



Public Health
England

Can WGS replace antibiotic susceptibility testing ?

Professor Neil Woodford

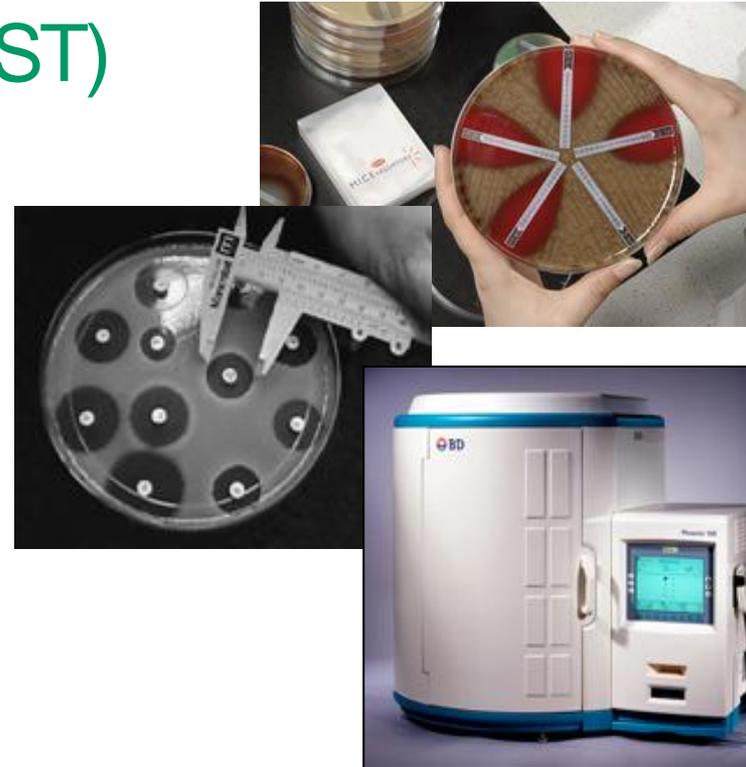
Antimicrobial Resistance & Healthcare
Associated Infections (AMRHAI) Reference Unit

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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” !***

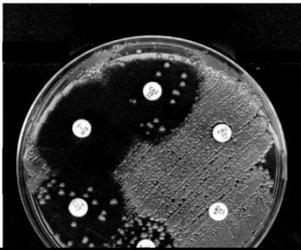




Testing for resistance mechanisms

BSAC

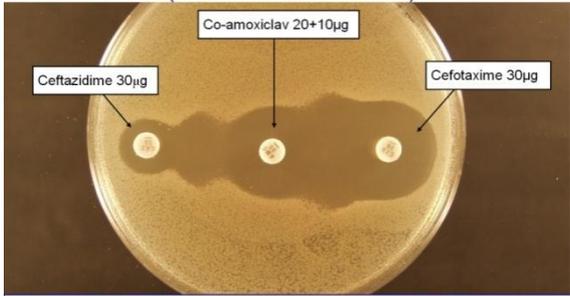
Derepressed AmpC



Derepressed colonies within cephalosporin zones (inducible AmpC-producing strains can become permanently derepressed)

BSAC

Double-disc tests for ESBLs (*E coli* with TEM-5)



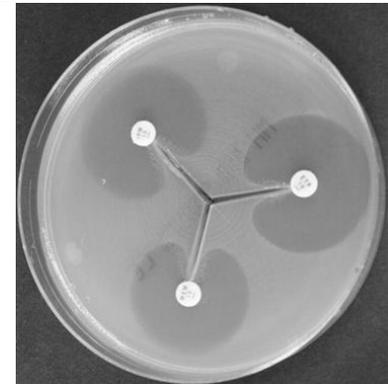
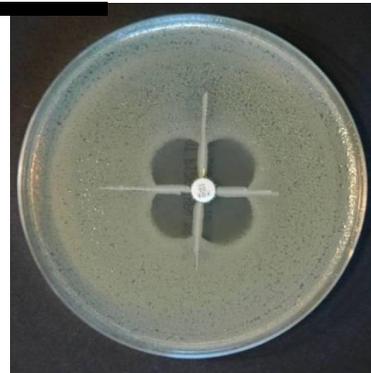
Use clavulanate (co-amoxiclav) discs in proximity to cephalosporin discs. The distance between discs is critical. Any distortion of zones indicates ESBL

BSAC

Detection of metallo-β-lactamase with the Etest

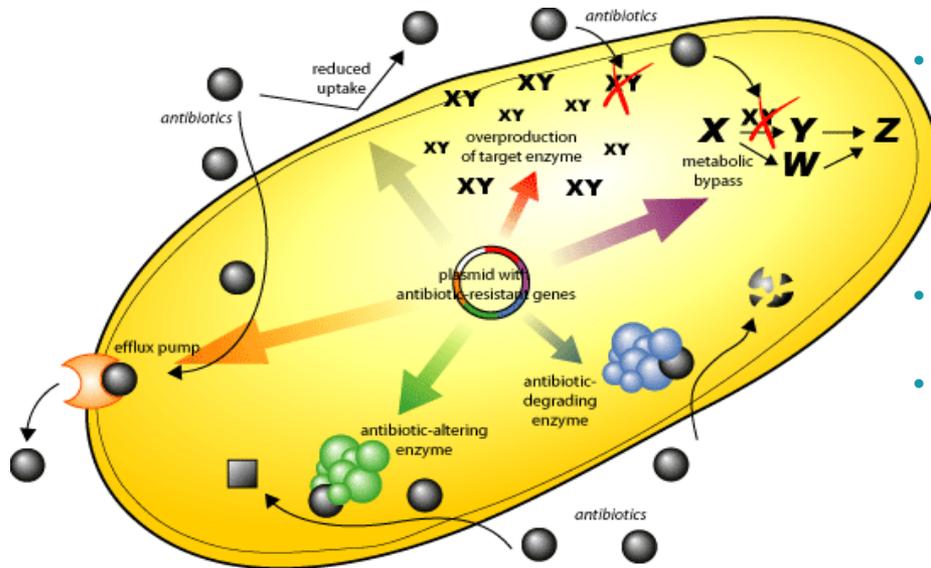


A metallo-β-lactamase in *Ps.aeruginosa* confers resistance to imipenem. The Zn-dependent enzyme is inactivated by the chelating agent EDTA, reducing the MIC by 8-fold or greater.





