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Can whole genome sequencing replace AST ?

Matthew J Ellington

Antimicrobial Resistance & Healthcare Associated Infections (AMRHAI) Reference Unit

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Antibiotic susceptibility testing (AST)

- Fundamental to diagnostic bacteriology
- Quantitative methods (MIC, mg/L)
  - agar or broth dilution
  - gradient strips (Etests, MICE)
- Qualitative methods (S/I/R)
  - disc diffusion
  - agar incorporation breakpoint method
- Automated methods

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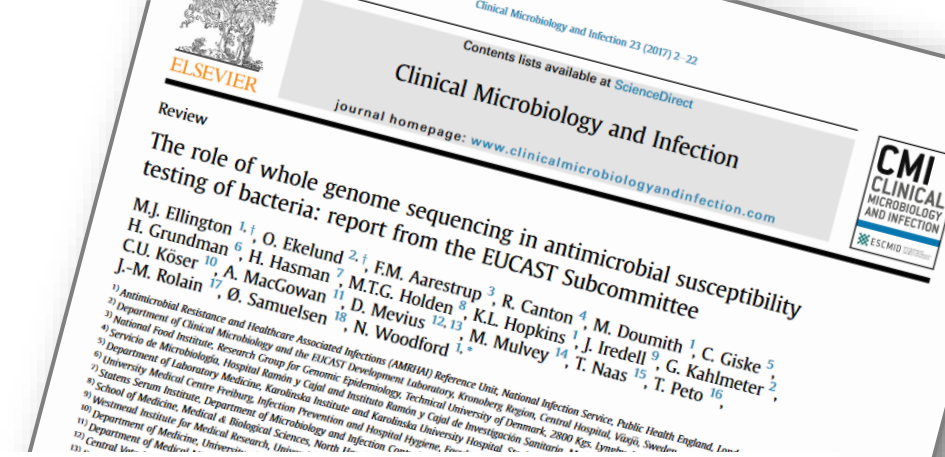
European Society of Clinical Microbiology and Infectious Diseases



EUCAST Subcommittee on the role of whole genome sequencing (WGS) in AST of bacteria

1. Review literature describing the role of WGS in AST of bacteria
2. Assess the sensitivity and specificity of WGS vs phenotypic AST
3. Consider how WGS for AST may be applied in clinical micro labs
4. Consider the epidemiological implications of using WGS
5. Consider the clinical implications of WGS for the selection of $\mathbf{R_x}$
6. To describe the drivers and barriers to routine use of WGS

http://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(16)30568-7/pdf

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Members bring diverse expertise

Frank M. Aarestrup (Denmark)	Gunnar Kahlmeter (Sweden)
Rafael Canton (Spain)	Claudio U. Koser (UK)
Michel Doumith (UK)	Alasdair MacGowan (UK)
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Most appropriate AST comparators


