



Public Health
England

Whole genome sequencing in AST (of bacteria):

...What's new?

Dr Matthew J Ellington

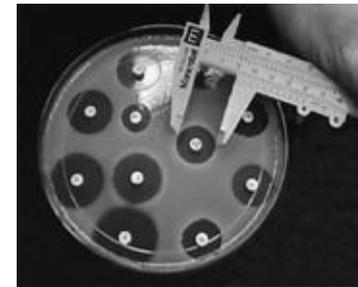
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Antibiotic susceptibility testing (AST)

- 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



EUCAST Sub-committee on the role of whole genome sequencing (WGS) in AST of bacteria v1

- Diverse expertise. Scientific and Clinical.

Frank M. Aarestrup (Denmark)	Gunnar Kahlmeter (Sweden)
Rafael Canton (Spain)	Claudio U. Koser (UK)
Michel Doumith (Saudi Arabia)	Alasdair MacGowan (UK)
Oskar Ekelund (Sweden)	Mike Mulvey (Canada)
Matthew J. Ellington (UK, Chair)	Thierry Naas (France)
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Matt Holden (UK)	Neil Woodford (UK)
Jon Iredell (Australia)	Dik Mevius (Netherlands)

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 R_x
6. To describe the drivers and barriers to routine use of WGS

Clin Microbiol Infect. 2017 Jan;23(1):2-22. doi:10.1016/j.cmi.2016.11.012.

Focus on WHO priority organisms

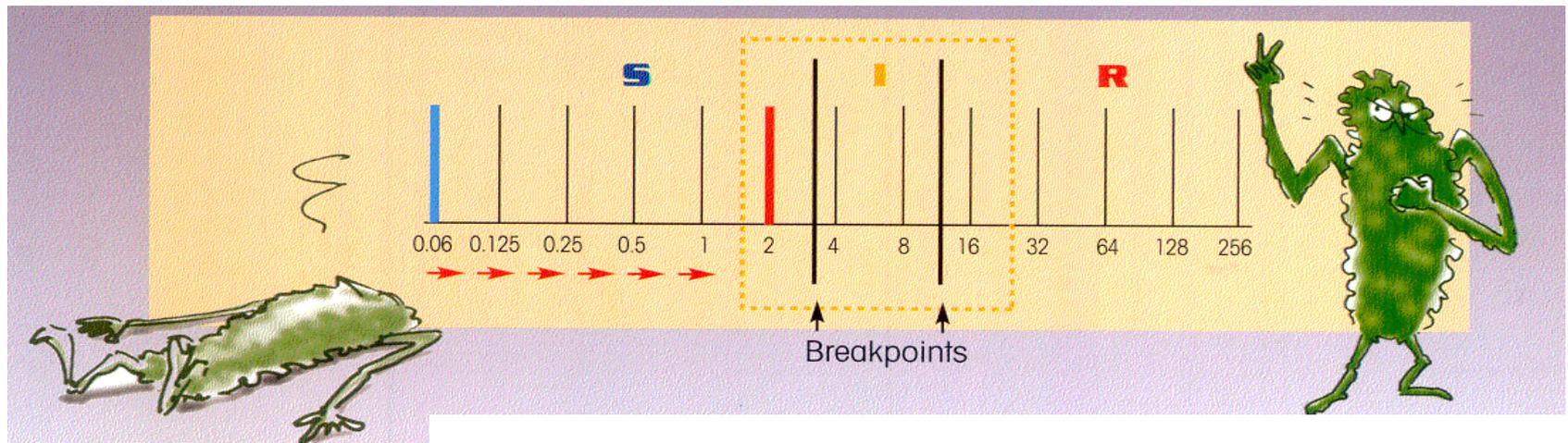


Organisms		Priority resistances
Enterobacteriaceae	<i>E. coli</i>	3GC, FQs
	<i>K. pneumoniae</i>	3GC, carbapenems
	Non-typhoidal <i>Salmonella</i>	FQs
	<i>Shigella</i> spp.	FQs
<i>S. aureus</i>	-	MRSA
<i>S. pneumoniae</i>	-	Penicillin
<i>N. gonorrhoeae</i>	-	3GCs