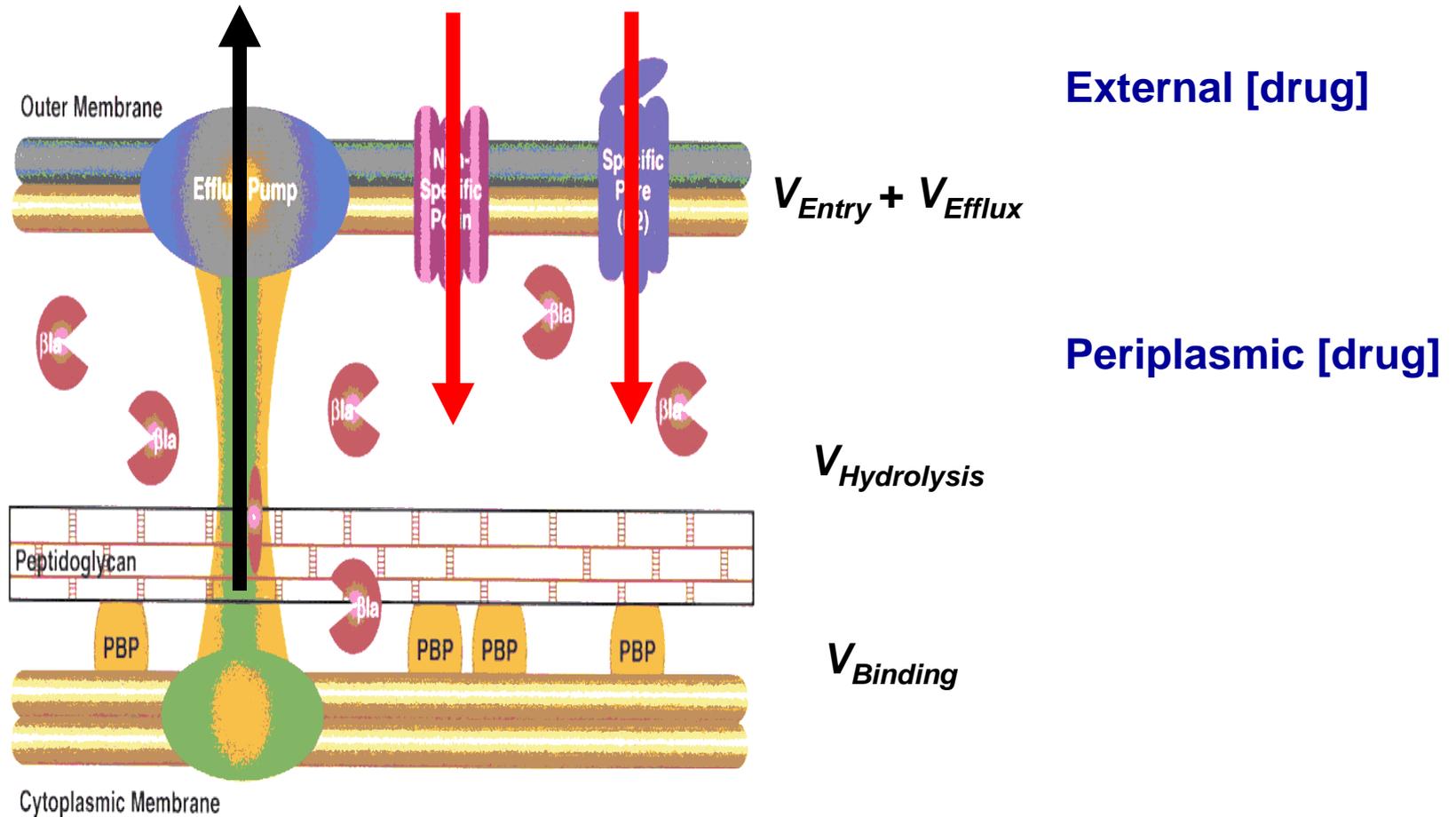
Mechanisms of antibiotic resistance



- Intrinsic or acquired
- Drug modification / inactivation
- Drug target modification
- Reduced accumulation of drug
 - reduced cell permeability (less in)
 - efflux (more pumped out)
- Alternative metabolic pathways (by-pass)
- Effective bioinformatics pipeline for prediction of resistance most likely:
 - family, genus or species specific
 - based on known genetic mechanisms

