Report

Advisory Committee on the Microbiological Safety of Food (ACMSF) Working Group on Antimicrobial Resistance (AMR)

Advisory Committee on the Microbiological Safety of Food (ACMSF) Working Group on Antimicrobial Resistance (AMR)

Recommendations on the use of AMR Terminology/Nomenclature when used in FSA Reports.

1. Introductory Statement

An issue concerning the use of antimicrobial resistance (AMR) nomenclature used in Food Standards Agency (FSA) research and survey reports has been raised with the FSA by the FSA Science Council.

The nature of the concern relates to FSA survey reports using the terms “resistant” or “resistance” to describe bacteria when ‘epidemiological cut off values’ (ECOFFs) are used to categorise susceptibility to antibiotics; the view expressed being that “resistant” should only be applied when susceptibility to such agents is determined using clinical breakpoints (CBPs), especially for foodborne pathogens.

Furthermore, that consideration should be given to using the term *‘less susceptible’* rather than *‘resistant’,* unless a CPB has been used or if the ECOFF has been shown to be the same as, or lower, than the CPB.

The FSA’s independent Advisory Committee on the Microbiological Safety of Food (ACMSF) AMR Sub-Group committed to investigating the issue.

A small Working Group[1] was convened in July 2023 to consider the issue and agreed to draft an initial set of terms/definitions for the ACMSF AMR Sub-Group to consider further.

The specific remit of the FSA AMR Working Group[1] was ‘to consider defining specific ‘AMR-related’ terms, including their applicability in different situations’.

It is self-evident that there are substantive differences in the clinical susceptibility of different organisms to antimicrobials.

For the purpose of this document, it was agreed that, as a starting point, recommendations should be targeted at bacterial pathogens which are for the most part related to infections spread by contaminated food.

In this respect the most common order of Gram-negative organisms would be the Enterobacterales, whilst Gram-positive organisms would include *Staphylococcus spp.* and *Listeria spp*.

Resistance in other organisms (e.g., parasites, fungi, etc.) could be addressed as and when appropriate.

Following extensive discussions, including consultation with a representative from the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the recommendations outlined below have been formulated specifically for FSA-funded projects.

In this context we have also considered susceptibility testing and the interpretation of results, definitions of ‘Intrinsic Resistance’ and ‘Multiple Drug Resistance’ (MDR), and genotypic *vs* phenotypic resistance, particularly in relation to the increasing use of methods such as whole Genome Sequencing (WGS) and metagenomics.

2. Background

2.1 Epidemiological cut-off values (ECOFFs) and Clinical Breakpoints (CBs)

Epidemiological cut-off values (ECOFFs) have been used for many years by the FSA and in AMR monitoring in the European Union (EU) to determine ‘resistance’, but ECOFFs do not necessarily indicate clinical resistance - i.e., resistance determined by applying clinical breakpoints (CPBs).

ECOFFs distinguish between individuals within a bacterial species which have or have not developed any phenotypically-detectable acquired resistance and are not necessarily indicative of clinical resistance.

EUCAST use the term “microbiological resistance” to describe resistance assessed by the application of ECOFFs.

The EUCAST definitions of clinical breakpoints (CBs) and ECOFFS are appended (Appendix 1).

The EU Summary Reports also follow this approach, and in the case of quantitative data have defined an isolate as ‘resistant’ to a selected antimicrobial when its minimum inhibitory concentration (MIC) value (in mg/L) was above the cut-off value, or the disc diffusion diameter (in mm) was below the cut-off value (EFSA 2022).

The Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) reports follow a slightly different approach, and state that *“ECOFFS classify isolates with acquired reduced susceptibility as non-wild-type’.*

In SVARM,, non-wild-type isolates are called ‘resistant’. This classification is relevant for monitoring purpose, but it should be understood that resistance defined in this manner not always implies clinical resistance.

ECOFFs are determined by a different approach than CPBs, and do not take into account the results of clinical efficacy studies, dosing and route of administration of the antimicrobial agents, nor the drug’s pharmacokinetic and pharmacodynamic parameters in humans or the animal species in which such substances are used.

The ECOFF and CBP can lie at the same minimum inhibitory concentration (MIC) value for some organism/antimicrobial combinations, despite the difference in their derivation.

For a number of antimicrobial/organism combinations EUCAST ECOFFs and EUCAST CBPs lie at the same value. There are nevertheless differences for some clinically-important antibiotics, with ECOFF levels below those of EUCAST CBPs.

In relation to the suggestion that the term *‘less susceptible’* rather than *‘resistant’,* should be used unless a CPB has been used or if the ECOFF has been shown to be the same as, or lower, we consider that at present the term’ *resistant’* is widely used both nationally and internationally in relation to reports

involving organisms from food, and changing this to '*less susceptible*' might lead to confusion.