REVIEW

EUCAST expert rules in antimicrobial susceptibility testing

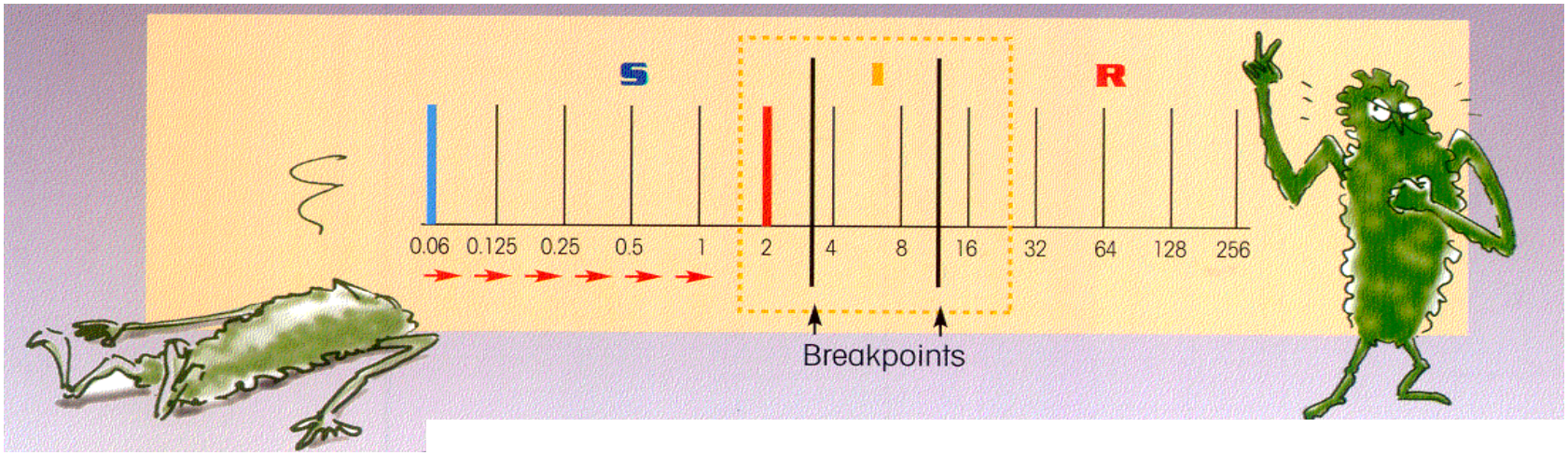
BACTERIOLOGY

Abstract

What criteria should WGS data be assessed against ?

clinical breakpoints indicate likelihood of therapeutic success (S) or failure (R) of antibiotic treatment based on microbiological findings

ECOFFs (epidemiological cut-off values) differentiate wild-type (WT) from non-wild-type (NWT) isolates with an acquired resistance mechanism



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What can WGS offer ?

	Phenotypic AST	WGS-based AST
Measures susceptibility	Y	N
Resistance mechanisms	Y (limited)	YYY
ECOFF (WT vs. non-WT)	Y	Y
Clinical resistance (S vs. R)	Y	**?** (must be inferred)
Additional data	N	YYY
Suitable speed	Y (most) N (e.g. TB)	N (most) Y (e.g. TB)
Cost	Y	N

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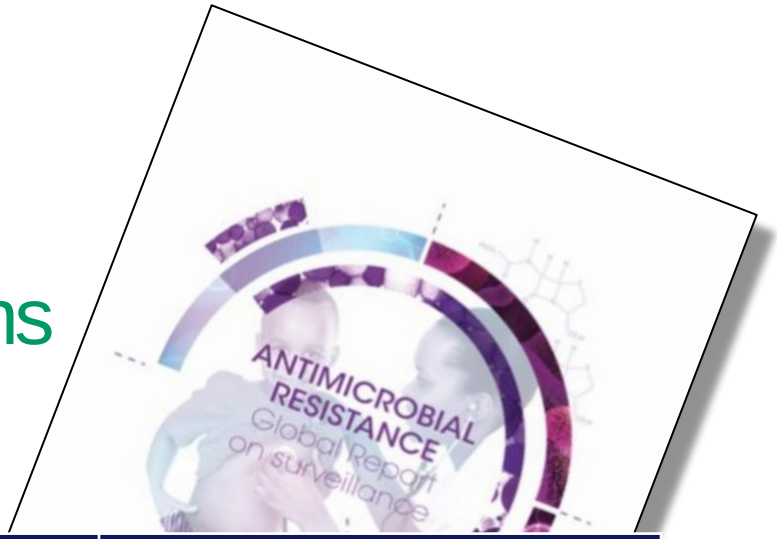
Focus on WHO priority organisms



Organisms		Priority resistances
Enterobacteriaceae	*E. coli*	3GC, FQs
	K. pneumoniae	3GC, carbapenems
	Non-typhoidal *Salmonella*	FQs
	Shigella spp.	FQs
S. aureus	-	MRSA
S. pneumoniae	-	Penicillin
N. gonorrhoeae	-	3GCs

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Expanded focus to include...