Plus: *P. aeruginosa*, *Acinetobacter*, *C. difficile* and Mtb

Most appropriate AST comparators

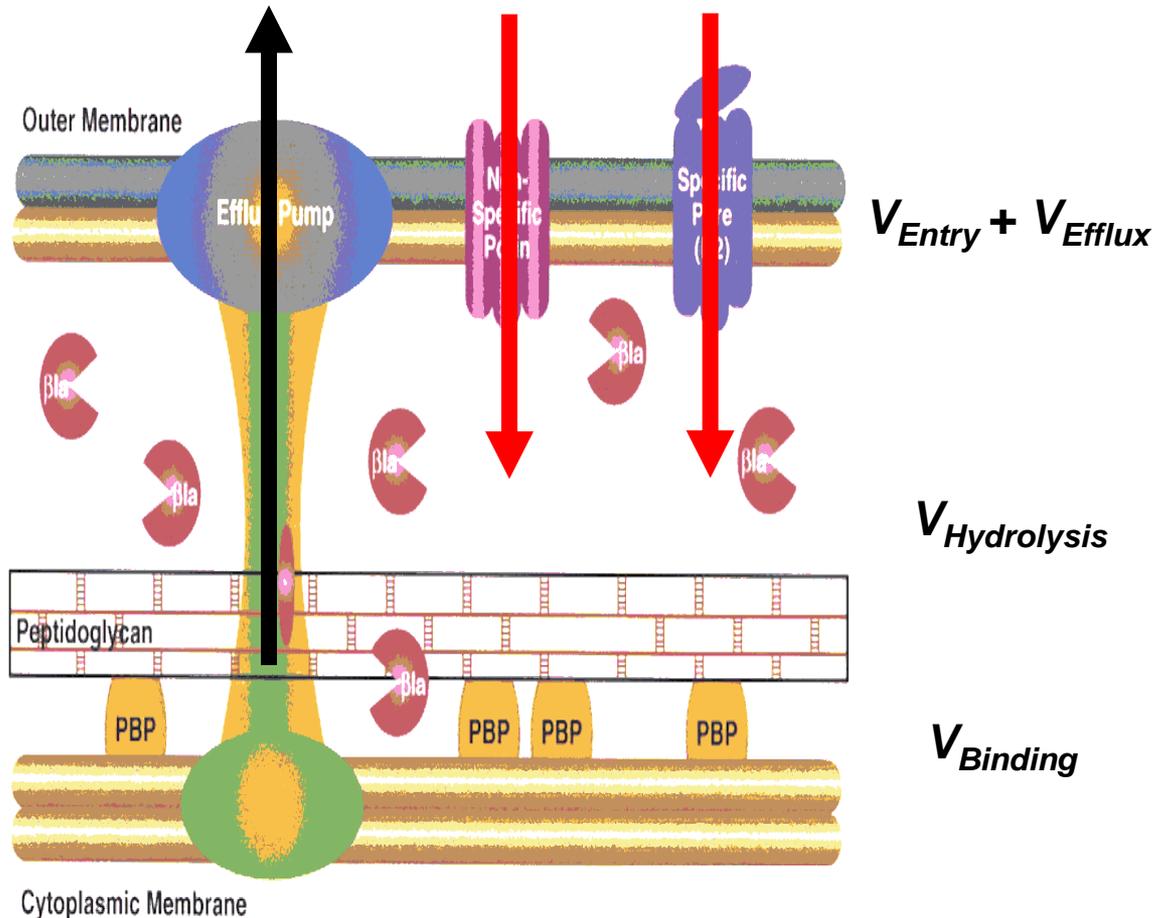
- What criteria should WGS data be assessed against ?
- **clinical breakpoints** indicate likelihood of therapeutic success (S) or failure (R) of antibiotic treatment based on microbiological findings
- **ECOFFs** (epidemiological cut-off values) differentiate wild-type (WT) from non-wild-type (NWT) isolates with an acquired resistance mechanism



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

Complex interplays determine an MIC



External [drug]

Periplasmic [drug]

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

Combinatorial resistance: WGS vs. AST

Table 1

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

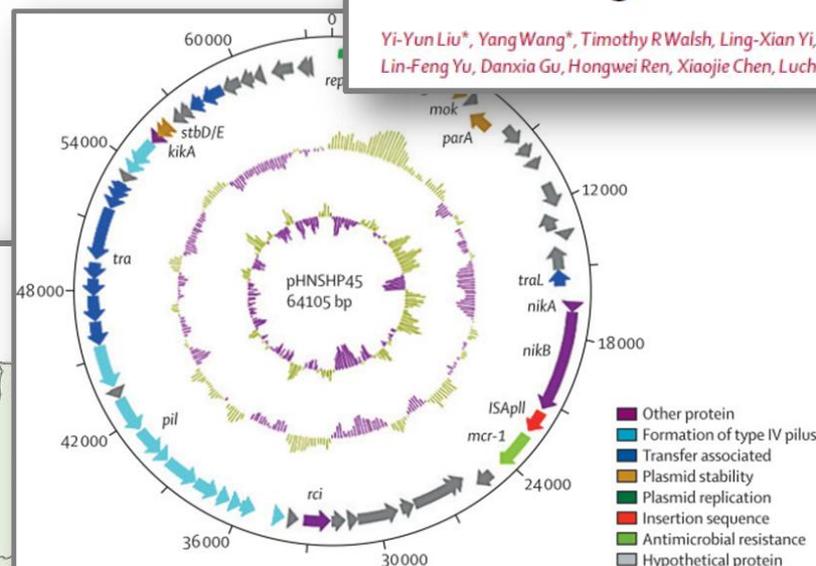
Organism	Isolate No.	Phenotypic Resistance to Carbapenems and Third-Generation Cephalosporins	Attributable Resistance Mechanism According to Reference Laboratory ^a	Dominant Resistance Mechanism Based on WGS ^b
<i>Acinetobacter baumannii</i>	AB223	MEM, IPM ^c	OXA-23 carbapenemase	OXA-23 carbapenemase
<i>Enterobacter cloacae</i>	EC1a ^d	ETP, MEM, IPM, CTX, CAZ	IMP-1 carbapenemase	IMP-1 carbapenemase
<i>E. cloacae</i>	EC302	ETP, CTX, CAZ	No carbapenemase genes detected. AmpC activity present	No carbapenemase genes detected. OmpF porin loss
<i>Klebsiella pneumoniae</i>	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
<i>Escherichia coli</i>	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, Ellington, et al., 2013. JAMA Intern Med 12;173:1397-404



