EFSA Infosession: Ready for EFSA’s new Guidance? Build a fit for purpose feed additive dossier—from microorganism to submission

24 June 2026

Table of contents

Introduction 2
General questions 2
Identification and characterisation 4
Whole Genome Sequencing (WGS) 5
Qualified Presumption of Safety (QPS) 6
Presence of cells and DNA 7
Toxigenicity and pathogenicity 9
Antimicrobial resistance and production 11

Introduction

This document lists the questions posed by attendees of the EFSA Infosession held on 24 June 2026 and the replies given by EFSA. A total of 49 questions were received, either before or during the meeting; some may be equivalent or similar, as they were intended to bring clarity to the points presented and discussed.

General questions

1. **Is a deposition in third-country culture collection possible?**

Yes, provided that the culture collection is internationally recognised.

2. **If the applicant is using a well-known yeast or mould, should data showing that there is no safety concern be submitted?**

All strains under assessment should be characterised in full. For strains belonging to species included in the QPS list some data requirements might be waived, but any argumentation should be based on the biology of the microorganism and not on the fact that it is commonly used. The assessment is carried out at strain level.

3. **How does EFSA distinguish microorganism-based and fermentation-derived products, especially when products are complex or not fully defined?**

The product and its manufacturing process should be fully described and characterised by the applicant. When the information provided is insufficient to determine the nature of the product or the relevance of the microorganism to the risk assessment, additional information may be requested.

4. **Are the only differences in the data requirements between GM and non-GM dossiers the genetic modification characterisation and the environmental risk assessment?**

The general data requirements apply to both GM and non-GM microorganisms. For GM microorganisms, additional information is required to characterise the genetic modification, including intended and unintended changes. Further requirements (e.g. presence of recombinant DNA, environmental risk assessment) may depend on the nature of the microorganism, the product and its intended use.

5. **Given that biomasses are included in the new guidance, are there any foreseen changes or implications regarding biomasses currently included as feed materials?**

This issue falls outside the scope of the session and of EFSA's remit. The classification of biomasses as feed materials is a risk management matter. Any potential discrepancies should therefore be addressed by the competent risk managers, including the European Commission or the relevant competent authority. It is important to note that the guidance applies to products for which risk assessment is needed as per regulatory requirement.

6. **How are GM, genome-edited, and non-GM microorganisms distinguished for regulatory purposes?**

Regulatory discussions regarding the applicability of legislation are outside of EFSA's remit. However, it should be noted that the definition of GM is provided in Article 2(2) of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms

and that regarding genome-edited microorganisms, further information may be available in the publication on the new developments in biotechnology applied to microorganisms[1].

7. **Please address risk categorisation, testing expectations, and the practical differences between GMO and non-GMO submissions.**

Regarding the testing expectations and practical differences, the data requirements for GM and non-GM microorganisms are quite similar and, as detailed in the Guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain, include their taxonomic identification and investigation for the presence of traits/genes of concern. In addition, and only for GM strains, the genetic modification(s) to which they are subject should be fully characterised. Some further specific analyses (e.g., environmental risk assessment (ERA), presence of DNA/viable cells) might also be required and are driven by type of microorganism and/or product type.

With respect to risk categorisation, the Guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain states that "the outcome of the risk assessment of the identified hazard(s) depends on the end use of the product. For instance, if a hazard (e.g. acquired genes encoding for resistance to therapeutic antimicrobials) is identified in the microorganism, this will be considered a risk if the microorganism is an active agent since exposure to the hazard is expected. Conversely, for a production strain when no viable cells nor DNA are detected in the final product, exposure to the hazard is not expected and the use of the product is not considered to be a risk."

Therefore, it is not possible to establish an initial risk hierarchy as the same hazard may be identified in different scenarios and, depending on the exposure, lead to different outcomes of the risk assessment.