Other considerations	
Other organisms	*M. tuberculosis, C. difficile, A. baumannii, P. aeruginosa*
Quality metrics for WGS	-
Categories of systematic errors in WGS predictions of AMR and the need for standardised, open-access databases	-
The epidemiological implications of using WGS	-
Clinical & wider impacts	-

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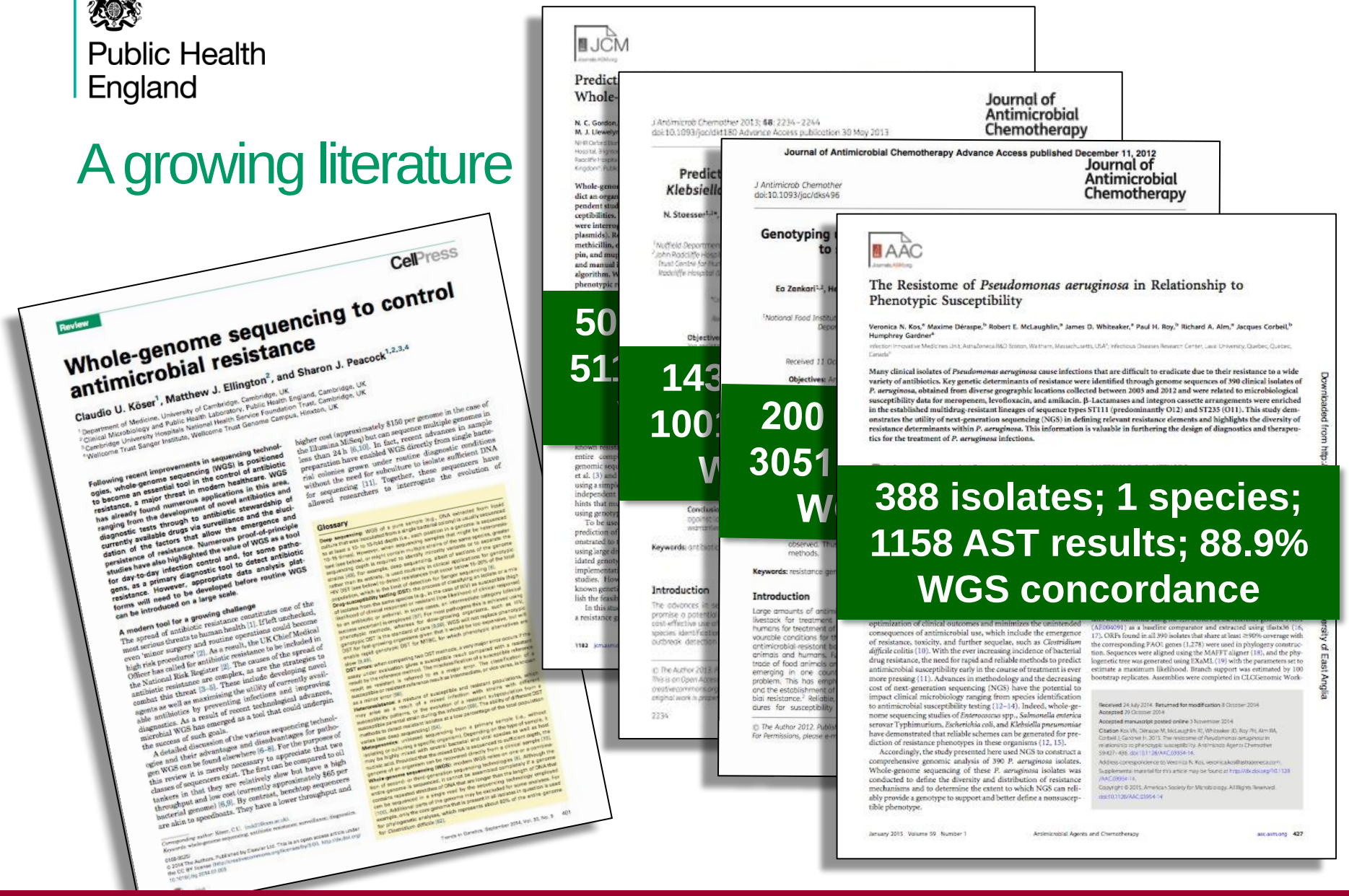
A growing literature



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Evidence reports – *e.g.* Enterobacteriaceae

- Relatively limited number of acquired resistance genes and resistance-associated mutations that dominate epidemiologically in the Enterobacteriaceae
- High levels of accuracy of genotype-phenotype correlation in published studies; means that well-informed screening approaches can be very accurate.
- Predicting AST results will be harder for some than for others
  - better understanding of the full range of mechanisms is required
  - …INCLUDING their interplay

