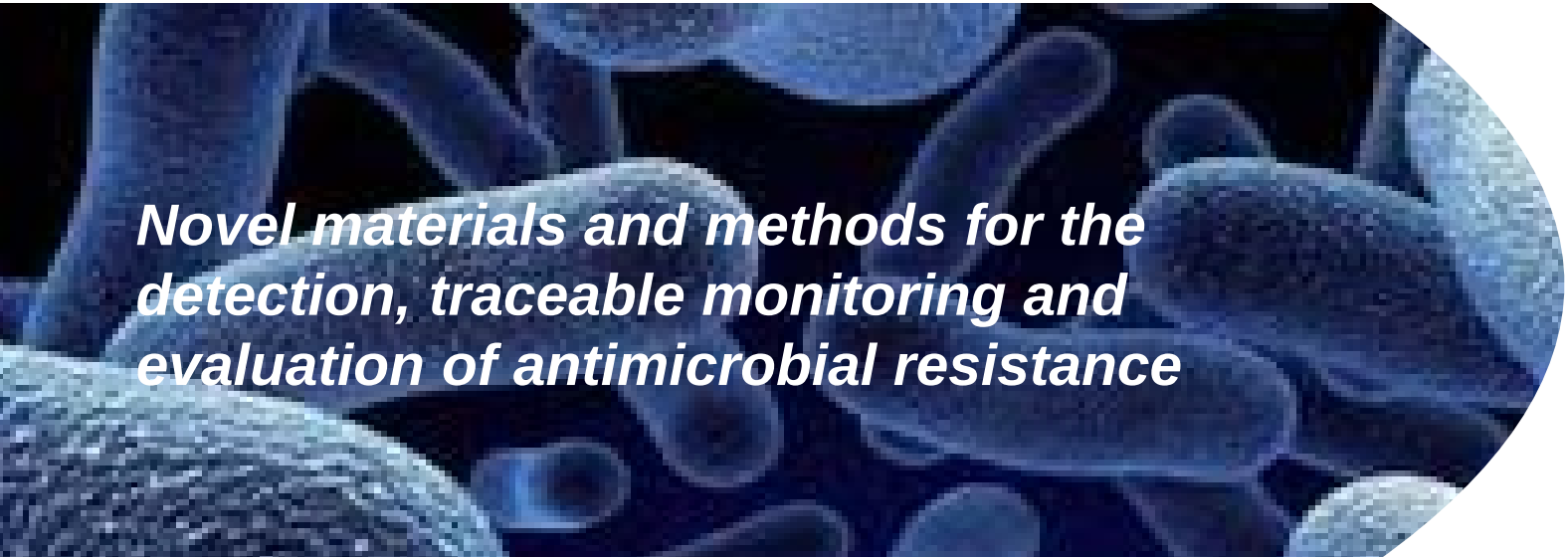
How can measurement science assist in improving the molecular detection and management of antimicrobial resistance?

Jim Huggett, LGC & University of Surrey



Antimicrobial resistance (AMR)

- World Health Organization
- CDC Centers for Disease Control and Prevention
  CDC 24/7: Saving Lives, Protecting People™
- Antibiotic / Antimicrobial Resistance
- About Antimicrobial Resistance
- Biggest Threats
- Protecting Yourself and Your Family
- Protecting Patients and Stopping Outbreaks
- For Laboratories: Testing & Resources
- Protecting the Food Supply
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New colistin resistance gene identified in China

Filed Under: Antimicrobial Stewardship; MCR-1

Chris Dall | News Reporter | CIDRAP News | Jun 28, 2017

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Researchers in China have discovered another gene that confers resistance to the last-resort antibiotic colistin.

In a study yesterday in *mBio*, the researchers report that the MCR-3 gene was discovered in a fecal sample obtained from an apparently healthy pig at a farm in Shangdong province during a routine surveillance study of antimicrobial resistant bacteria. The gene was located on a colistin-resistant *Escherichia coli* isolate, on a plasmid that contained 18 additional antibiotic

Erik Thor / Thinkstock

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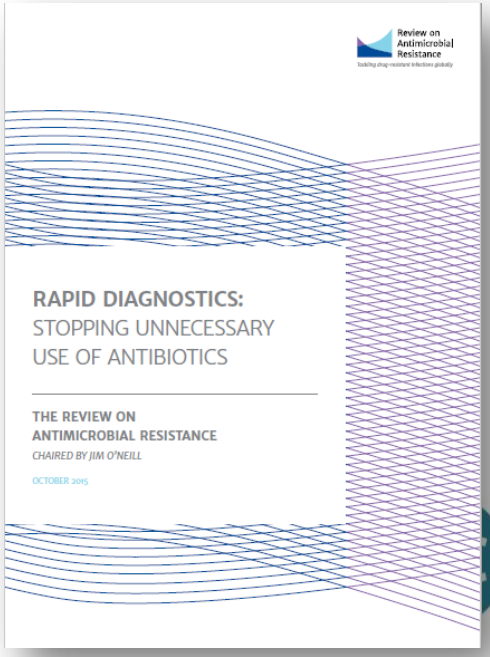
PRINCIPA UNDERWRITE

Threat from AMR

In 2014 WHO stated anti microbial resistance (AMR) is so serious it threatens the achievements of modern medicine

- new therapies to treat resistant pathogens are needed
- diagnostic tools required to guide their application are equally lacking



Diagnosis

Clinicians need methods to:

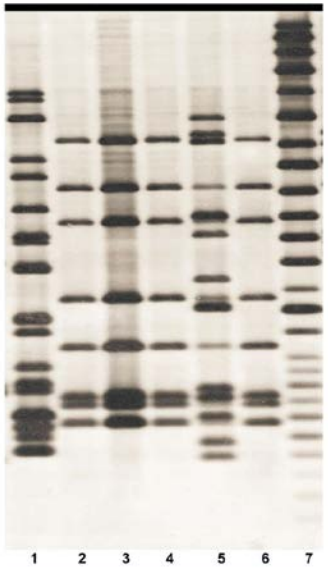
- Rapidly diagnose patients with infections that do need antimicrobials
- Detect infections that are already resistant
- Monitor patients for the development of resistance

Examples of methods for measuring bacterial resistance

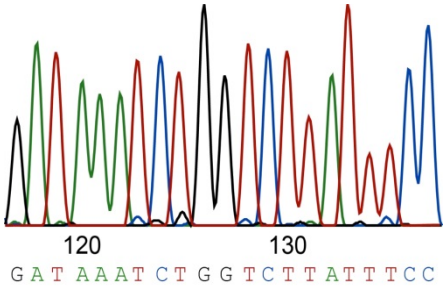
- Dilution method (broth and agar dilution method)
- Disk-diffusion method
- E-test
- Mechanism-specific tests such as beta-lactamase detection test and chromogenic cephalosporin test
- Molecular methods

