

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





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

Clinical Microbiology and Infection 23 (2017) 2-22 Contents lists available at ScienceDirect Clinical Microbiology and Infection journal homepage: www.clinicale The role of whole genome sequencing in antimicrobial susceptibility the role of whole serious sequencing in anumicrousal susc testing of bacteria: report from the EUCAST Subcommittee CMI M.J. Ellington ^{1,†} O. Ekelund ^{2,†} F.M. Aarestrup ³ R. Canton ⁴ M. Doumith ¹ C. Giske ⁵ H. Grundman ⁶ H. Hasman ⁷ M.T.G. Holden ⁸ K.L. Hopkins ^{1,†} I. Iredell ⁹ G. Kaklmeter C.U. Köser ¹⁰ A. MacGowan ¹⁴ D. Mevius ¹², 13' K.L. Hopkins ^{1,†} I. Iredell ⁹ G. Kaklmeter ⁵ J-M. Rolain ^{1,*} O. Samuelsen ¹⁸ D. Mevius ¹², 13' K.L. Hopkins ^{1,†} I. T. Naas ¹⁵ T. Peto ¹⁶

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

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





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)



Most appropriate AST comparators

What criteria should WGS data be assessed against ?

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

<u>ECOFFs</u> (epidemiological cut-off values) differentiate wild-type (WT) from nonwild-type (NWT) isolates with an acquired resistance mechanism



UCAST expert rules in antimicrobial susceptibility testing



What can WGS offer ?

	Phenotypic AST	WGS-based AST	
Measures susceptibility	Y	Ν	
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	Ν	



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



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	-



A growing literature

Whole-genome sequencing to control antimicrobial resistance Claudio U. Köser¹, Matthew J. Ellington², and Sharon J. Peacock^{1,2,3,4} Validio V. Noser, "Makterwy J. Elimyyun , and o Harvin V. rea Department of Mediane. Usiversity of Cardenders, Cardenders, W. "Cardendard Medianey and Paulic Health Started Health England, Cardender, W. "Sentendard Usiversity Homenas Issented Health Services Forenation Frank, Cardender, W. "Wellcome Tran Sanger Institute, Wellcome Trait Genome Carneou, Heneau, UK

neent improvements in sequencing technol-als genome sequencing (WOS) is positioned a esentia tool in the court beathcare. Tound numerous explanations in the sec-ne development of novel exhibition and users through to eaching experience of exercision to eaching exercision to exercision to exercision to experience of exercision to eaching exercision to exe on the development of novel antibiotics and institution to antibiotic stewardship of malable drogs via surveillance and the eluci-dual stators that allow the emergence of antibiotence Survey work-development of antibiotence dation of the factors that allow the emergence and surfacence of resistance. Numerous or Works as the surfacence and highlight with the value of the sector of day to day infection control and, orders authority resistance. Nowever, appropriate under an alwais forms will need to be developed before rearing WOS can be introduced on a large scale.

can be introduced on a large scale.

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Chemotherapy doi:10.1093/joc/dit180 Advance Acorss publication 30 May 2013 Journal of Antimicrobial Chemotherapy Advance Access published December 11, 2012 Journal of Antimicrobial J Antimicrob Chemother Chemotherapy doi:10.1093/jac/dks496 Genotyping AAC The Resistome of Pseudomonas aeruginosa in Relationship to Ea Zankari^{1,2}, H Phenotypic Susceptibility National Food Institu Veronica N. Kos," Maxime Déraspe," Robert E. McLaughlin," James D. Whiteaker," Paul H. Roy," Richard A. Alm," Jacques Corbell, Humphrey Gardner* mative Medicines Unit AstraZenera BSD Streets, Watham, Messachusetts, USA" Infectious Diseases Research Center, Laws University, Daebec, Oustee Received 11 0 Many clinical isolates of Pseudomonas aeroginosa cause infections that are difficult to eradicate due to their resistance to a wide Objectives: A variety of antibiotics. Key genetic determinants of resistance were identified through genome sequences of 390 clinical isolates of P. aeruginosa, obtained from diverse geographic locations collected between 2003 and 2012 and were related to microbiological susceptibility data for meropenem. levofloxacin, and amikacin. B-Lactamases and integron cassette arrangements were enriched 200 in the established multidrug-resistant lineages of sequence types ST111 (predominantly O12) and ST235 (O11). This study demonstrates the utility of next-generation sequencing (NGS) in defining relevant resistance elements and highlights the diversity of resistance determinants within P. aeruginosa. This information is valuable in furthering the design of diagnostics and therapeutics for the treatment of P. aeruginosa infections. 3051 W

Journal of Antimicrobial

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388 isolates; 1 species; 1158 AST results; 88.9% **WGS** concordance

consequences of antimicrobial use, which include the emergence of resistance, toxicity, and further sequelae, such as Clostridium difficile colitis (10). With the ever increasing incidence of bacterial drug resistance, the need for rapid and reliable methods to predict antimicrobial susceptibility early in the course of treatment is ever more pressing (11). Advances in methodology and the decreasing cost of next-generation sequencing (NGS) have the potential to impact clinical microbiology ranging from species identification to antimicrobial susceptibility testing (12-14). Indeed, whole-ge-nome sequencing studies of Enterococcus spp., Salmonella enterica serovar Typhimurium, Escherichia coli, and Klebsiella pneumoniae have demonstrated that reliable schemes can be generated for pre-diction of resistance phenotypes in these organisms (12, 15).

Accordingly, the study presented here used NGS to construct a comprehensive genomic analysis of 390 P. aeruginosa isolates. Whole-genome sequencing of these P. aeruginosa isolates was conducted to define the diversity and distribution of resistance mechanisms and to determine the extent to which NGS can reliably provide a genotype to support and better define a nonsusceptible phenotyp

17), ORFs found in all 390 isolates that share at least 250% coverage with the corresponding PAO1 genes (1,278) were used in phylogeny construc-tion. Sequences were aligned using the MAFFT aligner (18), and the phylogenetic tree was generated using EXAML (19) with the parameters set to estimate a maximum likelihood. Branch support was estimated by 100 bootstrap replicates. Assemblies were completed in CLCGenomic Work-

Received 24 July 2014. Returned for modification 8 October 2014 Accepted 29 October 2054 Accepted manuscript posted online 3 November 2014 Challon Kox VV, Diracon M, McLaughlin RI, Whitever, ID, Roy RI, Alm RA, Colbell J, Garoner H. 2015. The relations of Payatomena amaginasis in matterensis to phenotypic susceptibility. Antimicno Agents Diversible: 59.427-435 distantanta60.42 mase-14 Explorements installal for this article may be found at http:///dx.doi.org/10.110/ Copyright © 2015. American Society for Microbiology, All Bioth Tene

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Evidence reports – *e.g.* Enterobacteriaceae

- Relatively limited number of acquired resistance genes and resistanceassociated 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





Combinatorial resistance: WGS vs. AST

Table 1

Comparison of WGS and Reference Laboratory Testing of Carbapenem-Resistant

Gram-Negative Bacteria

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

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



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- 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.

Articles

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.



Public Health

England



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



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	<i>Salmonella</i> 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

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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 genotypephenotype 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.



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.



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.



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 <u>reference</u> 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



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





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