Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systems

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Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systems

Summary

Automated antimicrobial susceptibility testing devices are widely introduced in clinical microbiology laboratories in Spain, mainly using EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints. In 2007, a group of experts published recommendations for including antimicrobial agents and selecting concentrations in these systems. Under the patronage of the Spanish Antibiogram Committee (*Comité Español del Antibiograma*, COESANT) and the Study Group on Mechanisms of Action and Resistance to Antimicrobial Agents (GEMARA) from the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC), and aligned with the Spanish Plan against Antimicrobial Resistance (PRAN), a group of experts have updated this document. Main modifications from the previous version comprise the inclusion of new antimicrobial agents, adaptation of the ranges of concentrations to cover the EUCAST breakpoints and epidemiological cut-off values (ECOFFs), and the inference of new resistance mechanisms. This proposal should be considered by different manufacturers and users when designing new panels or cards. In addition, recommendations for selective reporting are also included. With this approach, the implementation of EUCAST breakpoints will be easier, increasing the quality of antimicrobial susceptibility testing data and their microbiological interpretation. It will also benefit surveillance as well as the clinical use of antimicrobials aligned with antimicrobial stewardship programs.

Key words: Antibiogram; Automated susceptibility testing systems; Antimicrobial concentrations; MICs.

Recomendaciones del Comité Español del Antibiograma (COESANT) para la selección de antimicrobianos y sus concentraciones en el estudio *in vitro* de la sensibilidad con métodos automáticos

Resumen

Los sistemas automáticos utilizados en el estudio de la sensibilidad a los antimicrobianos están introducidos en la mayoría de los laboratorios de Microbiología Clínica en España, utilizando principalmente los puntos de corte EUCAST (*European Committee on Antimicrobial Susceptibility Testing)*. En 2007, un grupo de expertos publicó unas recomendaciones para incluir antimicrobianos y seleccionar concentraciones en estos sistemas. Bajo el auspicio del Comité Español del Antibiograma (COESANT) y del Grupo de Estudio de los Mecanismos de Acción y Resistencia a los Antimicrobianos (GEMARA) de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC) y alineado con el Plan Nacional frente a la Resistencia a los Antibióticos (PRAN), un grupo de expertos ha actualizado dicho documento. Las principales modificaciones realizadas sobre la versión anterior comprenden la inclusión de nuevos agentes antimicrobianos, la adaptación de los rangos de concentraciones para cubrir los puntos de corte clínicos y los puntos de corte epidemiológicos (ECOFF) definidos por el EUCAST, y para la inferencia de nuevos mecanismos de resistencia. Esta propuesta debería ser considerada por los diferentes fabricantes y los usuarios cuando se diseñen nuevos paneles o tarjetas. Además, se incluyen recomendaciones para realizar informes selectivos. Con este enfoque, la implementación de los puntos de corte del EUCAST será más fácil, aumentando la calidad de los datos del antibiograma y su interpretación microbiológica. También será de utilidad para los estudios de vigilancia epidemiológica, así como para el uso clínico de los antimicrobianos, de acuerdo con los programas de optimización de uso de antimicrobianos (PROA).

Palabras clave. Antibiograma; Sistemas automáticos de detección de sensibilidad; concentraciones mínimas antimicrobianas; CMI.

Introduction

In 2007, the Study Group on Mechanisms of Action and Resistance to Antimicrobial Agents (GEMARA) and the Spanish Committee on Antimicrobial Susceptibility testing (named as MENSURA at that time) published, under the auspices of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC), "Recommendations for selecting antimicrobial agents for *in vitro* susceptibility studies using automatic and semiautomatic systems"[1]. Since then, significant efforts in Europe for harmonization of susceptibility testing methods and definition of breakpoint clinical criteria have been done led by the European Committee of Antimicrobial Susceptibility Testing (EUCAST)[2] and Spain has created the COESANT (*Comité Español del Antibiograma*) committee, which is the Spanish National Antimicrobial Committee (NAC) aligned with EUCAST[3]. Ever since, several new antimicrobials have been marketed, new resistance mechanisms have been described[4,5], and health authorities have promoted plans to address the problem of antimicrobial resistance[6]. In addition, professional societies, such as the SEIMC, have designed antimicrobial stewardship programs, for the better use of antimicrobial agents with the aim to curtail increasing prevalence of resistance[7]. Within these programs, the importance of antimicrobial susceptibility testing (AST), characterization of resistance mechanisms and analysis of clonal relationship are highlighted.

Unlike Northern European countries, but in common with many other countries worldwide, automated and semiautomated systems for AST are widely distributed in Spanish clinical microbiology laboratories. In a recent survey performed by the SEIMC in which 156 Spanish microbiology laboratories participated, 92.3% of them routinely used these systems (unpublished data). These data are consistent with those reported in recent multicentre quality control studies on antimicrobial susceptibility testing performed in Spain[8-10]. This wide distribution may have several advantages such as testing a high number of antimicrobial agents per isolate, and a better inference of resistance phenotypes with the aid of the so-called "expert systems" incorporated in these devices, the potential aggregation of data in MIC-based surveillance systems, and the reporting of MIC values to adapt patients' antimicrobial therapy applying pharmacokinetics-pharmacodynamics (PK-PD) criteria. Nevertheless, different manufacturers include diverse antimicrobials with different ranges of concentrations, which hinder some of these advantages, particularly the data aggregation in surveillance programs and in some cases, the inference of resistance mechanisms. In most cases, the design of panels or cards used in these systems does not follow a consensus procedure and only few documents address which antibiotics and concentrations should specifically be included[1,11,12].

In the current document we have updated the previous version of "Recommendations for selecting antimicrobial agents for *in vitro* susceptibility studies using automatic and semiautomatic systems"[1]. This new version has been led by COESANT, SEIMC and its study group GEMARA in the context of the Spanish Plan of Antimicrobial Resistance (PRAN, *Plan Nacional de Resistencia a los Antimicrobianos*) coordinated by the Spanish Agency of Medicines and Sanitary Products (AEMPS, *Agencia Española de los Medicamentos y Productos Sanitarios*)[13]. This manuscript was prepared by a group of experts and was submitted for public consultation through the COESANT and SEIMC websites. The manufacturers of automated AST devices marketed in Spain were also included in this consultation. The final version was constructed considering all these opinions.