Specific DNA detection methods

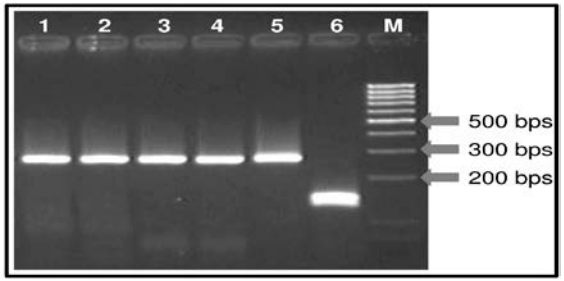
Hybridisation



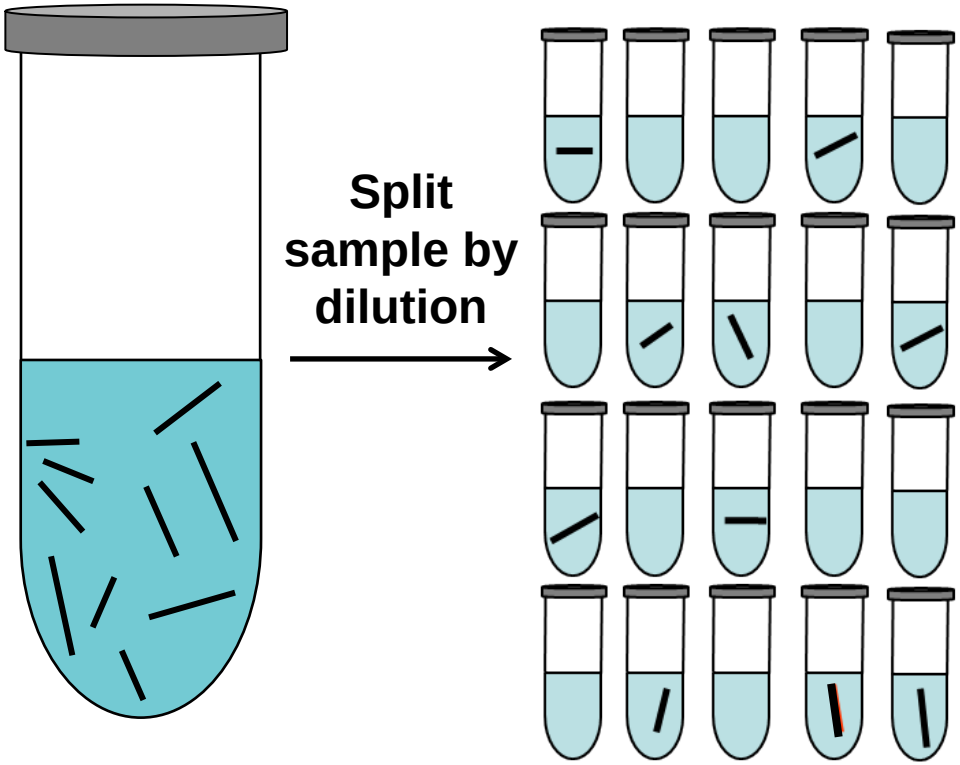
Sequencing



NAAT/PCR



Digital PCR



- Limiting dilution
  - Some reaction contain 0 templates
- PCR performed as normal using standard real-time PCR chemistry
- Absolute quantification
  - +ve or –ve reactions
  - Poisson statistics to account for multiple targets per partition (> 1)

Methicillin resistance Staph aureus (MRSA)

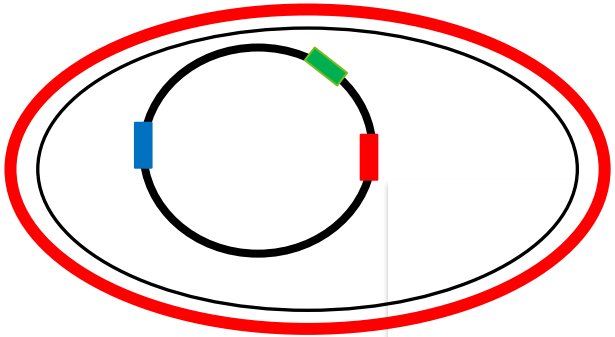
- ***S. aureus*** **is a common bacteria found on the human body**
- **Can be an important cause of disease (e.g. wounds, pneumonia, invasive infection)**
- **MRSA complicates treatment as first line antibiotics do not work**
- **Molecular methods can be used to determine if a patient is carrying/infected with MRSA**
- **Other organisms can carry resistance gene**