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

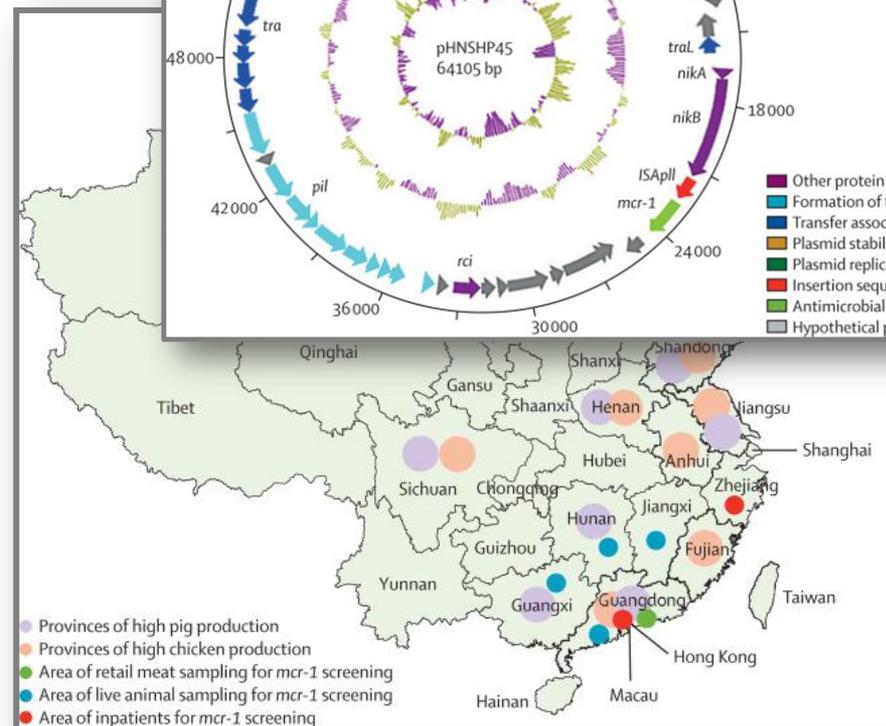
Yi-Yun Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen



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.doi.org/10.1016/S1473-3099\(15\)00424-2](http://dx.doi.org/10.1016/S1473-3099(15)00424-2)



PH investigation: *mcr-1*

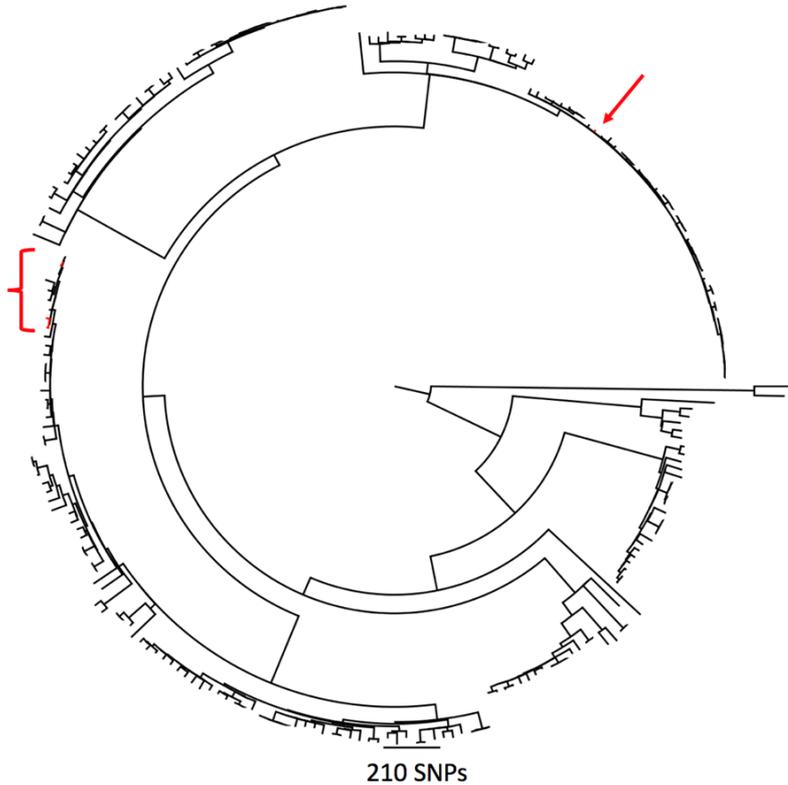
- Is *mcr-1* present in the UK?
 - How prevalent? Which species?
 - And where?
- In total ~24000 genomes
 - *Salmonella enteridis*
 - *Escherichia coli*
 - *Klebsiella pneumoniae*
 - Other Enterobacteriaceae
- Genefinder for *mcr-1* screening