Objectives and general recommendations for antimicrobial susceptibility testing using automated and semiautomated systems

The main objective in the elaboration of this document was to update the general recommendations for the selection of the antibiotics and their concentrations to be included in the AST panels used by automated or semiautomated systems commercialized in Spain that was published in 2007[1]. Likewise, suggestions for selective reporting of susceptibility testing results are also included (Table 1). The participating experts have also agreed on these recommendations of selective reporting. Recently, a European study has recognized this procedure as part of the stewardship programs in which clinical microbiology laboratories should actively participate through their informatics systems[14]. Obviously, this selective reporting can be facilitated with appropriate recommendations for antimicrobial testing against different microorganisms. In the European study, Spain was classified as a country with partial implementation of this procedure and the present document can facilitate criteria to enhance the number of laboratories with this practice.

However, although the document focuses on MIC-based automated systems, most of the established criteria related to the selection of the antibiotics to be included in the antibiogram and the reporting of the results can also be applied to the agar diffusion-based methods, either with disc or with MIC gradient strips. Since the first consensus document was published in 2007, a number of new antimicrobial agents have been approved, several indications have been changed or expanded, and different breakpoints have been significantly modified making it necessary to revise the previous document and to include new antimicrobials (Supplementary tables S1-S9). Moreover, the use of traditional susceptible clinical breakpoints does not necessarily recognize isolates with low-level resistance mechanisms[15,16] and recognition of wild-type populations and the definition of the epidemiological cut-off values (ECOFF) have been widely used.

More recently, EUCAST has modified definitions of interpretive clinical categories [susceptible (S), intermediate (I) and resistant (R)]. These new definitions mainly affect to the intermediate category, which is now interpreted as "susceptible, increased exposure" which occurs when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection[17.] As a consequence, EUCAST has modified some breakpoints and others only applied when high exposure of the microorganisms to the agent is considered (i.e. most β-lactams and *Pseudomonas aeruginosa*)[18]. In addition, EUCAST has introduced for some organism-agent combinations a new concept which has been designed as an Area of Technical Uncertainty (ATU). It corresponds to an MIC value and/or zone diameter interval where the categorisation is doubtful. Further explanations and how to deal with results in the ATU are explained in the EUCAST breakpoint tables[18].

Automated and semiautomated systems should have a minimal set of characteristics making them appropriate to fulfill the objectives for which they were designed, allowing the application of the general criteria used in the antibiogram interpretive reading[19,20]. These criteria are summarized in the following points:

a) Availability of the identification of the microorganism under study which is necessary for the antibiogram interpretive reading and for the inference of the resistance mechanisms[18,19]. This can be achieved through either biochemical tests included in the same panel/card or an additional panel/card or through any other method, including MALDI-TOF mass spectrometry. When the automated AST systems are linked to MALDI-TOF mass spectrometry devices, it would be desirable that this information could be also used for epidemiological purposes in the identification of antimicrobial resistance mechanisms and bacterial clones[21].

b) Incorporate an informatics application with the capacity to interpret MIC values (or inhibition zones) thus establishing the susceptible (S), intermediate (I) and resistant (R) clinical categories. This software should apply criteria recommended by EUCAST[18], although it is recommended that it may allow the access to the criteria established by other susceptibility testing committees, such as CLSI[12], or those specifically defined by COESANT (Supplementary tables S1-S9).

c) Incorporate the so-called “expert systems” for antibiogram interpretive reading, able to recognize phenotypes of resistance to multiple antibiotics from the same or different families and inferring the underlying resistance mechanisms[19,20].

d) Allow a bidirectional connection with the Laboratory Informatics System (LIS), required not only for the transference of AST data but also to receive the necessary information for the management of results, particularly with the aim of conducting epidemiological analysis, infection control studies, and antimicrobial stewardship programs[7]. Ideally, these systems should be compatible for the connection to national and international surveillance databases. The incorporation of these “expert” programs facilitates daily work and decreases the workload. Moreover, these devices should also be able to connect with programs using databases for infection control programs.

Antimicrobial selection criteria

The inclusion of antimicrobials in the panels of automated susceptibility testing systems is mainly conditioned by their clinical interest. However, other points should also be considered, such as the type of microorganism or the need of interpretation of resistance mechanisms. In our document, the selection of the different compounds was performed considering the following criteria:

Microbiological criteria

The antimicrobials to be included in the AST panels, regardless of the type of automated system, are those required for the interpretive reading of the susceptibility pattern and for the inference of underlying resistance mechanisms[22-24].The selection of antimicrobials is also intended to contribute to the inference of complex phenotypes causing multidrug-resistant profiles, such as those derived from the simultaneous presence of different resistance mechanisms affecting various members of a unique family, e.g. β-lactam antibiotics[19,25]. Moreover, certain antimicrobials, such as tetracycline or chloramphenicol, have been mainly selected for epidemiological monitoring purposes.

In the case of antimicrobials belonging to families with several members, e.g. cephalosporins and fluoroquinolones, selected compounds are considered as representative of the antimicrobial activity of the group, additionally allowing the deduction of the activity of those that are not included in the panels as well as the assumption of the presence of resistance mechanisms[23]. The only purpose of including certain antimicrobials, in some cases without clinical use such as nalidixic acid, ~~have the only purpose~~ is to act as a marker of a primary resistance step which indicates the presence of mutations that can preclude the use of fluoroquinolones in subsequent rounds of topoisomerase mutations[25]. Similarly, kanamycin resistance alerts for the presence of some aminoglycoside-modifying enzymes affecting amikacin while the association of clavulanic acid with a third or fourth generation cephalosporin helps to identify the presence of an extended-spectrum-β-lactamase[26]. Another example is cefoxitin in panels for the study of Enterobacterales, which help to predict the presence of AmpC β-lactamases (either chromosomally or plasmidic encoded) and/or a deficit in outer membrane permeability[24,27]. In the case of staphylococci, cefoxitin has been included as it performs better than oxacillin as a marker for detecting the presence of the *mec* genes causing methicillin resistance[28].

The emergence and sudden dispersion of a resistance mechanism may increase the interest for the study of a particular compound. This is the case of the acquired carbapenemases in gram-negative bacilli that has raised interest in aztreonam as an indicator of the presence of metallo-β-lactamases, particularly when the study is simultaneously performed with ceftazidime, the combination of ceftazidime-avibactam and carbapenems[29]. Additionally, tigecycline, ~~a~~ glycylcycline derivative of minocycline, has been included as it can be a therapeutic option against some multidrug-resistant gram-negatives[30].

