

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	
β-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 <i>E. coli</i> , <i>K. pneumoniae</i> and <i>P. mirabilis</i> 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 <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>P. mirabilis</i> , <i>Salmonella</i> spp., and <i>Shigella</i> 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 <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Proteus mirabilis</i> , <i>Salmonella</i> spp., and <i>Shigella</i> 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 <i>Enterobacter</i> spp., <i>Citrobacter freundii</i> complex, <i>Morganella morganii</i> , <i>Providencia stuartii</i> , <i>Serratia</i> spp., and <i>Hafnia alvei</i> . It is also useful for <i>E. coli</i> 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	

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				concentration of avibactam is fixed at 4 mg/L.
	Aztreonam	<u>0.25</u> - 0.5 -1-2-4- 8 -16-32	A	
	Imipenem	0.25- <u>0.5</u> -1-2-4- 8 -16	A	>1 mg/L has been defined as screening cut-off for carbapenemase production. Breakpoints for <i>M. morgani</i> , <i>Proteus</i> spp. and <i>Providencia</i> spp. are S ≤ 0.125 mg/L and R >4 mg/L
	Meropenem	0.125 - <u>0.25</u> -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 - <u>0.25</u> -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 - <u>0.125</u> -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	<u>2</u> -4-8	A	Breakpoints are based on once daily administration of high dose.
	Tobramycin	<u>2</u> -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 - <u>0.125</u> -0.25-0.5-1-2	A	
	Norfloxacin	<u>0.25</u> -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 <i>Salmonella</i> and <i>Shigella</i> spp. Breakpoints have not been defined by EUCAST, those shown are recommended by COESANT.
	Nitrofurantoin	32- 64 -128	D	Breakpoints defined for <i>E. coli</i> in uncomplicated urinary tract infection only.
	Cotrimoxazole	<u>1/19</u> -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- <u>2</u> -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 grey corresponds to concentrations within resistant (R) category.

Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systemsTABLE S2. Antibiotics and concentrations recommended for the susceptibility testing of *Pseudomonas* spp.

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
β-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

Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systemsTABLE S3. Antibiotics and concentrations recommended for the susceptibility testing of *Acinetobacter* spp.

Antimicrobial agent		Concentrations (mg/L)	Category	Comments
β-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>1-2-4-8-16</u>	A	Breakpoints are based on high dose therapy.
	Meropenem	0.25-0.5- <u>1-2-4-8-16</u>	A	
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	0.06-0.125-0.25- 0.5-1-2	A	
	Levofloxacin	0.25- 0.5-1-2-4-8	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>1/19-2/38-4/76-8/152</u>	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. 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.

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

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

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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
β-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 grey 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.

Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systems**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
β-lactams	Penicillin	0.06-0.125-0.25-0.5-1	A	
	Oxacillin (<i>S. aureus</i> , <i>S. lugdunensis</i> , <i>S. saprophyticus</i>)	0.25-0.5-1-2-4-8	A	<i>S. aureus</i> , <i>S. lugdunensis</i> and <i>S. saprophyticus</i> with oxacillin MICs >2 mg/L are mostly methicillin resistant due to the presence of the <i>mecA</i> or <i>mecC</i> genes.
	Oxacillin (CNS other than <i>S. lugdunensis</i> , <i>S. saprophyticus</i>)	0.25-0.5-1-2-4-8	A	Coagulase-negative staphylococci other than <i>S. saprophyticus</i> and <i>S. lugdunensis</i> with oxacillin MICs >0.25 mg/L are mostly resistant due to the presence of the <i>mecA</i> gene.
	Cefoxitin	2-4-8	E	<i>S. aureus</i> and <i>S. lugdunensis</i> with cefoxitin MIC values >4 mg/L and <i>S. saprophyticus</i> with cefoxitin MIC values >8 mg/L are methicillin resistant, mostly due to the presence of the <i>mecA</i> or <i>mecC</i> genes. For staphylococci other than <i>S. aureus</i> , <i>S. lugdunensis</i> and <i>S. saprophyticus</i> , 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	<i>S. aureus</i> 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 (<i>S. aureus</i>)	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 (<i>S. aureus</i>)	0.06-0.125-0.25	C	

