

## SCIENTIFIC OPINION<sup>1</sup>

### Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance<sup>2</sup>

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)<sup>3,4</sup>

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#### ABSTRACT

This Guidance document is intended to provide a method to identify resistance to antimicrobials of human and veterinary importance in bacterial strains intended for use as feed additives. Such tests should be made in a consistent manner using internationally recognised and standardised methods. As a basic requirement, the minimum inhibitory concentration of the antimicrobials should be determined for each of the following substances: ampicillin, vancomycin, gentamycin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol and, in specific cases, tylosine, apramycin, nalidixic acid, sulfonamide and trimethoprim. These antimicrobials are chosen to detect a wide range of determinants for resistance. The cut-off values identified by the FEEDAP Panel should be seen as a pragmatic response intended to introduce consistency in the separation of strains with acquired resistance from susceptible strains. These values are not intended for any purpose other than the assessment of microbial products for the possible presence of antimicrobial resistance. When a bacterial strain demonstrates higher resistance to a specific antimicrobial than the other strains of the same taxonomical unit, the presence of acquired resistance is indicated and additional information is needed on the genetic basis of the antimicrobial resistance. Any bacterial strain carrying an acquired resistance to antimicrobial that is shown to be due to the acquisition of genetic determinant presents the greatest potential for horizontal spread and should not be used as a feed additive.

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#### KEY WORDS

Antimicrobial, guidance, safety, resistance, bacteria

<sup>1</sup> This guidance document replaces the previous EFSA opinion on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance, adopted on 18 June 2008. (EFSA-Q-2008-004)

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## INTRODUCTION

The development of resistance amongst bacteria to antimicrobials remains a serious concern. For this reason, viable micro-organisms used as the active agent(s) in feed additives should not add to the pool of antimicrobial resistance genes already present in the gut bacterial population or otherwise increase the risk of transfer of drug resistance.

The European Food Safety Authority (EFSA) requires as part of its Qualified Presumption of Safety approach to the safety assessment of bacteria deliberately introduced in the food chain, that acquired resistance determinants to antimicrobials of clinical importance are absent.

When resistance to an antimicrobial is inherent to a bacterial species, it is generally referred to as 'intrinsic resistance' (sometimes called 'natural resistance') and is typical of all the strains of that species. In contrast, when a strain of a typically susceptible species is resistant to a given antimicrobial drug, it is considered to be 'acquired resistance'. Acquired resistance can be due either to added genes (genes acquired by the bacteria via gain of exogenous DNA) or to the mutation of indigenous genes (Ammor et al., 2007; van Reenen and Dicks, 2011).

The actual possibility of the transfer of resistance to human or animal pathogenic bacteria, which could result from the use of microbial products based on drug-resistant strains, is related to the genetic basis of resistance. Although it is reasonable to assume that gene transfer from viable micro-organisms to other micro-organisms will occur in an open environment such as the gastrointestinal tract, intrinsic resistance is presumed to present a minimal potential for horizontal spread, whereas acquired resistance mediated by added genes is considered as having a high potential for lateral spread (Devirgiliis et al., 2011; van Reenen and Dicks, 2011).

Added genes are the result of gene exchange between bacteria. The presence of added genes coding for antimicrobial resistance, particularly when carried by mobile genetic elements, presents the greatest risk for horizontal dissemination of resistance. Antibiotic resistance (AR) genes database ARDB (<http://ardb.cbcb.umd.edu/>) provides a centralized compendium of information on antibiotic resistance by providing a non-comprehensive list of AR gene sequences. Resistance by mutation of chromosomal genes presents a low risk of horizontal dissemination (Devirgiliis et al., 2011; van Reenen and Dicks, 2011).

In principle, the selection of micro-organisms for use as feed additives should be oriented towards the least resistant organism whenever possible.

This guidance document replaces the previous EFSA opinion on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance, adopted in 2008 (EFSA, 2008). It will be subject to regular updating when data from the scientific community and other relevant sources (e.g., the European Medicines Agency, the European Centre of Diseases Prevention and Control) become available.

