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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

COMMISSION STAFF WORKING DOCUMENT – DOES NOT NECESSARILY

REPRESENT THE VIEW OF THE COMMISION SERVICES

This guidance has been developed in co-operation with the Member States. It does not intend to produce legally binding effects and by its nature does not prejudice any measure taken by a Member State within the implementation prerogatives under Regulation (EC) No 1107/2009, nor any case law developed with regard to this provision. This document also does not preclude the possibility that the European Court of Justice may give one or another provision direct effect in Member States.

Introduction

Antimicrobial resistance (hereafter called "AMR") emerges when micro-organisms (such as bacteria, fungi) adapt in response to exposure to antimicrobial drugs. AMR is one of the adaptive traits that microbial subpopulations may possess or acquire, enabling them to survive and overcome host strategies aimed against them. Some micro-organisms are intrinsically resistant to certain types of antimicrobials. However, bacteria, in particular, may also acquire AMR via mutation(s) in chromosomal genes and/or via acquisition of AMR genes from other bacteria of the same or even of different species.

As a result, the microorganism can become resistant to an antimicrobial to which it was previously susceptible.

The WHO regards emergence of antimicrobial resistance in pathogenic micro-organisms as one of the biggest threats to human health and published the "Global action plan on antimicrobial resistance" with the five following objectives:

- To improve awareness and understanding of antimicrobial resistance through effective communication, education and training;
- To strengthen the knowledge and evidence base through surveillance and research;
- To reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures;
- To optimize the use of antimicrobial medicines in human and animal health; and
- To develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions.

It is acknowledged that there are knowledge gaps on how use of microbial pest control agents and products (MPCA/MPCP) could affect micro-organisms that are present in the ecosystem in regard to the triggering of specific antimicrobial mechanisms. The potential long-term consequences of releasing MPCAs potentially containing transferable AMR genes into the environment must thus be assessed.

AMR occurs naturally over time, in case of a certain selection pressure, usually through genetic mutations or exchanges of genetic material encoding resistance. However, the production of and (over)use of antimicrobials is favouring this process. For example, the use of antimicrobials in animal production means that opportunistic pathogens resistant to antimicrobials could be selected for, when placed under selection pressure by a certain compound or agent.

Applying micro-organisms in the environment by spreading them as plant protection products may potentially contribute to the antimicrobial resistance concern, through the spread of resistance genes which can be horizontally transmitted from the microbial pest control agent to pathogenic bacteria. The main objective of any policy to contain AMR is to reduce the above-mentioned risks related to spread of resistance genes and their impact on human and animal health.

Active substances must be approved under Regulation (EC) No 1107/2009 before they can be used in plant protection products. Uniform principles apply as regards the resistance to antimicrobial agents of importance for human and veterinary medicine. In addition to the approval criteria, the Regulation allows for the approval of an active substance as "low-risk substance" when it meets certain low-risk criteria, as specified in Annex II, point 5 of Regulation (EC) 1107/2009.

The Commission recently amended these low-risk criteria[1] to facilitate the identification of low-risk substances while ensuring a high level of protection of human health, animal health and the environment. They now provide distinct criteria for chemicals and micro-organisms.

Currently the only low-risk criterion for micro-organisms considers "multiple antimicrobial resistance". The text reads as follows:

"An active substance which is a microorganism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to antimicrobials used in human or animal medicine"

This guidance document explains how to assess antimicrobial resistance of microorganism, as well as the risk of increasing the spread of antimicrobial resistance of human and veterinary concern, in relation to the approval criteria and the low risk criteria set under Regulation (EC) 1107/2009.

Implementation schedule

This document has been finalised in the Standing Committee on Plants, Animals, Food and Feed on 23/10/2020. It will apply to applications submitted from 01/05/2021 onwards.