In the case of staphylococci, the simultaneous presence of a concentration of erythromycin together with one of clindamycin in the same well is intended to detect inducible macrolide-clindamycin resistance[31]. Moreover, daptomycin and linezolid have been included as they represent last-resort line therapeutic options against gram-positive cocci[32]. More recently, certain panel/card manufacturers have also included ceftaroline, a new cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*[33].

Pharmacokinetic/pharmacodynamic (PK/PD) criteria

EUCAST uses PK/PD Monte Carlo simulations as a key component of its breakpoints' setting process for old and new antimicrobials. The PK/PD breakpoint is the MIC value considered necessary to achieve a probability of target attainment of >95% and applies to specific dosage regimens[34]. The PD targets predicting maximum efficacy of the antimicrobial, for example 50% for the percentage of the dosing interval during which the serum concentration exceeds the MIC (%T>MIC) of a β-lactam, 100% for an area under the concentration-time curve/MIC ratio (AUC_{24}/MIC) of a fluoroquinolone, or 10 times for peak plasma concentration/MIC ratio (C_{max}/MIC) of an aminoglycoside, expressed as a function of the unbound drug concentration.

The magnitude of the PD target can vary among bacterial species[35]. A clinical breakpoint setting process requires knowledge of the wild-type distribution of MICs, assessment of the pharmacokinetic/pharmacodynamic (PK/PD) parameters, and study of the clinical outcome of the infected patient when the antimicrobial agent is used[34,36]. The use of PK parameters in the simulations considering different populations (healthy volunteers or critically ill patients with different degrees of renal function), various dose regimens and multiple infection sites (urinary concentrations of antimicrobial agents are higher than serum concentrations over a dosing interval) will result in different breakpoints. EUCAST has defined several breakpoints which are only valid for isolates from uncomplicated urinary tract infections (e.g. amoxicillin-clavulanic acid MIC breakpoint S ≤32 mg/L for Enterobacterales)[21].

PK/PD data and MIC distributions comprise the primary data to support decisions concerning revised breakpoints. For β-lactam antimicrobials and *P. aeruginosa*, susceptible and intermediate (susceptible, increased exposure) breakpoints are established to ensure optimal exposures with specific dosage regimens[17]. Additionally, the MIC and associated breakpoints are a better means for guiding selection of therapy for individual patients[37-39].

It is important to consider that accuracy of the automated susceptibility tests depends, among other factors, on the concentration of the antibiotics, as the lower the concentration, the higher the error rates[40].

Clinical criteria

Information about the bacterial susceptibility pattern is essential to guide the selection of antibiotic treatment. Furthermore, it is well known that there are many important host factors determining the clinical outcome. Several clinical data demonstrate that an *in vitro* susceptible result often predicts therapeutic success. However, even in patients with sepsis due to a microorganism with an *in vitro* resistant result, resistance *in vivo* with concomitant clinical failure cannot be always predicted[41-42]. Therefore, and from a clinical point of view, the most commonly used antibiotics or at least one representative of the antibiotic family that predicts the activity of the other members, should be included in the routine susceptibility report as occurs with first generation cephalosporins. This subrogated use is also claimed in the case of new antimicrobials when they are not yet included in testing devices. This is the case of tedizolid and linezolid or dalbavancin and vancomycin

In addition, when the MIC is high but within the susceptibility range suggesting the presence of a specific low-level resistance mechanism, or when clinical data indicate worse outcome when the MIC is high, alternative antibiotics should be tested. For instance, when MICs of carbapenems for *Klebsiella* spp. or *E. coli* are high, suggesting the presence of a carbapenemase, alternative antibiotics including colistin, tigecycline or fosfomycin should be tested[43]. A similar approach might occur when considering MICs of vancomycin >1 and ≤2 mg/l for *S. aureus* causing bacteremia, which has been associated in some studies to a worse outcome[44], it is recommended to report data concerning the susceptibility status of possible alternatives.

Nowadays, new antimicrobials, such as ceftazidime-avibactam or ceftolozane-tazobactam for gram-negatives as well as dalbavancin, telavancin or oritavancin for gram-positives, have been included in testing devices AST of these compounds are recommended not only to obtain information of new therapeutic alternatives but also to generate routine epidemiological data.

Criteria for the selection of antimicrobial concentrations

The selection of the concentrations proposed for each antimicrobial agent has been made with the objective of covering the breakpoints used for defining clinical categories (susceptible, intermediate and resistant) established by EUCAST[17]. For certain antimicrobials, specific COESANT recommendations have been considered (specified in the supplementary tables of this document). In addition, since the number of wells available in the different panels or cards varies from one manufacturer to another, more concentrations are also recommended. All these concentrations are classified in different groups. The first one (indicated in bold in (Supplementary tables S1-S9).) includes the concentrations that would be essential to respond to the previous objective (covering EUCAST breakpoints) and therefore, should always be included in the susceptibility testing panels. This range is mainly intended to include the concentration defining the resistance breakpoint and one dilution below the susceptible breakpoint. In addition, there are other concentrations (not indicated in bold) that could be added to encompass the ECOFF value to detect wild-type populations or to facilitate epidemiological surveillances, especially of microorganisms with low-level resistance mechanisms. This approach also contributes to a better interpretive reading of the antibiogram[19,20].

Definition of categories and groups of antimicrobial agents tested in the antibiogram

Five different categories of antimicrobials have been established (A to E) with the recommendation of inclusion in the panels and selective reporting depending on the clinical relevance of the antimicrobial tested, type of patient or type of infection. Moreover, these recommendations also consider the interest of the antimicrobials for the interpretive reading of the antibiogram and the inference of resistance mechanisms (Table 1). A specific category (category D) has been defined for antimicrobials that are recommended to be routinely studied and reported in urine isolates. These antimicrobials normally have clinical breakpoints specifically adapted for non-complicated urinary tract infections[12,21], and some manufacturers offer specific panels for microorganisms involved in these infections.

The last category (category E) is exclusively established for those antimicrobials recommended to be studied but not reported. They are useful for the detecion of antimicrobial resistance mechanisms, such as nalidixic acid and *gyrA* and topoisomerase IV mutations in gram-negative organisms, application of an expert rule or inference of a resistance mechanism, such as the combinations of third or fourth generation cephalosporins with clavulanic acid, or as subrogated markers of the susceptibility result of other antimicrobials[19,20,25,26]. Overall, they are not relevant for clinical purposes.