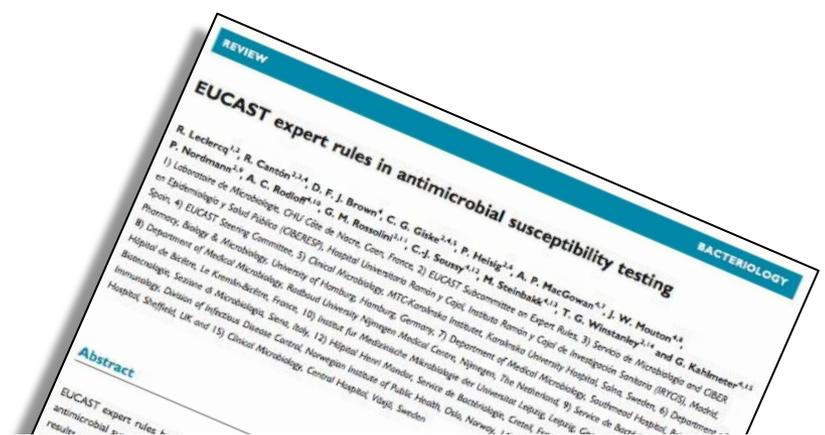


Susceptibility or resistance ? complex interplays



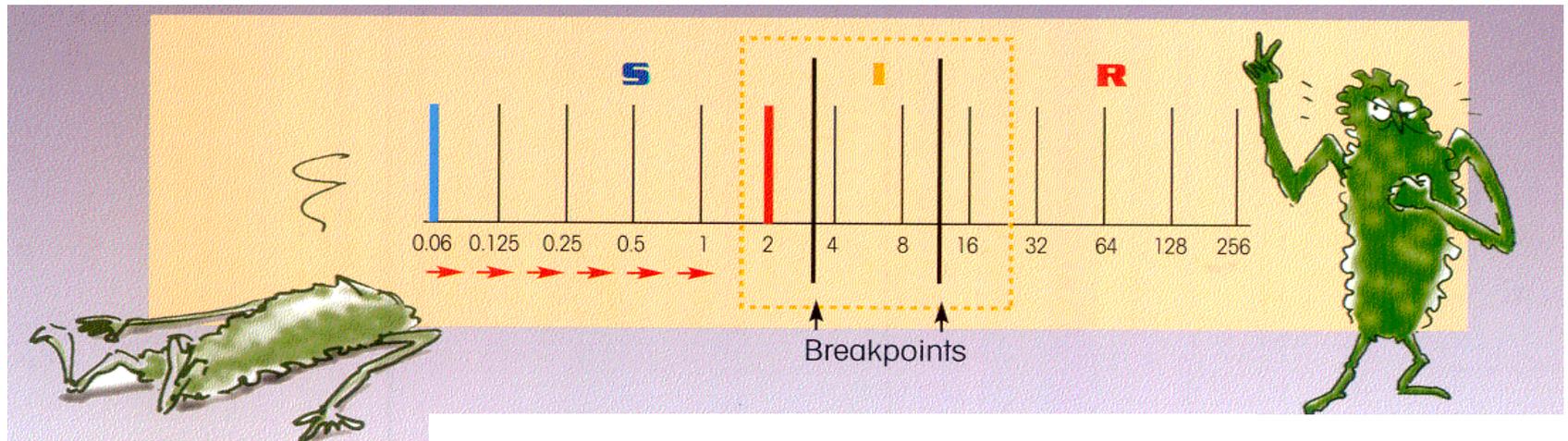


Interpreting AST data



MICs or zone sizes: meaningless unless interpretative criteria applied

- **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 from isolates with an acquired resistance mechanism



