STATEMENT

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Statement on how to interpret the QPS qualification on 'acquired antimicrobial resistance genes'

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Abstract

The qualified presumption of safety (QPS) approach was developed to provide a regularly updated generic pre-evaluation of the safety of microorganisms intended for use in the food or feed chains. Safety concerns identified for a taxonomic unit (TU) are, where possible, confirmed at the species/strain or product level and reflected by 'qualifications' which should be assessed at strain and/or product level by EFSA's Scientific Panels. The generic qualification 'the strains should not harbour any acquired antimicrobial resistance (AMR) genes to clinically relevant antimicrobials' applies to all QPS bacterial TUs. The different EFSA risk assessment areas use the same approach to assess the qualification related to AMR genes. In this statement, the terms 'intrinsic' and 'acquired' AMR genes were defined for the purpose of EFSA's risk assessments, and they apply to bacteria used in the food and feed chains. A bioinformatic approach is proposed for demonstrating the 'intrinsic'/'acquired' nature of an AMR gene. All AMR genes that confer resistance towards 'critically important', 'highly important' and 'important' antimicrobials, as defined by the World Health Organisation (WHO), found as hits, need to be considered as hazards (for humans, animals and environment) and need further assessment. Genes identified as responsible for 'intrinsic' resistance could be considered as being of no concern in the frame of the EFSA risk assessment. 'Acquired' AMR genes resulting in a resistant phenotype should be considered as a concern. If the presence of the 'acquired' AMR gene is not leading to phenotypic resistance, further case-by-case assessment is necessary.

Keywords: qualification, AMR, intrinsic resistance, acquired resistance, whole genome sequence, qualified presumption of safety, risk assessment

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Summary

To support the risk assessment of microorganisms carried out by the different EFSA risk assessment areas, EFSA has introduced the qualified presumption of safety (QPS) concept for microorganisms as a generic safety pre-assessment approach that covers safety concerns for humans, animals and the environment. In the QPS concept, a safety assessment of a defined taxonomic unit (TU) is performed independently of the legal framework under which the application is made during a market authorisation process.

Safety concerns for a TU are, where possible, reflected as 'qualifications', which should be assessed at strain and/or product level by the respective EFSA's Scientific Panel. The generic qualification 'the strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antimicrobials' applies to all QPS bacterial TUs.

The available definitions of 'acquired' and 'intrinsic' antimicrobial resistance (AMR) genes and, eventually, their distinction have resulted in certain ambiguity in the interpretation of that generic QPS qualification, both by the applicants submitting the dossier of a regulated product and the EFSA Units responsible for the respective risk assessment. The QPS working group and BIOHAZ Panel have decided to clarify the concepts of 'acquired' and 'intrinsic' AMR genes, considering the current body of knowledge on the genetic basis of these types of resistance and their clinical relevance. This assessment has been based on expert knowledge and published data.

QPS bacterial TUs are used in different areas of EFSA risk assessment: feed additives, pesticides (plant protection products – PPP), food ingredients and packaging, novel foods and genetically modified organisms (GMO). Although each area has its own sector specific guidance, they all use the QPS concept and generally the same approach to assess the qualification related to AMR genes. This assessment involves analysing the entire genome (WGS) to detect the existence of AMR genes. In most cases also phenotypic AMR testing, and in the pesticides area, the transferability to other microorganisms is considered. Classification of antimicrobials is reliant on the provision of guidance by relevant bodies (World Health Organization (WHO), World Organisation for Animal Health (WOAH), European Medicines Agency (EMA), European Commission (EC), etc.).

In this statement, the terms 'intrinsic' and 'acquired' AMR genes were defined for the purpose of EFSA's risk assessments, and they apply to bacteria used in the food and feed chains. A bioinformatic approach is proposed for demonstrating the 'intrinsic'/'acquired' nature of an AMR gene.