Concluding remarks

Spain is a country where automated susceptibility testing systems are widely distributed and every day, thousands of AST data are produced by clinical microbiology laboratories. These data, as it is quoted in a European survey and in quality control studies performed in Spain, are selectively reported by an important number of laboratories using EUCAST breakpoints[8-10,14]. All these data are mainly used for clinical purposes for patients' treatments. Moreover, they should also be useful for surveillance and for tracking the evolution of antimicrobial resistance at local or national level if compiled in a common database, which is an objective of the Spanish National Plan against Antimicrobial Resistance (PRAN)[13]. However, its development might be complex due to the lack of homogeneity in the number of antibiotics tested for each microorganism and also, importantly, in the concentrations tested for each antimicrobial, which precludes not only fully implementation of the EUCAST breakpoints but also data compilation.

Considering the criteria explained in the previous paragraphs, we propose ~~in this document~~ those antimicrobial agents and concentrations to be used in the study of *in vitro* susceptibility of the different microorganisms when automated systems are used (Supplementary tables S1-S9). Different manufacturers and users should consider this proposal when designing or using new panels. We believe that with this approach, the implementation of EUCAST breakpoints will be easier, increasing the quality of data and their microbiological interpretation[44]. Finally, it will benefit epidemiological surveillances as well as the clinical use of antimicrobials aligned with the stewardship programs.

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Table 1. Categories used for the inclusion of the antimicrobial agents in susceptibility testing panels for automated systems

Categories	Definitions
A	Antimicrobials that must be routinely studied and reported. They are relevant for both clinical purpose and for the process of interpretive reading of the antibiogram.
B	Antimicrobials that must be routinely studied but selectively reported. They are useful for the process of interpretive reading of the antibiogram and should be selectively reported according to the type of patient, type of infection or the inferred resistance mechanism.
C	Antimicrobials that should be selectively studied and reported acording to the type of patient, type of infection or to the inferred resistance mechanism.
D	Antimicrobials that are recommended to be routinely studied and reported in urine isolates
E	Antimicrobials that should be studied but not reported. They are useful for the detecion of antimicrobial resistance mechanisms, aplication of an expert rule or as subrogate markers of the susceptibility testing result of other antimicrobials.

Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systems

TABLE S1. Antibiotics and concentrations recommended for the susceptibility testing of Enterobacterales

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
ß-lactams	Ampicillin	2-**4**-**8**-**16**-32	A	Report as amoxicillin.
	Amoxicillin-clavulanic acid	**2/2-4/2-8/2-16/2-32/2**	A	For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L. ECOFF has not yet been defined. Breakpoints for uncomplicated urinary tract infections has been defined as S ≤32/2 mg/L and R >32/2.
	Ticarcillin	4-**8**-**16**-**32**-64	E	It can be useful to infer the presence of resistance mechanisms, such as TEM-1, chromosomal AmpC hyperproduction or plasmid-mediated AmpC.
	Piperacillin-tazobactam	4/4-**8/4-16/4-32/4**-64/4	A	
	Cefazolin	2-4-**8**-**16**-32	D	It can be used as a surrogate test for uncomplicated urinary tract infection treated with oral cephalosporins. Breakpoints have not been defined by EUCAST; those shown are recommended by COESANT. ECOFF has not yet been defined.
	Cefuroxime	1-2-**4**-**8**-**16**-32	A	Breakpoints for iv and oral (uncomplicated urinary tract infections) formulations are the same. iv defined for *E. coli*, *K. pneumoniae* and *P. mirabilis* only. Oral breakpoints defined for uncomplicated urinary tract infection only.
	Cefoxitin	4-**8**-**16**-32	E	Breakpoints have not been defined by EUCAST. Cefoxitin MIC >8 mg/L may indicate high-level expression of AmpC β-lactamases (with the exception of ACC β-lactamases) or, in some organisms, porin deficiency.
	Ceftazidime	**0.5-1-2-4-8**-16-32	A	
	Ceftazidime-clavulanic acid	**1/4**-2/4-4/4-**8/4**	E	Recommended for confirmation of ESBL production in *Escherichia coli*, *Klebsiella* spp., *P. mirabilis*, *Salmonella* spp., and *Shigella* spp.
	Cefotaxime	**0.25-0.5-1-2-4-8**-16-32	A	
	Cefotaxime-clavulanic acid	**1/4**-2/4-4/4-**8/4**	E	Recommended for confirmation of ESBL production in *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Salmonella* spp., and *Shigella* spp.
	Cefixime	0.5-**1-2-4-8**-16	C	Breakpoints defined for uncomplicated urinary tract infection only. ECOFF has not yet been defined.
	Cefepime	0.125-**0.25-0.5-1-2-4-8**-16-32	A	
	Cefepime-clavulanic acid	**1/4**-2/4-4/4-**8/4**	E	Recommended for confirmation of ESBL production in *Enterobacter* spp., *Citrobacter freundii* complex, *Morganella morganii*, *Providencia stuartii*, *Serratia* spp., and *Hafnia alvei*. It is also useful for *E. coli* hyperproducing chromosomal AmpC or producing plasmidic AmpC.
	Ceftolozane-tazobactam	0.5/4-**1/4-2/4-4/4**-8/4	C	ECOFF has not yet been defined. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
	Ceftazidime-avibactam	**0.5/4-1/4-2/4-4/4-8/4**-16/4	C	ECOFF has not yet been defined. It can be used to infer the presence of class A and class D carbapenemases in isolates that are resistant to carbapenems. For susceptibility testing purposes, the