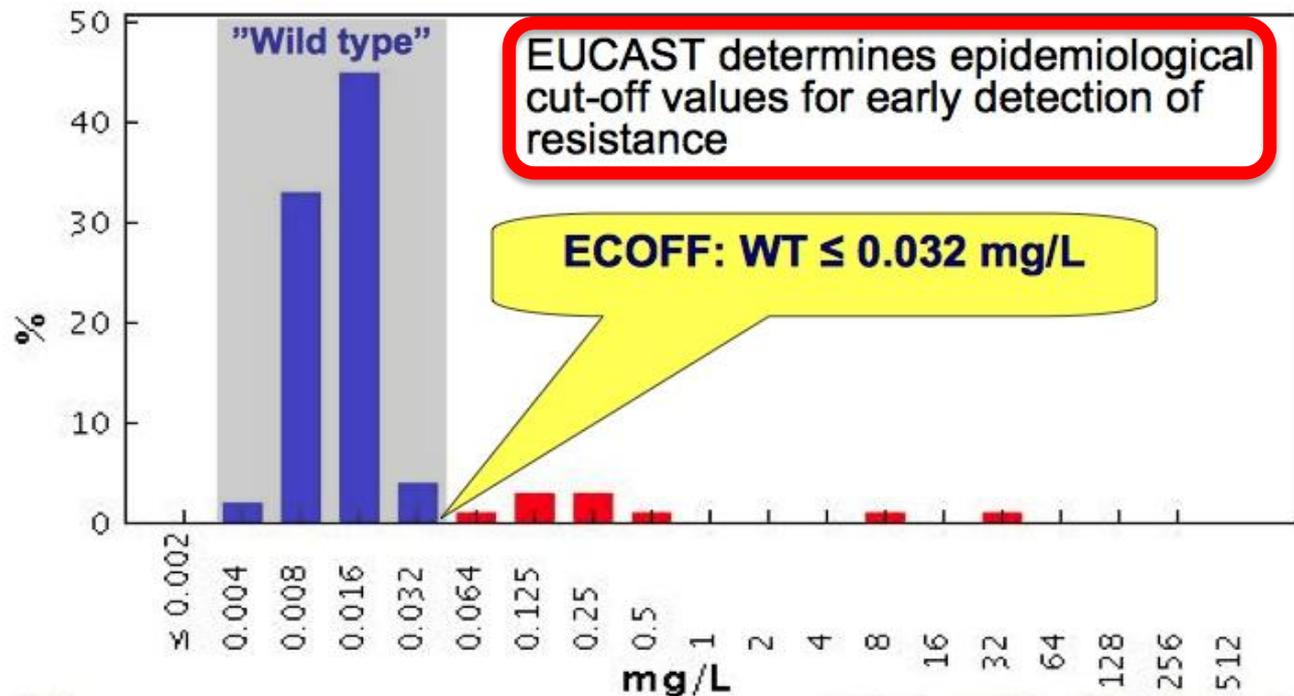


ECOFFs are lower than clinical breakpoints

Ciprofloxacin / *Escherichia coli*

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST



MIC
Epidemiological cut-off: WT ≤ 0.032 mg/l

8011 observations (14 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L



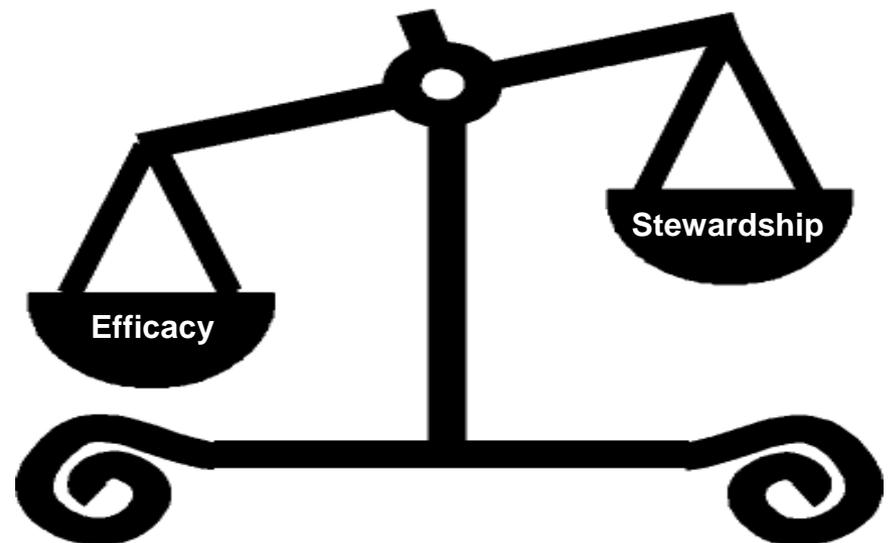
Need to revolutionize AST

- Much antibiotic prescribing is empiric (= best guess)
 - ? likely bacterial infection; ? likeliest pathogens; ? likely susceptibility
- We need faster, accurate diagnosis of (resistant) infections
 - better antibiotic stewardship
 - reduce length of or eliminate need for broad-spectrum empiric therapy
 - reduce selection pressure and collateral damage
- Molecular detection of resistance genes / mutations
 - a surrogate for rapid AST



Gonorrhoea: a paradigm for better AMR diagnostics

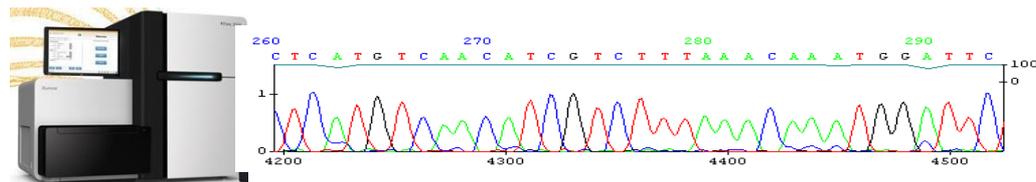
- International approach to treatment
- Recommendation changes when resistance rate exceeds 5%
- **Many patients are over-treated to prevent under-treating a few**
- Gonococci in UK (2013):
 - >80% are PEN-susceptible
 - >70% are CIP-susceptible
- Neither drug is used
- **Can't detect AMR at presentation**





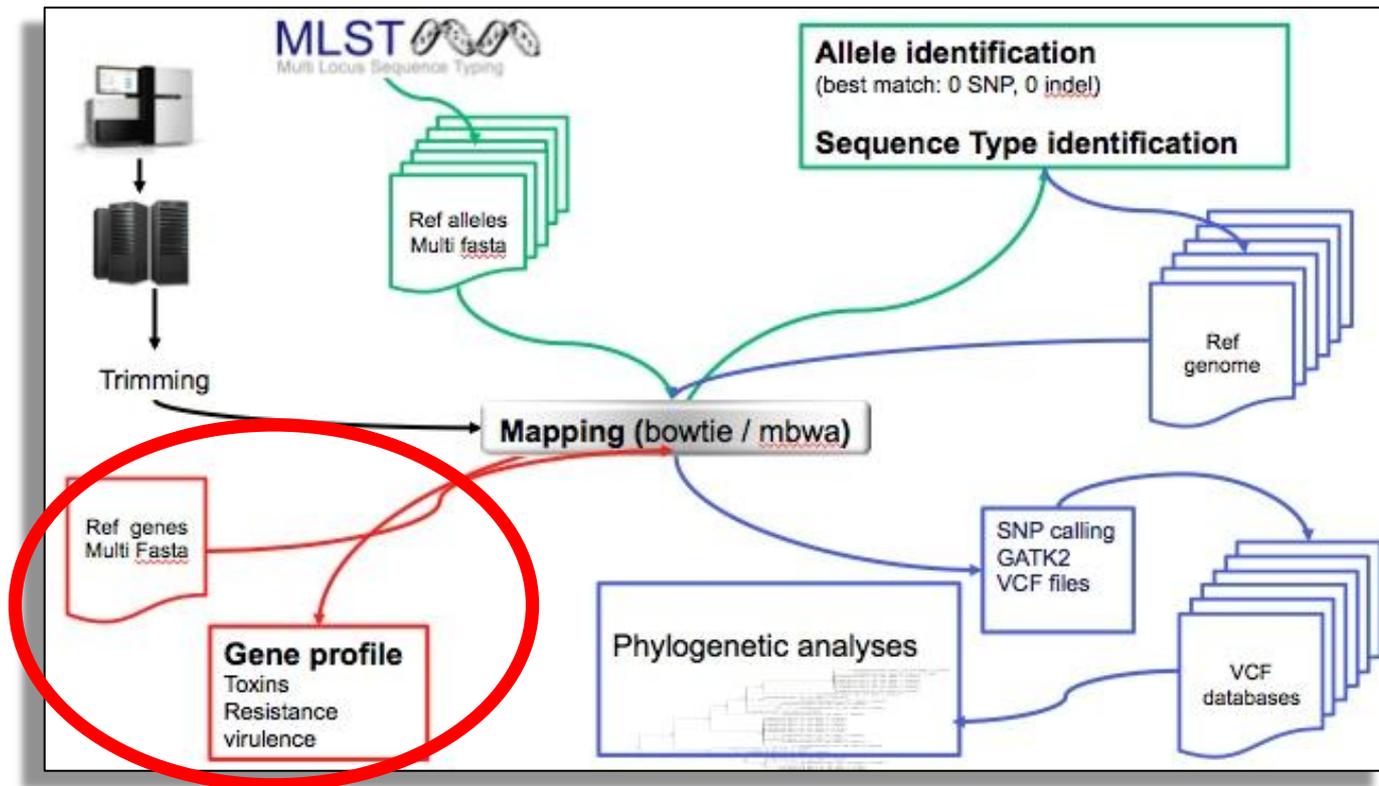
What can WGS offer ?