PHE WGS archive screen for *mcr-1*

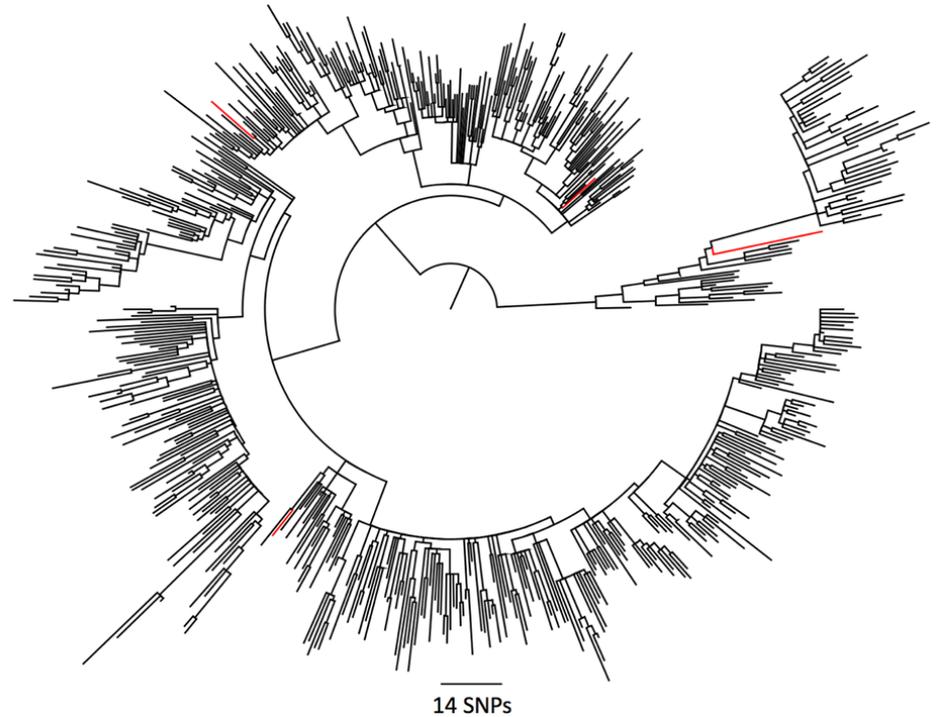
Date of isolation	Source of isolate	Travel reported ^a	Clinical illness	Organism identified	MLST	Colistin MIC mg/L
Aug-12	Human-1, Faeces	Nil	Gastroenteritis	<i>Salmonella</i> Typhimurium (monophasic) DT193	34	4
Oct-13	Human-2, blood ^c	Nil	Bacteraemia	<i>E. coli</i>	457	4
Nov-13	Human-3, Stool	Egypt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

Doumith M, et al. J Antimicrob Chemother. 2016 71:2300-5.

Positive-*mcr-1* *S. Typhimurium* phylogeny



phylogenetic tree of 241 *Salmonella* Typhimurium ST36



phylogenetic tree of 601 *Salmonella* Typhimurium ST34

Doumith M, et al. J Antimicrob Chemother. 2016 71:2300-5.

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 (*e.g.* *mcr-1*)
 - ...INCLUDING their interplay (*e.g.* some beta-lactams)
 - Will require more study if improved levels of accuracy across large genetically diverse datasets are to be achieved.