				concentration of avibactam is fixed at 4 mg/L.
	Aztreonam	0.25-**0.5-1-2-4-8**-16-32	A	
	Imipenem	0.25-**0.5-1-2-4-8**-16	A	>1 mg/L has been defined as screening cut-off for carbapenemase production. Breakpoints for *M. morganii*, *Proteus* spp. and *Providencia* spp. are S ≤ 0.125 mg/L and R >4 mg/L
	Meropenem	**0.125-0.25-0.5-1-2-4-8**-16	A	>0.125 mg/L has been defined as screening cut-off for carbapenemase production.
	Meropenem-vaborbactam	**0.125-0.25-0.5-1-2**-4-8-16	C	ECOFF has not yet been defined. It can be used to infer the presence of class A carbapenemases in isolates that are resistant to carbapenems. For susceptibility testing purposes, the concentration of vaborbactam is fixed at 8 mg/L.
	Ertapenem	**0.06-0.125-0.25-0.5-1-2**-4	A	>0.125 mg/L has been defined as screening cut-off for carbapenemase production. ECOFF has not yet been defined.
Aminoglycosides	Gentamicin	**2-4-8**	A	Breakpoints are based on once daily administration of high dose.
	Tobramycin	**2-4-8**	A	
	Amikacin	2-4-**8-16-32**	A	
Quinolones	Nalidixic acid	8-**16**-32	E	Breakpoints have not been defined. It can be useful to infer the presence of mutations in topoisomerases and/or plasmid-mediated fluoroquinolone resistance genes.
	Ciprofloxacin	**0.06-0.125-0.25-0.5-1**-2	A	
	Norfloxacin	**0.25-0.5-1-2**-4	D	Breakpoints defined for uncomplicated urinary tract infection only.
Tetracyclines	Minocycline	0.5-**1-2-4-8**	C	ECOFF has not yet been defined. Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
	Tigecycline	0.25-**0.5-1-2**-4	B	ECOFF has not yet been defined.
	Eravacycline	0.25-**0.5-1-2**-4	C	ECOFF has not yet been defined.
Others	Azithromycin	**16-32**	C	Only for *Salmonella* and *Shigella* spp. Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
	Nitrofurantoin	32-**64**-128	D	Breakpoints defined for *E. coli* in uncomplicated urinary tract infection only.
	Cotrimoxazole	1/19-**2/38-4/76**-8/152	A	
	Fosfomycin	**8-16-32-64**-128	B	Breakpoints for oral (uncomplicated urinary tract infections) and iv formulations are the same.
	Chloramphenicol	4-**8-16**-32	C	It can be useful to infer the presence of certain efflux pumps or to study in multi-drug resistant isolates.
	Colistin	0.5-1-**2-4**-8	B	

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. Underlined numbers indicate the ECOFF values, when lacking, this is due to the absence of definition by EUCAST. When different ECOFF values exist for the different enterobacterial species, the *E. coli* ECOFF value is indicated in the table. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category.

TABLE S2. Antibiotics and concentrations recommended for the susceptibility testing of *Pseudomonas* spp.

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
ß-lactams	Ticarcillin	8-**16-32-**64	E	Breakpoints are based on high dose therapy. Not currently used in the clinical setting but useful for the inference of resistance mechanisms such as acquired β-lactamases and/or efflux pump overexpression. ECOFF has not yet been defined.
	Piperacillin	4-**8-16-32-**64	C	Breakpoints are based on high dose therapy.
	Piperacillin-tazobactam	4/4-**8/4-16/4-32/4-**64/4	A	Breakpoints are based on high dose therapy. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
	Ceftazidime	1-**2-4-8-16-**32	A	Breakpoints are based on high dose therapy.
	Cefepime	1-**2-4-8-16-**32	A	Breakpoints are based on high dose therapy.
	Ceftolozane-tazobactam	0.25/4-0.5/4-**1/4-2/4-4/4-8/4-**16/4	C	Useful for the detection of resistance mechanisms, particularly acquired β-lactamases. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
	Ceftazidime-avibactam	0.5/4-1/4-**2/4-4/4-8/4-16/4-**32/4	C	ECOFF has not yet been defined. Useful for the detection of resistance mechanisms, particularly acquired β-lactamases.
	Aztreonam	**1-2-4-8-16-**32	A	Breakpoints are based on high dose therapy. Useful for the detection of resistance mechanisms such as acquired MBLs.
	Imipenem	0.5-**1-2-4-8-**16	A	Breakpoints are based on high dose therapy.
	Meropenem	0.25-0.5-**1-2-4-8-**16	A	
	Meropenem-vaborbactam	**0.125-0.25-0.5-1-2**-4-8-16	C	ECOFF has not yet been defined. For susceptibility testing purposes, the concentration of vaborbactam is fixed at 8 mg/L.
Aminoglycosides	Gentamicin	**2-4-8**	A	Breakpoints are based on once daily administration of high dose therapy.
	Tobramycin	**1-2-4-8**	A	
	Amikacin	2-4-**8-16-32**	A	
Fluoroquinolones	Ciprofloxacin	0.125-**0.25-0.5-1-2**-4	A	Breakpoints are based on high dose therapy.
	Levofloxacin	0.25-0.5-**1-2-4**-8	C	Breakpoints are based on high dose therapy.
Others	Fosfomycin	16-**32-64-128**-256	C, D	Breakpoints are not defined. Infections caused by wild type isolates (ECOFF 128 mg/L) have been treated with combinations of fosfomycin and other agents.
	Colistin	0.5-1-2-**4-8**	B	

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. Underlined numbers indicate the ECOFF values, when lacking is due to the absence of definition of this value by EUCAST. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark grey corresponds to concentrations within resistant (R) category. MBL: metallo-β-lactamases

TABLE S3. Antibiotics and concentrations recommended for the susceptibility testing of *Acinetobacter* spp.

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
ß-lactams	Ampicillin-sulbactam	4/2-**8/4-16/8**-32/16	B	Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
	Piperacillin-tazobactam	4/4-**8/4-16/4-32/4**-64/4	B	Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
	Ceftazidime	2-**4-8-16**-32	B	Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
	Imipenem	0.5-**<u>1</u>-2-4-8**-16	A	Breakpoints are based on high dose therapy.
	Meropenem	0.25-0.5-**1-<u>2</u>-4-8**-16	A	
Aminoglycosides	Gentamicin	2-**<u>4</u>-8**	A	Breakpoints are based on once daily administration of high dose therapy.
	Tobramycin	1-**2-<u>4</u>-8**	A	
	Amikacin	2-**4-<u>8</u>-16**-32	A	
Fluoroquinolones	Ciprofloxacin	0.06-0.125-0.25-**0.5-<u>1</u>-2**	A	
	Levofloxacin	0.25-**<u>0.5</u>-1-2-4**-8	C	
Tetracyclines	Doxycycline	**2-4-8**-16	A	ECOFF has not yet been defined Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
	Minocycline	**2-4-8**-16	A	
	Tigecycline	0.25-**0.5-1-2**-4	A	
Others	Cotrimoxazole	**<u>1/19</u>-2/38-4/76**-8/152	B	
	Colistin	0.5-**1-2-4**-8	A	
	Rifampicin	**2-4-8**	C	Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. <u>Underlined numbers</u> indicate the ECOFF values, when lacking is due to the absence of definition of this value by EUCAST. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category.