	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	✓	✗





AMRHAI and WGS



- *S. aureus* ; Enterobacteriaceae; *P. aeruginosa*



S. aureus - I

□ Acquired antimicrobial resistance genes

- Beta-lactams : *blaZ, mecA, mecC*
- Aminoglycosides : *aacA-aphD, aphA3, aphD, aadE, aadD, spc, str*
- Chloramphenicol : *cfr, cat*
- MLS : *ermA, ermB, ermC, ermT, ermY, mphC, msrA, lsaB, lnuA, lnuB, vgaA, vgaB, vgaA*
- Fusidic acid : *fusB, fusC*
- Glycopeptides : *vanA, vanB, vanC*
- Tetracyclines : *tetk, tetL, tetM, tetO*
- Trimethoprim : *dfrA, dfrC, dfrD, dfrG, dfrK*
- Mupirocin : *mupA, mupB*
- Linezolid : *cfr*



S. aureus - II

□ Chromosomal mutations associated with antimicrobial resistance

- Ciprofloxacin : *gyrA, glrA*
- Trimethoprim : *dfrB*
- Mupirocin : *ileS*
- Fusidic acid : *fusA*
- Rifampicin : *rpoB*
- Linezolid : *23S rRNA*



S. aureus: WGS genotype vs. AST

	Phenotype S		Phenotype R		Error%
	Genotype S	Genotype R	Genotype R	Genotype S	
Streptomycin	357	6	17	14	4.88
Gentamicin	307	3	68	16	4.74
Ciprofloxacin	304	0	75	15	3.81
Moxifloxacin	304	0	90	0	0.00
Penicillin	65	6	319	4	2.54
Oxacillin	209	7	171	7	3.55
Trimethoprim	114	0	33	5	2.99
Tetracycline	315	5	71	3	2.01
Erythromycin	254	6	131	3	2.28
Fusidic acid	319	1	68	6	1.76
Mupirocin	370	5	14	0	1.29
Rifampicin	374	0	16	1	0.25
Linezolid	394	0	0	0	0.00
	3686	39	1073	74	2.32

Major errors, 0.8% Very major errors, 1.5%



AST error ratings

- Major error = a susceptible isolate reported resistant
 - **steers prescribing unnecessarily to other options**
- Very major error = a resistant isolate reported susceptible
 - **potential for inappropriate therapy !**



S. aureus: WGS genotype vs. AST

Major errors				
	Phenotype S		Potential reasons	
	Genotype S	Genotype R		
Antibiotics	0	39	Expression, stability, MICs	
Very major errors				
	Phenotype R		MIC mg/L	Potential mechanisms
	Genotype R	Genotype S		
Gentamicin	68	16	2-4 (16/16)	Efflux
Ciprofloxacin	75	15	2 (15/15)	Efflux
Moxifloxacin	90	0		
Penicillin	319	4		PBPs, Efflux
Oxacillin	171	7	4 (4/7)	PBPs, Efflux
Trimethoprim	33	5	2-4 (4/5)	Efflux, Ribosomal proteins
Tetracycline	71	3		Efflux, Ribosomal proteins
Erythromycin	131	3		Ribosomal proteins
Fusidic acid	68	6	1-4 (5/6)	Permeability
Rifampicin	16	1	1 (1/1)	Permeability



Enterobacteriaceae: Panel of targets

- In house database of ~1600 resistance genes
 - covers all classes of antibiotics
- Chromosomal mutations associated with antimicrobial resistance
 - ciprofloxacin : *gyrA*, *glrA*
 - rifampicin : *rpoB*



CPOs in the UK

Department of Health
Department for Environment Food & Rural Affairs
UK Five Year Antimicrobial Resistance Strategy 2013 to 2018