All AMR genes encoding resistance to clinically 'critically important' (CIAs), 'highly important' (HIAs) or 'important' antimicrobials (IAs) as defined by the WHO,[1] need to be considered as hazards, and need further assessment. Genes conferring 'intrinsic' resistance could be considered as of no concern in the frame of the EFSA risk assessment purpose. 'Acquired' AMR genes coinciding with the corresponding antimicrobial phenotypic resistance should be considered as a hazard/ concern. If the presence of the 'acquired' AMR gene is not leading to phenotypic resistance, further case-by-case assessment is necessary.

[1] Critically important antimicrobials for human medicine, 6th revision. Geneva: World Health Organisation; 2019 https://www.who.int/publications/i/item/9789241515528 According to the most recent update, still under public discussion (https://www.who.int/news-room/articles-detail/public-discussion-on-the-who-medically-important-antimicrobial-list–7th-revision) within the new term 'Medically important antimicrobials' there will be 4 categories: (i) important, (ii) highly important, (iii) critically important, and (iv) High priority critically important.

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1. Introduction

A wide variety of microorganisms are intentionally used at different stages of the food and feed chains. In the context of applications for pre-market authorisation, EFSA is requested to assess the safety of microorganisms used 'as such' or as production organisms for food and feed additives, food enzymes and food flavourings, plant protection products (PPPs), novel foods (NFs) or genetically modified microorganisms (GMMs). In each area, the risk assessment falls under specific legislations and separate EFSA guidance documents are available/apply.

In 2011, the scientific secretariat staff of several EFSA risk assessment areas collaborated to summarise the 'EFSA approaches to risk assessment in the area of antimicrobial resistance (AMR), with an emphasis on commensal microorganisms' (EFSA, 2011). At that time, the assessment of AMR was based on the analysis of phenotypic AMR, after which the genotypic determinants were analysed. The availability of whole genome sequence (WGS) data fundamentally changed this approach.

To support the risk assessment of microorganisms carried out by the different EFSA risk assessment areas, in 2004 EFSA introduced the qualified presumption of safety (QPS) concept for microorganisms as a generic safety pre-assessment approach that covers safety concerns for humans, animals and the environment (EFSA, 2005). In the QPS concept, a safety assessment of a defined taxonomic unit (TU) is performed independently of the legal framework under which the application is made during a pre-market authorisation process. Strains belonging to QPS TUs still require an assessment based on data analysis by the relevant EFSA Scientific Panel, but the QPS status facilitates this evaluation (EFSA BIOHAZ Panel, 2023).

Safety concerns for a TU are, where possible, reflected as 'qualifications', which should be assessed at strain and/or product level by EFSA's Scientific Panels. The generic qualification 'the strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antimicrobials' applies to all QPS bacterial TUs (EFSA BIOHAZ Panel, 2023).

1.1. Background and Terms of Reference as provided by the requestor

The overarching mandate related to the QPS activities is included in the latest QPS opinion (EFSA BIOHAZ Panel, 2023[2]). The scope of this Panel Statement is very specific and related to the clarification of a QPS qualification that may be considered linked to part of ToR2 (*…'to verify if the qualifications still effectively exclude safety concerns'.*).

1.2. Scope of this document

The generic qualification for bacterial TUs that 'the strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antimicrobials' applies to all QPS bacterial TUs (EFSA BIOHAZ Panel, 2023). This document focuses on QPS bacteria but may also be used for the evaluation of non-QPS bacteria. The available definitions of 'acquired' and 'intrinsic' AMR genes and, eventually, their distinction have triggered certain ambiguity in the interpretation of that generic QPS qualification both by applicants submitting the dossier of a regulated product and the EFSA Units responsible for the respective risk assessment. This document aims to clarify this qualification.