mecA

coA

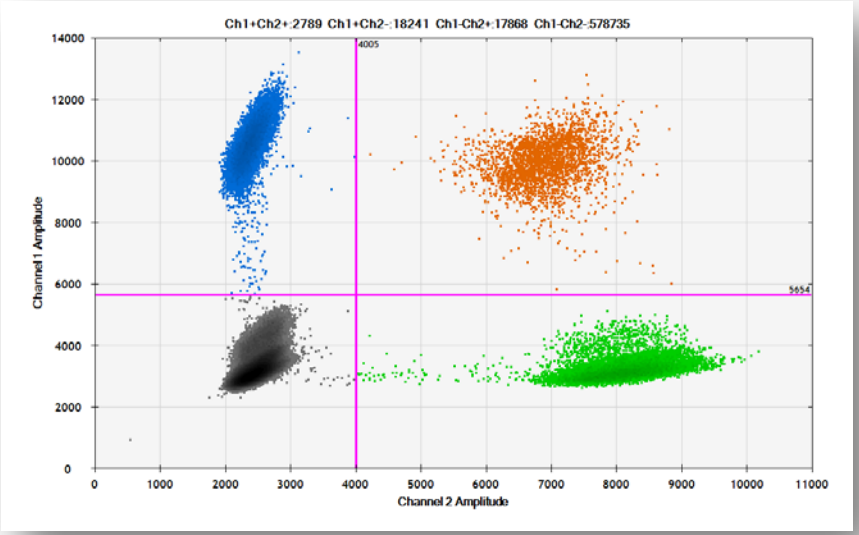
sa442

MRSA

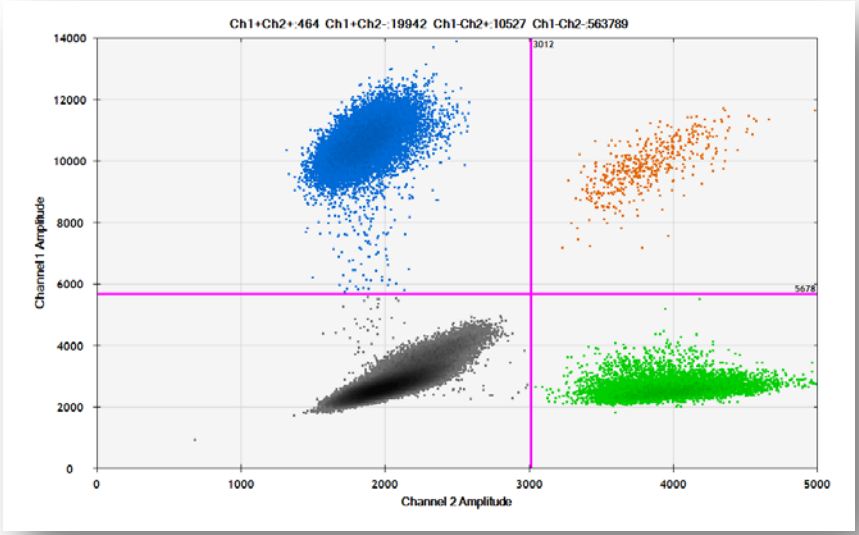


MRSA

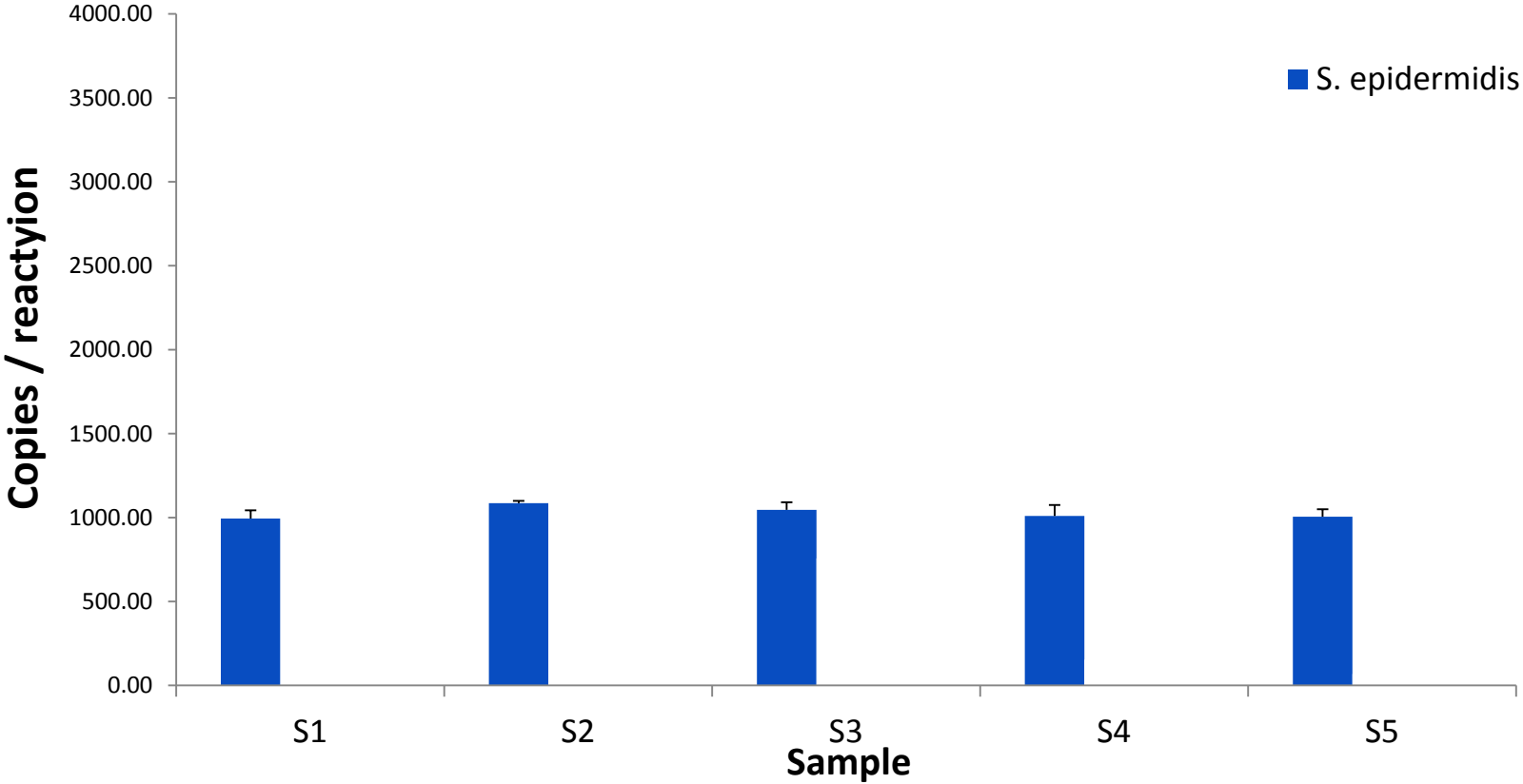
S. aureus & Mec assays



S. epidermidis & Mec assays



