

EUCAST expert rules in antimicrobial susceptibility testing

R. Leclercq^{1,2}, R. Cantón^{2,3,4}, D. F. J. Brown⁴, C. G. Giske^{2,4,5}, P. Heisig^{2,6}, A. P. MacGowan^{4,7}, J. W. Mouton^{4,8}, P. Nordmann^{2,9}, A. C. Rodloff^{4,10}, G. M. Rossolini^{2,11}, C.-J. Soussy^{4,12}, M. Steinbakk^{4,13}, T. G. Winstanley^{2,14} and G. Kahlmeter^{4,15}

1) Laboratoire de Microbiologie, CHU Côte de Nacre, Caen, France, 2) EUCAST Subcommittee on Expert Rules, 3) Servicio de Microbiología and CIBER en Epidemiología y Salud Pública (CIBERESP), Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain, 4) EUCAST Steering Committee, 5) Clinical Microbiology, MTC-Karolinska Institutet, Karolinska University Hospital, Solna, Sweden, 6) Department of Pharmacy, Biology & Microbiology, University of Hamburg, Hamburg, Germany, 7) Department of Medical Microbiology, Southmead Hospital, Bristol, UK, 8) Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 9) Service de Bactériologie-Virologie, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France, 10) Institut für Medizinische Mikrobiologie der Universität Leipzig, Leipzig, Germany, 11) Dipartimento di Biotecnologie, Sezione di Microbiologia, Siena, Italy, 12) Hôpital Henri Mondor, Service de Bactériologie, Creteil, France, 13) Department of Bacteriology and Immunology, Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway, 14) Department of Microbiology, Royal Hallamshire Hospital, Sheffield, UK and 15) Clinical Microbiology, Central Hospital, Växjö, Sweden

Abstract

EUCAST expert rules have been developed to assist clinical microbiologists and describe actions to be taken in response to specific antimicrobial susceptibility test results. They include recommendations on reporting, such as inferring susceptibility to other agents from results with one, suppression of results that may be inappropriate, and editing of results from susceptible to intermediate or resistant or from intermediate to resistant on the basis of an inferred resistance mechanism. They are based on current clinical and/or microbiological evidence. EUCAST expert rules also include intrinsic resistance phenotypes and exceptional resistance phenotypes, which have not yet been reported or are very rare. The applicability of EUCAST expert rules depends on the MIC breakpoints used to define the rules. Setting appropriate clinical breakpoints, based on treating patients and not on the detection of resistance mechanisms, may lead to modification of some expert rules in the future.

Keywords: Antimicrobial susceptibility testing, breakpoints, EUCAST, expert rules, interpretive reading

Clin Microbiol Infect

Corresponding author: R. Cantón, Servicio de Microbiología, Hospital Universitario Ramón y Cajal, Madrid, Spain
E-mail: rcanton.hrc@salud.madrid.org

Introduction

Antimicrobial susceptibility testing is a daily task in clinical microbiology laboratories worldwide. In view of the increasing complexity and widespread increase in antimicrobial resistance mechanisms and the clinical implications of the resistance, expert knowledge is desirable for interpretation of tests. An expert rule in antimicrobial susceptibility testing describes an action to be taken on the basis of specific antimicrobial susceptibility test results. The rules are based on current clinical breakpoints and knowledge of resistance mechanisms. Expert rules for antimicrobial susceptibility testing can assist clinical microbiologists in the interpretation of antimicrobial susceptibility tests [1], but, with changes in breakpoints and the discovery of new resistance mechanisms,

rules may become redundant or require modification. Rules can also contribute to quality assurance by highlighting anomalous or unlikely results [2–5]. The EUCAST expert rules in antimicrobial susceptibility testing, first published in 2008 (<http://www.eucast.org>), are divided into intrinsic resistance, exceptional phenotypes, and interpretive rules. In this document, we present the second version of these rules, which has been updated in line with current EUCAST breakpoints.