[1] Commission Regulation (EU) 2017/1432, OJ L 205, 8.8.2017, p. 59

0. Definitions

For the purpose of this guidance document the following terms have been defined:

Adverse health effect: An undesirable outcome in humans and animals. For the purpose of this guidance document, this refers to human infections caused by micro-organisms in food from use as plant protection product or acquired from food of animal/crop origin as well as increased frequency of infections and treatment failures, loss of treatment options, and increased severity of infections manifested by prolonged duration of disease, increased hospitalization and mortality[2].

Antimicrobial agent: Any substance of natural, semi-synthetic, or synthetic origin that at specific concentrations kills or inhibits the growth of micro-organisms by interacting with a specific target. The term antimicrobial is a collective for antibacterial, antiviral, antifungal, anthelmintic and antiprotozoal agents.

Antimicrobial resistance (AMR): The ability of a microorganism to multiply in the presence of an antimicrobial substance at relevant therapeutic concentrations, making that substance therapeutically ineffective. AMR may be an intrinsic or an acquired property of the microorganism.

Antimicrobial resistance determinant: A mutation, a gene or a set of genes, that mediates antimicrobial resistance. The genetic element(s) are located either chromosomally or extra-chromosomally and may be transferable, if located on mobile genetic elements (MGE) such as plasmids, bacteriophages, integrative conjugative elements, transposable elements, integrons, gene cassettes or genomic islands. Identified antimicrobial resistance genes are collected in international databases.

Acquired antimicrobial resistance: The acquisition of novel resistances enables a microorganism to survive or multiply in the presence of an antimicrobial agent at concentrations higher than that, which inhibits growth of other strains of the same species without this acquired resistance. An acquired resistance can be caused either by a mutation (which may or may not be transferable) or by uptake of a resistance gene, located on a chromosomal or extrachromosomal mobile genetic element (**MGE**) such as plasmids, bacteriophages, integrative conjugative elements, transposable elements, integrons, gene cassettes or genomic islands. For the purpose of this guidance acquired resistances are of concern if they are transferable.

Intrinsic antimicrobial resistance: All inherent properties of a microbial species that limit the action of antimicrobials thereby allowing them to survive and multiply at relevant therapeutic concentrations of an antimicrobial substance. Inherent properties of micro-organisms are considered not transferable and can include structural characteristics like lack of drug targets, the impermeability of cellular envelopes, activity of multidrug efflux pumps, or metabolic enzymes. An antimicrobial resistance gene is considered intrinsic if it is located on a chromosome in the absence of MGE and shared by the majority[3] of wild type strains of the same species.

[2] Second Joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: scientific assessment (2004): see report at http://apps.who.int/iris/handle/10665/68701

[3] In line with the following publication: Sandner-Miranda et al. (2018) "The Genomic Basis of Intrinsic and Acquired Antibiotic Resistance in the Genus Serratia". Front. Microbiol. 9:828.

Medically important antimicrobials (MIA): All antimicrobial agents important for therapeutic use in humans and animals as described in the World Health Organisation (WHO) list[4] of Critically Important Antimicrobials (CIA) and Highly Important Antimicrobials (HIA) and Important Antimicrobials (IA) for Human Medicine[5]. Please note that the latest version of these lists should be used.

[4] Critically Important Antimicrobials for human medicine 6th revision 2018, WHO 2019 https://apps.who.int/iris/bitstream/handle/10665/312266/9789241515528-eng.pdf?ua=1

[5] Please note that the World Organisation on Animal Health (OIE) listed also veterinary CIA, HIA and IAs for food-producing animals as antimicrobial agents of veterinary importance, OIE 2018 https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/AMR/A_OIE_List_antimicrobials_May2018.pdf. By alignment with the latest guidance elaborated by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) the additional agents from the OIE list are not to assessment in the context of this guidance document.

1. Scope of this guidance document

The scope of the antimicrobial resistance characterisation described in this guidance document addresses **mainly bacteria**.