MRSA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S. epidermidis
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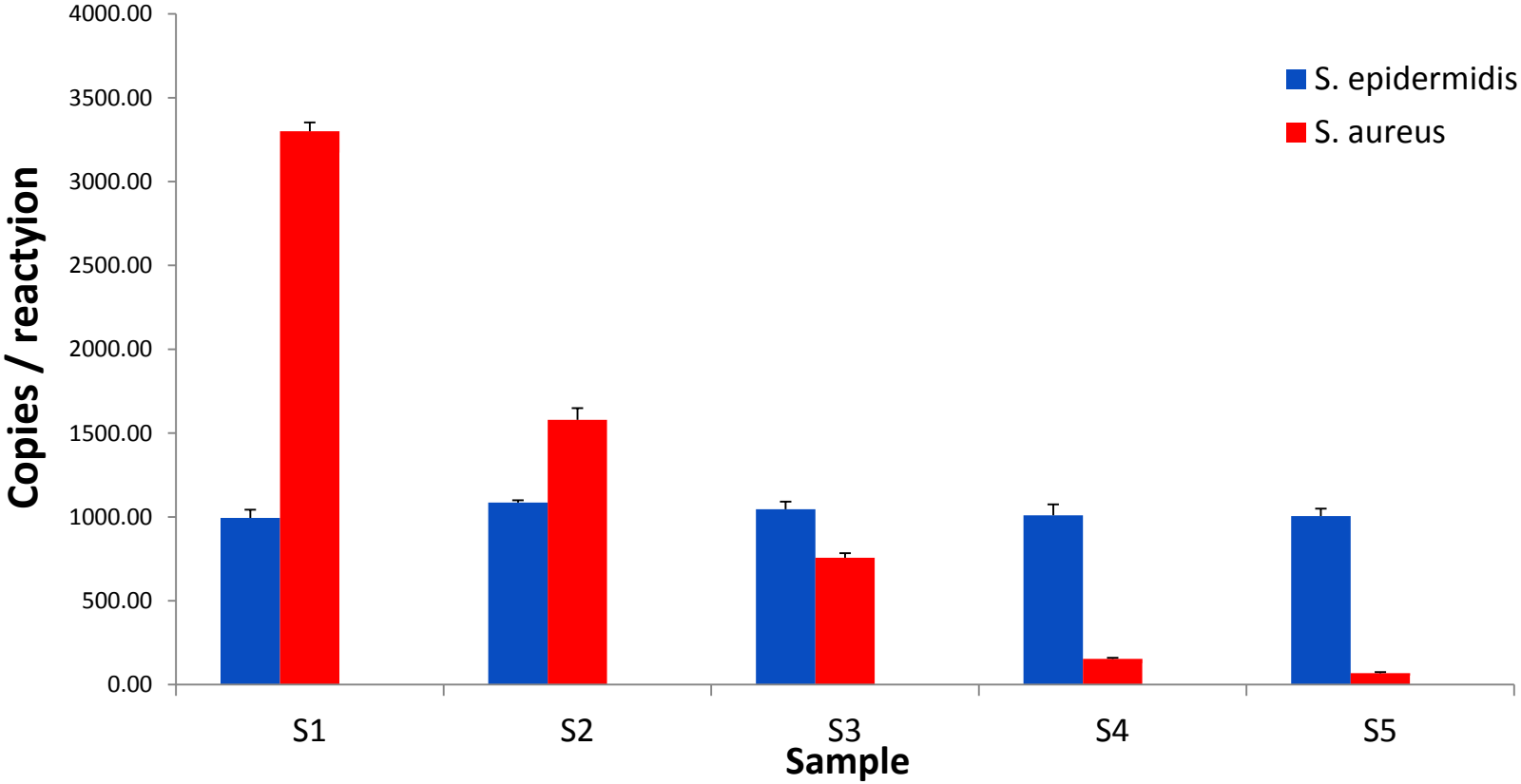
Sample

MRSA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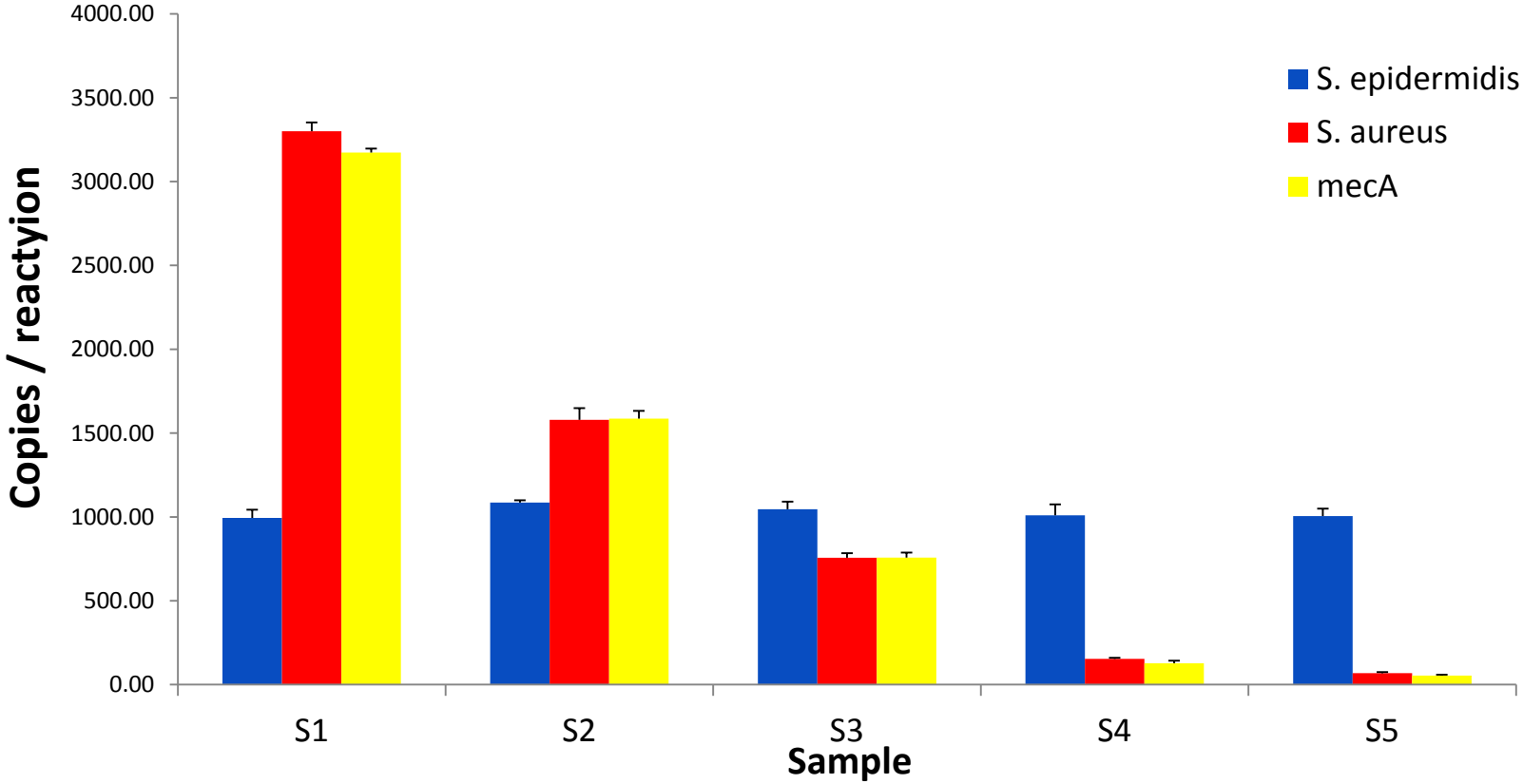
Sample