Conclusions from previous report

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

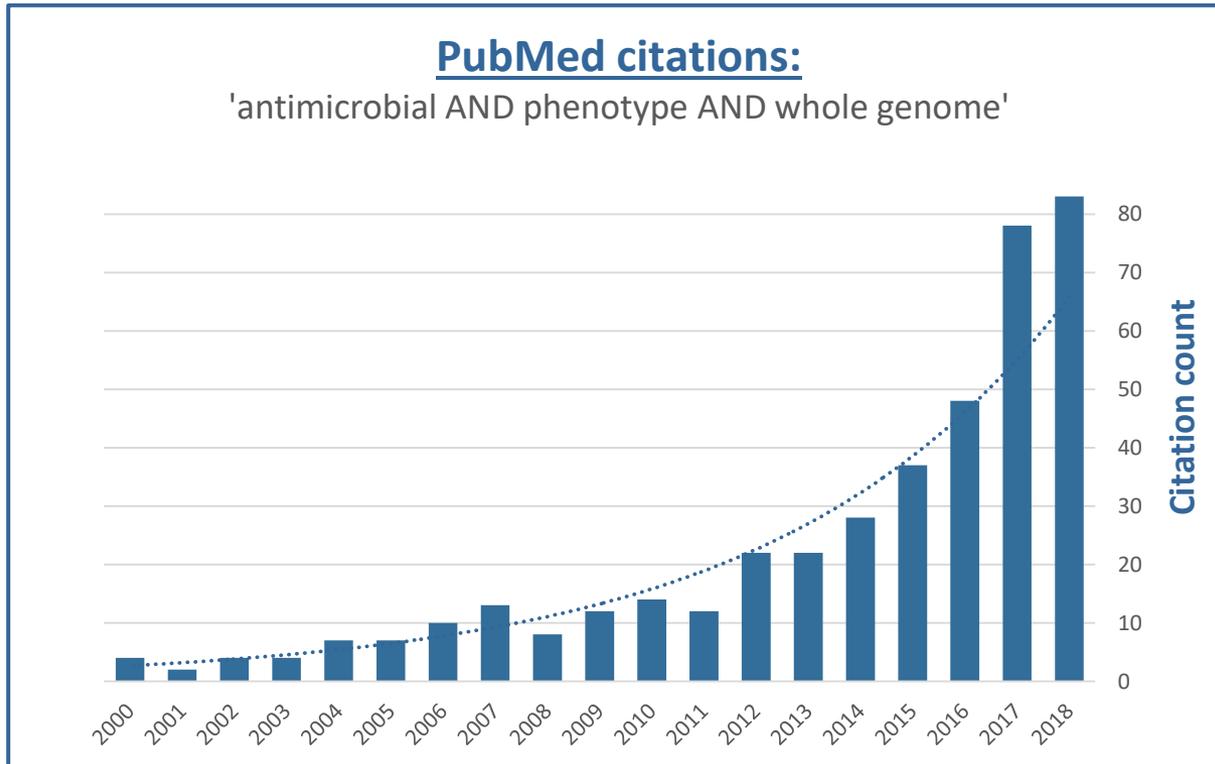
EUCAST Sub-committee on the role of whole genome sequencing (WGS) in AST of bacteria v2

- Personnel almost same, still diverse expertise. Scientific and Clinical.

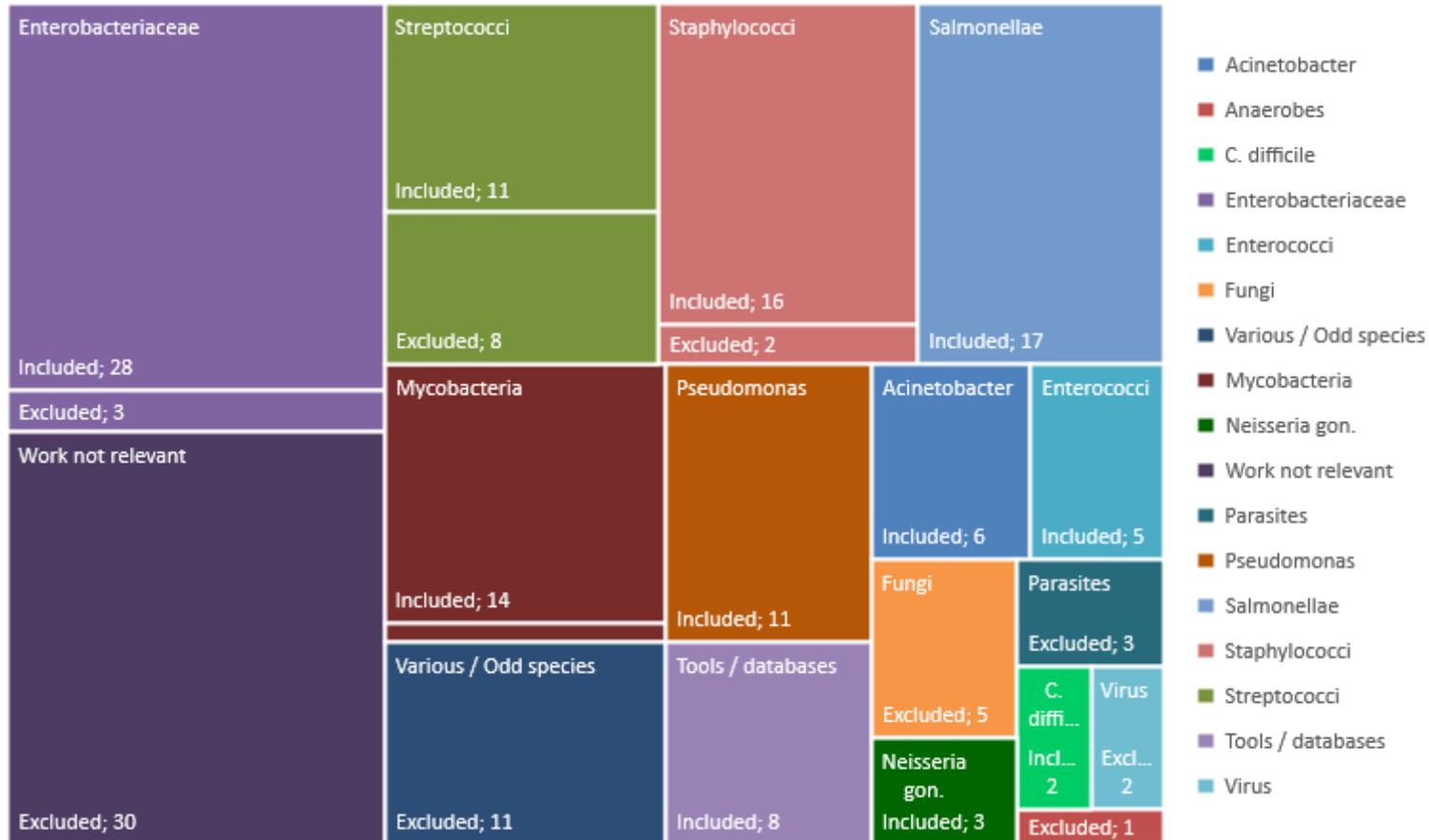
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Jon Iredell (Australia)	+ Mike Brouwer (Netherlands)