8. **Can the safety of a strain similar to *E. coli* K12 be presumed for the production of feed additives?**

The safety of the strain must be supported by data. A statement indicating that the strain is similar to *E. coli* K12 would not be sufficient; data supporting relatedness and safety would still need to be provided.

9. **In a biomass fermentation process, if all production strains have been successfully inactivated and subsequent plating reveals the presence of other microbial colonies that are confirmed by PCR not to belong to the production strain, is there an established acceptance threshold for the amount or proportion of these non-production microorganisms that may be present in the inactivated biomass?**

This issue relates mainly to good manufacturing practices, which should be respected by the applicant and are out of the scope of this guidance.

10. **What type of questions are considered suitable to be discussed in the pre-submission advice?**

The advice that can be provided during pre-submission advice is subject to limitations set by the legislation. Nevertheless, questions may be submitted through pre-submission advice and askEFSA services, and they will be addressed where possible.

Identification and characterisation

[1] https://doi.org/10.2903/j.efsa.2024.8895

11. Is average nucleotide identity (ANI) the method for strain identification or is there some other method which is the basis for strain identification?

If the species of the microorganism is known, ANI analysis may be performed against the type strain of the expected species and closely related species. Alternatively, digital DNA–DNA hybridisation tools may be used to compare the genome against available reference genomes. ANI requires an a priori expectation of the taxonomic assignment, whereas database-based approaches may support broader taxonomic placement.

High similarity should be obtained with an appropriate reference genome; however, if the results are ambiguous, additional analyses (e.g. phylogenomic analysis) may be needed. Reference sequences should be carefully selected, preferably from type material or otherwise well-characterised strains.

For bacteria, ANI values of approximately 95% are commonly used to support species-level assignment. The applicability of such thresholds to fungi should be considered with caution.

For fungi, fixed ANI thresholds are not generally established. However, strains assigned to the same species would be expected to show high genomic similarity, and the interpretation should be made on a case-by-case basis using appropriate reference data.

12. Is an adaptive lab evolution strain considered GMO? If it is not, then what is it? It is known that during laboratory adaptation, strains might acquire non-intended characteristics.

The legislation provides a clear definition of what is considered GMO and what is considered non-GMO. GMO means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. This includes recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by any means outside an organism into any virus, bacterial plasmid or other vector system, and their incorporation into a host organism in which they do not occur naturally.

13. In case of an adaptive laboratory evolution study where the microbe acquired resistance for an antibiotic after many generations, what would be the criteria to characterise the strain?

The microorganism would need to be characterised in accordance with the requirements set out in the EFSA Guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain. Regarding the susceptibility profile of the strain, the genetic basis of the resistance should be elucidated.

Whole Genome Sequencing (WGS)

14. What does missing sequence data mean?

The EFSA statement on the requirements for whole genome sequence analysis includes a dedicated chapter on the type of WGS raw data and processed data that should be submitted as part of an application involving a microorganism. In particular, sequencing reads should be submitted as part of the technical dossier alongside the assembled sequences.

15. Are there any requirements on the design of primers used for analytical gene sequencing testing?

Primers may be used provided that they are fit for purpose. They should be specific for the strain under assessment, and appropriate positive and negative controls should be included. The performance of the method should be demonstrated for the relevant matrix and microorganism. In general, higher specificity is preferable to minimise the detection of non-target microorganisms.

The methodology should be fully described in the dossier to allow its assessment.

16. What are EFSA expectations regarding genome quality, assembly, annotation and reporting in dossiers?

Please refer to the EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain[2].

17. What raw data (e.g. FASTA files) should be submitted, and how should large datasets and confidentiality concerns be handled?

Concerning the raw data and datasets to be submitted as part of an application, please refer to the EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain[3].
The topic of confidentiality is not within the scope of this infosession and applicants should refer to the information available in OpenEFSA on Confidentiality and sanitisation[4]. For further advice, applicants can contact EFSA at confidentialityrequestassessment@efsa.europa.eu.