Intrinsic Resistance

Intrinsic (inherent) resistance, as opposed to acquired and/or mutational resistance, is a characteristic of all or almost all isolates of the bacterial species. The antimicrobial activity of the

drug is clinically insufficient or antimicrobial resistance is innate, rendering it clinically useless. Antimicrobial susceptibility testing is therefore unnecessary, although it may be performed as part of panels of test agents. In these species, 'susceptible' results should be viewed with caution, as they most likely indicate an error in identification or susceptibility testing. Even if a susceptible result is confirmed, the drug should preferably not be used or, when no alternative is available, should be used with caution. In some cases, intrinsic resistance to an agent may be expressed at a low level, with MIC values close to the susceptible breakpoint, although the agent is not considered to be clinically active. There are also situations where the agent appears to be fully active *in vitro* (MIC values cannot be separated from those of the wild type) but is inactive *in vivo*. These are generally not mentioned in the tables, as they are rather a matter of therapeutic recommendations. Examples of intrinsic resistance are *Enterobacteriaceae* resistant to glycopeptides or linezolid, *Proteus mirabilis* resistant to nitrofurantoin and colistin, *Serratia marcescens* resistant to colistin, *Stenotrophomonas maltophilia* resistant to carbapenems, Gram-positive organisms resistant to aztreonam, and enterococci resistant to fusidic acid (Tables 1–4).

Exceptional Resistance Phenotypes

Exceptional resistance phenotypes are phenotypes of resistance of some bacterial species to particular antimicrobial agents that have not yet been reported or are very rare. Exceptional resistance phenotypes should be checked, as they may also indicate an error in identification or susceptibility testing. If they are confirmed locally, the isolate should be further studied to confirm the exceptional phenotype, and sent to a reference laboratory or other laboratory with expertise in resistance mechanisms for independent confirmation. Exceptional resistance phenotypes may change, as resistance may develop and increase over time. There may also be local, regional or national differences, and a very rare resistance phenotype in one hospital, area or country may be more common in another. Examples of exceptional phenotypes are *Streptococcus pyogenes* resistant to penicillin, *Staphylococcus aureus* resistant to vancomycin, *Enterococcus faecium* susceptible to ampicillin, *Enterobacteriaceae* resistant to carbapenems (rare but increasing), and anaerobes resistant to metronidazole (Tables 5–7).

Interpretive Reading and Expert Rules

Interpretive reading is another type of expert rule, and involves inference of resistance mechanisms from susceptibil-

TABLE 1. Intrinsic resistance in *Enterobacteriaceae*; *Enterobacteriaceae* are also intrinsically resistant to benzylpenicillin, glycopeptides, fusidic acid, macrolides (with some exceptions^a), lincosamides, streptogramins, rifampicin, daptomycin, and linezolid

Rule no.	Organisms	Ampicillin	Amoxicillin-clavulanate	Ticarcillin	Piperacillin	Cefazolin	Cefoxitin	Cefamandole	Cefuroxime	Aminoglycosides	Tetracyclines/tigecycline	Polymyxin B/colistin	Nitrofurantoin
1.1	<i>Citrobacter koseri</i>	R	I	R	R	I	I	I	I	I	I	I	I
1.2	<i>Citrobacter freundii</i>	R	R	I	I	R	R	I	I	I	I	I	I
1.3	<i>Enterobacter cloacae</i>	R	R	I	I	R	R	I	I	I	I	I	I
1.4	<i>Enterobacter aerogenes</i>	R	R	I	I	R	R	I	I	I	I	I	I
1.5	<i>Escherichia hermannii</i>	R	I	R	I	I	I	I	I	I	I	I	I
1.6	<i>Hafnia alvei</i>	R	R	I	I	R	I	I	I	I	I	I	I
1.7	<i>Klebsiella</i> spp.	R	I	R	I	I	I	I	I	I	I	I	I
1.8	<i>Morganella morganii</i>	R	R	I	I	R	I	R	R	R	R	R	R
1.9	<i>Proteus mirabilis</i>	I	I	I	I	I	I	I	I	I	I	I	I
1.10	<i>Proteus vulgaris</i>	R	I	I	I	R	R	R	R	R	R	R	R
1.11	<i>Proteus penneri</i>	R	I	I	I	R	R	R	R	R	R	R	R
1.12	<i>Providencia rettgeri</i>	R	R	I	I	R	I	I	I	I	I	I	I
1.13	<i>Providencia stuartii</i>	R	R	I	I	R	I	I	I	I	I	I	I
1.14	<i>Serratia marcescens</i>	R	R	I	I	R	I	R	R	Note ^b	R	R	R
1.15	<i>Yersinia enterocolitica</i>	R	R	R	I	R	R	R	R	Note ^c	I	I	I
1.16	<i>Yersinia pseudotuberculosis</i>	I	I	I	I	I	I	I	I	I	I	R	I