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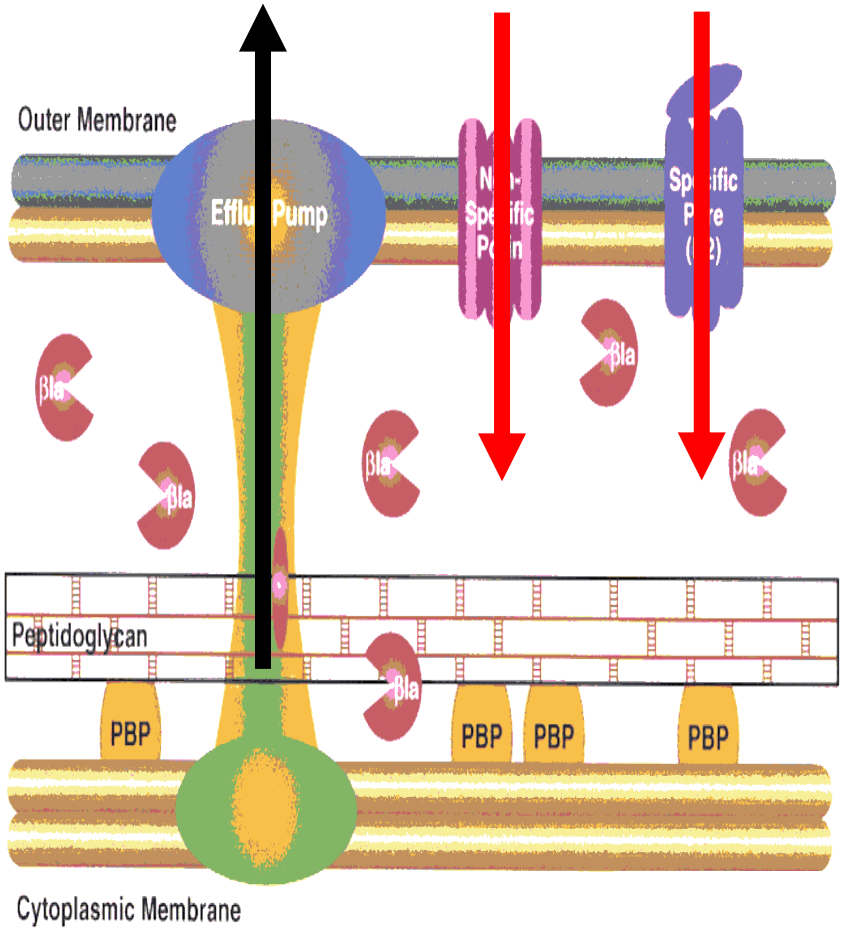
Complex interplays determine an MIC



$V_{Entry} + V_{Efflux}$

External [drug]

Periplasmic [drug]

$V_{Hydrolysis}$

$V_{Binding}$

It's a lot more complex than gene presence / absence

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Combinatorial resistance: WGS vs. AST

Table 1

Comparison ofWGS and Reference Laboratory Testing of Carbapenem-Resistant Gram-Negative Bacteria

Organism	Isolate No.	Phenotypic Resistance to Carbapenems and Third-Generation Cephalosporins	Attributable Resistance Mechanism According to Reference Laboratory[a]	Dominant Resistance Mechanism Based on WGS[b]
Acinetobacter baumannii	AB223	MEM, IPM[c]	OXA-23 carbapenemase	OXA-23 carbapenemase
Enterobacter cloacae	EC1a[d]	ETP, MEM, IPM, CTX, CAZ	IMP-1 carbapenemase	IMP-1 carbapenemase
E cloacae	EC302	ETP, CTX, CAZ	No carbapenemase genes detected. AmpC activity present	No carbapenemase genes detected. OmpF porin loss
Klebsiella pneumoniae	KP652	ETP, CTX, CAZ	No carbapenemase genes detected. ESBL activity consistent with CTX-M. ETP resistance consistent with porin loss	No carbapenemase genes detected. CTX-M-15 ESBL with OmpK36 porin loss
Escherichia coli	Eco216	ETP, CTX, CAZ	No carbapenemase genes detected. ESBL activity present. ETP resistance consistent with porin loss	No carbapenemase genes detected. CTX-M-15 ESBL with OmpF porin loss

Reuter et al., 2013. JAMA Intern Med 12;173:1397-404

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- High levels of accuracy of genotype-phenotype correlation in published studies; means that well-informed screening approaches can be very accurate.
- Predicting AST results will be harder for some than for others
  - better understanding of the full range of mechanisms is required
  - …INCLUDING their interplay
  - Will require more study if improved levels of accuracy across large genetically diverse datasets are to be achieved.