Multi-Drug Resistant Bacteria

Klebsiella spp - carbapenem

E. coli - carbapenem

E. coli - cephalosporin

E. coli - fluoroquinolone

Pseudomonas - carbapenem

N. gonorrhoeae - ceftriaxone

Klebsiella spp - cephalosporin

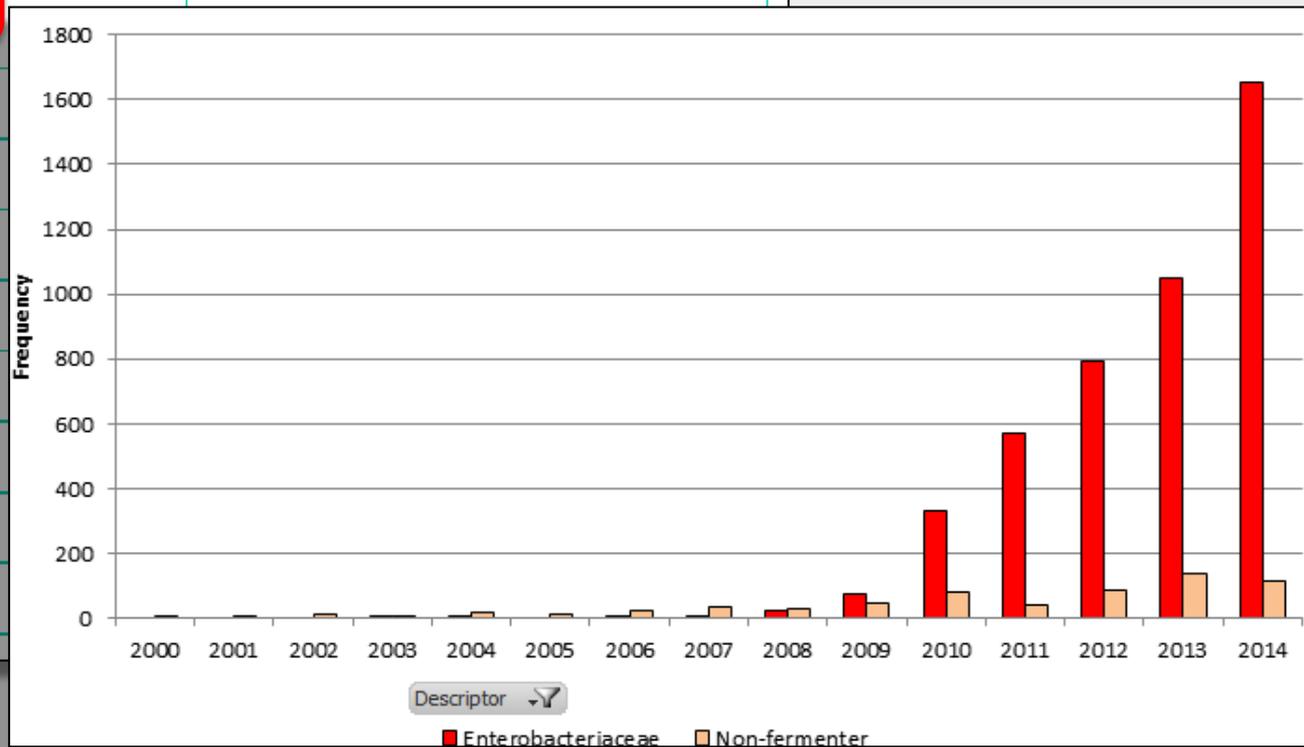
Pseudomonas - cephalosporin

E. coli - gentamicin

S. pneumoniae - penicillin

Metric

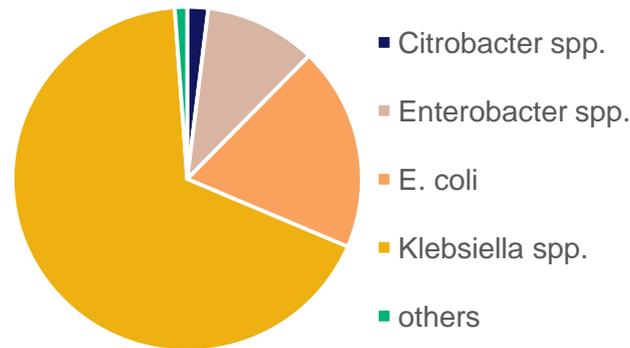
% non-susceptible to imipenem and/or meropenem





WGS for CPE

- First CPE of different bacterial ID from each new patient
- Excludes KPC-producers from two NW England hospitals
- Isolates with unexplained carbapenem resistance



Enzyme	2014 (WGS)
GES	12 (4)
IMI	4 (4)
IMP	12 (6)
KPC	844 (198)
NDM	290 (201)
OXA-48-like	383 (284)
NDM + OXA-48-like	28 (21)
VIM	75 (48)
Total	1651 (766)



WGS vs. gene-specific PCRs for carbapenemases

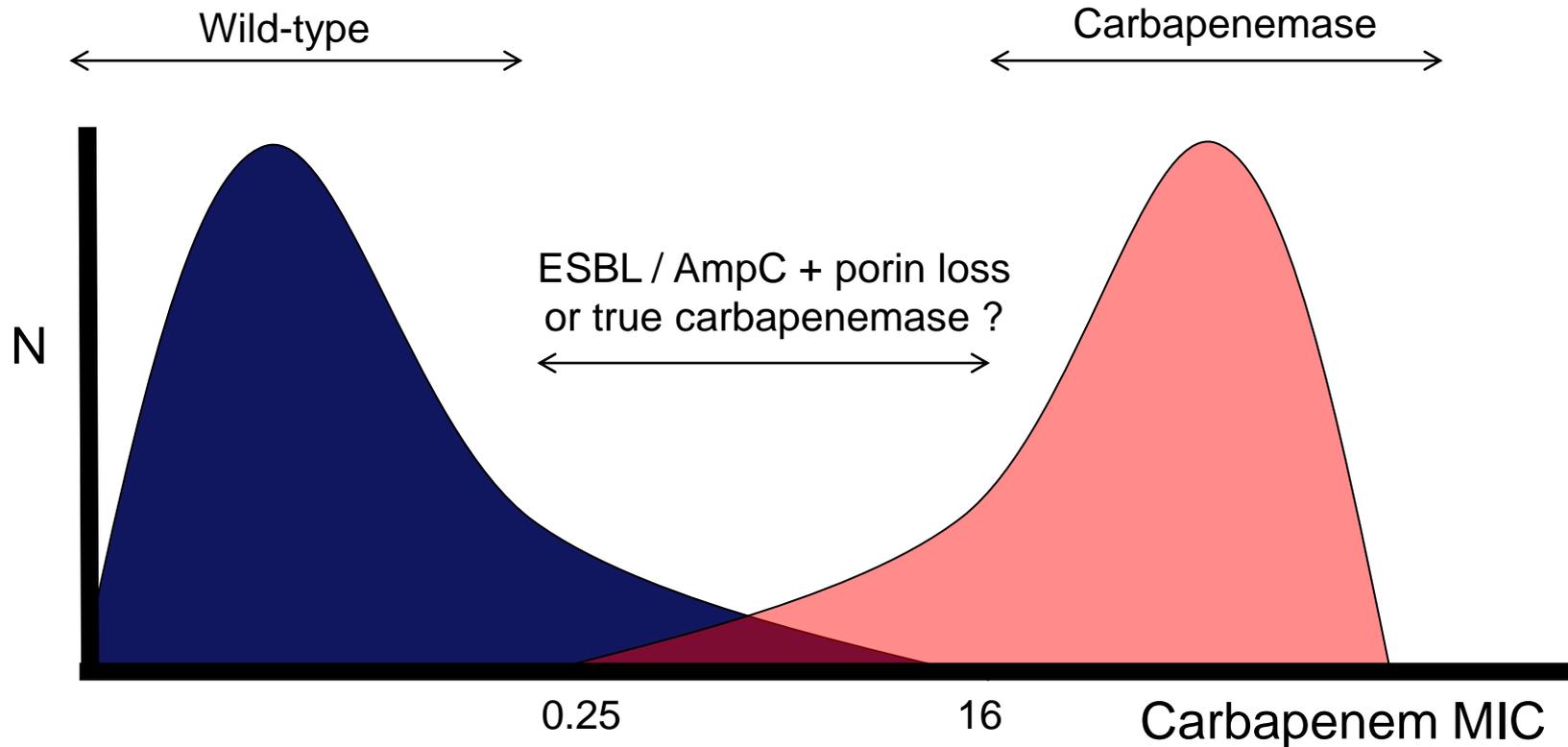
- Overall excellent agreement

Carbapenemase	Genotype +ve	PCR +ve	Genotype -ve	PCR -ve	
NDM	154	154	0	0	NDM-1, NDM-5, NDM-7
OXA-48 like	152	152	0	0	OXA-48, OXA-181, OXA-244, OXA-232
KPC	92	92	0	0	KPC-2, KPC-3
VIM	29	29	0	0	VIM-4
IMP	4	3	0	1	IMP-1, IMP-8
GES	2	2	0	0	GES-5
SPM	2	2	0	0	SPM-1
Others	0	0	12	12	