For some 'critical' antibiotics such as ciprofloxacin, when used for the treatment of systemic *Salmonella* spp. infections, although the breakpoint level for resistance is low (MIC >0.06 mg/L), there is in some cases clinical evidence to indicate a poor response (Møller Aarestrup *et al.*, 2003).

In contrast, for *Campylobacter spp.,* the breakpoint level is higher (0.5 mg/L).

It should be noted that in Europe, the term "full susceptibility" has been suggested to being replaced with "zero resistance" on the grounds that this is more easily understood by readers.

We consider that the introduction of the term 'zero resistance' can be confusing, particularly when genes encoding resistance to several antibiotics may be present but not expressed (see under Item 6 below).

For the time being, we recommend that the term *'full susceptibility'* (to the panel of antibiotics tested) may *be used in reports for organisms which did not show resistance to any* of antibiotics tested.

2.2 AMR in pathogens and non-pathogens from animals

Although AMR in pathogens and non-pathogens possibly associated with the use of antimicrobials in animals is not fundamentally the responsibility of the FSA and falls within the remit of the Department for Environment, Food & Rural Affairs (DEFRA), the Veterinary Medicines Directorate (VMD) and the Animal & Plant Health Agency (APHA), we consider that within the 'One Health'[2] context, such usage is relevant to the occurrence of resistance in organisms from food originating in animals bred for food.

For example, some antibiotics which are exclusively used in animals, can result in the development of resistance to important' antibiotics used in human medicine, an example of this is the use of apramycin, which can result in resistance to the antibiotic gentamicin, which is used to treat serious infections in humans (Threlfall et al., 1986).

It should be noted that because of genetic linkage of resistance (that antibiotics used exclusively in animals can co-select for resistance to antibiotics which are not used in animals.

We note that Feßler *et al.* (2023) have concluded that to assess the susceptibility of bacteria causing animal infections for treatment purposes, it is critical that approved standards for antimicrobial agents and bacteria are used and have recommended that standards from the USA Clinical Laboratory Standards Institute Veterinary Antimicrobial Susceptibility Testing Subcommittee (CLSI-VAST) should be applied.

In this respect It should be noted that CLSI clinical veterinary breakpoints may have been derived using those dosage regimens which are applied in the USA and that CBPs are also available from other breakpoint setting organisations.

We do not consider that the exclusive use of clinical breakpoints is, at the present time, appropriate for the epidemiological surveillance of AMR in bacterial pathogens related to infections primarily spread by contaminated food. Rather we suggest that both the ECOFF and CBP have a role in a comprehensive analysis of surveillance data.

3. Recommendations for FSA-funded projects involving AMR

As this is a rapidly evolving field, any recommendations should be reviewed at regular intervals to ensure that they are 'fit for purpose'.

3.1 Terminology

Although several definitions of 'resistance' are available the term "resistance" should be used in accordance with the Codex definition of 2005, amended 2021, which is as follows:

Antimicrobial resistance (AMR) - the ability of a microorganism to multiply or persist in the presence of an increased level of an antimicrobial agent relative to the susceptible counterpart of the same species.

In the context of FSA reports, 'AMR' refers to resistance to antibiotics, and not to 'non-antibiotic' compounds such as, e.g. biocides.

A statement should be included as a precursor to all FSA reports involving AMR surveillance activities, that when 'resistance' is mentioned which involves the use of ECOFF terminology, then it should be made clear that such microbiological resistance has been determined using ECOFFs and is not necessarily at clinical levels.

“Microbiological resistance” may be abbreviated to “resistance” for brevity once the position has been set out which is relevant to a particular section of a report.

Reports may analyse data by applying both CPBs and ECOFFs. In this case, they should clearly indicate which interpretive criteria have been used in different sections of the report.

3.2 Methodology

- Human clinical breakpoint tables for interpretation of MICs and zone diameters, produced by EUCAST version 14.0, valid from 2024-01-01 be used for FSA-sponsored surveys involving Enterobacterales and for specific Gram-positive organisms such as *Staphylococcus spp.*, *Enterococcus spp.* and *Listeria spp.*
- Data should also be analysed by applying EUCAST ECOFFs to provide information on microbiological resistance. This may provide an indication of emerging resistance, where the ECOFF is set at a lower value than the CBP and will also provide harmonisation with many neighbouring European countries.
- As a general rule, FSA-sponsored AMR project specifications should include a simplified list of antimicrobials to be used for test, together with the recommended EUCAST clinical breakpoint and ECOFF levels for the relevant organisms for the surveys to be undertaken. Such specifications should be the responsibility of the FSA, and not the research applicant.
- Materials and methods sections of reports should provide tabulated details of CBPs and/or ECOFFs applied.
- For antibiotics that lack published definitions of sensitivity/resistance such as azithromycin and sulphonamides, and for which there are no definitive breakpoints from organisations which set interpretive criteria, an approach could be based on applying tentative breakpoints available in the literature or elsewhere, so that some degree of interpretation / context is possible.
- For reports involving AMR, inclusion of the percentage resistance to antimicrobials surveilled should only be for surveys where AMR has been phenotypically confirmed and not for AMR detected by genotypic methods such as Whole Genome Sequencing (WGS) – see under Item 6 below.
- The classification of levels of resistance as very low, low, moderate, high, etc. throughout surveillance reports involving should be consistent with EFSA definitions for these terms – see EFSA & ECDC 2022 as are summarised in UK-VARSS 2022. These definitions are presented in Appendix 2.