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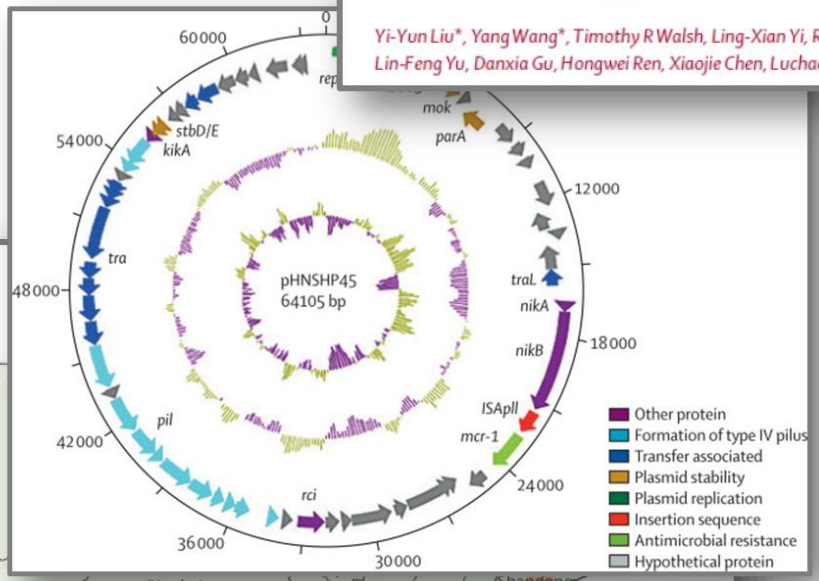
Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen*



Discussion

Until now, colistin resistance has occurred via chromosomal mutations and, although clonal outbreaks have been reported, the resistance is often unstable, imposes a fitness cost upon the bacterium and is incapable of spreading to other bacteria.[7] The rapid dissemination of previous resistance mechanisms (eg, NDM-1) indicates that, with the advent of transmissible colistin resistance, progression of Enterobacteriaceae from extensive drug resistance to pan-drug resistance is inevitable and will ultimately become global.[5] In this context the emergence

www.thelancet.com/infection **Published online November 18, 2015** http://dx.

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Rapid screening for resistance determinants

- In total ~24000 genomes
  - *Salmonella enteridis*
  - *Escherichia coli*
  - *Klebsiella pneumoniae*
  - Other Enterobacteriacaea

- Genefinder for *mcr-1* screening

Doumith et al., *JAC* 2016

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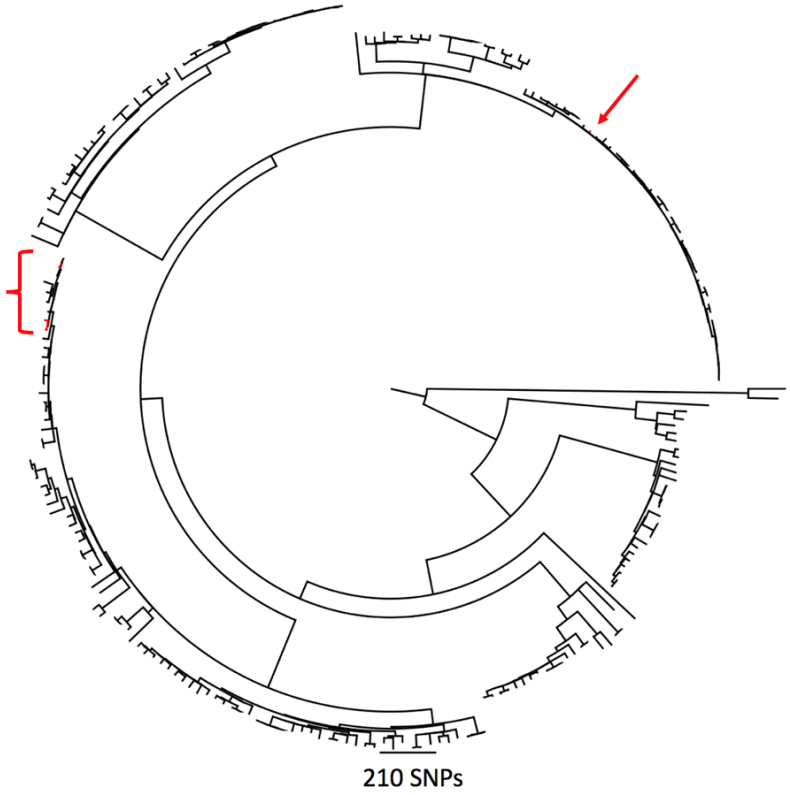
PHE WGS archive screen for *mcr-1*