### 1. CRITERIA FOR IDENTIFYING BACTERIAL STRAINS WITH ACQUIRED RESISTANCE TO ANTIMICROBIALS

All bacterial products intended for use as feed additives must be examined to establish the susceptibility of the component strain(s) to a relevant range of antimicrobials of human and veterinary importance. It is essential that such tests are made in a consistent manner using internationally recognised and standardised methods. As a basic requirement, the minimum inhibitory concentration (MIC) of the antimicrobials expressed as mg/L or µg/mL should be determined for each of the following substances: ampicillin, vancomycin, gentamycin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol and, in specific cases, tylosine, apramycin, nalidixic acid,

sulfonamide and trimethoprim (see Table 1). These antimicrobials are chosen to detect a wide range of determinants for resistance.

### 1.1. Microbiological cut-off values

For the purpose of distinguishing resistant from susceptible strains, the FEEDAP Panel defines microbiological cut-off values. Microbiological cut-off values are set by studying the distribution of MICs of the chosen antimicrobials in bacterial populations belonging to a single taxonomical unit (species or genus). The part of the population that clearly deviates from the normal susceptible populations is categorised as resistant. The data used for the definition of microbiological cut-off values, as reported in Table 1, were derived from the published body of research, the European Committee on Antimicrobial Susceptibility Testing (EUCAST, <http://www.eucast.org/>), and from national and European monitoring programmes.

For the assessment of bacteria used as feed additives, strains can be categorised as susceptible or resistant to antimicrobials:

- Susceptible (S): a bacterial strain is defined as susceptible when it is inhibited at a concentration of a specific antimicrobial equal or lower than the established cut-off value ( $S \leq x$  mg/L).
- Resistant (R): a bacterial strain is defined as resistant when it is not inhibited at a concentration of a specific antimicrobial higher than the established cut-off value ( $R > x$  mg/L).

The cut-off values identified should be seen as a pragmatic response intended to introduce consistency in the separation of strains with acquired resistance from susceptible strains. The cut-off values are not intended for any purpose other than the assessment of microbial products for the possible presence of antimicrobial resistance.

**Table 1.** Microbiological cut-off values (mg/L)

	ampicillin	vancomycin	gentamycin	kanamycin	streptomycin	erythromycin	clindamycin	tetracycline	chloramphenicol	
<i>Lactobacillus</i> obligate homofermentative <sup>a</sup>	1	2	16	16	16	1	1	4	4	
<i>Lactobacillus acidophilus</i> group	1	2	16	64	16	1	1	4	4	
<i>Lactobacillus</i> obligate heterofermentative <sup>b</sup>	2	n.r.	16	32	64	1	1	8	4	
<i>Lactobacillus reuteri</i>	2	n.r.	8	64	64	1	1	16	4	
<i>Lactobacillus</i> facultative heterofermentative <sup>c</sup>	4	n.r.	16	64	64	1	1	8	4	
<i>Lactobacillus plantarum/pentosus</i>	2	n.r.	16	64	n.r.	1	2	32	8	
<i>Lactobacillus rhamnosus</i>	4	n.r.	16	64	32	1	1	8	4	
<i>Lactobacillus casei /paracasei</i>	4	n.r.	32	64	64	1	1	4	4	
<i>Bifidobacterium</i>	2	2	64	n.r.	12 8	1	1	8	4	
<i>Pediococcus</i>	4	n.r.	16	64	64	1	1	8	4	
<i>Leuconostoc</i>	2	n.r.	16	16	64	1	1	8	4	
<i>Lactococcus lactis</i>	2	4	32	64	32	1	1	4	8	
<i>Streptococcus thermophilus</i>	2	4	32	64	64	2	2	4	4	
<i>Bacillus</i> spp	n.r.	4	4	8	8	4	4	8	8	
<i>Propionibacterium</i>	2	4	64	64	64	0.5	0.25	2	2	
Other Gram +	1	2	4	16	8	0.5	0.25	2	2	
n.r. not required.										
<sup>a</sup> including <i>L. delbrueckii</i> , <i>L. helveticus</i>										
<sup>b</sup> including <i>L. fermentum</i>										
<sup>c</sup> including the homofermentative species <i>L. salivarius</i>										
	ampicillin	vancomycin	gentamycin	kanamycin	streptomycin	erythromycin	clindamycin	tylosine	tetracycline	chloramphenicol
<i>Enterococcus faecium</i>	2	4	32	1024	128	4	4	4	4	16
	ampicillin	gentamycin	kanamycin	streptomycin	tetracycline	chloramphenicol	nalidixic acid	sulfonamide	trimethoprim	apramycin
<i>Escherichia coli</i>	8	2	8	16	8	16	16	256	2	8