The use of a QPS microorganism, viable/inactivated or its product(s) should not add to the overall pool of AMR genes nor increase the spread of AMR. The potential of an AMR gene to be transferable/ mobilisable is a key element in this respect. All bacterial genomes contain a number of mobile or mobilisable genetic elements that, with different frequencies, may be involved in horizontal gene transfer (Andam and Gogarten, 2011). However, the analysis of mobile or mobilisable genetic elements in the WGS of a bacterium and/or experiments for testing transferability of genes is not considered applicable as a basis for the EFSA safety assessment of AMR genes, as any genetic element can be transferred at a higher or lower frequency. It has been recognised that low frequency events are difficult to reproduce in lab-designed experiments. Although improvements have been obtained by recent developments in WGS technology, currently analysing the WGS data for the presence of mobile or mobilisable genetic elements may still lead to false-positive and/or false-negative results.

The definitions and principles included in this document in relation to AMR genes are intended to contribute towards the assessment of bacteria notified to EFSA for pre-market authorisation to be used in the food and feed chains.

[2] https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.7747

This document aims to clarify the definitions of 'intrinsic' and 'acquired' AMR genes and provide some general principles on the risk assessment of AMR genes present in QPS bacterial TUs. The definitions and principles are intended to harmonise and support the risk assessment performed in the different EFSA risk assessment areas. They should therefore be generally applicable and of practical use.

The terms 'intrinsic' and 'acquired' AMR genes are in this document specifically defined for the purpose of EFSA's risk assessments and they apply to bacteria used in the food and feed chains.

The tools and methodologies recommended in this document are formulated based on the current knowledge in an area that is constantly evolving. Therefore, updates will be required in the future.

In this document, the following topics are covered:

1) Identification of the purposes of use of QPS bacterial TUs in the different risk assessment areas under the remit of EFSA and a summary of the existing EFSA guidance documents in these areas related to the assessment of AMR and AMR genes.
2) Definition of the terms 'intrinsic' and 'acquired' AMR genes under the scope of EFSA's risk assessment.
3) Definition of general principles and criteria for guiding the EFSA risk assessment of AMR genes.

2. Data and methodologies

As this Panel Statement is intended to clarify certain concepts and to support the level of applicability of a QPS qualification associated with all bacteria included in the QPS list, expert knowledge and data from the literature were used to support the content of this BIOHAZ Panel Statement. For literature data, relevant databases, such as PubMed, Web of Science, CAB Abstracts or Food Science Technology Abstracts (FSTA) and Scopus, were used, based on expert judgement.

In line with EFSA's policy on openness and transparency, and to receive comments on its work from the scientific community and stakeholders, EFSA engages in public consultations on key issues for which external input would be desirable.

EFSA's Panel on Biological Hazards (BIOHAZ) carried out an open consultation to receive input from interest parties on this Panel Statement on how to interpret the QPS qualification for all QPS bacterial taxonomic units 'the strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antimicrobials'. This public consultation gave stakeholders the opportunity to provide any relevant evidence relevance not yet included in the scientific assessment, and feedback on the methods, results, conclusions and recommendations. All details may be found in Annex A – Outcome of the Public consultation on the draft Statement on how to interpret the QPS qualification on 'Acquired antimicrobial resistance genes'.

3. Assessment

3.1. Assessment of the AMR qualification for QPS bacterial TUs in the different EFSA's areas and summary of guidance documents on the assessment of AMR and AMR genes

3.1.1. Feed additives safety assessment area

The EFSA unit responsible for this area (FEEDCO Unit) applies the QPS evaluation on the assessment of biological agents intended for use as feed additives or as a source of a feed additive, as defined in Regulation (EC) 1831/2003.[3] The opinions of the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) dealing with QPS recommended microorganisms, consider that the use of the microorganism as a feed additive is presumed safe for the target species, consumer and the environment without the need for further evidence, provided that the microorganism is unambiguously identified and the existing qualifications are met. In the case of feed additives produced by fermentation with microorganisms, when the latter qualify for the QPS approach, the safety assessment of the fermentation product would not need specific toxicological test/data for consumers and the environment. However, if residues, impurities or degradation products

[3] Regulation (EC) 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, pp. 29–43.

linked to the total feed additive process (production, recovery and purification) could give rise to concern, toxicological data may be requested.