It should be realised that the significance of resistance levels are dependent on the organism in question. For example, in terms of public health importance high levels of resistance to certain non-therapeutic antimicrobials may be less significant than low levels of resistance to Critically Important Antimicrobials (CIAs), in accordance with WHO definitions (WHO 2019).

4. Intrinsic resistance

The ‘intrinsic antibiotic resistome’ is a naturally-occurring phenomenon that predates antibiotic chemotherapy and can be observed in almost all bacterial species.

‘Intrinsic resistance’ is considered to be when a significant proportion (>90%) of a bacterial species is naturally resistant to a certain antibiotic or family of antibiotics, without the need for mutation or gain of further genes (EUCAST, 2020). This means that these antibiotics can never be used at normal therapeutic doses to treat infections caused by that species of bacteria.

Such resistance frequently results from properties of the cell membrane, making the organism naturally ‘resistant’ to certain antibiotics.

Examples of ‘intrinsic resistance’ include:

Escherichia coli:	Macrolides
Klebsiella spp:	Ampicillin
Serratia marcescens:	Macrolides
Campylobacter spp:	Trimethoprim
Pseudomonas aeruginosa:	Sulphonamides, Ampicillin, 1st and 2nd generation cephalosporins, Chloramphenicol, Tetracycline
Listeria spp.:	Polymyxins (e.g., colistin)

Although EUCAST has decided to replace the term “intrinsic” with the terms “ **expected susceptible phenotype**” and “**expected resistant phenotype**”, in our opinion these terms are designed for clinical microbiology laboratories reporting to physicians dealing with medical patients, whereas ‘intrinsic resistance’ is still widely used in the scientific literature, and we consider that this designation will be more easily understood by readers of FSA reports.

We consider that the term ‘intrinsic resistance’ is meaningful when applied to bacteria associated with foodborne infections, and should be retained as such for FSA-funded projects.

Consideration may be given to replacing the term *‘intrinsic’*, with *‘natural’,* as this term may be better understood by the general public in relation to AMR terminology.

5. Multiple Drug Resistance (MDR)

Following a joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the USA Centers for Disease Control and Prevention (CDC), to create a standardised international terminology with which to describe acquired resistance profiles (Magiorakos *et al.*, 2012), we recommend that for FSA surveillance purposes, **MDR[3] is considered as non-intrinsic resistance to three or more different classes of antibiotics’***.* This terminology is now widely used globally.

6. Genotypic *vs* phenotypic resistance

There is an additional challenge in interpreting/presenting the outcome of methods used to detect the presence of AMR genes such as PCR.

Methods such as Whole Genome Sequencing (WGS) and metagenomics, etc. are increasingly being used, either independently or in conjunction with 'traditional' phenotypic methods. However, quality aspects, such as the depth of sequencing and plasmid coverage must also be considered.

In the particular, WGS of single organisms, or indeed communities, offers the potential to predict antimicrobial susceptibility (predictive MICs) from a single assay, and may be particularly useful in identifying genes conferring resistance by small changes in susceptibility, as is the case with some MDR genes.

Such usage could also potentially identify the presence of resistance determinants identified by genotypic means, to a wider number of antimicrobial classes than might be screened by phenotypic testing.

This may be problematic in that such usage could result in organisms being classified as ‘resistant’ or ‘multi-resistant’ when some genes identified by genotypic methods are either not relevant, or not expressed in the resistance phenotype.

The use of these tools is likely to increase, especially in combination with artificial intelligence (AI) and Machine Learning (ML) algorithms. Therefore, it will be important to find a way to explain what detection of resistance genes using these tools actually means, and how the findings can be described in terms of "resistance" and "multiple resistance", with appropriate caveats.

In 2017, EUCAST established a subcommittee to review the current development status of WGS for bacterial antimicrobial susceptibility testing (AST).

They considered that at the time of their report, the published evidence for using WGS as a tool to infer antimicrobial susceptibility accurately was either poor or non-existent and the evidence/knowledge-base required significant expansion.