The case of fungi and viruses is not addressed extensively here for the following reasons:

- Viruses excluding bacteriophages have not been reported in the scientific literature as contributor to the AMR concern.
- The acquisition of antimicrobial resistance in fungi is multifactorial. Therefore, horizontal transfer of AMR genes between fungi appears to be very rare and is not associated with specific mechanisms, as described for bacteria (for instance through plasmid exchange).

For **fungi and viruses** there is no need to assess the potential transfer of genes for resistance to antimicrobials. However, some information regarding the antimicrobial susceptibility of fungi still needs to be provided because it has to be demonstrated that there are sufficient treatment options in case infections with the fungal microorganism may occur (e.g. in immunocompromised people) (data requirement 5.2.6 of Regulation (EC) 283/2013 part B).

The case of **bacteriophages** (viruses that infect bacteria) is not currently addressed by this guidance document. Nevertheless, bacteriophages harness the "machinery" of the host bacterial cell to infect it: recombination may then occur between bacterial DNA and the bacteriophage genome. The interest in the use of bacteriophages as plant protection products might be reason to develop a specific guidance in the future.

2. Approval criteria related to antimicrobial resistance

Several aspects shall be assessed in view of the approval of a microorganism used in plant protection products:

- Overall the micro-organisms shall **not** be **infective** or **pathogenic** to humans. This aspect is not further developed in this guidance document, as it is not linked to its central topic of antimicrobial resistance.
- **Antimicrobial resistance**: The Uniform Principles outline that it should be demonstrated that, if the micro-organisms are resistant to antimicrobial(s), this resistance or its possible transfer does not interfere with the effectiveness of antimicrobials used in human and animal health care or that this possible transfer does not lead to adverse effects on human and animal health. **When the resistance can be transferred to other micro-organisms, including human and animal pathogens, the microorganism should not be approved** [6].

3. Reference to EFSA guidance document on micro-organisms used for feed additives

Without being fully applicable to the case of micro-organisms used as plant protection products, it is recommended to applicants and evaluators to pay particular attention to the updated EFSA "Guidance

[6] This is in line with the EFSA Guidance on feed additives referred in the next footnote which provides that "bacterial strains carrying acquired genes that confer resistance to relevant antimicrobial(s) [i.e. CIA/HIA's] are considered to represent a risk for those exposed to the microorganism"

on the characterisation of micro-organisms used as feed additives or as production organisms"[7] published on 28 March 2018. It is recommended to use its methodology in the evaluation, with appropriate interpretation and adaptation, as outlined below, for plant protection products, taking into account that the exposure of consumers and the environment is different between feed additives and plant protection products.

The EFSA feed additives guidance document aims indeed at assisting applicants in the preparation and presentation of dossiers for feed additives containing micro-organisms or produced by micro-organisms by fermentation. It therefore covers aspects related to AMR that can be relevant to microbial plant protection products. However, only part of the information, principles and procedures that the EFSA feed additives guidance document described are considered as relevant. To assist applicants and evaluators the relevant parts will be explained more specifically in the following sections.

The EFSA feed additives guidance document provides lists of antimicrobial agents to test for and breakpoint values to discern between susceptible and resistant strains of bacteria commonly used in feed additives. The EFSA table has been amended to add medically important antimicrobials defined by WHO (Appendix 1).

4. Stepwise approach to evaluate and decide upon the approvability or the low-risk status of a bacterium with regard to antimicrobial resistance

The assessment of the approvability and the low-risk qualification of bacteria as regards the specific aspect of antimicrobial resistance can be carried out according to a "step-by-step" procedure.

To address the micro-organism's capacity to transfer genetic material relevant to AMR to other organisms as well as its capacity of being pathogenic for plants, animals or man, the applicant should follow a similar approach as in the EFSA feed additives guidance document, combining information collected from Whole Genome Sequencing ('WGS') data screening to identify known gene(s) responsible for resistance to class(es) of antimicrobial(s) with further phenotypic testing to confirm the resistance to the given antimicrobial(s).