Committee is reconvened for v2

- Not a whole new report
- Update of existing sections



Literature scan vs. species $n=187$



Existing sections – early update findings

- Not a whole new report
- Update of existing sections:

- Good concordances highlighted in previous version for bug / drug combinations
- Developed since....

Existing sections – early update findings

Species	Antibiotic(s)	No. of genomes tested	Diversity/ no. of STs	Primary database(s)	Software	Input data	Sensitivity (%)	Specificity (%)	Reference
<i>E. coli</i>	Amoxicillin-clavulanate	76	NR	Custom	Blastx, ClustalW	Assembly, FASTQ	100	100	Tyson et al. (71)
	Trimethoprim	48	19+ STs	ResFinder	ResFinder	Assembly or FASTQ	100	100	Zankari et al. (115)
	Gentamicin	74	NR	>100 loci	Blastn	Assembly	100	100	Stoesser et al. (70)
<i>S. aureus</i>	Fusidic acid	491	61 STs	Custom	Blastn, tblastn	Assembly	91	100	Gordon et al. (66)
	Vancomycin	NR	16 CCs	Custom	Blastn, mapping software	Assembly, FASTQ	100	100	Aanensen et al. (67)
	Mupirocin	340	25 CCs	Modified from Gordon et al. (66)	Mykrobe	FASTQ	100	100	Bradley et al. (5)
	Ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, methicillin, mupirocin, penicillin, rifampin, tetracycline, trimethoprim, vancomycin	1,379	111 STs	Custom	Mykrobe, GeneFinder, Typewriter	FASTQ, Assembly	97	99	Mason et al. (42)
<i>Salmonella enterica</i> serovar Typhi	Chloramphenicol	332	ST1 and ST2	CARD, ResFinder, literature	GeneFinder	FASTQ	100	100	Day et al. (61)
Non-serovar Typhi <i>S. enterica</i>	Ceftriaxone	640	NR	Tyson et al. (71)	Blastx, ClustalW	Assembly, FASTQ	100	99.8	McDermott et al. (59)
	Ciprofloxacin	3,491	227 serovars	CARD, ResFinder	GeneFinder	FASTQ	99.28	99.97	Neuert et al. (60)
<i>S. pneumoniae</i>	Erythromycin	210	90 STs	SRST2	SRST2	FASTQ	100	100	Deng et al. (69)
<i>Campylobacter jejuni</i> , <i>Campylobacter coli</i>	Erythromycin	32/82	NR	Tyson et al. (71), ARDB, ResFinder	Blastx, ClustalW	Assembly, FASTQ	100	100	Zhao et al. (62)
<i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i>	Kanamycin	50	12 STs 17 STs	ResFinder	ResFinder	Assembly or FASTQ	100	100	Zankari et al. (115)
<i>Pseudomonas aeruginosa</i>	Levofloxacin	390	175 STs	Custom	NR	Assembly	91.9	93.7	Kos et al. (68)
<i>Klebsiella pneumoniae</i>	Gentamicin	69	NR	Custom	Blastn	Assembly	96	100	Stoesser et al. (70)
<i>Shigella sonnei</i>	Ampicillin	341	NR	CARD, ResFinder	GeneFinder	FASTQ	100	100	Sadouki et al. (65)

^aNR, not reported; ST, sequence type; CC, clonal complex.

^bAID, Autoimmun Diagnostika GmbH.