Available guidance on the assessment of the AMR QPS qualification requires the analysis of the WGS of the bacterial strain under investigation for the presence of genes coding for or contributing to resistance to critically important antimicrobials (CIAs) or highly important antimicrobials (HIAs)[4] (EFSA FEEDAP Panel, 2018). The specification on how to carry out the WGS assessment is available in an EFSA Statement (EFSA, 2021). The assessment of the phenotypic resistance based on the determination of a minimum inhibitory concentration (MIC) value is also mandatory, regardless of the outcome of the genome query. Interpretation of the MIC values is based on cut-off values provided in the guidance document for a selected group of antimicrobials, which are based on published data (EFSA FEEDAP Panel, 2018).

3.1.2. Pesticides area

The EFSA unit responsible for this area (PREV Unit) organises the peer review of the microorganisms that are submitted for approval under Regulation (EC) No 1107/2009[5]. In this regulation, data are required at strain level, including investigations of the effects on human health and on non-target organisms (in the environment), assessment of residues in or on treated crops and information on the fate and behaviour of PPPs in the environment after application. Microorganisms recommended for the QPS list and proposed as PPPs are often exempted from certain data requirements, such as oral toxicity data.

As part of approval criteria defined under Regulation (EC) No 1107/2009, strains of bacteria shall only be approved if it is concluded that they do not have any known, functional and transferable gene coding for resistance to relevant antimicrobial agents. As part of the uniform principles for evaluation and authorisation of plant protection products containing microorganisms,[6] it is also recommended to implement the most updated scientific and technical knowledge on the transferability of AMR, in relation to the risk of transfer of AMR genes to pathogenic microorganisms, potentially affecting the effectiveness of antimicrobials used in human or veterinary medicine. Therefore, transferability is an important factor for the risk assessment during pesticide peer review.

Available guidance (European Commission, 2020)[7] recommends an approach similar to the EFSA feed additives guidance document (EFSA FEEDAP Panel, 2018), combining information collected from WGS data screening to identify known gene(s) responsible for resistance to class(es) of antimicrobial (s) with further testing to confirm phenotypic resistance to the given antimicrobial(s).

3.1.3. Food ingredients and packaging area

The EFSA unit responsible for this area (FIP Unit) applies the QPS approach to production strains during the risk assessment of food enzymes, food additives and food flavourings falling under Regulations (EC) No 1332/2008[8], 1333/2008[9] and 1334/2008[10], respectively. If the microorganism used in the production of the substance under assessment qualifies for the QPS approach, the safety assessment of the product would not need specific toxicological test data. However, if residues,

[4] The guidance is currently being updated and whether important antimicrobials should also be considered is being discussed.

[5] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, pp. 1–50.

[6] Part B of the Annex to Commission Regulation (EU) No 546/2011 as regards specific uniform principles for evaluation and authorisation of plant protection products containing microorganisms. OJ L 227, 1.9.2022, pp. 70–116, as amended by Commission Regulation (EU) 2022/1441.

[7] European Commission, 2020. Guidance on the Approval and Low-risk Criteria linked to 'Antimicrobial Resistance' applicable to Microorganisms used for Plant Protection in Accordance with Regulation (EC) No 1107/2009. SANTE/2020/12260, 23 October 2020. Available online: https://food.ec.europa.eu/system/files/2020-11/pesticides_ppp_app-proc_guide_180652_microorganism-amr_202011.pdf

[8] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. J L 354, 31.12.2008, pp. 7–15.

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, pp. 16–33.

[10] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, pp. 34–50.

impurities or degradation products linked to the total food enzyme production process (production, recovery and purification) could give rise to concerns, toxicological data may be requested.

Available guidance for the assessment of the AMR QPS qualification requires the analysis of the WGS data of the bacterial strain under investigation for the presence of genes coding for or contributing to resistance to CIAs or HIAs (EFSA CEP Panel, 2021).