Table S4. Antibiotics and concentrations recommended for the susceptibility testing of *Stenotrophomonas maltophilia.*

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
ß-lactams	Imipenem	0.5-**1**-**2**-**4**-**8**-16	E	*S. maltophilia* is intrinsically resistant to all β-lactams. Imipenem MIC values >8 mg/L supports identification.
Fluoroquinolones	Levofloxacin	0.25-0.5-**1**-**2**-**4**-**8**	A	ECOFF has not yet been defined. Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
Tetracyclines	Minocycline	1-2-**4**-**8**-**16**	A	Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
Others	Cotrimoxazole	1/19-**2/28**-**4/76**-**8/152**	A	

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. Underlined numbers indicate the ECOFF values, when lacking is due to the absence of definition of this value by EUCAST. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category.

Table S5. Antibiotics and concentrations recommended for the susceptibility testing of non-fermentative Gram-negative bacilli other than *Pseudomonas* spp., *Acinetobacter* spp. and *Stenotrophomonas maltophilia*. The ECOFF values are not indicated due to this table is for miscellaneous microorganisms for which in many cases ECOFFs have not been defined.

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
ß-lactams	Ticarcillin	8-**16**-**32**-64	E	Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT. For their definition, general criteria included in the EUCAST guidance document "Antimicrobial susceptibility tests on groups of organisms or agents for which there are no EUCAST breakpoints (http://www.eucast.org/clinical_breakpoints/when_there_are_no_breakpoints) have been followed It is also recommended to consult the EUCAST intrinsic resistance tables for those species included in these tables (http://www.eucast.org/expert_rules_and_intrinsic_resistance/)
	Piperacillin-tazobactam	4/4-**8/4**-**16/4**-**32/4**-64/4	A	
	Ceftazidime	1-**2**-**4**-**8**-**16**-32	A	
	Cefepime	1-**2**-**4**-**8**-**16**-32	B	
	Aztreonam	0.5-1-2-**4**-**8**-**16**-32	B	
	Imipenem	0.5-1-2-**4**-**8**-**16**	A	
	Meropenem	0.5-1-2-**4**-**8**-**16**	A	
Aminoglycosides	Gentamicin	**2**-**4**-**8**	E	
	Tobramycin	**1**-**2**-**4**-**8**	A	
	Amikacin	2-4-**8**-**16**-**32**	A	
Fluoroquinolones	Ciprofloxacin	0.125-**0.25**-**0.5**-**1**-**2**-**4**	A	
	Levofloxacin	**0.25**-**0.5**-**1**-**2**-**4**-8	A	
Tetracyclines	Minocycline	2-**4**-**8**-**16**	A	
Others	Cotrimoxazole	**1/19**-**2/38**-**4/76**-**8/152**	A	
	Chloranfenicol	4-**8**-**16**-**32**	C	
	Colistin	0.5-1-**2**-**4**-8	C	

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category. Breakpoints have not been defined by EUCAST for these microorganisms; PK/PD breakpoints were used when available and when not COESANT recommendations were followed.

TABLE S6. Antimicrobial agents and concentrations for testing and reporting the susceptibility for *Staphylococcus* spp. ECOFF values in this table are those from *S. aureus.*

Antimicrobials		**Concentrations (mg/L)**	**Category**	**Comments**
ß-lactams	Penicillin	**0.06-0.125-0.25**-0.5-1	A	
	Oxacillin (*S. aureus*, *S. lugdunensis*, *S. saprophyticus*)	**0.25-0.5-1-2**-4-8	A	*S. aureus*, *S. lugdunensis* and *S. saprophyticus* with oxacillin MICs >2 mg/L are mostly methicillin resistant due to the presence of the *mecA* or *mecC* genes.
	Oxacillin (CNS other than *S. lugdunenis*, *S. saprophyticus*)	**0.25-0.5-1-2-4**-8	A	Coagulase-negative staphylococci other than *S. saprophyticus* and *S. lugdunensis* with oxacillin MICs >0.25 mg/L are mostly resistant due to the presence of the *mecA* gene.
	Cefoxitin	2-**4**-8	E	*S. aureus* and *S. lugdunensis* with cefoxitin MIC values >4 mg/L and *S. saprophyticus* with cefoxitin MIC values >8 mg/L are methicillin resistant, mostly due to the presence of the *mecA* or *mecC* genes. For staphylococci other than *S. aureus*, *S. lugdunensis* and *S. saprophyticus*, the cefoxitin MIC is a poorer predictor of methicillin resistance than the disk diffusion test.
	Ceftaroline	**0.25-0.5-1-2**-4	B	Methicillin-susceptible isolates can be reported susceptible to ceftaroline or ceftobiprole without further testing.
	Ceftobiprole	**0.5-1**-2-**4**-8	B	
Aminoglycosides	Gentamicin	0.5-**1-2-4**-8	A	Breakpoints are based on once daily administration of high dose therapy.
	Tobramycin	0.5-**1-2-4**-8	A	
Glycopeptides	Vancomycin	**0.5-1-2-4-8**-16	A	*S. aureus* with vancomycin MIC values of 2 mg/L are on the border of the wild type distribution and there may be an impaired clinical response.
	Teicoplanin (*S. aureus*)	**1-2-4-8**-16-32	A	
	Teicoplanin (CNS)	**1-2-4-8**-16-32	A	ECOFFs have not yet been defined.
Lipoglycopeptides	Telavancin (MRSA)	0.06-**0.125-0.25**	C	Only approved for MRSA. MICs must be determined in the presence of polysorbate-80 (0.002% in the medium for broth dilution methods; agar dilution methods have not been validated).
	Dalbavancin	0.06-**0.125-0.25**	C	MICs must be determined in the presence of polysorbate-80 (0.002% in the medium for broth dilution methods; agar dilution methods have not been validated).
	Oritavancin (*S. aureus*)	0.06-**0.125-0.25**	C	