However, this recommendation does not prevent applicants to propose other relevant technical methods as powerful as WGS to demonstrate absence of transferable genetic material associated with antimicrobial resistance.

4.1. Stepwise approach

To determine whether the strain carries AMR and, if so, whether resistance is 'intrinsic' or 'transferable`, two sets of data should be provided[8]:

1. Whole Genome Sequencing (**WGS**) data screening for the presence of known AMR genes
2. Phenotypic testing based on determination of a minimum inhibitory concentration (**MIC**) for a selected group of antimicrobials.

[7] Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

[8] It is to be noted that also strains belonging to species with a QPS status have to be tested as other presumptive PPPs.

4.1.1. Step 1: Whole Genome Sequencing (WGS) data screening

Technologies of DNA sequencing and bioinformatics enable the screening of genomic data for phenotypical traits encoded in a genome of interest. The so-called "whole-genome sequencing" (WGS) is an essential method that may prove helpful in the detection of genetic determinants for antimicrobial resistance. Both chromosomal and extrachromosomal genetic elements such as plasmids must be included in the whole genome sequencing.

WGS data should be interrogated for the presence of genetic material coding for or contributing to resistance to antimicrobials relevant to their use in humans and animals (MIAs), specifically complete genes coding for resistance to antimicrobials that were reported to have been transferred between bacteria to which it conferred an acquired resistance are of high concern. For this purpose, a comparison against at least two up-to-date curated international databases (e.g. CARD[9], ARG-ANNOT[10], ResFinder[11]) should be performed and documented in accordance to the EFSA statement[12] on the requirements for whole genome sequence analysis of micro-organisms intentionally used in the food chain. The outcome of the analysis should be presented as a table focusing on complete genes coding for resistance to antimicrobials. The table should include at least the gene identification, the function of the encoded protein, the percentage of identity and the e-value. Regions up- and downstream of the gene should be analysed in order to assess their potential transferability.

In case a resistance gene has been identified, the applicant should also provide information if this resistance gene is located on a chromosomal or extrachromosomal mobile genetic element (MGE), such as plasmids, bacteriophages, integrative conjugative elements, transposable elements, integrons, gene cassettes or genomic islands. Further information regarding definition and screening for mobile genetic elements within the WGS data are provided in the review by Partridge et al 2018[13].

Possible outcomes:

- if the screening of the WGS results in "hits" for a MIA resistance gene, the respective resistance shall be phenotypically tested in step 2.
- If the screening of the WGS results in "hits" for a MIA resistance gene and WGS confirms that the resistance gene identified is located on a mobile genetic element (MGE), the gene is considered to be transferable.

4.1.2. Step 2: Phenotypic testing

The phenotypic testing should be performed with the antimicrobial agent(s):

- for which a known AMR gene was identified in step 1.
- for determining whether the species is susceptible to compounds of several antimicrobial classes to ensure treatment options in any case of opportunistic infection: susceptibility shall

[9] https://card.mcmaster.ca/
[10] http://en.mediterranee-infection.com/article.php?laref=283%26titre=arg-annot
[11] https://cge.cbs.dtu.dk/services/ResFinder/
[12] To be added when available
[13] Partridge SR. et al 2018. Mobile Genetic Elements Associated with Antimicrobial Resistance Clinical Microbiology Reviews Aug 2018, 31 (4) e00088-17; DOI: 10.1128/CMR.00088-17

be demonstrated for compounds of at least two classes of antimicrobials selected among medically important antimicrobials listed in Appendix 1.

Phenotypic tests shall be carried out in a consistent manner by an officially established facility[14] using recognised or standardised methods and when they exist, using validated methods. Justification should be provided for the testing method used. As a basic requirement, the minimum inhibitory concentration (MIC expressed as mg/L) should be determined for the antimicrobial agents for which a known AMR gene was identified and for at least two different classes of antimicrobials defined as MIA by WHO to provide treatment options. The determination of MICs shall be carried out according to the principles outlined in the EFSA feed additives guidance document under its section 2.2.1.