- Good for finding rarer carbapenemases or novel variants
 - GES-5, novel IMI variant



The problem with spotting the carbapenemase producers



- Not all CPE are resistant to carbapenems
- Not all carbapenem-resistant Enterobacteriaceae are CPE
- Human experts, subjective : computer algorithms, poor specificity



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



A growing literature



Predict Whole

N. C. Gordon, M. J. Dewey, ...

Whole-genome predict an organ... independent stud... ceptibilities, were inter... plasmids, B... methicillin, c... pin, and mu... and manual... algorithm, W... phenotypic i...

50
51

143
100

200
3051

W

1182

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doi:10.1093/jac/dkt1150 Advance Access published 30 May 2013

Predict Klebsiella

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¹Welford Department of John Radcliffe Hospital, Trust Centre for Human Microbiology, Oxford, UK

Objective

143
100

200
3051

W

1182

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Journal of Antimicrobial Chemotherapy

Journal of Antimicrobial Chemotherapy Advance Access published December 11, 2012

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Genotyping to

Ea Zenkari^{1,2}, ...
¹National Food Institute, Denmark

Received 11 October 2012

Objectives: Antimicrobial resistance genes (ARGs) are often found in plasmids, which can be transferred between bacteria. The presence of ARGs in plasmids is a major concern for public health. The aim of this study was to determine the prevalence of ARGs in plasmids from *Escherichia coli* isolates from a pig-breeding farm in Denmark.

200
3051

W

1182

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Journal of Antimicrobial Chemotherapy



The Resistome of *Pseudomonas aeruginosa* in Relationship to Phenotypic Susceptibility

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Many clinical isolates of *Pseudomonas aeruginosa* cause infections that are difficult to eradicate due to their resistance to a wide variety of antibiotics. Key genetic determinants of resistance were identified through genome sequences of 390 microbial 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. β -Lactamases and integron cassette arrangements were enriched in the established multidrug-resistant lineages of sequence types DT11 (predominantly DT11 and ST235 (DT11)). 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.

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

optimization of clinical outcomes and minimizes the unintended 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-genome sequencing studies of *Enterococcus* spp., *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, and *Klebsiella pneumoniae* have demonstrated that reliable schemes can be generated for prediction 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 non-susceptible phenotype.

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Whole-genome sequencing to control antimicrobial resistance
Claudio U. Köser¹, Matthew J. Ellington², and Sharon J. Peacock^{1,2,3,4}

Review

Following recent improvements in sequencing technologies, whole-genome sequencing (WGS) is positioned to become an essential tool in the control of antibiotic resistance, a major threat in modern healthcare. WGS has already found numerous applications in the study of resistance, from the development of novel antibiotics and diagnostic tests through surveillance and the elucidation of the factors that allow the emergence and persistence of resistance. Numerous proof-of-principle studies have also highlighted the value of WGS as a tool for day-to-day infectious control and, for some pathogens, as a primary diagnostic tool to detect antibiotic resistance. However, appropriate data analysis platforms will need to be developed before routine WGS forms will be introduced on a large scale.

A modern tool for a growing challenge

The spread of antibiotic resistance constitutes one of the most serious threats to human health [1]. If left unchecked, the emergence of antibiotic resistance could become an 'minor surgery and routine operations' could become high risk procedures [2]. As a result, the UK Chief Medical Officer has called for antibiotic resistance to be included in the National Risk Register [2]. The causes of the spread of antibiotic resistance are complex, as are the strategies to combat this threat [3–5]. These include developing novel antibiotics as well as maximizing the utility of currently available antibiotics by preventing infections and improving diagnostics. As a result of recent technological advances, microbial WGS has emerged as a tool that could underpin the success of such goals.

A detailed discussion of the various sequencing technologies and their advantages and disadvantages for pathogen WGS can be found elsewhere [6–8]. For the purposes of this review, it is merely necessary to appreciate that two types of WGS exist. The first can be compared to a high-throughput and low cost (currently approximately \$65 per sample) and the second to a low-throughput and high cost (currently approximately \$150 per sample) and the second to a low-throughput and high cost (currently approximately \$150 per sample).

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University of East Anglia



Meta-analysis: WGS for resistance prediction

Ref	Species	Number isolates	Phenotype R		Phenotype S		Total ASTs
			WGS = R	WGS = S	WGS = R	WGS = S	
Kos	<i>P. aeruginosa</i>	388	398	73	56	631	1158
Stoesser	<i>E. coli</i>	74	166	2	13	337	518
Stoesser	<i>K. pneumoniae</i>	69	176	10	8	289	483
Gordon	<i>S. aureus</i>	501	1069	22	9	4087	5187
AMRHAI	<i>S. aureus</i>	?	1073	74	39	3686	4872
AMRHAI	<i>Salmonella</i>	622	953	40	43	7700	8736
			3835	221	168	16730	20954

Concordance = 98.1%

Major errors = 0.8%

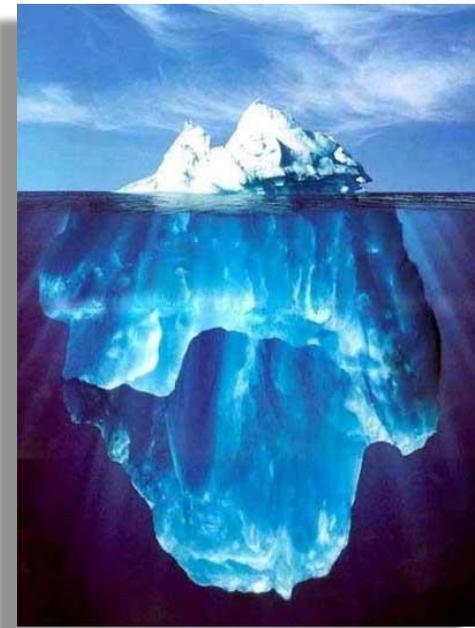
Very Major errors = 1.1%

Sensitivity	94.6%	(93.8 - 95.2%)
Specificity	99.0%	(98.8 - 99.2%)
Positive Predictive Value	95.8%	(95.1 - 96.4%)
Negative Predictive Value	98.7%	(98.5 - 98.9%)



WGS-based detection of resistance

- Detects known mechanisms well
 - but only as good as available sequence data
 - need curated and iterative databases
- Most WGS errors vs. AST are *very major* (= failure to find mechanism)
 - need better algorithms to predict resistance in 'hit-negative' isolates
- Unexpressed resistance mechanisms seem uncommon
- Would you base treatment on a negative WGS result ?