The content of Table 1 is reviewed on a regular basis and modified as necessary when new data are made available.

## 1.2. Quantitative methods for the MIC determination

For the assessment of the susceptibility to antimicrobials of bacterial strains, serial two-fold dilution procedures in agar or broth should be used and include relevant quality control strains. The tests should be performed according to internationally recognised standards such as the Clinical and Laboratory Standard Institute (CLSI; [www.clsi.org](http://www.clsi.org)), ISO standard or similar. An ISO standard is currently available for bifidobacteria and non-enterococcal lactic acid bacteria (ISO 10932:2010 (IDF 223:2010)). After incubation, the MIC is defined as the lowest concentration of the antimicrobial that inhibits bacterial growth. Qualitative or semi-qualitative methods to determine MIC indirectly, such as diffusion methods, are generally not acceptable. The existing body of scientific information related to that specific or related bacterial species must be considered when the procedure for MIC determination (dilution method, growth media and incubation conditions) is chosen, keeping in mind the possible interference of media and growth conditions.

## 2. DEFINING THE GENETIC BASIS OF RESISTANCE

The detection of the MIC above the cut-off values, identified by the FEEDAP Panel for one or more antimicrobials, requires further investigation to determine the nature of the resistance. Initially, it may be necessary to make the distinction between acquired and intrinsic resistance. This would only arise when there is limited or no information on the MIC distribution within the considered taxonomical unit. In these cases the structural nature and genetic basis of the resistance must be demonstrated analysing a representative selection of strains belonging to that taxonomical unit. Since intrinsic resistance is specific for a bacterial species or genus, an indispensable pre-requisite is the correct identification of the strain at species level by means of molecular taxonomy methods. Where all strains within a given taxonomic group show phenotypic resistance to an antimicrobial, such resistance can be considered intrinsic to the taxonomic group.

When a bacterial strain demonstrates higher resistance to a specific antimicrobial than the other strains of the same taxonomical unit, the presence of acquired resistance is indicated and additional information is needed on the genetic basis of the antimicrobial resistance. A single exception to this approach is *E. faecium* and ampicillin. Strains with ampicillin MIC >2 µg/mL are not considered suitable for feed use since this is a marker for the hospital associated clade and not considered safe.<sup>5</sup>