R, resistant.

^aAzithromycin is effective *in vivo* for the treatment of typhoid fever, and erythromycin may be used to treat travellers' diarrhoea.

^b*Providencia stuartii* produces a chromosomal AAC(2)-IIa enzyme and should be considered to be resistant to clinically available aminoglycosides, except amikacin, arbekacin, and streptomycin. Some isolates express the enzyme poorly and can appear to be susceptible to netilmicin *in vitro*, but should be reported as resistant, as mutation can result in overproduction of this enzyme.

^cAll *Serratia marcescens* isolates produce a chromosomal AAC(6)-Ic enzyme that affects the activity of clinically available aminoglycosides, except streptomycin, gentamicin, and arbekacin.

TABLE 2. Intrinsic resistance in non-fermentative Gram-negative bacteria; non-fermentative Gram-negative bacteria are also intrinsically resistant to benzylpenicillin, cefoxitin, cefamandole, cefuroxime, glycopeptides, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin, daptomycin, and linezolid

Rule no.	Organisms	Ampicillin	Amoxicillin-clavulanate	Ticarcillin	Ticarcillin-clavulanate	Piperacillin	Piperacillin-tazobactam	Cefazolin	Cefotaxime	Ceftaxone	Ceftazidime	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim-sulphamethoxazole	Fosfomycin	Tetracyclines/tigecycline	Polymyxin B/collistin
2.1	<i>Acinetobacter baumannii</i>	R ^a	R ^a	-	-	-	-	R	R	R	-	R	-	-	-	-	-	R	-	-	-
2.2	<i>Acinetobacter calcoaceticus</i>	R	-	-	-	-	-	R	R	R	-	R	-	-	-	-	-	-	-	-	-
2.3	<i>Achromobacter xylosoxidans</i>	R	-	R	R	-	-	R	R	-	-	R	-	-	-	-	-	-	-	-	-
2.4	<i>Burkholderia cepacia</i> complex ^b	R	-	R	R	-	-	R	R	R	-	R	R	R	R	R	R ^c	-	-	-	-
2.5	<i>Elizabethkingia meningoseptica</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	-	-	-	-	-	-	-
2.6	<i>Ochrobactrum anthracis</i>	R	R	R	R	R	R	R	R	R	R	R	R	R	-	-	-	-	-	-	-
2.7	<i>Pseudomonas aeruginosa</i>	R	R	R	-	-	-	R	R	R	R ^f	R	R	R	-	-	-	R ^e	-	-	-
2.7	<i>Stenotrophomonas maltophilia</i>	R	R	R	-	R	R	R	R	R	R	R	R	R	-	-	R ^c	R ^g	-	-	-

R, resistant.

^a*Acinetobacter baumannii* may appear to be susceptible to ampicillin-sulbactam, owing to the activity of sulbactam against this species.

^b*Burkholderia cepacia* complex includes different species. Some strains may appear to be susceptible to some β -lactams *in vitro*, but they are clinically resistant and are shown as R in the table.