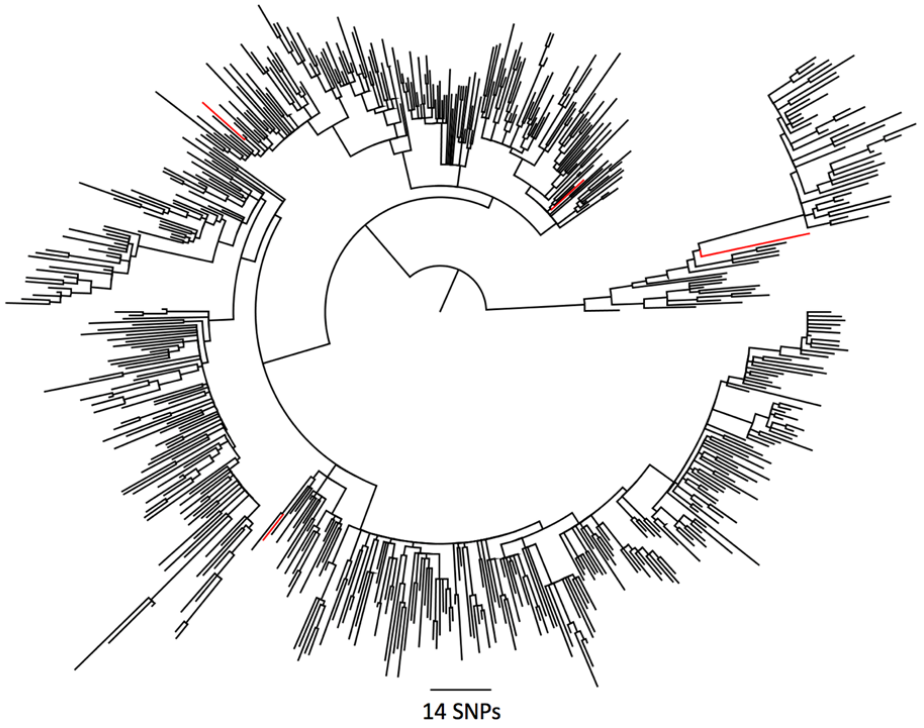
Date of isolation	Source of isolate	Travel reported[a]	Clinical illness	Organism identified	MLST	Colistin MIC mg/L
Aug-12	Human-1,Faeces	Nil	Gastroenteritis	*Salmonella* Typhimurium (monophasic) DT193	**34**	4
Oct-13	Human-2, blood[c]	Nil	Bacteraemia	*E. coli*	457	4
Nov-13	Human-3, Stool	Egypt	Gastroenteritis	*E. coli*	Novel	**N/A**
Feb-14	Human-2, blood [c]	Nil	Bacteraemia	*E. coli*	457	4
Jun-14	Human-4, faeces	Egypt	Gastroenteritis	*Salmonella* Virchow PT131	16	4
Jul-14	Human-5, Faeces	**Malaysia, Singapore, Hong Kong**	Febrile Gastroenteritis	*Salmonella* Typhimurium (monophasic) DT136	**36**	4
Oct-14	Poultry meat	Imported from Europe	N/A	*Salmonella* Paratyphi B var Java PT Colindale	28	4
Oct-14	Poultry meat	Imported from Europe	N/A	*Salmonella* Paratyphi B var Java, PT Colindale	28	4
Nov-14	Human-6,Faeces	Nil	Gastroenteritis	*Salmonella* Typhimurium (monophasic) DT179	**34**	4
Feb-15	Human-7,Faeces	**Thailand, United Arab Emirates**	Gastroenteritis	*Salmonella* Typhimurium, DT120	**36**	4
Mar-15	Human-8,Faeces	**Malaysia**	Gastroenteritis	*Salmonella* Typhimurium (monophasic) PT untypable	**36**	4
Jul-15	Human-9,Faeces	**Thailand**	Gastroenteritis	*Salmonella* Paratyphi B var Java	42	4
Aug-15	Human-10,Faeces	**Borneo**	Gastroenteritis	*Salmonella Typhimurium,* DT120	**36**	4
Sep-15	Human-11,faeces	Nil	Gastroenteritis	*Salmonella* Typhimurium (monophasic) DT20a	**34**	8
Sep-15	Human-12,faeces	**Thailand,Cambodia**	Gastroenteritis	*Salmonella* Typhimurium (monophasic) DT193	**34**	4

