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Assess drug-resistance phenotypes, not just genotypes

Standfirst: Whole genome sequencing is becoming the starting point for assessing the presence of known antimicrobial drug-resistance genes and identifying new resistance mechanisms in laboratory mutants and clinical isolates. However, without phenotypic characterization to confirm drug-resistance, caution needs to be taken in attributing relevance to any genes hitherto not shown to confer drug resistance.

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Whole genome sequencing (WGS) is increasingly advocated as a diagnostic tool to identify the cause/s of infection and/or drug resistance.[1] With rapid advances in sequencing technologies that address technical barriers to providing data about microorganisms in real time, WGS will soon be able to identify the cause of infections within a few hours of taking a sample from a patient. Known drug resistance genes, and virulence genes (e.g. that encode toxins), will also be quickly identified. For this technology to be fully embraced and embedded into clinical practice, the current challenge is to provide the non-specialist with user-friendly data analysis and reporting platforms that can reveal the presence of a pathogen, or pathogens in the case of polymicrobial infections, and resistance to achievable drug concentrations of clinically relevant drugs using internationally recognised standardised tests.[2] For samples from some body sites, the ability to distinguish a pathogenic from commensal microorganisms of the same species is essential. This will

facilitate rapid reporting to healthcare practitioners and by informing them of the pathogen and drug resistance genes facilitate decision making in antimicrobial treatment options.

There are numerous examples of whole genome sequencing and it's utility in identifying the source of outbreaks of drug-resistant bacterial infections and/or their spread within the hospital environment, community or animals e.g. MRSA, *Mycobacterium tuberculosis, Klebsiella pneumoniae* and *Salmonella*.[3] Quickly establishing the cause of hospital and community outbreaks of infection helps clinical decisions and infection control practices thereby helping to both treat the patient and eradicate microbial transmission.

WGS is an extremely powerful tool that reveals in fine detail the evolution and transmission of drug resistant pathogens (e.g. de Been *et al.*[4]). WGS is also excellent at identifying previously described transmissible drug resistance factors and single nucleotide polymorphisms (SNPs) and rearrangements in chromosomal genes. Searchable databases of bacterial drug resistance genes and SNPs have been developed, such as the comprehensive antibiotic resistance database (https://card.mcmaster.ca/),[5] which allow researchers and others to quickly identify known mechanisms of drug resistance in their strains or isolates of interest. However, caution should be exercised in concluding that the presence of known drug resistance genes in a whole genome sequence means that this confers the phenotype. This is because a drug resistance gene may not cause a clinically relevant level of resistance, meaning that treatment with the antimicrobial may still be possible. Furthermore, our knowledge of drug resistance mechanisms is incomplete and there are occasions when the WGS of a microorganism does not reveal the mechanism. For this reason, a genome sequence should be seen as only the first stage in uncovering new drug-resistance mechanisms. For instance, we recently described a post-therapy multidrug resistant clinical

isolate of *Salmonella enterica* serovar Typhimurium;[6] despite it containing known resistance genes, none were shown to confer the phenotype. We uncovered a SNP in a multidrug resistance (MDR) transporter gene, *acrB.* However, it took several years' research by a multidisciplinary team to show that this SNP conferred MDR.

Another example where the WGS did not reveal any known drug resistance genes was in our study of fluoroquinolone-resistant *Streptococcus pneumoniae*. We and two other research groups independently described SNPs in a gene encoding a predicted attenuator of transcription of the downstream genes encoding the ABC transporters PatA and PatB [7-9], but it was the laboratory experiments that confirmed this new mechanism of drug-resistance.[7] However, more challenging was identification of a gene duplication in *S. pneumoniae* that conferred drug resistance,[10] again via increased production of PatA and PatB. Increasing gene copy number by duplication to confer resistance has been described in transmissible elements from numerous bacterial species and for numerous drugs (see Table 1 ref. 11). Likewise, genomic duplications are now a recognised mechanism of drug resistance for several drugs and bacterial species, including sulphonamides, trimethoprim and beta-lactams in *Escherichia coli*[11]. Identifying this type of mechanism will be challenging for those who wish to use using WGS routinely in the clinic as a means to identifying drug resistances and will require a different type of data analysis.

Genome-wide association studies (GWAS) bring together the phenotype of all members of a (large) collection of cell types (eukaryotic and prokaryotic) and their WGSs. These data are statistically analysed to identify specific genetic traits that associate with particular phenotypes. It has therefore been suggested that GWAS is a tool that will allow us to identify new drug resistance mechanisms. A proof of principle GWAS with *S. pneumoniae*

indicated how genes associated with drug resistance can be identified.[12] Retrospective analysis of stored genome sequences in several different countries revealed that the transmissible colistin-resistance gene, *mcr-1,* was widely disseminated prior to first description in 2015. This gene may have been found sooner if GWAS had been applied; such analysis could still reveal the true origin of *mcr-1* as it may not have emerged in China, or in animals, and then spread globally. It could have emerged on several occasions, as have the CTX-M family of extended-spectrum beta-lactamase (ESBL) genes.[13]

However, when a drug resistance trait occurs rarely, only once it has been amplified in a population by bacterial spread or horizontal gene transmission will GWAS be useful. For instance, if WGS was the sole method to detect drug resistance the early identification of NDM-1 mediated carbapenem-resistance in Gram-negative bacteria would have been unlikely. Data arising from WGS would have indicated such bacteria as being drug-susceptible leading to the continued use of ineffective drugs.