For the purpose of distinguishing resistant from susceptible strains, European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) defined MIC breakpoint values for different microorganism species based on published data.

Table of Appendix 1 can be used as a reference as it lists medically important antimicrobials of all relevant classes defined by WHO, for which either genus-specific or non-species specific microbiological break-point values are available.

For the cases concerning non-listed microbial species, it is recommended to use the non-species specific cut off values of the latest EUCAST clinical breakpoint table as listed in Appendix 1. However, it should be noted that genus-specific cut off values such as epidemiological cut off values (ECOFF) are preferable if available.

The applicant may therefore decide to refine the non-species specific breakpoint values, for example by:

- using a scientifically documented breakpoint value more specific to the microorganism to be tested, e.g. available in the database[15] of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), or peer-reviewed literature.
- determining a provisional MIC breakpoint value specific to the species of the microorganism to be tested using the appropriate EUCAST Guidance Documents on susceptibility testing.

Possible outcomes: on this basis, a strain can be categorised as:

- **susceptible** when its growth is inhibited at a concentration of a specific antimicrobial equal to or lower than the established breakpoint value (MIC ≤ x mg/L) => **no resistance**
- **resistant** when it is able to grow at a concentration of a specific antimicrobial higher than the established breakpoint value (MIC > x mg/L). When a species-specific break-point value is used, the identified resistance is considered an acquired resistance. When non-species specific

[14] By reference to a testing facility which are either working according to ISO/IEC 17025 standard, or to accreditation or to good laboratory practices, where possible.
[15] http://www.eucast.org/clinical_breakpoints/ ;
http://www.eucast.org/expert_rules_and_intrinsic_resistance/ ;
http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Expert_Rules/Expert_rules_intrinsic_exceptional_V3.1.pdf
http://www.eucast.org/ast_of_bacteria/guidance_documents/

breakpoints are used an identified resistance may be either caused by an acquired or an intrinsic resistance gene or an inherent characteristic. To distinguish whether the gene is transferable or not, please refer to section 5.1., hereafter.

5. Decision making

Reference is made in this sub-chapter to current Uniform Principles - decision making criteria 2.2.2 and 2.6.1.4 (which are under revision at the moment of adoption of this guidance document) and to EFSA FEEDAP Guidance Document section 2.2.3 and 5.1 (first bullet point).

5.1. Approval criteria - Antimicrobial resistance

The following principles apply:

- When a **MIA resistance gene** has been identified from the WGS screening step and it is located on a mobile genetic element (MGE), **the resistance is considered as transferable and the strain may not be approved**, **unless** the applicant demonstrates that the resistance gene is not functional by phenotype testing (demonstrating absence of phenotypic resistance) and genomic information demonstrating that the gene is not functional (e.g. by demonstrating key changes in the gene sequence).
- When a MIA resistance gene has been identified from the WGS screening step and the phenotypic testing results show that the strain of an otherwise typically susceptible species is resistant to the respective MIA, **the resistance is considered as acquired and the strain may not be approved, unless** the applicant demonstrates that the antimicrobial resistance gene is not transferable, e.g. located on a chromosome in the absence of MGE**.**
- When the **phenotypic testing** for determining susceptibility to antimicrobials (or literature information) reveals that a strain of an otherwise typically susceptible species is **resistant to a MIA** that has **not been identified by genotypic testing**, there may be an unknown gene responsible for this resistance. The **strain may be approved**, but applicants are reminded of the obligations provided for in Article 56 of Regulation (EC) 1107/2009 and thus shall monitor and notify to the authorities when new information becomes available on the genetic basis of the particular phenotypic resistance (e.g. when a new resistance gene is identified coding for that phenotypic resistance).
- When a MIA resistance **gene** has been identified that is not located on an MGE and the **phenotype tests** show **no resistance** to that respective MIA, the gene is not considered to be of concern. Based on that information the **strain may be approved**.
- When a MIA resistance **gene** has been identified from the WGS screening step that is **not located on a MGE** but the **phenotype** tests **show a resistance** to that respective MIA, the gene is **not** considered to be **of concern** as far as it is an **intrinsic resistance** shared by the majority of other **wild type strains** of the same species. Based on that information the **strain may be approved**.