Table adapted from: Su *et al.*, JCM 2019

- Some species where good evidence base now exists for many key drugs
 - Limited extension beyond the previously investigated antimicrobials

Existing sections – early update findings

- Tb sequencing at PHE for diagnostic testing

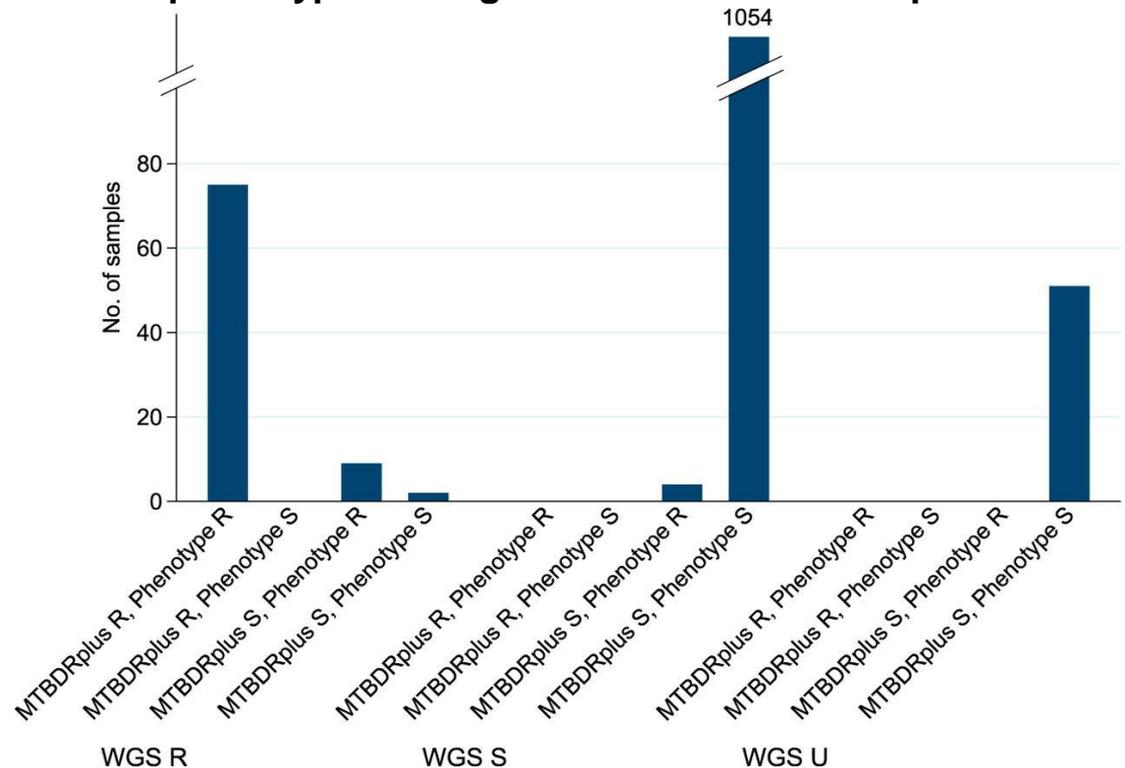
WGS susceptibility predictions vs those of MTBDRplus and phenotypic testing for isoniazid and rifampin.

2626 WGS vs pheno results:

Concordance: 99.2%

Sensitivity: 94.2%

Specificity: 99.4%

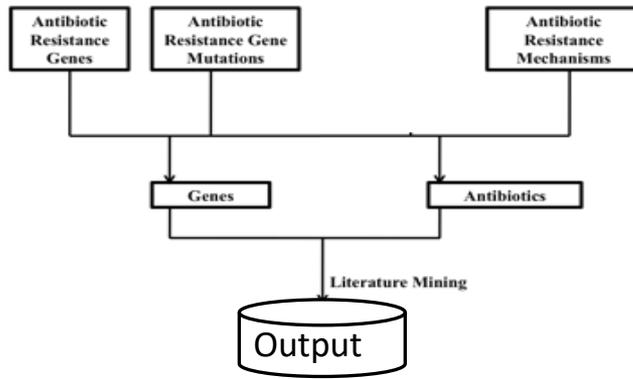


T. Phuong Quan et al. *J. Clin. Microbiol.* 2018; doi:10.1128/JCM.01480-17

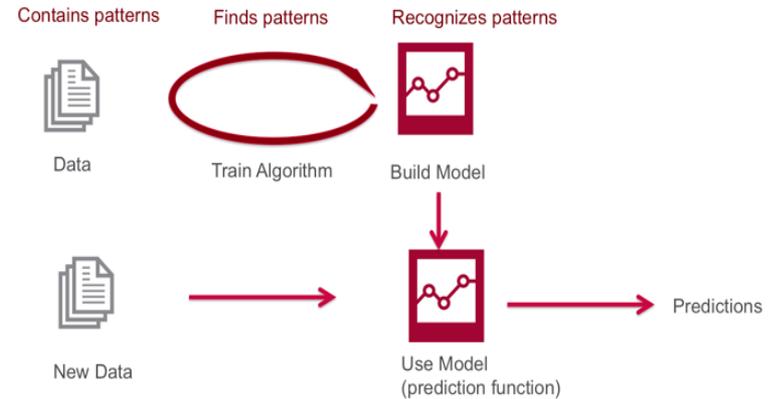
Systematic sources of error affecting phenotypic / WGS correlation

- Incomplete understanding of genotypic basis of phenotypic resistance
 - affects sensitivity of WGS prediction (resulting in very major errors)
 - problematic bacteria; problematic antibiotics
 - Major gaps in the knowledge base – e.g. *mcr-1* & *combinatorial Rs*
- *Different databases of R loci used – comparisons need to be standardised*
- 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

New sections – AI / machine learning



Vs.



