Published in "Journal of Antimicrobial Chemotherapy doi: 10.1093/jac/dkw204, 2016" which should be cited to refer to this work.

Comment on: Resistance gene naming and numbering: is it a new gene or not?

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Sir,

Hall and Schwarz[1] recently proposed in this Journal a scheme for naming antibiotic resistance genes. Although there may be advantages to their approach in some cases, we think it is unworkable with respect to β-lactamase nomenclature where single mutations impact enzyme function.[2]

The argument advanced is that a new allele number should be assigned when the amino acid sequence differs by ≥2% from a known sequence. As a result, genes differing by <2% will receive the same designation. In the case of β-lactamases, this approach does not reflect our understanding of how these enzymes have behaved. β-Lactamases have been distinguished by a single amino acid change since TEM-2 was differentiated from TEM-1 by isoelectric focusing in 1976.[3] More importantly, single amino acid changes within a β-lactamase family can markedly affect the substrate spectrum or response to β-lactamase inhibitors. A single substitution changes the penicillinase SHV-1 to the extended-spectrum cephalosporinase SHV-2[4] and a single amino acid differentiates inhibitor-resistant SHV-49 from SHV-1.[5] Indeed, most of the >220 numbered bla_{TEM} alleles or >190 bla_{SHV} alleles in a β-lactamase database[6] encode enzymes differing from each other by <2% and so by the Hall and Schwarz[1] proposal would be given the same allele number.

The first TEM β-lactamase differing from TEM-1 by ≥2% is TEM-162 with seven amino acid changes and so by the new proposal TEM-162 would become TEM-2, while the preceding 160 TEM varieties would need to be distinguished as TEM-1 subtypes by such inelegant nomenclature as TEM-1-1 to TEM-1-160. Furthermore, the effect of a few mutations on the resistance phenotype is not limited to β-lactamases. Only two mutations separate an acetyltransferase active only on aminoglycosides from one that also confers quinolone resistance by modifying ciprofloxacin and norfloxacin.[7] Consequently, we believe that a single amino acid difference suffices to define a new resistance allele.

We also take issue with assigning allele numbers only to resistance genes that have been mobilized and not to chromosomally determined or intrinsic genes. A plasmid location is not always easy to prove and a chromosomal gene may be just as significant as a mobilized one in determining a clinically important resistance phenotype. For example, SHV β-lactamase is commonly encoded on the chromosome of *Klebsiella pneumoniae*,[8] but can also be plasmid-borne. In both locations, particular bla_{SHV} alleles can provide a range of resistance phenotypes from resistance to penicillins only, to activity against oxyimino-β-lactams, to carbapenemase activity.[9,10] Distinct resistance alleles should not be denied an allele designation due to their genetic location.

Lastly, we share the concern that much of the variation uncovered by today's facile sequencing technology will have uncertain functional consequences. We urge that allele distinctions be based on protein and not nucleotide sequence variation where synonymous codons could create trivial differences. We anticipate that as WGS becomes the method of choice for pathogen outbreak surveillance, delivering high-resolution understanding of genomic relationships at low cost, resistance phenotypes will be increasingly predicted from sequence data. Already, this approach is being used as a surveillance and even diagnostic tool.[11] Improving predictive methods will require a nomenclature that provides amino acid-level resolution and that also is robust to bioinformatics manipulation (e.g. lacks italic and other non-ASCII characters).

Accordingly, we agree that:

(1) A resistance gene should be shown to encode a product that decreases antibiotic susceptibility.
(2) To establish uniqueness, the nucleotide sequence must be determined in full, including a signal sequence if present (the promoter sequence is not taken into account).
(3) The gene should have been isolated from a natural source and not generated in the laboratory as by mutation or recombination.

And add:

(4) A single amino acid difference in the protein product is sufficient to define a new numbered allele, independent of whether the responsible gene has a plasmid or chromosomal location and independent of any documented change in phenotype.

(5) These rules do not preclude subgroups within an existing category such as the currently heterogeneous OXA group of β-lactamases with >400 members having a variety of resistance phenotypes and species of origin, which are currently being considered as a basis of subgrouping.

NOTE: The National Center for Biotechnology Information (NCBI) has taken over the β-lactamase allele assignment from the Lahey Clinic site and bases its curation efforts and allele assignments for β-lactamases on these principles so as to encourage phenotypic characterization (NCBI β-lactamase submissions: http://www.ncbi.nlm.nih.gov/projects/pathogens/submit_beta_lactamase/).

For other resistance gene classes, NCBI encourages the resistance community to reach a consensus on nomenclature by building on the principles outlined in this communication. In particular, we agree that some gene families, such as tetracycline and macrolide resistance genes, where cut-offs are presently >80% identity, should be subjected to debate with the possibility of some genes being renamed.

Transparency declarations

G. A. J. has served as a consultant or advisor for Achaogen and Gladius. P. A. B. is employed by AstraZeneca. K. B. has served as a consultant or advisor for Achaogen, Allecra, Entasis, Fedora, Gladius, Merck, Melinta, Naeja, Roche, Tetraphase and Warp Drive. Y. D. has served as a consultant or advisor for Achaogen, Meiji, Tetraphase and Merck, and has received research funding from The Medicines Company. P. N. has served as a consultant or advisor for Allecra and Antabio. T. P. has served as a consultant for Synthetic Biologics. G. M. R. has received support from or advised Accelerate, Achaogen, Alifax, Angelini ACRAF, AstraZeneca, Basilea, Becton-Dickinson, bioMérieux, Biotest, Cepheid, Checkpoints, Curetis, Liofilchem, Medivir, Menarini, Merck-Cubist, Nordic Pharma, Novartis, Pfizer, Rempex and Zambon. All other authors: none to declare.

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