18. How should applicants interpret WGS outputs for taxonomic identification, antimicrobial resistance (AMR), virulence factors and metabolites?

Please refer to the EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain[5].

19. What are the expectations regarding bioinformatic tools, databases and methodological consistency, and what are the limits of WGS in risk assessment? How should the requirement for up-to-date bioinformatic analyses be applied across new submissions, updates and existing authorisations?

To maintain the reliability of bioinformatic analyses, it is crucial to ensure the use of curated databases in which the reference sequences undergo at least basic quality control for both sequence quality (e.g. integrity and contamination) and annotation accuracy. Moreover, it is important to provide up-to-date data which, in EFSA's view, should not be older than 2 years from the date of submission of the application.
Although WGS is essential for the characterisation of all microorganisms, some limitations, such as technical constraints (e.g. analysis of highly repetitive sequence regions), lack of appropriate databases and/or incomplete information on reference genomes, may still trigger the need for additional data to complement the assessment (e.g. phenotype, literature search, toxicological data).
Regarding the request for up-to-date bioinformatic analyses, this requirement applies to new submissions and is not applied retroactively.

Qualified Presumption of Safety (QPS)

20. Is antifungal susceptibility testing necessary for yeasts having QPS status and used for fermentation purposes?

If a fungus is used as a production strain and the presence of its cells in the final product is excluded, antifungal susceptibility testing is not required. However, if the study is inconclusive, cells are present or their presence cannot be excluded, antifungal susceptibility testing is required.

---

[2] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2024.8912.

[3] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2024.8912.

[4] Confidentiality and sanitisation

[5] https://doi.org/10.2903/j.efsa.2024.8912

21. For species that are not currently included on the QPS list but that may be eligible for inclusion, do applicants need to submit a separate application for QPS assessment, or will the species be evaluated internally as part of the assessment process?

QPS is an internal EFSA risk assessment tool and is not a service that applicants can request directly. A QPS assessment is only initiated when EFSA receives an application involving a microorganism. Therefore, to have a species considered for QPS status, an application must be submitted. Once an application is received, the QPS assessment process is triggered automatically as part of EFSA's evaluation. The assessment process is conducted regularly, and the outcome is typically available during the preparation of the scientific opinion.

22. Are strains with QPS status exempted from antimicrobial susceptibility and antimicrobial production testing?

It should be noted that QPS is assessed and granted at species level, while the risk assessment is done at strain level. Regarding the production of antimicrobials, if the species has QPS status this assessment is not required, unless a qualification indicating that this analysis should be performed exists. For antimicrobial resistance, the situation is different: interrogation of the WGS should be performed, even when the strain belongs to a QPS species. The following steps depend on whether any hits are identified. If no hits are identified, the assessment may stop after interrogation of the two up-to-date databases. If hits are identified, their nature must be established, including whether they are intrinsic or acquired for the species. If intrinsic, there is no concern; if acquired or uncertain, a phenotypic test is required. One main difference with the previous guidance is that phenotypic tests do not need to be performed for the full battery of antimicrobials listed in the table, but only for those for which a hit is identified.

Presence of cells and DNA

23. If the production organism is a fungus, are applicants allowed to use antibacterial substances in the plates for the analysis of the presence of viable cells?

A growth medium appropriate for the strain being tested should be used. If the strain is resistant to a particular antimicrobial, that antimicrobial can be included in the medium to facilitate detection or selective growth of the strain. The key requirement is that the same medium is used consistently for the controls and the test samples.

24. Regarding residual DNA: what is considered genes of concern? Are these only acquired AMR and genes coding for secondary metabolites of concern?

According to the guidance glossary, a gene of concern is a gene known to contribute to the production of toxins, harmful metabolites, therapeutic antimicrobials, or acquired resistance to therapeutic antimicrobial agents. For active agents, virulence factors are also included in this definition.

25. What analytical methods, detection limits, and reports are needed for purified and fermentation-derived products?

Please refer to the EFSA guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain[6].