Lipopeptides	Daptomycin	**0.5-1-2-4**	A	MICs must be determined in the presence of Ca^{2+} (50 mg/L) in the medium for broth dilution methods; agar dilution methods have not been validated.
Fluoroquinolones	Ciprofloxacin	**0.5-1-2**-4	A	Breakpoints are based on high dose therapy.
	Levofloxacin	**0.5-1-2**-4	A	
	Moxifloxacin	**0.125-0.25-0.5-1**-2-4	C	
Macrolides and lincosamides	Erythromycin	**0.5-1-2-4**	A	Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin.
	Clindamycin	0.125-**0.25-0.5-1-2**	A	
	Erythromycin-Clindamycin	**4/0.5**	E	Inducible clindamycin resistance test. In a positive test, report as clindamycin resistant and consider adding this comment to the report: "Clindamycin may still be used for short-term therapy of less serious skin and soft tissue infections as constitutive resistance is unlikely to develop during such therapy".
Tetracyclines	Tetracycline	0.5-**1-2**-4-8	B	Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, although some resistant to tetracycline may still be susceptible to minocycline and/or doxycycline.
	Minocycline	0.125-**0-25-0.5-1-2**	C	
	Tigecycline	0.25-**0.5-1**-2	C	
	Eravacycline	0.125-**0.25-0.5-1**	C	ECOFFs have not yet been defined.
Oxazolidinones	Linezolid	1-**2-4-8**	A	Isolates susceptible to linezolid can be reported susceptible to tedizolid.
	Tedizolid	**0.25-0.5-1-2**	B	
Others	Fosfomycin	**8-16-32-64**-128	B	Use in combination in serious infections (i.e endocarditis). Breakpoints are not defined for oral use.
	Cotrimoxazole	0.25/4.75-**0.5/9.5-1/19-2/38-4/76**-8/152	A	
	Rifampicin	0.01-**0.03-0.06-0.125-0.25-0.5-1**-2	B	
	Mupirocin	0-5-**1-2-4-256**	B	Breakpoint related to nasal decolonization of *S. aureus*. Intermediate isolates are associated to short term suppression (useful preoperatively) but unlike susceptible isolates, long-term eradication rates are low.
	Fusidic acid	0.25-**0.5-1-2**-4	B	
	Nitrofurantoin	16-**32-64-128**	D	

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. Underlined numbers indicate the ECOFF values, when lacking is due to the absence of definition of this value by EUCAST. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category. CNS: coagulase negative staphylococci.

Table S7. Antimicrobial agents and concentrations for testing and reporting the susceptibility for *Streptococcus pneumoniae* and other streptococci (including viridans streptococci and β-haemolytic groups A, B, C and G). Unless indicated in comments, breakpoints in this table are those recommended for *S. pneumoniae.* ECOFF values have not been indicated in this table as different values have been defined for different species/group.

Antimicrobial agents		Concentrations (mg/L)	Category			Comments
			S. pneumoniae	**β-haemolytic streptococci**	**Viridans group streptococci**	
ß-lactámicos	Penicillin	**0.06-0.12-0.25-0.5-1-2-4**	A	A	A	Breakpoints (*S. pneumoniae*) are those recommended for meningitis. For infections other than meningitis oral penicillin V breakpoints are S ≤0.06 mg/L / R>2 mg/L, and penicillin parenteral breakpoints are S ≤2 mg/L / R>4 mg/L.
	Ampicillin	0.25-**0.5-1-2-4**	A	A	A	Breakpoints (*S. pneumoniae*) are those recommended for infections other than meningitis.
	Cefuroxime	0.125-**0.25-0.5-1-2**	C	C	C	Breakpoints (*S. pneumoniae*) defined for oral administration are one dilution step lower than those for i.v administration.
	Cefotaxime	**0.125-0.25-0.5-1-2-4**	A	A	A	
	Cefepime	**0.25-0.5-1-2-4**	C	C	C	
	Ceftaroline	**0.25-0.5-1**	B	C	C	
	Meropenem	**0.125-0.25-0.5-1-2-4**	B	C	C	Meropenem is the only carbapenem recommended for meningitis.
	Ertapenem	**0.25**-0.5-**1-2-4**	C	C	B	
	Imipenem	0.06-**0.125-0.25-0.5-1-2**	C	C	C	
Glycopeptides	Vancomycin	**0.5-1-2-4**	C	C	A	
Lipopeptides	Daptomycin	**0.5-1-2**	-	C	C	Breakpoints have not been defined by EUCAST for *S. pneumoniae*, those shown are recommend by COESANT, which are also the same for β-haemolytic groups A, B, C and G.
Lipoglycopeptides	Dalbavancin	**0.06-0.125-0.25-0.5**	-	C	C	Breakpoints have not been defined by EUCAST for *S. pneumoniae*, those shown are for *S. anginosus* group and β-heamolytic groups A, B, C and G.
Quinolones	Levofloxacin	**0.5-1-2-4**	A	A	A	Breakpoints are based on high dose therapy. Breakpoints

						have not been defined by EUCAST for viridans group streptococci, those shown are recommended for *S. pneumoniae*.
	Moxifloxacin	**0.25-0.5-1-2**	C	C	C	
Macrolides and lincosamides	Erithromycin	**0.25-0.5-1-2**	A	A	A	Breakpoints have not been defined by EUCAST for viridans group streptococci, those shown are recommended for *S. pneumoniae*.
	Erithromycin-Clindamycin	4/0.5	E	E	E	Inducible clindamycin resistance test.
	Josamycin	**0.5-1-2**	C	C	C	Breakpoints have not been defined by EUCAST, those shown are recommend by COESANT
	Clindamycin	**0.5-1-2**	A	A	A	
Tetracyclines	Tetracycline	0.5-1-2-4	A	C	A	Breakpoints have not been defined by EUCAST for viridans group streptococci, those shown are recommend for *S. pneumoniae*
Others	Linezolid	**1-2-4-8**	C	C	C	
	Tedizolid	**0.125-0.25-0.5**	C	C	C	Breakpoints have not been defined by EUCAST for *S. pneumoniae*, those shown are recommend for *S. anginosus* group
	Chloramphenicol	**4-8-16**	C	C	C	Breakpoints have not been defined by EUCAST for viridans group streptococci, those shown are recommend for *S. pneumoniae*
	Cotrimoxazole	**0.5/9.5-1/19-2/38-4/76**	B	B	C	
	Rifampicin	0.03-**0.06-0.125-0.25-0.5**-1-2	A	B	C	Breakpoints have not been defined by EUCAST for viridans group streptococci, those shown are recommend for *S. pneumoniae*

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category. Unless indicated, breakpoints in this table are for *S. pneumoniae*.

TABLE S8. Antibiotics and concentrations recommended for the susceptibility testing of *Enterococcus* spp.