3.1.4. Novel foods area

The EFSA unit responsible for this area (NIF Unit) takes into consideration the QPS evaluation for the assessment of novel foods (NFs) consisting of, isolated from or produced from microorganisms as defined in Regulation (EC) 2015/2283[11]. The risk assessment of microorganisms intended as NFs or used in their production follows the guidance document on the characterisation of microorganisms issued by the EFSA FEEDAP Panel (2018) and the EFSA statement on WGS analysis (EFSA, 2021). Therefore, the above-mentioned scientific requirements for feed additives (Section 3.1.1) are considered for the assessment of the generic QPS qualification on acquired AMR genes, i.e. phenotypic testing (MIC determination) and interrogation of the WGS of the bacterial strain under investigation for the presence of genes coding for or contributing to resistance to CIAs or HIAs. If a NF consists of or is isolated or produced from a microorganism included in the QPS list, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel) will not challenge the safety of the microbial strain under evaluation, as long as its TU is unequivocally demonstrated at species level as corresponding to the QPS TU and the relevant QPS qualifications are met following the requirements set in relevant EFSA guidance documents. Toxicological studies (e.g. genotoxicity and subchronic toxicity) may still be needed for the risk assessment of the NF to rule out potential safety concerns arising from other NF-related components (e.g. the source of the NF or the production process), according to the NF guidance (EFSA NDA Panel, 2021).

3.1.5. Genetically modified organisms (GMO) area

Genetically modified microorganisms (GMMs) used as viable microorganisms and/or microbial-derived biomass, fall under Regulation (EU) No 1829/2003 on GM food and feed. Such products may also fall under other sectoral legislation depending on the nature and intended use of the product (e.g. feed additive, food enzyme, additives and flavourings, novel food).

For the products that fall under the GMO legislation, the EFSA GMO Panel issued the 'Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use' (EFSA GMO Panel, 2011). This guidance covers the molecular characterisation of GMMs, as well as other aspects to be considered for the safety evaluation of GMMs and GMM-derived products (i.e. compositional analysis, nutritional evaluation, allergenicity assessment and environmental safety). With the later incorporation of WGS information to the risk assessment, the section on molecular characterisation of the guidance document became outdated and is no longer used, but the rest of the guidance still applies. The QPS concept is introduced as a useful tool for the comparative assessment: If the parental strain of a GMM qualifies for QPS, the assessment can focus on the changes introduced by the genetic modification only, as any other trait of the strain can be considered safe. This concept was further elaborated by the BIOHAZ Panel (EFSA BIOHAZ Panel, 2020), who established that the QPS concept is also applicable to GMMs used for production purposes if the recipient strain qualifies for QPS status, and if the genetic modification does not indicate a concern.

3.2. Definition of 'intrinsic' and 'acquired' antimicrobial resistance genes under the scope of EFSA's risk assessment

3.2.1. Definition of 'intrinsic' and 'acquired' AMR genes

This statement focuses on the hits corresponding to AMR genes detected from the WGS analysis for the presence of AMR genes (as explained in the WGS Statement (EFSA, 2021)). These AMR genes need to be classified as 'intrinsic' or 'acquired' for the purpose of EFSA's risk assessments, and they apply to bacteria used in the food and feed chains:

[11] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, pp. 1–22.

- 'Intrinsic' AMR genes are considered as genes, inherent to strains of a bacterial species, that limit the action of antimicrobial agents thereby allowing them to survive and multiply in the presence of the antimicrobial agents. An AMR gene is considered 'intrinsic' if it is shared by the vast majority of wild type strains of the same species (or subspecies) and restricted to those located on the chromosome (see Section 3.2.2).
- The term 'acquired' AMR gene is interpreted as a novel resistance gene for the strain under assessment, acquired through horizontal transfer, enabling a bacterial strain to survive or multiply in the presence of concentrations of an antimicrobial agent higher than those that inhibit the growth of the majority of wild-type strains of the same species without this AMR gene (EFSA FEEDAP Panel, 2018) (see Section 3.3). 'Acquired' AMR genes could be integrated in the bacterial chromosome or harboured on a separate genetic element.