[6] https://doi.org/10.2903/j.efsa.2025.9705

A suitable, culture-based method enabling the recovery of stressed cells, as well as a PCR-based method capable of detecting ≤10 ng of total DNA per gram or mL of product, should be provided. A detailed study report should also be submitted, including high-quality images of relevant gels and culture plates, covering both controls and any samples where growth is observed.
Attention should be paid to the selection of the test item, which should be representative of the product as placed on the market in all its forms. Where multiple formulations are available, testing should be conducted on the most concentrated form(s), as these are expected to provide the most robust demonstration of the absence of viable cells and/or DNA.

26. How should residual DNA from production strains or genetically modified microorganisms (GMMs) be handled?

The assessment should be conducted on a case-by-case basis. Where DNA from the production strain is present in the final product, its relevance for the risk assessment depends on whether genes of concern are present. If no genes of concern are identified, the presence of residual DNA may have regulatory implications outside EFSA's remit.

27. And in case the test on presence of DNA shows that there is no DNA from the production organism, isn't that a proof that there are also no viable cells?

The absence of detectable DNA does not necessarily demonstrate the absence of viable cells, as the limits of detection for DNA-based methods and culture-based methods are different.

28. In case of a postbiotic produced using a GMM in which no live bacterial cells are detected, is there the need to ensure that there's no DNA present in the final product? Or is it the same as non-GMOs?

As described in Section 4 of the guidance, for feed additives, demonstration of the absence of viable cells is requested irrespective of whether the production strain is genetically modified. For genetically modified production strains, the absence of DNA in the final product should be demonstrated.

29. The slides describe the use of genomic DNA dilutions spiked into the product before DNA extraction. Would an alternative approach, whereby the final product is spiked with whole production strain cells prior to DNA extraction, followed by extraction of total DNA, be acceptable? The use of intact cells may provide a more representative assessment of the overall process, including DNA extraction efficiency.

Adding DNA extracted from the genome of the production strain is preferable to spiking with production cells because in the latter case it is not possible to establish whether cells in the final product are lysed or destroyed.
The protocol aims to reduce uncertainty in the generated data. Deviations from the guidance may be acceptable, if they are scientifically justified and the alternative approach is shown to be fit for purpose. The data and the justification will be assessed by the relevant experts.

30. In the analysis of viable cells, if colonies are found, should every colony be analysed for its identity or only the suspected colonies?

In principle, colonies recovered during testing should be analysed to determine whether they correspond to the production strain. Where a high number of colonies is obtained, a preliminary selection based on colony morphology or other relevant criteria may be used, provided that the approach is justified and supports a robust conclusion.

31. How should products be assessed when viable cells are absent but genetic material or metabolites remain?

A full assessment is required and should be conducted on a case-by-case basis. The outcome depends on whether the production strain harbours genes of concern and/or produces compounds of concern that may remain in the final product. Where viable cells are absent, the

potential presence of residual DNA or metabolites should be considered in relation to the intended use and characteristics of the product. Regulatory implications outside EFSA's remit may also apply.

Toxigenicity and pathogenicity

32. Regarding the assessment of toxigenicity and pathogenicity in yeasts and fungi, is the submission of both a comprehensive literature search and whole genome sequencing data required in all cases?

In terms of data requirements, the guidance requires both the interrogation of WGS for the presence of any known metabolic pathway involved in toxigenicity in the body of knowledge and the literature search. Regarding the literature search, the body of knowledge may be more restricted compared with bacteria, but any information that can support the characterisation of the strain should be submitted.

In this case, it would be important to distinguish which species is involved. For a few very well-known models, obtaining WGS is not problematic, and the same applies to the body of knowledge regarding toxigenicity and pathogenicity. The situation is different for entirely new production strains for which good reference sequence data are not available. In such cases, genome data would be useful for identification but would not provide all the details that may be needed for risk assessment. For example, where genes involved in toxigenicity and pathogenicity are not known, a literature search would then be an appropriate way to address the questions and compile the supporting arguments.