For **bacteria[16] which cannot be cultured in conventional media as described by EUCAST guidance[17]**, determination of susceptibility to antimicrobials has to be performed in the culture medium used for manufacturing of the end-use product (e.g. TGAI). In these cases, activity of the antimicrobial in the test growing medium should be confirmed.

These approval criteria above also apply to active substances consisting of **inactivated/dead bacteria,**

[16] Typical example is *Pasteuria nishizawae*

[17] Broth microdilution - EUCAST reading guide (v 1.0, 19 January, 2019) available at: https://www.eucast.org/ast_of_bacteria/mic_determination/?no_cache=1

because genetic material and in particular AMR genes can be present. In this case, active/ living individuals of the same strain shall be tested as described above. Strains with transferable resistance may not be approved, unless the applicant demonstrates that the identified genes of concern are not present in the product (e.g. via an appropriate PCR protocol) and thus there is no hazard and no risk to be expected from the inactivated/dead bacterium.

5.2. Approval criteria - Sufficient treatment options

In order to be approved, phenotypic susceptibility of the bacterium to at least two classes of antimicrobial agents of the CIA or HIA groups in the WHO list, has to be demonstrated, to secure two therapeutic options in case of human infection.

Phenotypic susceptibility for at least two antimicrobial agents with different modes of action has to be demonstrated for a fungus as well.

5.3. Low-risk criterion for micro-organisms

The low-risk criterion is considered to be met when it can be demonstrated that the approval criteria for the **bacterial strain** (section 5.2.) and the approval criteria related to AMR (section 5.1.) are fulfilled.

For fungi, viruses, inactivated/dead bacteria and other micro-organisms other than bacteria (e.g. protozoa) the low-risk criterion is considered as met when it is demonstrated that the approval criteria for micro-organisms (section 5.2.) are fulfilled (AMR does not apply).

6. Review and implementing period

Considering that several years will pass between the application of this guidance and actual decision making, a re-evaluation of these guidelines would need to be considered at that point.

In the meantime, the above proposed definition of low-risk substance can be used, taking also into account that current dossiers that are already in the approval process will come to the Commission's desk for the decision making process in at least three more years. These dossiers will not yet contain the data as described in the EFSA (2018) feed additive guidance document.

Based on the data requirements, applicants already have to provide data on resistance to antimicrobial agents. In previous cases for which decisions have been made the provided data was not always suitable to determine whether any resistances found were transferable. In those cases, this did not preclude approval. For the sake of legal certainty, this line of decision making will be continued for dossiers submitted before the implementation date of this guidance. However, where applicable, a condition in the approval regulation will be included to deliver confirmatory data in accordance with the requirements set in this guidance document.

Appendix 1

Table of Minimum Inhibitory Concentrations Break-Point values related to phenotypical characterisation of the bacterial plant protection active agent regarding antimicrobial resistance