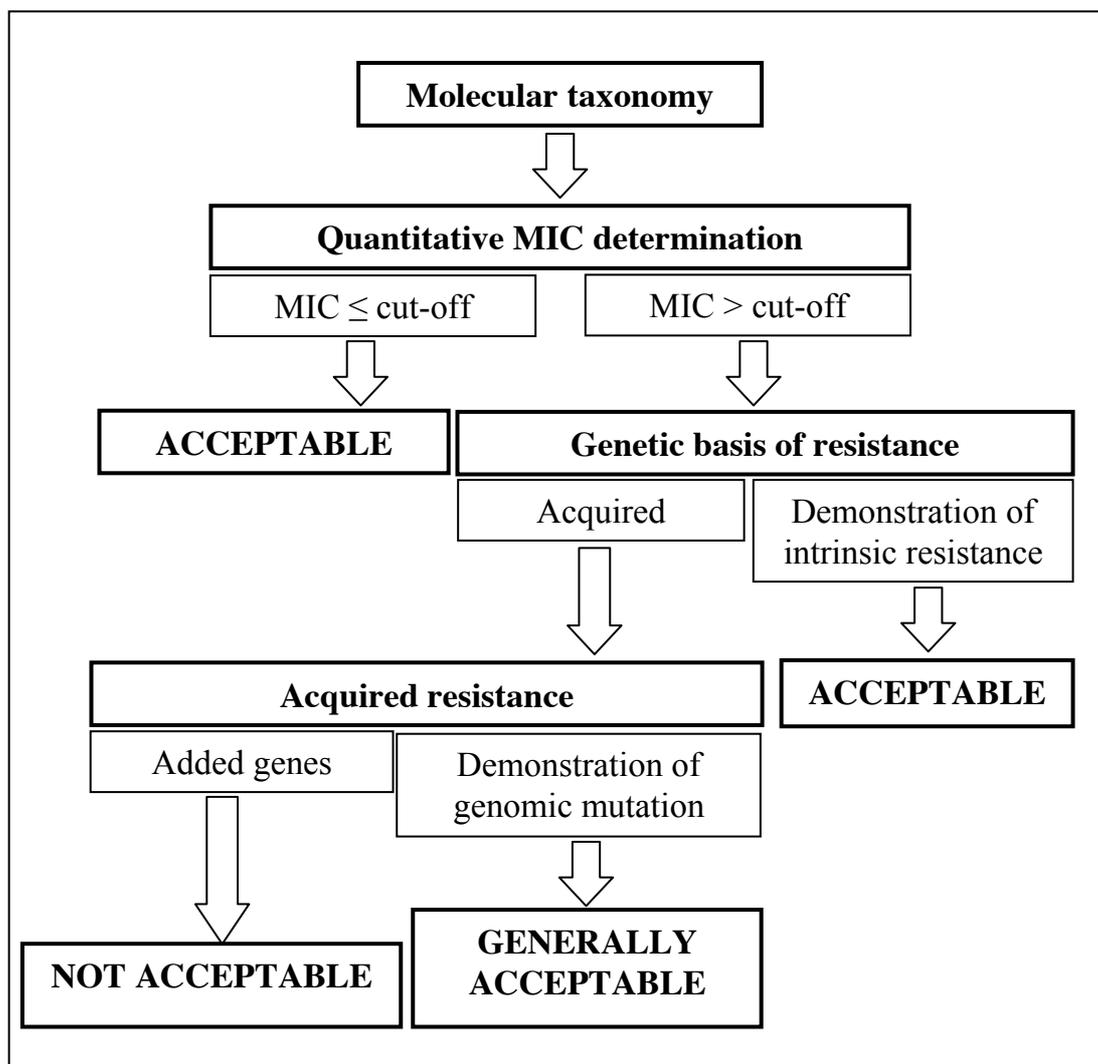
Acquired resistance can be due either to acquired genes (genes acquired by the bacteria via gain of exogenous DNA) or to the mutation of indigenous genes. The absence of known antimicrobial resistance genes (e.g. based on analysis utilising the ARBD) is not sufficient to explain the nature of the detected resistance.

The scheme proposed by the FEEDAP Panel for the antimicrobial resistance assessment of a bacterial strain used as a feed additive is shown in Figure 1.

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<sup>5</sup> See Guidance on the safety assessment of *Enterococcus faecium* in animal nutrition at <http://www.efsa.europa.eu/en/efsajournal/pub/2682.htm>

**Figure 1.** Proposed scheme for the antimicrobial resistance assessment of a bacterial strain used as a feed additive



The FEEDAP Panel considers that:

- Any bacterial strain carrying an intrinsic resistance to antimicrobial(s) presents a minimal potential for horizontal spread and thus, may be used as a feed additive
- Any bacterial strain carrying an acquired resistance to antimicrobial(s) that is shown to be due to chromosomal mutation(s) presents a low potential for horizontal spread and generally may be used as a feed additive
- Any bacterial strain carrying an acquired resistance to antimicrobial(s) that is shown to be due to the acquisition of genetic determinant(s) presents the greatest potential for horizontal spread and should not be used as a feed additive
- In the absence of information on the genetic nature of a demonstrated resistance, the strain should not be used as a feed additive

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