33. When analysing toxigenicity and pathogenicity in yeasts and moulds using antiSMASH, is there a recommended threshold for deciding when a biosynthetic gene cluster should be flagged for further investigation?

The recommended thresholds are described in the EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. The thresholds considered are those for coverage and identity.

In fungi, especially when working with organisms that are not well characterised, matches may be weak simply because the available genome annotations are limited. Nevertheless, these thresholds represent the current state of knowledge and are used to avoid overly vague predictions.

If there are concerns and good matches are identified, the next step is to assess whether viable cells are present in the final product and to test the relevant phenotype under conditions that closely resemble the fermentation process. It is also important to determine whether the expected metabolite or activity can be detected in the final product.

34. When a biosynthetic gene cluster associated with a secondary metabolite is identified based on sequence similarity and gene content analyses, what follow-up phenotypic studies are expected to confirm the relevance of the genomic finding? Specifically, is it sufficient to demonstrate the absence of viable cells in the final product, or are dedicated assessments of toxicity, toxigenicity, or pathogenicity also required?

If the strain is supposed to produce a toxin, its presence in the final product should be tested. Identifying a potentially relevant gene cluster is only one part of the safety assessment. WGS analysis is mandatory, but it is not sufficient on its own. Additional studies, including toxicological data, may be required depending on the product, its intended use, and other case-specific factors. Therefore, the final safety evaluation relies on evidence beyond genome sequence analysis alone.

35. How should metabolites of concern and undesirable by-products be identified and assessed in final products?

The assessment should be performed on a case-by-case basis, taking into account the production organism, the manufacturing process, and the nature and intended use of the final product. The strategy to identify and assess metabolites of concern and undesirable by-products should follow the relevant provisions of the guidance for the applicable taxonomic group.

36. Is there an available database or list of harmful metabolites?

A comprehensive and universally applicable list of relevant compounds or virulence factors cannot be established for all microorganisms. The assessment should therefore be based on the existing body of knowledge for the relevant taxonomic group to which the microorganism belongs. Likewise, the significance of potential virulence factors is species-dependent and must be evaluated within the specific biological and product context. As some databases may classify traits related to colonisation, attachment, or survival as virulence factors, the relevance of identified genes or compounds should be interpreted on a case-by-case basis, taking into account the microorganism, the available scientific evidence, and the characteristics of the final product.

Antimicrobial resistance and production

37. When acquired antimicrobial resistance is identified through whole genome sequencing (WGS) in a bacterial strain, but the corresponding antimicrobial is not associated with a microbiological cut-off value in the EFSA guidance, is follow-up phenotypic testing still required? If so, what approach should be used to determine the minimum inhibitory concentration (MIC) and assess the relevance of the genomic finding?

When EFSA cut-off values are unavailable for a specific antimicrobial-species combination, relevant information may be sourced from the scientific literature, EUCAST resources, or applicant-generated data. If no suitable published data exist, a microbiological cut-off value may be established based on a newly generated MIC dataset. However, this cannot be derived from a single strain and requires testing a sufficiently large collection of non-clonal strains of the same species. The resulting MIC distribution can then be analysed to identify the wild-type population and detect potential resistant subpopulations. Guidance on the establishment and interpretation of microbiological cut-off values is available through EUCAST.

38. If antimicrobial production pathways are identified in a strain through genome analysis, is it sufficient to demonstrate through phenotypic testing that there is no detectable activity, or would additional evidence still be required?

If a biosynthetic pathway for the production of a therapeutic antimicrobial is identified, demonstrating the absence of antimicrobial activity through a standard inhibition assay is not sufficient. In such cases, additional targeted analyses are required, including analytical methods such as mass spectrometry, to determine whether the therapeutic antimicrobial compound is actually present in the culture supernatant of the strain, for active agents, or final product, for example for production strains or strains used to produce biomasses.