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
ß-lactams	Ampicillin	**1-2-4-8-16**	A	Susceptibility to ampicillin-sulbactam and to amoxicillin or piperacillin with and without β-lactamase inhibitors can be inferred from ampicillin. *E. faecium* resistant to penicillins can be considered resistant to all other ß-lactam agents including carbapenems. β-lactamase-producing isolates have been very unfrequently reported in some countries. These isolates may present ampicillin MIC values ≤4 mg/L and can be detected by the nitrocefin test.
Aminoglycosides	Gentamicin	**128**-500	A	High-level resistance to gentamicin (MIC >128 mg/L) determines resistance to all aminoglycosides, except streptomycin. It also determines loss of synergism of all aminoglycosides (except streptomycin) with ß-lactams and glycopeptides.
	Streptomycin	**512**-1000	A	High level-resistance to streptomycin (MIC >512 mg/L) determines the lost of synergy of this aminoglycoside with ß-lactams and glycopeptides.
	Kanamycin	**1000**	E	This antibiotic can be used to predict high-level resistance to amikacin in non-high-level gentamicin resistant enterococci.
Glycopeptides	Vancomycin	**1-2-4-8**-16-32	A	
	Teicoplanin	**1-2-4-8**-16-32	A	
Lipoglycopeptides	Dalbavancin	**0.125-0.25-0.5**	C	ECOFFs have not been defined. Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
Lipopeptides	Daptomycin	**1-2-4-8**	B	MICs must be determined in the presence of Ca^{2+} (50 mg/L in the medium for broth dilution methods; agar dilution methods have not been validated). Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
Quinolones	Ciprofloxacin	1-**2-4-8**	D	Defined only for uncomplicated urinary tract infections
	Levofloxacin	1-**2-4-8**	D	
Macrolides	Erythromycin	**0.5-1-2-4**-8	E	Breakpoints have not been defined by EUCAST. The ECOFF (4 mg/L) is used to infer resistant population for epidemiological purposes.
Tetracyclines	Tetracycline	2-**4-8-16**	E	Breakpoints have not been defined by EUCAST. The ECOFF is used to infer resistant population for epidemiological purposes.
	Tigecycline	0.12-**0.25-0.5-1-2-4**	B	Isolates with MIC values above the susceptible breakpoint are very rare.
	Eravacycline	0.06-**0.125-0.25**	C	ECOFFs have not yet been defined.

Others	Linezolid	0.5-**1-2-<u>4</u>-8**	A	
	Fosfomycin	32-64-**128**-256	D	ECOFFs have not yet been defined. Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT. ECOFF has not yet been defined.
	Cotrimoxazole	0.5/9.5-**<u>1/19</u>-2/38**-4/76-8/152	E	The activity of trimethoprim-sulfamethoxazole is uncertain against enterococci due to their ability to incorporate exogenously produced folates (which may be found in highly variable concentrations in the urine), so the wild type population is categorized as intermediate (susceptible, increased exposure). ECOFF value has been only defined for *E. faecium*.
	Nitrofurantoin	16-**<u>32</u>-64-128**	D	Breakpoints apply to *E. faecalis* only.

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. <u>Underlined numbers</u> indicate the ECOFF values (most of them from *E. faecalis*), when lacking is due to the absence of definition of this value by EUCAST. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category.

TABLE S9. Antibiotics and concentrations recommended for the susceptibility testing of *Haemophilus* spp. These recommendations have been mainly performed for *H. influenzae* however they can be also applied for *H. parainfluenzae*

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
ß-lactams	Ampicillin	0.25-**0.5-1-2-4-8-16**	A	BLNAR* strains are usually referred to ampicillin. Breakpoints are based on intravenous administration.
	Amoxicillin	0.25-0.5-**1-2-4-8-16**	B	
	Amoxicillin/ clavulanic acid	**0.25-0.5-1-2-4-8**	A	For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
	Cefuroxime	**0.125**-**0.25-0.5-1**-2-**4-8-16**	A	This concentration range is useful both i.v and oral cefuroxime. Indicated breakpoints are those for oral administration. Breakpoints for i.v administration are S= 1 mg/L and R >2 mg/L.
	Cefotaxime	**0.06-0.125-0.25-0.5-1-2-4**	A	Reported in invasive infections. Cefotaxime susceptibility can be used to infer that of ceftriaxone.
	Cefepime	0.06-**0.125-0.25-0.5-1-2-4**	B	
	Meropenem	0.06-**0.125-0.25-0.5-1-2-4**	B	This concentration range is useful for meningitis and other infections. Only reported in nervous central infections. Indicated breakpoints are those for meningitis. Breakpoints for infections other than meningitis are S ≤ 2 mg/L and R >2 mg/L.
Quinolones	Nalidixic acid	4	E	Breakpoints have not been defined by EUCAST. Breakpoints for screening purposes have been defined by COESANT. It can be useful to infer the presence of mutations in topoisomerases. Isolates categorized as S to nalidixic acid (<4 mg/L) can be reported S to ciprofloxacin, levofloxacin and moxifloxacin. Isolates categorized as non-susceptible may have fluoroquinolone resistance and should be tested against the appropriate agent. Ciprofloxacin can better detect the presence of mutations in topoisomerases than levofloxacin.
	Ciprofloxacin	0.03-**0.06-0.125-0.25-0.5-1-2-4**	A	
	Levofloxacin	0.03-**0.06-0.125-0.25-0.5-1-2-4**	A	
MLS_B	Azithromycin	**0.125-0.25-0.5-1-2-4-8**-16	A	Correlation between macrolide MICs and clinical outcome is weak for *H. influenzae*. Therefore, breakpoints for macrolides and related antibiotics have been set to categorize wild type *H. influenzae* as intermediate (susceptible, increased exposure).
Tetracyclines	Tetracycline	0.25-**0.5-1-2-4-8-16**	B	Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, although some resistant to tetracycline may still be susceptible to minocycline and/or doxycycline.
	Minocycline	0.25-**0.5-1-2-4-8-16**	E	
Others	Cotrimoxazole	0.25/4.75-**0.5/9.5-1/19-2/38-4/76**	A	
	Rifampicin	0.5-**1-2**-4	C	Only for prophylaxis.
	Chloramphenicol	0.5-**1-2-4-8**	C	

Bold numbers indicate the minimum number of concentrations that are recommended to be included in the study of susceptibility testing to address the objectives explained in the text. Underlined numbers indicate the ECOFF values, when lacking is due to the absence of definition of this value by EUCAST. Greyed numbers indicate clinical categories: light grey corresponds to concentrations within intermediate (I) category and dark gray corresponds to concentrations within resistant (R) category. BLNAR*: β-negative ampicillin-resistant.