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Lipopeptides	Daptomycin	0.5-<u>1</u>-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-<u>1</u>-2-4	A	Breakpoints are based on high dose therapy.
	Levofloxacin	0.5-<u>1</u>-2-4	A	
	Moxifloxacin	0.125-<u>0.25</u>-0.5-1-2-4	C	
Macrolides and lincosamides	Erithromycin	0.5-<u>1</u>-2-4	A	Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin.
	Clindamycin	0.125- <u>0.25</u> -0.5-1-2	A	
	Erithromycin-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- <u>1</u> -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- <u>0.25</u> -0.5-1-2	C	
	Tigecycline	0.25- <u>0.5</u> -1-2	C	
	Eravacycline	0.125- <u>0.25</u> -0.5-1	C	ECOFFs have not yet been defined.
Oxazolidinones	Linezolid	1-2- <u>4</u> -8	A	Isolates susceptible to linezolid can be reported susceptible to tedizolid.
	Tedizolid	0.25-<u>0.5</u> -1-2	B	
Others	Fosfomicin	8-16-<u>32</u>-64-128	B	Use in combination in serious infections (i.e endocarditis). Breakpoints are not defined for oral use.
	Cotrimoxazole	0.25/4.75- <u>0.5/9.5</u> -1/19-2/38- <u>4/76</u> -8/152	A	
	Rifampicin	0.01- <u>0.03</u> -0.06-0.125-0.25-0.5- <u>1</u> -2	B	
	Mupirocin	0-5- <u>1</u> -2-4-256	B	Breakpoint related to nasal decolonization of <i>S. aureus</i> . Intermediate isolates are associated to short term suppression (useful preoperatively) but unlike susceptible isolates, long-term eradication rates are low.
	Fusidic acid	0.25- <u>0.5</u> -1-2-4	B	
	Nitrofurantoin	16- <u>32</u> -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.

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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
			<i>S. pneumoniae</i>	β -haemolytic streptococci	Viridans group streptococci	
β -lactámicos	Penicillin	0.06-0.12-0.25-0.5-1-2-4	A	A	A	Breakpoints (<i>S. pneumoniae</i>) are those recommended for meningitis. For infections other than meningitis oral penicillin V breakpoints are S \leq 0.06 mg/L / R>2 mg/L, and penicillin parenteral breakpoints are S \leq 2 mg/L / R>4 mg/L.
	Ampicillin	0.25- 0.5-1-2-4	A	A	A	Breakpoints (<i>S. pneumoniae</i>) are those recommended for infections other than meningitis.
	Cefuroxime	0.125- 0.25-0.5-1-2	C	C	C	Breakpoints (<i>S. pneumoniae</i>) 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 <i>S. pneumoniae</i> , 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 <i>S. pneumoniae</i> , those shown are for <i>S. anginosus</i> group and β -haemolytic groups A, B, C and G.
Quinolones	Levofloxacin	0.5-1-2-4	A	A	A	Breakpoints are based on high dose therapy. Breakpoints

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						have not been defined by EUCAST for viridans group streptococci, those shown are recommended for <i>S. pneumoniae</i> .
	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 <i>S. pneumoniae</i> .
	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 <i>S. pneumoniae</i>
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 <i>S. pneumoniae</i> , those shown are recommend for <i>S. anginosus</i> group
	Chloramphenicol	4-8-16	C	C	C	Breakpoints have not been defined by EUCAST for viridans group streptococci, those shown are recommend for <i>S. pneumoniae</i>
	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 <i>S. pneumoniae</i>

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 grey corresponds to concentrations within resistant (R) category. Unless indicated, breakpoints in this table are for *S. pneumoniae*.

Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systemsTABLE S8. Antibiotics and concentrations recommended for the susceptibility testing of *Enterococcus* spp.

Antimicrobial agent	Concentrations (mg/L)	Category	Comments
β-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. <i>E. faecium</i> resistant to penicillins can be considered resistant to all other β-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 β-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 β-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.

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

Others	Linezolid	0.5- 1-2-4-8	A	
	Fosfomicin	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- 1/19-2/38-4/76-8/152	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 <i>E. faecium</i> .
	Nitrofurantoin	16- 32-64-128	D	Breakpoints apply to <i>E. faecalis</i> 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.

Underlined numbers 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 grey corresponds to concentrations within resistant (R) category.

Recommendations of the Spanish Antibiogram Committee (COESANT) for selecting antimicrobial agents and concentrations for *in vitro* susceptibility studies using automated systems**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	
β-lactams	Ampicillin	0.25- 0.5 - <u>1</u> -2-4-8-16	A	BLNAR* strains are usually referred to ampicillin. Breakpoints are based on intravenous administration.
	Amoxicillin	0.25-0.5- <u>1</u> -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 <i>H. influenzae</i> . Therefore, breakpoints for macrolides and related antibiotics have been set to categorize wild type <i>H. influenzae</i> as intermediate (susceptible, increased exposure).
Tetracyclines	Tetracycline	0.25- 0.5 - <u>1</u> -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 - <u>1</u> -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- <u>1</u> -2-4	C	Only for prophylaxis.
	Chloramphenicol	0.5- 1 - <u>2</u> -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 grey corresponds to concentrations within resistant (R) category. BLNAR*: β-negative ampicillin-resistant.