MRSA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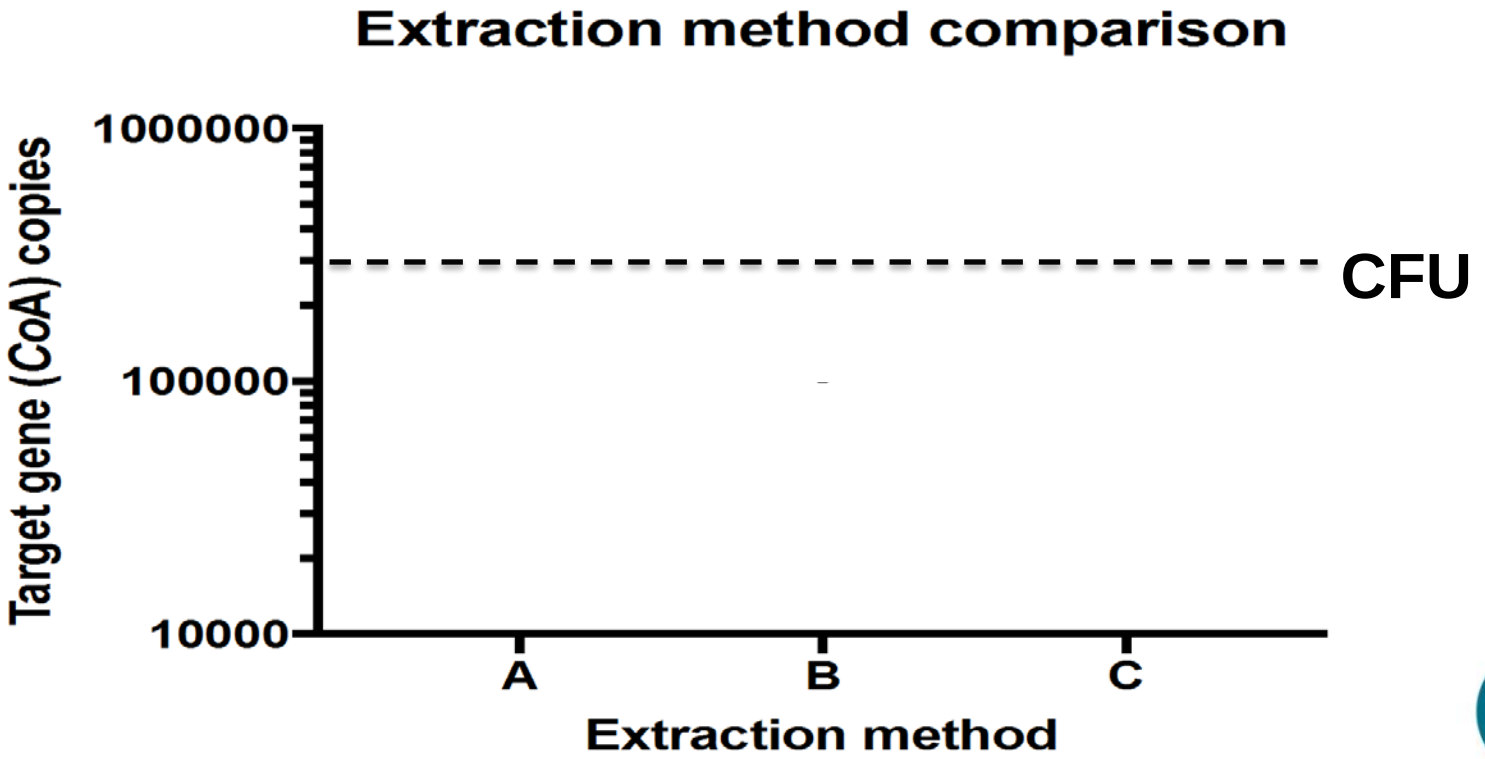
S. epidermidis
S. aureus
mecA

Comparison of extraction methods when quantifying *Staphylococcus aureus*



Molecular diagnostics

Gene Xpert

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Devonshire *et al. BMC Infectious Diseases* (2016) 16:366
DOI 10.1186/s12879-016-1696-7

BMC Infectious Diseases

RESEARCH ARTICLE — Open Access

The use of digital PCR to improve the application of quantitative molecular diagnostic methods for tuberculosis

Alison S. Devonshire[1†], Denise M. O'Sullivan[1†], Isobella Honeyborne[2], Gerwyn Jones[1], Maria Karczmarczyk[3], Jernej Pavšič[4], Alice Gutteridge[1], Mojca Milavec[4], Pablo Mendoza[5], Heinz Schimmel[3], Fran Van Heuverswyn[3], Rebecca Gorton[2], Daniela Maria Cirillo[6], Emanuele Borroni[6], Kathryn Harris[7], Marinus Barnard[8,9], Anthenette Heydenrych[8,9], Norah Ndusilo[10], Carole L. Wallis[11], Keshree Pillay[11], Thomas Barry[12], Kate Reddington[12], Elvira Richter[13], Erkan Mozioğlu[14], Sema Akyürek[14], Burhanettin Yalçınkaya[14], Muslum Akgoz[14], Jana Žel[4], Carole A. Foy[1], Timothy D. McHugh[2] and Jim F. Huggett[1,2,15*]

Inside
News
Event
Press
Global
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(WHO) earlier today issued a recommendation that Ultra can be used as an alternative to the existing Xpert MTB/RIF test for the diagnosis of TB and detection of rifampicin resistance in all settings[1].

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Diagnosis of drug resistance