One strength of GWAS is that it uses isolates from humans, animals and/or the environment, and which have arisen due to evolutionary pressures such as drug exposure. Recently, GWAS became a realistic option to find new traits not previously associated with drug resistance[14] with the identification of an outer membrane protein involved in resistance to the drug cefazolin in *E. coli*. GWAS will be particularly useful to identify those genetic traits that give rise to microbial survival in low drug concentrations that accumulate and then predispose strains to full drug resistance[15]. Another strength of GWAS is that it will identify those traits that individually have a small impact upon drug susceptibility, but together act epistatically to give drug resistance. Nonetheless, where GWAS is employed, careful experimentation will be required to confirm that the identified genomic changes

confer the drug-resistance phenotype and that it is clinically relevant. Some traits may be compensatory genomic changes that allow any fitness cost associated with drug resistance to be ameliorated.

The provision of WGS datasets as freely accessible, large reference libraries of genomes, and the rapid advance in data analysis tools will ultimately allow the identification of the full repertoire of mechanisms of drug resistance including for bacteria that undergo frequent genetic recombination. With the technological advances reducing time and cost of WGS, this will then facilitate the rapid identification of drug resistances, increased adoption of genomics in clinical microbiology laboratories and give healthcare practitioners confidence in using genomic information to guide antibiotic treatment. This will help improve antimicrobial stewardship and so preserve the effectiveness of current and new drugs. Until that time, however, conventional drug susceptibility testing (phenotyping) using recognized standardized procedures (e.g. EUCAST or CLIS) will remain the mainstay in identifying novel resistances of clinical relevance in many bacteria, particularly Gram-negatives. This is because these methods use cut-off values that distinguish drug-susceptibility or intermediate-drug-susceptibility from clinically relevant levels of drug-resistance. However, for species where there is little or no horizontal gene transfer and drug resistance is due to a genomic change, such as in *M. tuberculosis*, and where time is of the essence to inform drug treatment decisions, where it is available the use of WGS will replace conventional methodologies.

With the likelihood of the increasing use of genomic datasets to identify known and new drug resistances, it is now important that a guiding set of principles is introduced that indicate the steps that should be taken to confirm that any newly described genetic

change/s confer the associated or predicted drug resistance phenotype in any microorganism. In the same way that it was necessary to identify the properties that define a pathogen, Koch's postulates,[16] the same is true for new drug resistances. Having identified the putative drug-resistance trait, four antimicrobial drug resistance postulates are proposed. It is recommended that a drug resistance trait should only be described as such if the criteria of all postulates are met. These are that the genetic trait must be present in all drug-resistant microorganisms, and not present/not expressed in drug-susceptible strains. Replacing the genetic trait with the wild type DNA sequence should confer drug-susceptibility, and introduction of the genetic trait into a drug susceptible strain should confer drug-resistance (Box 1). Where two or more traits are proposed to be necessary to confer drug-resistance, either because they work in an additive manner or synergistically, the postulates should be met for all traits in the predicted combination. Adhering to these postulates will prevent misidentification or mis-attribution of genetic traits as conferring drug resistance.

References

1. Loman N.J. & Pallen, M. *Nat. Rev. Microbiol*. **13**: 787-794 (2015).
2. Goldberg B. *et al. mBio* **6,** e01888-15 (2015).
3. Koser C.U. *et al*. *Trends Genet*. **30,** 401 -407 (2014).
4. De Been M. *et al. PLOS Genet*. **10,** e1004776 (2014).
5. McArthur A. G. *et al*. *Antimicrob. Agents Chemother.* **57**, 3348-3357 (2013).
6. Blair J. M. *et al. Proc. Natl. Acad. Sci U S A.* **112**, 3511-6 (2015)
7. Baylay, A. J. & Piddock, L.J.V. *J. Antimicrob. Chemother.* **70**, 670-9 (2015)

8. Billal D. S. *et al*. *BMC Genomics* **12**, 512 (2011)

9. Croucher N.J. *et al*. *PLOS Genetics* **9**: e1003868 (2013)

10. Baylay, A.J. & Piddock, L. J. V. *Antimicrob. Agents Chemother.* **59**, 3098-108 (2015)

11. Sandegren L. & Andersson D. *Nat. Rev. Microbiol*. **7**, 578-88 (2009).

12. Chewapreecha C. *et al*. *PLOS Genet*. **10**, e1004547 (2014)

13. Livermore D. M. *et al*. *J. Antimicrob. Chemother*. **59,** 165-74 (2006)

14. Earle *et al*. *Nat. Microbiol*. **1**, 16041 (2016).

15. Andersson, D. I. & Hughes, D. *Nat. Rev. Microbiol*. **12**, 465–478 (2014)

16. Koch R. *Beitr. Biol. Pflanz*. **2,** 277–310 (1876).

Box. Proposed antimicrobial drug resistance postulates.

A drug resistance trait should only be described as such if the criteria of these four postulates are met:

1. Show genetic trait is present in drug-resistant microbes.
2. Demonstrate that genetic trait is not present/not expressed in drug-susceptible strains.
3. Replace the genetic trait with wild type DNA sequence and show that it confers drug-susceptibility.
4. Introduce the genetic trait into a drug susceptible strain and show that it confers drug resistance.