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England

Can whole genome sequencing replace AST ?

Neil Woodford

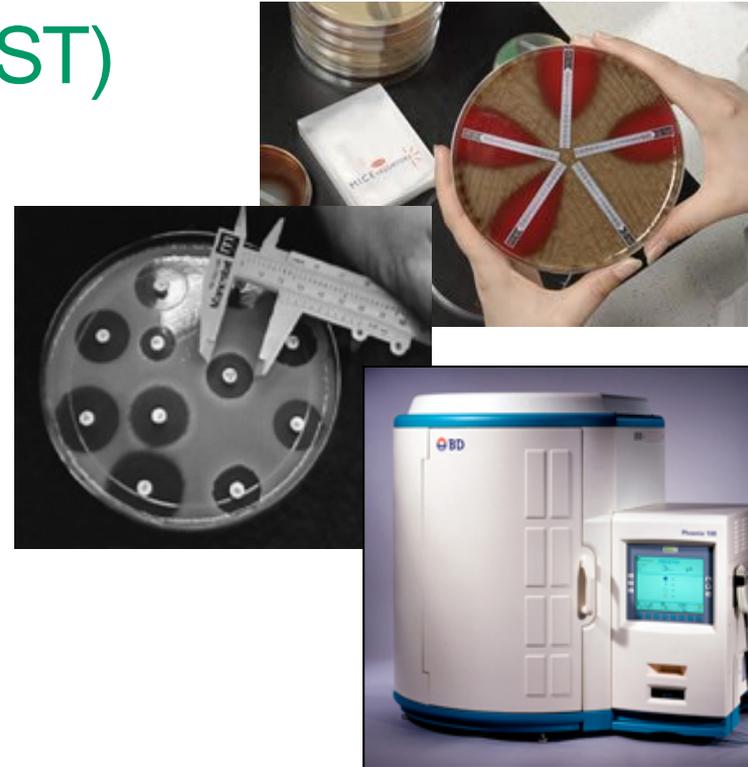
Antimicrobial Resistance & Healthcare
Associated Infections (AMRHAI) Reference Unit

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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
- ***“Microbiologists do it with culture and sensitivity” !***



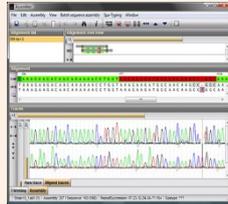


Towards WGS for reference services

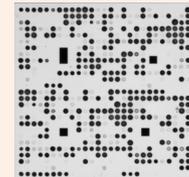
Define lineage
& surveillance



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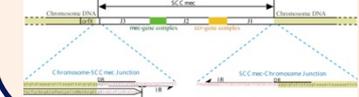


Biomarkers



sasX

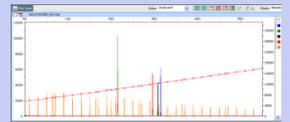
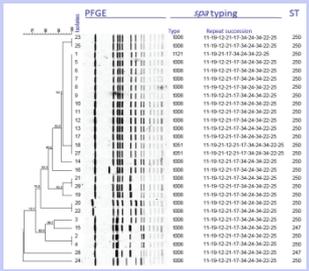
φSPβ-like prophage



ID



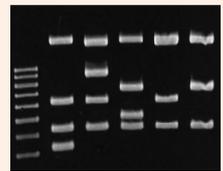
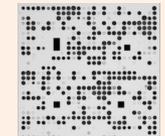
Outbreak
investigations



WGS



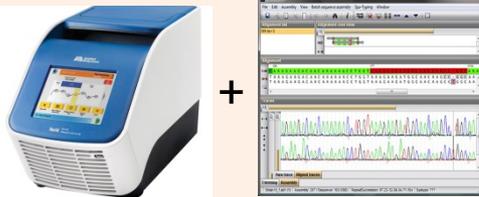
Assess virulence



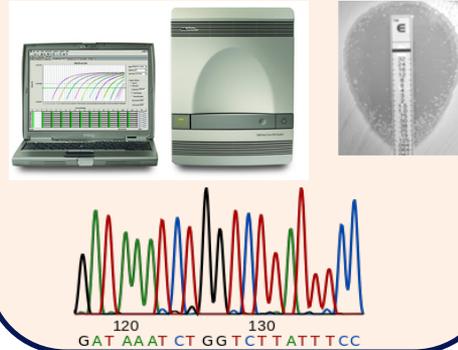


Towards WGS for reference services

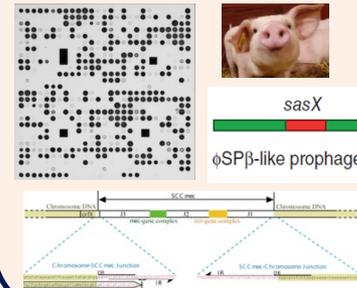
Define lineage
& surveillance



Predict resistance



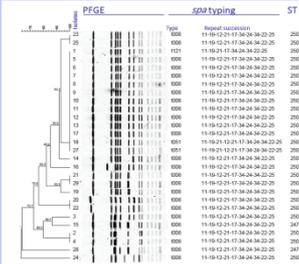
Biomarkers



ID



Outbreak investigations



WGS



Assess virulence





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A new EUCAST subcommittee

EUCAST Subcommittee on the role of whole genome sequencing (WGS) in antimicrobial susceptibility testing of bacteria