- **Culture**
- **PCR based molecular methods**

Diagnosis of drug resistance

Illumina MISEQ

ION Proton

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Press release

England world leaders in the use of whole genome sequencing to diagnose TB

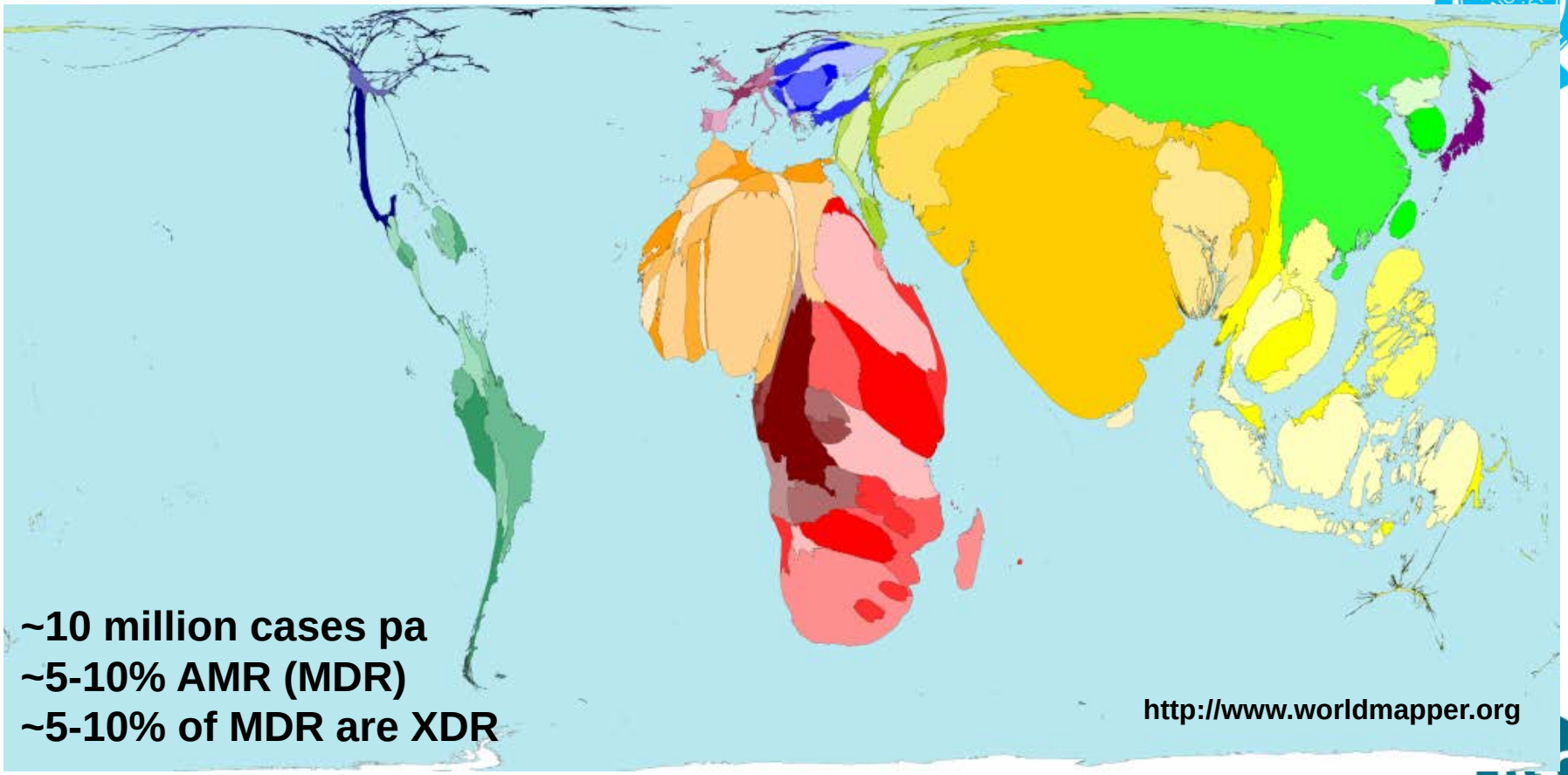
From: Public Health England
Published: 28 March 2017

Whole genome sequencing (WGS) is now being used to identify different strains of tuberculosis (TB), announced Public Health England today.

This is the first time that WGS has been used as a diagnostic solution for managing a disease on this scale anywhere in the world. This builds on WGS based services for public health investigation of infectious diseases, which offer the opportunities for faster, cheaper and more accurate diagnostics than other testing methods.

The technique, developed in conjunction with the University of Oxford, means patients can be treated with precisely the right medication more quickly. Where previously it could take up to a month to confirm a diagnosis of TB, confirm the treatment choices and to detect spread between cases, this can now be done in just over a week by PHE's Birmingham laboratory. This slows the spread of the disease and boosts the fight against anti-microbial resistance (AMR).

Tuberculosis (TB)



Territory size shows the proportion of worldwide TB cases found there.

Experience from pharmaceutical clinical trials: relapse v re-infection

REMoxTB

- **1931 patients**
  - **17 treatment failures**
  - **122 relapses**
  - **58 re-infections**

***Gillespie* et al NEJM 2014**

***Bryant* et al LRM 2013**

RIFAQUIN

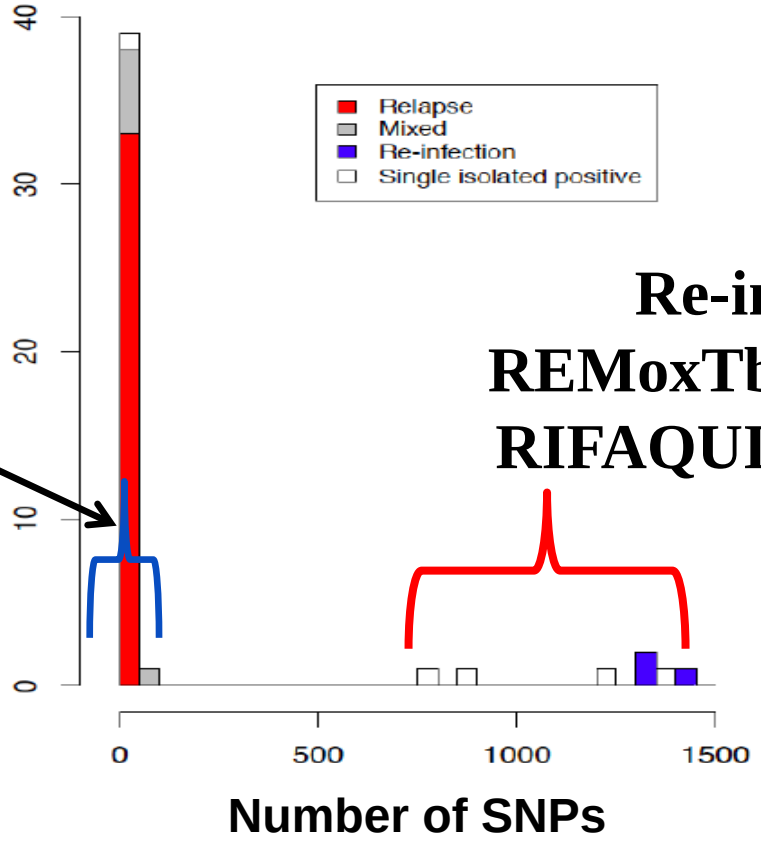
- **827 patients randomised**
  - **33 relapse**
  - **9 re-infection**
  - **4 culture confirmed treatment failure**