Rules based

Model based

		# tested	Sens (%)	Spec (%)		# tested	Sens (%)	Spec(%)	Overall
E. coli	Gent	74	100	100		564	87	99	
Mtb	Etham	752	100	98.5		3526	91.9	90.3	
S. enterica	Ceftriax	648	100	99.8					80, 95%(±1 MIC)
P. aeruginosa	Levo	390	91.9	93.7					

Table adapted from: Su *et al.*, JCM 2019

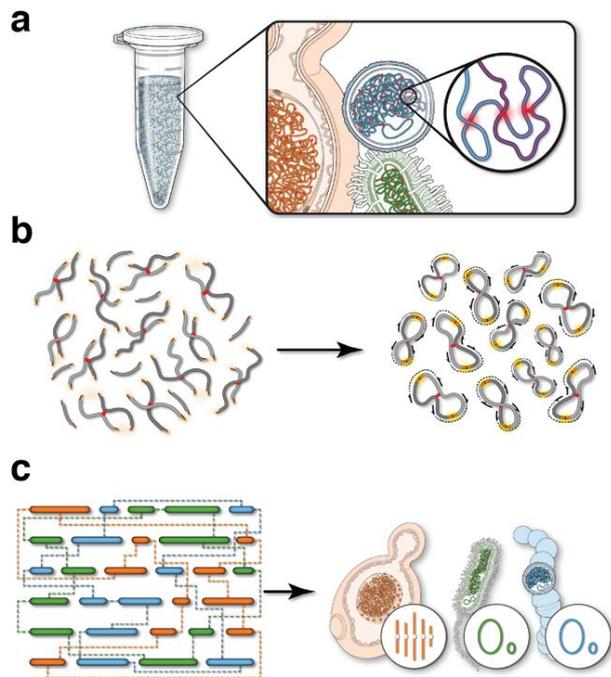
New sections – AI / machine learning

AI: Questions arising – listed by Su *et al.*, JCM 2019

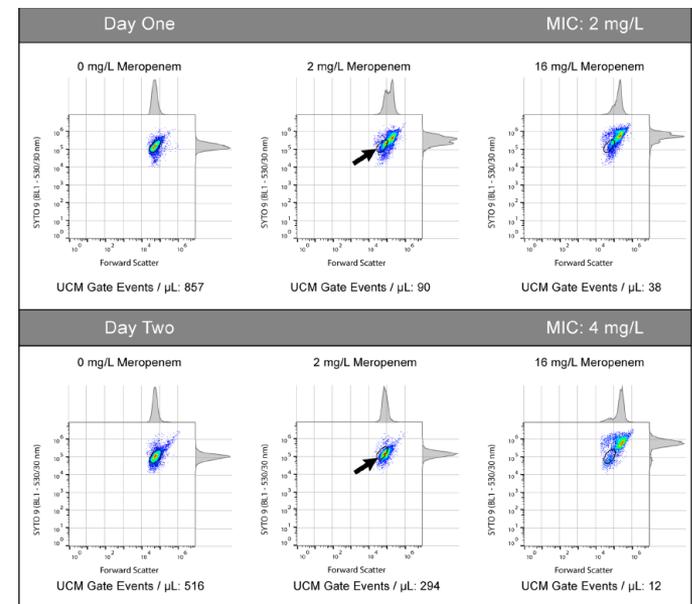
- At what price and turnaround time will WGS-AST replace culture-based sequencing for routine use in clinical microbiology labs?
- How do we interpret the presence of an antimicrobial resistance determinant gene if the susceptibility of the strain is below the MIC?
- Can genome prediction be used to detect hetero-resistance? Or to detect polygenic phenotypes?
- How important is epistasis in determining the resistance to different classes of antibiotics?
- Can gene amplification as a mechanism of resistance be accurately determined from WGS data?
- How efficiently can WGS-AST prediction software be ported to metagenomic-AST

New sections ...incl' metagenomes

- Metagenomic approaches – Positive early stages for finding AMR, challenges faced include: with seq yield, fragment length, locating / localizing mobile resistances
- New Tech – orthogonal approaches to help with gaps in knowledge base and delineating heteroresistance



Press *et al.*, Biorxiv: <https://doi.org/10.1101/198713>



Mulrone *et al.*, SCIENTIFIC REPORTS |
7:1903 | DOI:10.1038/s41598-017-02009-3

WGS based AST - Conclusions – v1

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 - surveillance of treatment failure +/- novel resistance mechanisms

Still no consensus, but...

- Overall increased evidence base for key bug/drugs identified previously
 - Previous per organism/drug patterns persist
 - Limited number of impactful studies
- Scope extended: AI, (LR-)WGS, metagenomics
 - Mostly early stages and some positives
- Reconvened committee for update of the report
 - Anticipate general consultation doc' 2019, Q4

....on behalf of the sub-committee...

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Thank you for your attention