39. Question for AMR: how exactly should the activity of an AMR gene be evaluated? What would be the accepted evidence of inactivity?

If a gene of concern is present but appears to be inactive, the underlying genetic change should be characterised on a case-by-case basis. The key question is why the gene is not functional—for example, whether it is affected by a minor mutation that could potentially revert, or whether a substantial part of the gene is missing. The risk assessment should consider how easily the gene could become functional again. If only a single point mutation is responsible for the loss of

function, reactivation may be possible. In contrast, if a large portion of the gene is missing, reactivation is considered highly unlikely.

40. Please explain how to distinguish intrinsic from acquired resistance and how to evaluate transferability risk.

Indications on how to conduct the search for AMR genes are provided in the referred EFSA WGS Statement (EFSA, 2024a). When a hit is identified, the applicant can follow the EFSA BIOHAZ Panel (2023a) strategy to demonstrate whether the gene is intrinsic to a taxonomic unit or acquired. Acquired AMR genes are of greater concern due to their higher transfer potential. While transferability is an important consideration, current scientific understanding does not support reliable experimental modelling of horizontal gene transfer in complex environments (Nielsen *et al*., 2014).

41. Please define when phenotypic MIC testing is required and what to do when cut-off values are unavailable.

For bacteria, phenotypic testing is required only in specific cases, such as when acquired antimicrobial resistance genes are identified, as the scope of the analysis for antimicrobial susceptibility in bacteria is to identify acquired AMR genes that have the potential for horizontal transfer. For fungi, the scope of antifungal susceptibility testing is to ensure that effective therapeutic options remain available in case of fungal infections occurring in humans and animals. Therefore, genomic analyses are not relevant for yeasts and filamentous fungi.
When the genomic analysis identifies a hit to an AMR gene for which its intrinsic nature cannot be confirmed and for which phenotypic resistance is not shown, a case-by-case assessment is needed.
When cut-off values for the specific or closely related species are not available, see Question 37.

42. Please identify which antimicrobial by-products may be of regulatory concern.

The risk assessment focuses on antimicrobials of medical and veterinary importance, as defined by the World Health Organization (WHO) and World Organisation for Animal Health (WOAH) (see Section 3.2.1 of the guidance). This classification is maintained and regularly updated by the respective organisations, and EFSA will apply the most up-to-date versions available at the time of the assessment. Given the complexity and diversity of microorganisms assessed and microbial metabolites potentially produced by them, it is not possible to provide a comprehensive list of examples or case studies.

43. Please clarify how undesirable antimicrobial by-products should be detected, characterised, and monitored in final products.

WGS analysis can rule out the production of well-known antimicrobial substances for which the biosynthetic gene clusters are well-characterised. However, for antimicrobial compounds with unknown or not fully characterised genetic determinants, WGS alone may not detect their potential production. For this reason, a combined approach using both WGS and phenotypic analysis is recommended. If a positive result is obtained in the phenotypic test, the guidance recommends further investigation of the nature of the activity. Only if the active agent is shown to produce therapeutic antimicrobials is it considered to be a risk. The test item to be analysed will depend on the specific nature of the product under assessment, its intended use and other factors such as the manufacturing process.

44. If genes encoding for the production of antimicrobial substances are found, must phenotypic testing be conducted?

If the strain does not qualify for the QPS approach, testing for production of antimicrobial substances must always be done. In such cases, phenotypic testing should always be conducted as part of the assessment and is not limited to situations where genes involved in antimicrobial biosynthesis are identified by WGS analysis. Even when the genes were identified in a WGS analysis, these genes may not be activated under the production conditions. Therefore, the

guidance foresees a combined approach based on both WGS-based analysis and phenotypic testing.

45. The guidance requires evidence of susceptibility to therapeutic antifungals. However, for many non-pathogenic filamentous fungi used in food and feed applications, species-specific susceptibility thresholds are not available. What is the scientific justification for requiring antifungal susceptibility testing in such cases, and how should the results be interpreted when no validated criteria exist for the microorganism under assessment?