***Jindani* et al NEJM 2014**

Thank you Professor Tim McHugh

Defining the groups: trail data

Relapse:
REMoxTb: 0 – 6
RIFAQUIN: 0- 65



Re-infection:
REMoxTb: 1306 – 1419
RIFAQUIN: 720 - 1400

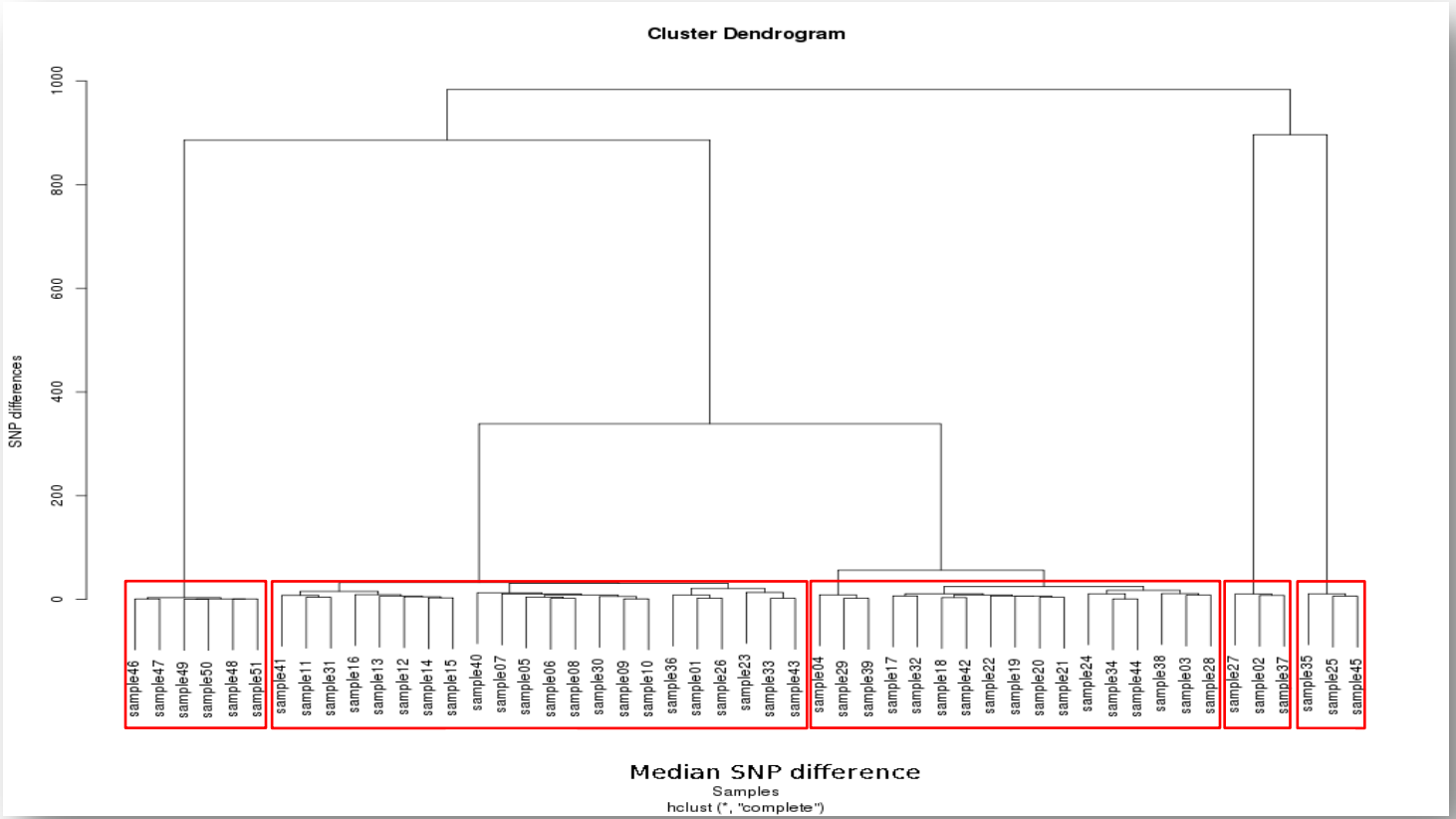
Phelan *et al. Genome Medicine* (2016) 8:132
DOI 10.1186/s13073-016-0385-x

Genome Medicine

RESEARCH Open Access

The variability and reproducibility of whole genome sequencing technology for detecting resistance to anti-tuberculous drugs

Jody Phelan[1†], Denise M. O'Sullivan[2†], Diana Machado[3†], Jorge Ramos[3], Alexandra S. Whale[2], Justin O'Grady[4], Keertan Dheda[5], Susana Campino[1], Ruth McNerney[5†], Miguel Viveiros[3†], Jim F. Huggett[2,6†] and Taane G. Clark[1,7*†]


Cluster Dendrogram
SNP differences
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600
800
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sample24
sample34
sample44
sample38
sample03
sample28
sample27
sample02
sample37
sample35
sample25
sample45
Median SNP difference
Samples
hclust (*, "complete")

Same data, different pipeline

Phenotypic resistance vs predicted using different informatics tools for assigning resistance from sequence data

Sample	Year[a]	Lineage	Spoligo. family	Drug susceptibility test phenotype														
				INH	RIF	STR	ETB	PZA	RFB	ETH	AMK	CAP	OFX	MOX	PAS	LZ	KAN[b]	Resistance phenotype
POR1	2007	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	R	S	R	XDR-TB
POR2	2007	4.1.1.1	X2	R	R	S	S	S	R	R	S	S	S	S	S	S	-	MDR-TB
POR3	2007	4.3.4.2	LAM1	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR4	2007	4.3.4.2	LAM1	R	R	R	R	R	R	R	R	S	R	R	S	S	R	XDR-TB
POR5	2007	4.3.4.2	LAM4	R	R	R	R	R	R	R	S	S	S	S	S	S	-	MDR-TB
POR6	2008	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR7	2009	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR8	2012	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	S	S	R	XDR-TB
POR9	2011	4.3.4.2	LAM4	R	R	R	R	R	R	R	R	R	R	R	R	S	R	XDR-TB
POR10	2013	4.2.1	Ural H3/4	R	R	R	R	R	R	R	S	S	S	S	S	S	R	MDR-TB
H37Rv	-	4.9	H37RV	S	S	S	S	S	S	S	S	S	S	S	S	S	-	Pan-susceptible