3.2.2. Genome-based identification of 'intrinsic' AMR genes

The risk assessment of AMR genes should contain a bioinformatic analysis of the WGS of the bacterial strain under investigation. For this, the requirements for sequencing and data quality control included in the most recent update of the EFSA Statement on WGS analysis of microorganisms intentionally used in the food chain are recommended to be followed (EFSA, 2021 and future updates). The document sets the principles and minimum criteria for the conducting and reporting of the AMR analysis at the time of drafting the document and provides guidance on the specifications for the bioinformatic analysis.

To demonstrate the 'intrinsic' nature of an AMR gene, a sufficient number of high-quality complete genomes of strains of the single species should be interrogated for the presence of the AMR gene under investigation (see next paragraph for more details). The genome sequences present in publicly available databases (ENA, SRA from NCBI, DDBJ)[12] or provided by the applicant could be used.

Generally, the analysis should start with all genome sequences of the strains of the species available in the databases/repositories (at least ENA or SRA should be included). The databases used and the year of release should be documented. The reason why any strains would not be included for further analysis needs to be documented. If fewer than 30 strains are available, it is recommended to include sequences of more strains based on the applicant's own research. The quality of the WGS sequence and assembly should be provided according to EFSA (2021 and future updates). RefSeq sequences as available in the NCBI database[13] are curated and would not need further quality control. The species identity of the strains should be verified based on the available WGS according to the specifications set in the WGS Statement (EFSA, 2021 and future updates). This includes an identification preferentially based on digital DNA–DNA hybridisation (dDDH) and/or average nucleotide identity (ANI) or phylogenomic methods (EFSA, 2021 and future updates). The isolates of the same species should be epidemiologically and/or ecologically unrelated. The non-clonal relationship can, in most cases, be demonstrated by providing a phylogenomic tree based on the available WGS (possible tools are FastANI with a phylogenomic tree, based on ANI values, BLAST (Basic Local Alignment Search Tool) Microbial Genomes and Tree Viewer provided by NCBI). This phylogenomic information should be carefully interpreted based on species-specific taxonomic information to avoid any bias in the analysis.

The assessment should be based on an identity search (e.g. BLAST) of the collected sequences for the presence of genes homologous to the AMR gene under investigation. For this assessment, the gene sequence as present in the bacterial strain under investigation should be used. A positive control gene expected to be present in all strains of the target group (e.g. a DNA replicon initiator) must be included. In general, query sequence hits with at least 80% identity (at the protein level or nucleotide level as reported in the database) and 70% length of the subject sequence should be taken into account (EFSA, 2021 and future updates).

Based on a proper analysis performed as described above, an AMR gene can be considered 'intrinsic' if it is present in the chromosome of the vast majority of the wild-type strains of a bacterial species (or subspecies). The interpretation and conclusion will be species and gene dependent and will be made on a case-by-case basis, performed in the different areas of EFSA risk assessment.

[12] EMLB-EBI (which hosts ENA), NCBI (which hosts SRA) and DDBJ work closely together, and are interconnected, as partners in the International Nucleotide Sequence Database Collaboration (INSDC). Available online: https://www.insdc.org/

[13] Pruitt K, Murphy T, Brown G, et al. RefSeq Frequently Asked Questions (FAQ) 2010 Nov 15 [Updated 2020 Jan 31]. In: RefSeq Help [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2011. Available online: https://www.ncbi.nlm.nih.gov/books/NBK50679/

3.3. General principles and criteria for guiding the EFSA risk assessment of AMR genes

All identified hits corresponding to genes associated with AMR towards HIA, CIAs or IAs (WHO, 2019[1]) (see Section 3.2.2) are, in principle, considered as hazards and need further assessment. This assessment is based on the following general principles:

- The discrimination between 'intrinsic' or 'acquired' AMR genes (see Section 3.2.2). Hits identifying 'intrinsic' resistance will be considered as of no concern in the frame of the EFSA risk assessment purpose.
- Any 'acquired' AMR gene with corresponding antimicrobial phenotypic resistance should be considered as a hazard/concern. This phenotypic resistance enables a bacterium to survive or multiply in the presence of an antimicrobial agent at concentrations higher than those tolerated by other strains of the same species without this acquired resistance. This analysis is based on phenotypic antimicrobial susceptibility testing, with MIC determination (the MIC cut-off values, EFSA FEEDAP Panel, 2018).
- If the presence of the 'acquired' AMR gene does not lead to phenotypic resistance, further assessment is considered necessary. A case-by-case assessment is necessary to evaluate the likelihood that the AMR gene will be expressed.

Phenotypic antimicrobial resistance not directly linked to the presence of a known AMR mechanism should on a case-by-case be assessed in the context of the EFSA risk assessment.

4. Conclusions

- The QPS status of bacterial TUs is used in different areas of EFSA risk assessment: feed additives, pesticides, food ingredients and packaging, novel foods and genetically modified organisms. Although each area has its own sector-specific guidance, they all use the QPS concept and generally the same approach to assess the qualification related to AMR genes. This assessment involves analysing the entire genome (WGS) to detect the existence of AMR genes. In most cases phenotypic AMR susceptibility testing, and in the pesticides area, the transferability to other microorganisms is also considered. Available guidance relies upon a classification of antimicrobials by relevant bodies (WHO1, WOAH, EMA, EC).
- The terms 'intrinsic' and 'acquired' AMR genes were defined for the purpose of EFSA's risk assessments, and they apply to bacteria used in the food and feed chains. A bioinformatic approach is proposed for demonstrating the 'intrinsic'/'acquired' nature of an AMR gene.
- All AMR genes encoding resistance to CIAs, HIAs or IAs as defined by the WHO[1], found as hits in the bioinformatic analysis, should be considered as hazards for humans, animals and environment and need further assessment. Hits identifying genes conferring 'intrinsic' resistance could be considered as 'of no concern' in the frame of the EFSA risk assessment. 'Acquired' AMR genes with corresponding antimicrobial phenotypic resistance should be considered as a concern. If the presence of the 'acquired' AMR gene is not leading to phenotypic resistance, further case-by-case assessment is necessary.

5. Recommendation

- It is recommended that this document is regularly reviewed and revised as required in response to any developments in the knowledge about, and the tools for defining and discriminating, 'intrinsic' and 'acquired' AMR genes.

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Abbreviations

AMR	Antimicrobial Resistance
ANI	Average Nucleotide Identity
BIOHAZ Panel	EFSA Panel on Biological Hazards
BLAST	Basic Local Alignment Search Tool
CAB Abstracts	Centre for Agriculture and Biosciences.
CIAs	Critically Important Antimicrobials
dDDH	digital DNA–DNA Hybridisation
DDBJ	DNA Data Bank of Japan
DNA	Deoxyribonucleic acid
ENA	European Nucleotide Archive
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed.
FIP	EFSA Food Ingredients and Packaging Unit
FSTA	Food Science Technology Abstracts
GC	Guanine-cytosine
GMMs	Genetically Modified Microorganisms
GMO Panel	EFSA Panel on Genetically Modified Organisms
HIAs	Highly Important Antimicrobials
IAs	Important Antimicrobials
MIC	Minimal Inhibitory Concentration
NDA Panel	EFSA Panel on Nutrition, Novel Foods and Food Allergens

NF	Novel Food
NIF	EFSA Nutrition and Food Innovation Unit
PPPs	Plant Protection Products
PREV	Pesticides Peer Review
QPS	Qualified Presumption of Safety
SRA	Sequence Read Archive
ToR	Terms of Reference
TU	Taxonomic Unit
WGS	Whole Genome Sequence
WHO	World Health Organization

Annex A – Outcome of the public consultation on the draft Statement on how to interpret the QPS qualification on 'acquired antimicrobial resistance genes'

Annex A is available under the Supporting Information section on the online version of the scientific output.