Chair: Neil Woodford, London UK

Formed after 2015 ECCMID, Copenhagen

Report to be issued for consultation after 2016 ECCMID, Amsterdam





Members bring diverse expertise

Frank M. Aarestrup (Denmark)	Gunnar Kahlmeter (Sweden)
Rafael Canton (Spain)	Claudio U. Koser (UK)
Michel Doumith (UK)	Alasdair MacGowan (UK)
Oskar Ekelund (Sweden)	Dik Mevius (Netherlands)
Matthew J. Ellington (UK)	Mike Mulvey (Canada)
Christian Giske (Sweden)	Thierry Naas (France)
Henrik Hasman (Denmark)	Tim Peto (UK)
Katie L. Hopkins (UK)	Jean-Marc Rolain (France)
Matt Holden (UK)	Ørjan Samuelsen (Norway)
Jon Iredell (Australia)	Neil Woodford (UK, Chair)



The remit - 1

1. to perform a review of the literature describing the role of WGS in antimicrobial susceptibility testing (AST) of bacteria;
2. to assess the sensitivity and specificity of WGS compared with standard phenotypic AST
3. to consider how WGS for AST may be applied in clinical microbiology laboratories and the likely implications for phenotypic and other genotypic methods in use
4. to consider the epidemiological implications of using WGS



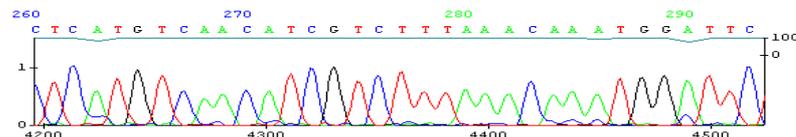
The remit - 2

5. to consider the clinical implications of WGS for the selection of antimicrobial therapy
6. to consider the principles of how the results of WGS for AST could be presented to clinical users to describe the drivers and barriers to routine use of WGS
7. to report at ECCMID 2016



What can WGS offer ?

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





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



Expanded focus to include...

Other considerations	
Other organisms	<i>M. tuberculosis, C. difficile, A. baumannii, P. aeruginosa</i>
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	-