Opinion: WGS-based genotypic antibiograms

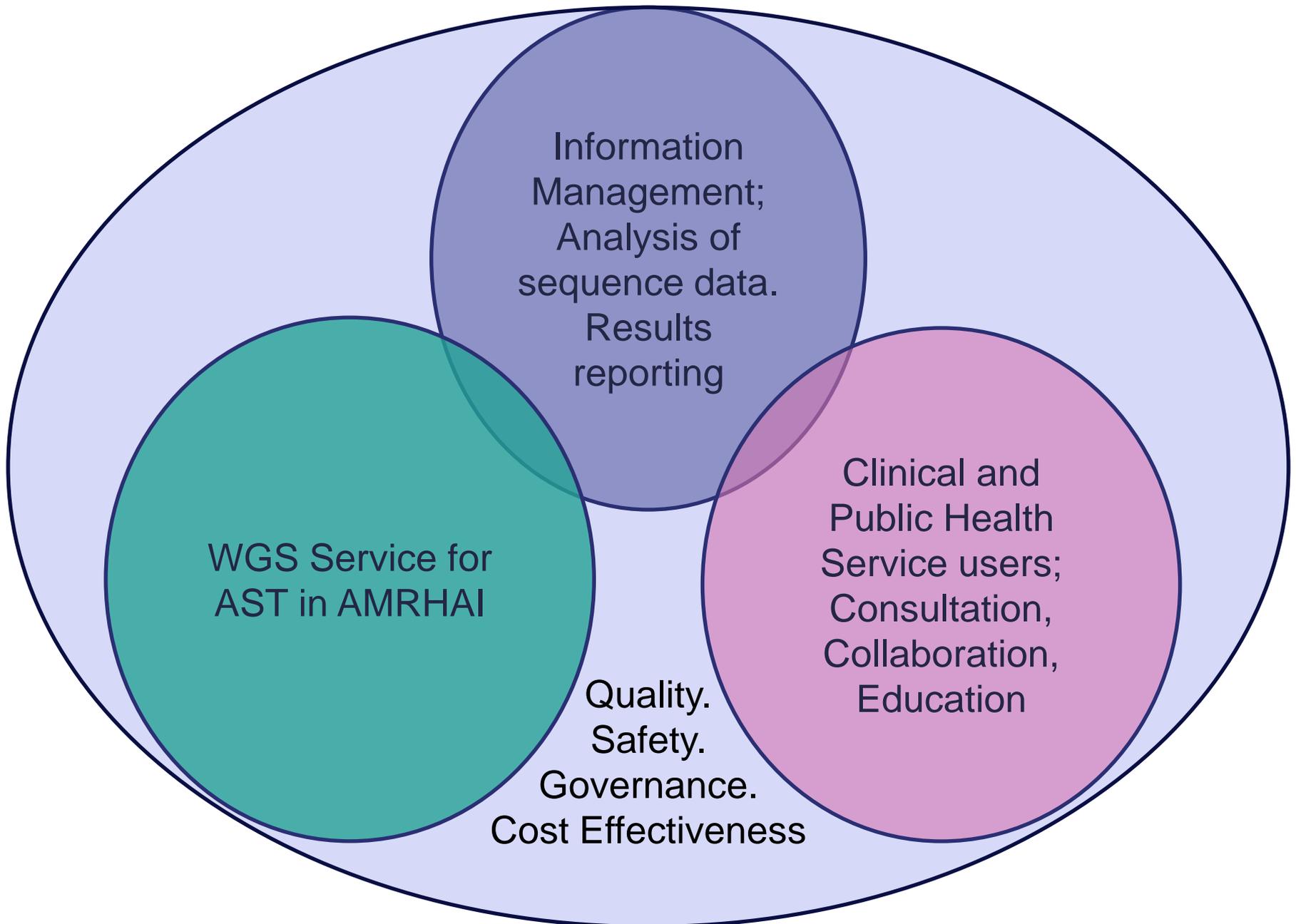
- should 'soon' replace much AST for surveillance purposes
 - low impact of the low error rate
- could 'soon' reduce need for AST in reference laboratories unless
 - to guide treatment
 - for agents with poorest genotypic/phenotypic concordance
 - comparative in-vitro activity of new agents





Opinion: WGS-based genotypic antibiograms

- '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
 - surveillance of treatment failure +/- novel resistance mechanisms
 - education needed and major behavioural change for prescribers





Determine the approach to a change of method, document it, provide assurance that is performing as determined

Process

- Inform of proposed change of method
- Likely to be visited

Information

- Performance characteristics of the assay
- Verification/Implementation
- SOPs-read and understood!
- Competency records
- Quality Control and Quality Assurance
- Security, robustness



Engaging with users

- **NHS labs and microbiologists-** providing material for testing, plus patient information.
 - Turnaround time for results-
 - Impact on existing reference service
 - Impact on reports to clinicians and for surveillance and notification
- **Clinical users-** Report format, interpretation, evaluation, management of discordant results
- **Public Health users-** All of the above plus managing outbreaks and incidents, investigating clusters
 - Format for reporting relatedness between isolates
 - Nomenclature for describing clusters
 - Information available during clinical episode



Public Health
England

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

Invites for membership sent

Report due mid-2016





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