Antimicrobial class	Anti-microbial	Non species related break-points (mg/L)*		Genus-specific resistance break-points R > (mg/L)									
		S* ≤	R** >	*Bacillus spp.*	*Bifidobacterium*	*Corynebacterium*	*Enterobacteriaceae*	*Enterococcus. faecium*	*Lactobacillus acidophilus group*	*Lactococcus lactis*	*Leuconostoc*	*Pseudomonas spp.*	*Propionibacterium*
Cephalo-sporins of 3rd, 4th and 5th generation	Cefepime	4	8				4	-				8	
	Cefotaxime	1	2	32[c]			2	-				32	
	Ceftaroline	0.5[a]	0.5[a]				0.5	-				-	
	Ceftazidime	4	8	32[c]			4	-				8	
	Ceftobiprole	4	4				0.25	-				IE	
	Ceftriaxone	1	2				2	-				-	
Cephalo-sporins 1st/2nd generation	Cefazolin	1	2	8[c]			4					-	
	Cefuroxime	4	8				8					-	
Carbapenem	Ertapenem	0.5	0.5				0.5	-				-	
	Imipenem	2	4	4[c]			4	4				4	
	Meropenem	2	8	8[d]			8	-				16	
Glycylcycline	Tigecycline	0.5	0.5				0.5	0.25				-	
Oxazolidinone	Linezolid	2	2	2[c]		2	-	4				-	
Monobactams	Aztreonam	4	8				4	-				16	
Quinolones	Levofloxacin	0.5	1	1[c]			1	4				1	
	Moxifloxacin	0.25	0.25			0.5	0.25	1				-	
	Ofloxacin	0.25	0.5				0.5	-				-	
	Ciprofloxacin	0.25	0.5	1[d]	-	1	0.5	4	-	-	-	0.5	-
Amino-glycosides	Gentamicin	0.5	0.5	4	64	4	4	32	16	32	16	4	64
	Kanamycin	IE	IE	8	-	16	8	1024	64	64	16		64
	Streptomycin	IE	IE	8	128	8	16	128	16	32	64		64
Macrolides and Ketolides	Erythromycin	IE	IE	4	1	1	-	4	1	1	1	-	0.5
	Tylosin	IE	IE	-	-	-		-	-	-			-
Lincosamides	Clindamycin	IE	IE	4	1	4	-	4	4	1	1	-	0.25
Tetracyclines	Tetracycline	IE	IE	8	8	2	8	4	4	4	8	-	2
Amphenicols	Chlor-amphenicol	IE	IE	8	4	4	8	16	4	8	4	-	2
Polymyxins	Colistin	IE	IE	-	-	-	2	-	-	-	-	2	-
Phosphonic acid derivate	Fosfomycin	IE	IE		-	-	32	-	-	-	-	-	-
Glycopeptide	Vancomycin	IE	IE	4	2	4	-	4	2	4	-	-	4
Penicillins (aminopenicillin)	Ampicillin	2	8	8[c]	2	1	8	-	1	2	2	-	2
	Amoxicillin	2	8			8	8					-	

Penicillins (with beta-lactamase inhibitors)	Amoxicillin-clavulanic acid[b]	2	8				8					-	
Penicillins (antipseudomonal)	Piperacillin	4	16				16					16	

Blue MIC values are derived from the EUCAST clinical breakpoint table version 10.0 2020[18]. **Black values** are genus-specific MICs taken from the EFSA feed additives guidance document (EFSA 2018)[19]. Non-species related MIC breakpoints are used only, if no genus-specific breakpoints are available. Please consider the latest version of the EUCAST clinical breakpoint table[17] and the EFSA feed additives guidance document[18].

Legend:

* : S: Susceptible: A microorganism is categorized as susceptible to an agent when MIC value is lower or equal to susceptible breakpoint.
**: R: resistant: A microorganism is categorized as resistant to an agent when a MIC value is higher than the resistance breakpoint.
***:IE: insufficient evidence;
"-": No breakpoint. Susceptibility testing is not recommended/not required. Empty fields, no MICs were determined.
a: Based on PK-PD target for Gram-negative organisms.
b: For susceptibility testing purposes to Amoxicillin clavulanic acid, the concentration of clavulanic acid is fixed at 2 mg/L
c, d: MIC value for Bacillus strains derived from scientific literature c) Ikeda et al. 2015[23] and d) Torkar et al. 2018[24]

[18] http://www.eucast.org/clinical_breakpoints/
[19] Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16 (3):5206, 24 pp.
[23] Ikeda, M., et al. (2015). Ann Clin Microbiol Antimicrob 14: 43.
[24] Torkar, K. G. and B. Bedenic (2018). Microb Pathog 118: 140-145.