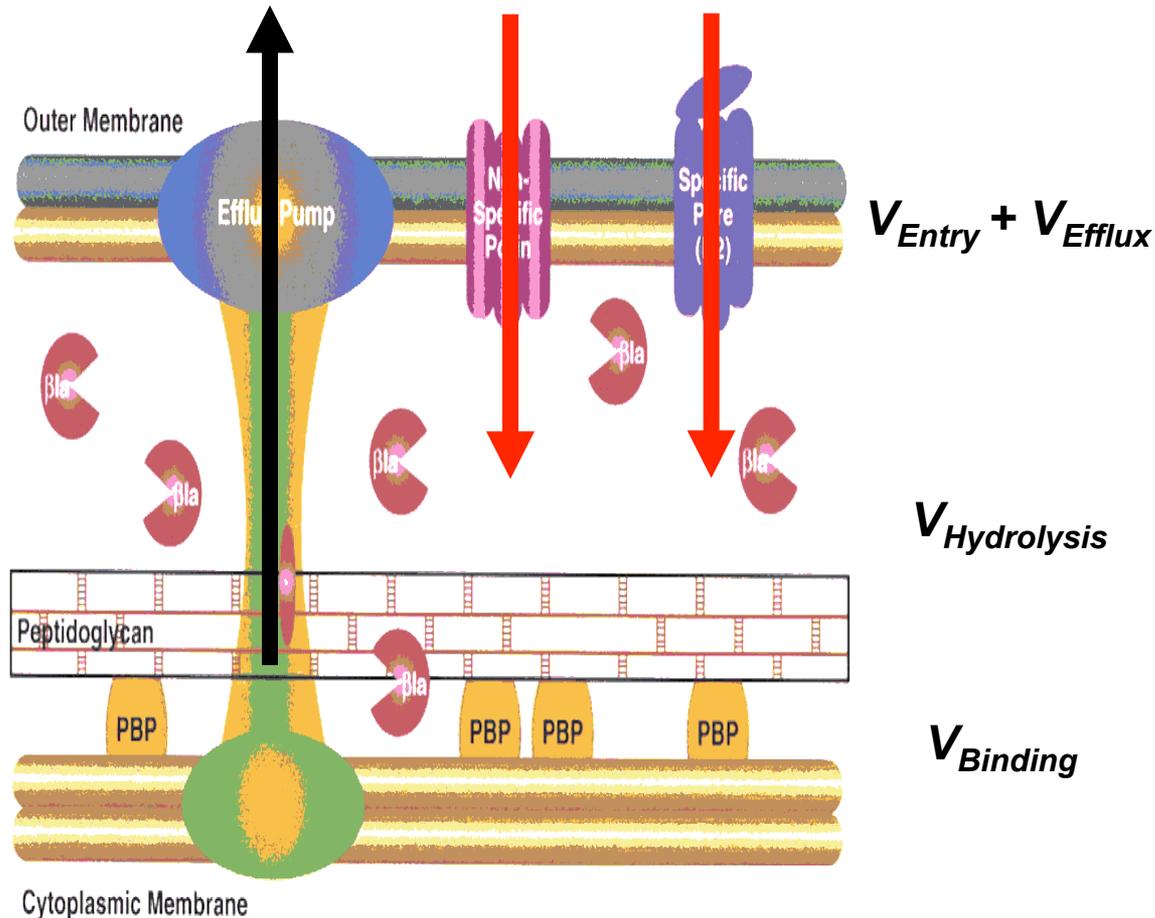


Evidence reports – e.g. Enterobacteriaceae

- The 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 and their interplay will require more study if improved levels of accuracy across large genetically diverse datasets are to be achieved.



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



Not always straightforward: e.g. gonorrhoea

- 'Easy'
 - PPNG: TEM-1 or TEM-135
 - TRNG: Tet(M)
 - CIP-R: SNPs in *gyrA* or *parC*
 - High-level Azi-R: 23S rRNA mutations or Erm
- More difficult – cumulative mechanisms
 - PBP2 (*penA*) mosaicism and SNPs (penicillin, cephalosporins)
 - mutations upregulating Mtr efflux
 - mutations affecting porin activity



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
- Currently there are no international QC standards
- 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.



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.



Systematic sources of error affecting phenotypic / WGS correlation

- 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.
- Incomplete understanding of genotypic basis of phenotypic resistance
 - affecting sensitivity of WGS prediction (resulting in very major errors)
 - 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
 - problematic bacteria; problematic antibiotics



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



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
 - education needed and major behavioural change for prescribers



Will new AMR diagnostics rise out of WGS data ?

- *Hypothesis:*
 - *a small subset of genes from the global resistome will be prevalent and will account for most resistance locally / regionally / nationally*
- Tests for these might be clinically useful ‘quick wins’
 - they would still be surrogates for rapid AST
 - will rapid AST become possible sooner ?



Concluding comments - 1

- Taken 50+ years for ‘harmonization’ of breakpoints
 - Learn lessons from the past and avoid multiple parallel systems
- 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



Concluding comments - 2

- At present there are insufficient data to present a definitive document on the topic.
- We have reviewed the state-of-the-art as a first approach.
- Our report is presented as a baseline discussion document; it marks where we are now.
- it will require updating as sequencing technologies become more affordable and more widely applied.



Concluding comments - 3

- The quantity and quality of evidence for AST phenotypic / genotypic concordance (or lack thereof) must improve.
 - poor use of ECOFFs in published literature
- Must balance need for analysis to be more rigorous and 'standardised' (ISO accreditation etc.) with academic drivers for bioinformaticians to develop and improve their own tools
 - advocating a global standard method is unrealistic at this time
- Pragmatic to accept that algorithms can vary, but that they:
 - should use the same centralised database of all known resistance genes / mutations (NCBI, ResFinder, CARD, others)
 - should have been calibrated and shown equivalent



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Questions for you

- How many have used WGS of bacteria for any purpose ?



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- How many have analysed WGS data for resistance genes ?
- How many have compared AST phenotype with WGS genotype ?
 - How many work primarily in a reference or research laboratory ?
 - How many work in a diagnostic laboratory ?



Report for consultation

- Draft will be posted 'soon' on EUCAST website
- Final revision made after close of consultation period
- Preparation for publication

- For future versions
 - systematic review of literature ?
 - grading the quality of evidence
 - proposing QC metrics and analytical criteria



Acknowledgements

- Gunnar Kahlmeter and Derek Brown – setting us the challenge
- All subcommittee members – excellent engagement and debate
- Oskar Ekelund and Matt Ellington – editorial duties