For most bacterial species, they considered that **at that time,** the major limitations to widespread adoption of WGS-based AST in clinical laboratories was the current high-cost and limited speed of inferring antimicrobial susceptibility from WGS data, in addition to the dependency on previous culture-based methods.

For most bacterial species they concluded that at that time there was currently insufficient evidence to support the use of WGS-inferred AST to guide clinical decision making, and that resistance genotypic profiles should not be considered for the management of patients or used as definitions of resistance, and that they were purely for surveillance purposes) (Ellington *et al.*, 2017).

There are however numerous databases such as PointFinder or for resistance genes, ResFinder, CARD, AMRFinder, ARG-ANNOT and MEGARes. Such databases are now being increasingly used to investigate WGS -generated sequences for the presence of resistance-mediating mutations.

A suggested way forward for FSA surveillance activities is to describe/list any such genes identified by WGS/metagenomics, etc., with an indication of the antimicrobial resistances potentially conferred, whether or not such ‘resistances’

are clinically significant in the organism(s) in which they are identified, and whether or not the genes have the capacity to be transferred to other organisms, including key foodborne pathogens.

Metagenomics is a rapidly developing technology, and its usage in relation to the detection of AMR genes should be regularly reviewed.

7. Citations

Codex Alimentarius International Food Standards Code of Practice to minimize and contain foodborne antimicrobial resistance CXC 61-2005., Adopted 2005, Amended 2021).

Ellington, M.J. *et al.* (2017) 'The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee', *Clinical Microbiology and Infection*, 23(1), pp. 2–22. Available at: https://doi.org/10.1016/j.cmi.2016.11.012.

The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021–2022. EFSA Journal. 2024;22:e8583. https://doi.org/10.2903/j.efsa.2024.8583

EUCAST 2020, EUCAST News - intrinsic resistance and unusual phenotypes update.03.03.2020.

European Medicines Agency (EMA). Categorisation of antibiotics in the European Union. Answer to the request from the European Commission for updating the scientific advice on the impact on public health and animal health of the use of antibiotics in animals.12 December 2019 EMA/CVMP/CHMP/682198/2017. Committee for Medicinal Products for Veterinary use (CVMP), Committee for Medicinal Products for Human Use (CHMP).

Feßler, A. *et al.* (2023) 'Antimicrobial susceptibility testing in veterinary medicine: performance, interpretation of results, best practices and pitfalls', *One Health Advances* [Preprint]. Available at: https://doi.org/10.1186/s44280-023-00024-w.

Magiorakos, A.-P. *et al.* (2012) 'Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance', *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 18(3), pp. 268–281. Available at: https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Moellering, R.C. (2010) ‘NDM-1 — A Cause for Worldwide Concern’, *New England Journal of Medicine*, 363(25), pp. 2377–2379. Available at: https://doi.org/10.1056/NEJMp1011715.

Møller Aarestrup, F. *et al.* (2003) ‘Is It Time To Change Fluoroquinolone Breakpoints for *Salmonella* spp.?’, *Antimicrobial Agents and Chemotherapy*, 47(2), pp. 827–829. Available at: https://doi.org/10.1128/AAC.47.2.827-829.2003.

Threlfall E.J, Rowe B, *et al* (1986) Characterization of plasmids conferring resistance to gentamicin and apramycin in strains of *Salmonella typhimurium* phage type 204c isolated in Britain. Journal of Hygiene (London) Dec; 97(3):419-26.

Veterinary Medicines Directorate. UK Veterinary Antibiotic Resistance and Sales Surveillance Report. UK-VARSS 2023. Update March 2024. Veterinary Antibiotic Resistance and Sales Surveillance Report New Haw, Addlestone: Veterinary Medicines Directorate.

World Health Organisation 2019. Critically Important Antimicrobials for Human Medicine, 6th Revision, WHO March 2019.

[1] WG Membership: Roberto La Ragione, Rohini Manuel, Christopher Teale and John Threlfall. Secretariat: Kathryn Callaghan, Bobby Kainth and Elaine Pegg (FSA).

[2] The One Health High Level Expert Panel (OHHLEP) have defined One Health as an integrated unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent. The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate changes and contributing to sustainable development.

[3] ‘MDR’ should not be confused with ‘Multiple Resistance Genes’ (MRGs), which are defined as situations when one gene mediates resistance to several unrelated antibiotic classes (AMEG, 2018). One such gene is the New Delhi metallo-β-lactamase-1 gene (NDM-1), first discovered in an isolate of *Klebsiella*

pneumoniae in 2008. This gene, which is plasmid-encoded and readily transmissible to other Enterobacterales, encodes metallo-β-lactamase-mediated resistance to carbapenems and to almost all β-lactam antibiotics, and is also an efflux pump capable of promoting resistance to additional antimicrobials and growth promoters (Moellering, 2010). Further examples include *cfr* genes, which confer resistance to phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A antibiotics.