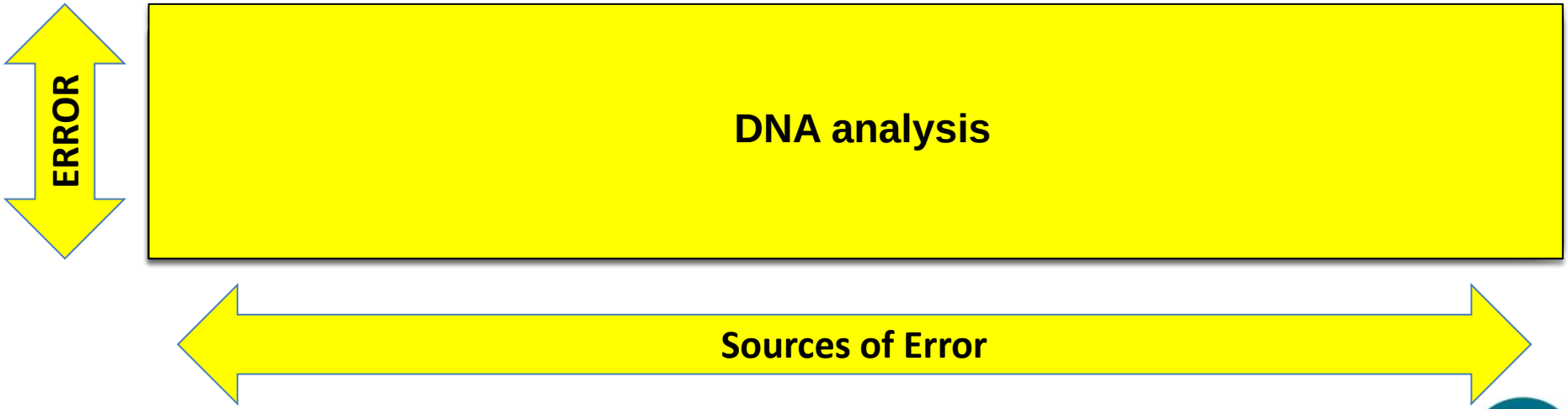
Informatics tool

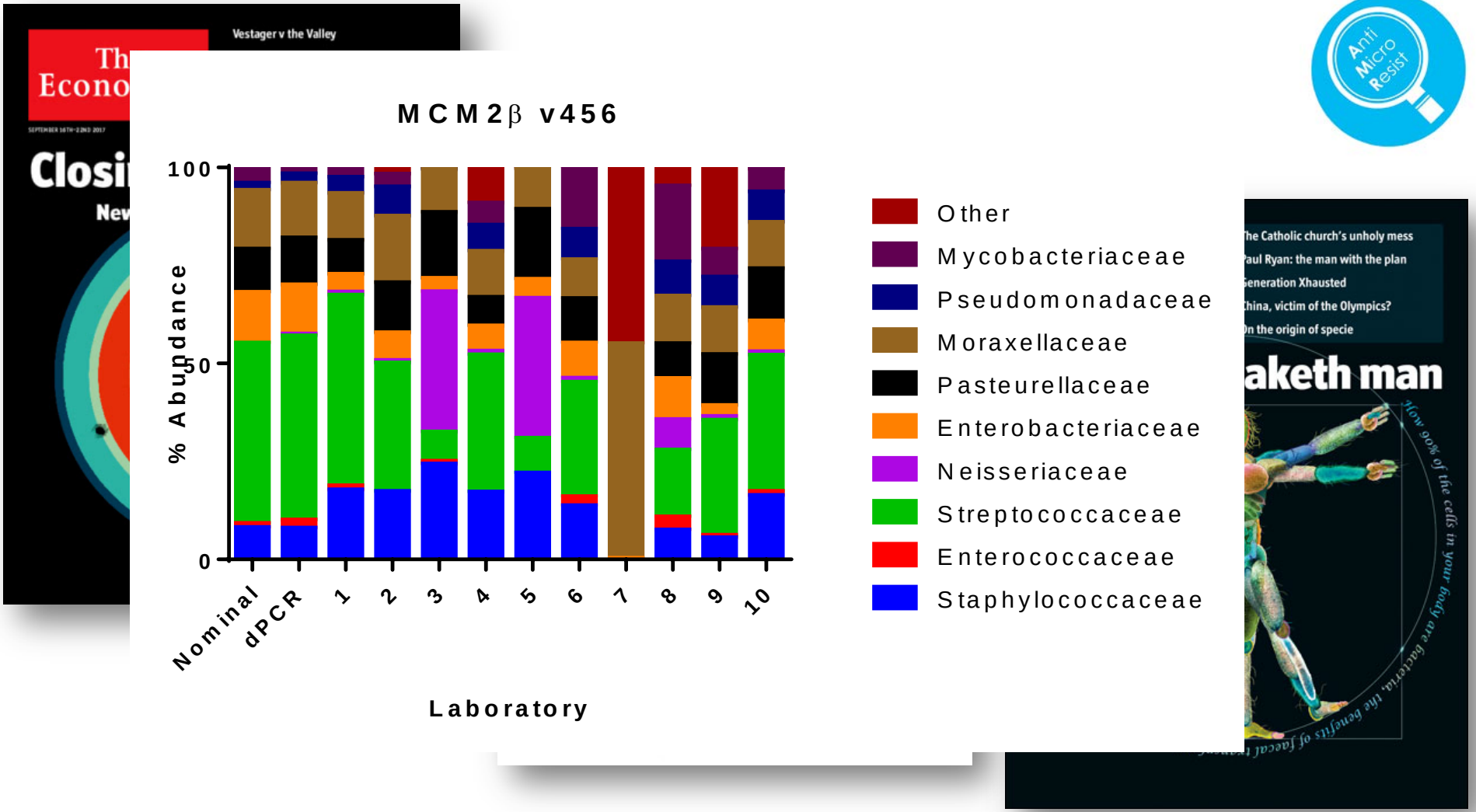
- **Mykrobe Predictor**
- **TBProfiler**

Conclusion

- Molecular methods offers the potential for monitoring of bacteria and guide treatment providing many new diagnostic opportunities

Technical vs Biological error




Vestager v the Valley
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Other
Mycobacteriaceae
Pseudomonadaceae
Moraxellaceae
Pasteurellaceae
Enterobacteriaceae
Neisseriaceae
Streptococcaceae
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he Catholic church's unholy mess
aul Ryan: the man with the plan
eneration Xhausted
hina, victim of the Olympics?
n the origin of specie
aketh man
How 90% of the cells in your body are bacteria. the benefits of faecal

Acknowledgements

LGC

- **Denise O'Sullivan**
- **Eloise Busby**
- **Gerwyn Jones**
- **Alison Devonshire**
- **Alexandra Whale**
- **Simon Cowen**
- **Carole Foy**

University College London

- **Tim McHugh**

WHO/TDR

- **Andrew Ramsay**

Vircell

- **Pablo Mendoza**

LSHTM

- **Jody Phelan, Taane Clark**

Great Ormond Street Hospital

- **Kathryn Harris, Ronan Doyle**

Cepheid

- **Sushma Patel**
- **Inis Hormann**

GHTM

- **Miguel Viveiros**
- **Diana Machado**
- **Jorge Ramos**

Thank you

15HLT07 AntiMicroResist

EURAMET

Publishable Summary for 15HLT07 AntiMicroResist
Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance

Overview

In 2014 a World Health Organisation (WHO) report stated that antimicrobial resistance (AMR) is so serious, that it threatens the achievements of modern medicine, and while new therapies to treat resistant pathogens are needed, the diagnostic tools required to guide their application are equally lacking. This clinically focussed project will apply innovative metrological concepts for developing quantitative higher order methodologies and materials to support the improved application of diagnostic testing to the detection and management of AMR.

15HLT07 AntiMicroResist

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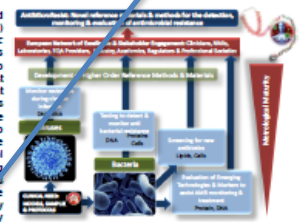
Publishable Summary for 15HLT07 AntiMicroResist
Novel materials and methods for the detection, traceable monitoring and evaluation of antimicrobial resistance

Overview



jim.huggett@lgcgroup.com