The objective of antifungal susceptibility testing is to provide evidence that effective therapeutic options remain available in the event of an accidental infection caused by the microorganism under assessment.

As species-specific cut-off values are currently not available for all fungal species, EFSA has published cut-off values for a range of fungal taxa and antifungal compounds. For taxa or antimicrobials not covered by this table, the guidance indicates that existing cut-off values (e.g. EUCAST or CLSI) may be used. When cut-off values for the specific or closely related species are not available, MIC distributions retrieved from the literature and/or generated in-house may be used as a basis for defining cut-off values.

EFSA is currently working on an update of the available cut-off values to expand their coverage to additional antifungal compounds and filamentous fungal species, and this update will be repeated regularly.

46. Given that increasing the concentration of an antimicrobial agent will eventually inhibit the growth of organisms such as *Trichoderma*, isn't the more relevant question whether the concentration required for inhibition can be achieved safely in patients? In other words, how should susceptibility results be interpreted in relation to clinically achievable and safe drug concentrations?

Clinical outcome is influenced by multiple factors, including the host, the site of infection, and the pharmacokinetics of the antifungal compound. However, the purpose of the susceptibility assessment in this guidance is not to evaluate whether therapeutic concentrations can be achieved in specific clinical scenarios. Rather, the objective is to demonstrate susceptibility of commonly used antifungals and thereby provide assurance that effective treatment options are available in the event of an accidental infection. The results should therefore be considered as part of the overall risk assessment of products used in the food chain and not as direct predictors of clinical outcome.

47. How does EFSA approach AMR assessment in cases where fewer than 30 strains are available, particularly for novel or underexplored species?

For novel or underexplored species, additional strains may need to be collected and assessed to investigate the distribution of the AMR gene within the species. Particular attention should be paid to ensuring that the strains analysed are independent and representative of the species, rather than clonal isolates.

EFSA applies a case-by-case assessment, considering the available genomic, phenotypic and literature evidence. When the available data are insufficient to demonstrate that an AMR gene is intrinsic to the species, a conservative approach is followed, and the gene is considered acquired for the purpose of the risk assessment.

48. In practice, how is 'vast majority' defined? >50%, >95% of strains? Additionally, how is 'non-clonal' defined as an ANI percentage, 99.99% or number of SNPs?

No fixed numerical thresholds are defined for determining either the "vast majority" of strains or the degree of relatedness required to consider strains as non-clonal. The assessment is

performed on a case-by-case basis, taking into account the available body of evidence and the characteristics of the AMR gene under assessment. For instance, if a gene is widely recognised as acquired in several other species, it is difficult to argue that it is intrinsic in a particular species.

To support the conclusion that an AMR gene is intrinsic to a species, evidence should demonstrate that the gene is consistently present across a sufficiently diverse set of independent strains representative of that species. The clonality must be assessed using whole-genome sequence data. Multiple isolates may actually be the same strain, especially if a strain has been widely distributed commercially. It is not meaningful to draw conclusions about acquired resistance from multiple clones of the same strain. The origin, distribution, and diversity of the strains must therefore be taken into account.

49. Please clarify why a gene widely recognised as acquired in several species is unlikely to be intrinsic in another given species.

A resistance gene such as *erm(C)* is a good example. It is widely distributed among Gram-positive bacteria, including QPS species such as *Bacillus* and *Lactobacillus*, and is known to be associated with mobile genetic elements that confer macrolide-lincosamide-streptogramin (MLS) resistance. Therefore, even if the gene is found in many strains of a species, it is generally still considered a potential risk because it is known to be mobile and widely acquired. While such genes may originally have emerged in environmental microorganisms, they are currently treated as acquired resistance determinants. More broadly, guidance documents cannot cover every microorganism and every possible situation. As a result, some cases require a case-by-case assessment based on the totality of the available evidence.