Doumith et al., *JAC* 2016

Positive-*mcr-1 S. Typhimurium phylogeny*



phylogenetic tree of 241 *Salmonella* Typhimurium ST36



phylogenetic tree of 601 *Salmonella* Typhimurium ST34

Doumith et al., *JAC* 2016

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Systematic sources of error affecting phenotypic / WGS correlation

- Incomplete understanding of genotypic basis of phenotypic resistance
  - affecting sensitivity of WGS prediction (resulting in very major errors)
  - problematic bacteria; problematic antibiotics
  - at this relatively early stage of development of WGS based genotype-phenotype comparisons it can be anticipated that there may be many gaps in the knowledge base – e.g. *mcr-1*
- Flaws with phenotypic AST
- An inadequate limit of detection of WGS
  - when detection is direct from clinical specimens e.g. TB
  - for most organisms WGS is likely to use cultured (high titre) bacteria.

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A single, standardised AMR reference database

- Need better standardisation of annotation of AMR genes
  - BLAST analysis retrieves hits that are inconsistently annotated even where the actual sequences are identical.
- Need a single, regularly updated ‘challenge database’ containing all validated AMR genes and chromosomal point mutations linked with AMR
- Need international consensus on the criteria used to define genes as “new” or as variants of known genes.
- There should be minimum standards for inclusion of new resistance determinants in the standardised database.
- This is inextricably linked to issues of gene nomenclature.

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Data quality

- Only datasets passing QC metrics should be used for AST predictions, since resistance genes or mutations might be missed in sequences of poor quality.
- Before WGS can be routinely implemented into accredited clinical practice there is a need to establish necessary minimum QC-thresholds
- The Global Microbial Identifier initiative is currently collaborating with the US-FDA and the COMPARE project in proficiency testing of WGS data and isolates that have been distributed to 50 laboratories worldwide.
- This and similar initiatives are important first steps towards setting objective QC thresholds.

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WGS-based genotypic antibiograms - 1

- Need for further evidence, but could ‘soon’ replace much AST for surveillance purposes
  - low impact of the low error rate

- Need for further evidence, but could ‘soon’ reduce need for AST in *reference* laboratories unless
  - to guide treatment
  - for agents with poorest genotypic/phenotypic concordance
  - comparative in-vitro activity of new agents

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WGS-based genotypic antibiograms - 2

- ‘longer’ for a paradigm shift to WGS to guide clinical decision making
  - very major errors - gene absence cannot always predict susceptibility
  - robust evidence will be needed
  - probably first for TB (for bacteria)
  - surveillance of treatment failure +/- novel resistance mechanisms

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Concluding comments

- An MIC reflects more than gene presence / absence
- Primary AST comparator for WGS-based prediction should be an ECOFF, wherever possible.
  - categorisation of WT vs. non-WT
- Clinical breakpoints should be used as secondary comparators.
  - tougher criterion, but will ultimately be needed
- Insufficient data to present a definitive document on the topic.
  - We reviewed the state-of-the–art as a first approach.
  - Baseline discussion document; state of art to March 2016

Report available: **http://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(16)30568-7/pdf**

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