^c*Burkholderia cepacia* and *Stenotrophomonas maltophilia* are intrinsically resistant to all aminoglycosides. Intrinsic resistance is attributed to poor permeability and putative efflux. In addition, most *Stenotrophomonas maltophilia* isolates produce the AAC(6)-I₂ enzyme.

^d*Pseudomonas aeruginosa* is intrinsically resistant to kanamycin and neomycin, owing to low-level APH(3)-IIb activity.

^e*Pseudomonas aeruginosa* is typically resistant to trimethoprim and moderately susceptible to sulfonamides. Although it may appear to be susceptible *in vitro* to trimethoprim-sulphamethoxazole, it should be considered to be resistant.

^f*Stenotrophomonas maltophilia* may show low ceftazidime MIC values but should be considered to be resistant.

^g*Stenotrophomonas maltophilia* is typically susceptible to trimethoprim-sulphamethoxazole but resistant to trimethoprim alone.

TABLE 3. Intrinsic resistance in Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria; Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria listed are also intrinsically resistant to glycopeptides, lincosamides, daptomycin, and linezolid

Rule no.	Organisms	Macrolides	Fusidic acid	Streptogramins	Trimethoprim	Nalidixic acid
3.1	<i>Haemophilus influenzae</i>	I	R	–	–	–
3.2	<i>Moraxella catarrhalis</i>	–	–	–	R	–
3.3	<i>Neisseria</i> spp.	–	–	–	R	–
3.4	<i>Campylobacter fetus</i>	–	R	R	R	R
3.5	<i>Campylobacter jejuni</i> , <i>Campylobacter coli</i>	–	R	R	R	–

R, resistant; I, intermediate.

ity test results, and interpretation of clinical susceptibility on the basis of the resistance mechanism [1–4]. The applicability of such rules is limited by the range of agents tested, so individual laboratories will need to choose which agents to test for their local requirements. The applicability of any rule will also depend on the MIC breakpoints used to define the rule. EUCAST interpretive rules may be simple—for example, IF *S. aureus* is resistant to oxacillin or ceftioxin, THEN report as resistant to all β -lactams—or more complicated—for example, IF *Enterobacteriaceae* are intermediate to tobramycin, resistant to gentamicin, and susceptible to amikacin, THEN report as resistant to tobramycin. The evidence supporting interpretive rules is often not conclusive, and there may be differences of opinion regarding the most appropriate clinical action. Hence, these rules should be based on current published evidence, the quality of evidence should be assessed, and exceptions to any rules should be noted. In the EUCAST tables (Tables 8–13), the evidence for rules has been graded as follows:

1. There is good clinical evidence that reporting the test result as susceptible leads to clinical failures.
2. Evidence is weak and based on only a few case reports or on experimental models. It is presumed that reporting the test result as susceptible may lead to clinical failures.
3. There is no clinical evidence, but microbiological data suggest that clinical use of the agent should be discouraged.

Actions to be taken by laboratories on the basis of EUCAST expert rules include recommendations on reporting, such as inferring susceptibility to other agents from results with one, suppression of results that may be inappropriate, and editing of results from susceptible to intermediate/resistant or from intermediate to resistant on the basis of an inferred resistance mechanism. Rules never recommend editing intermediate or resistant to susceptible or resistant to intermediate, because even if resistance has never been reported, there

may be new resistance mechanisms that have not been previously recognized, and treatment is likely to fail. Comments may also be added to explain actions or warn of resistance of particular epidemiological significance. Advice may be given on further tests that may be appropriate or on the need for referral of isolates to a reference laboratory for checking susceptibility or identification.

Application of EUCAST expert rules may impose some testing requirements on clinical laboratories. Many rules require the full identification of the organism even if it is not essential for clinical management. There may be a need to test an extended range of appropriate agents, as interpretive rules may require testing of agents that may not be required clinically. There is also a clinical need for access to a set of expert rules, as there are many expert rules, and few individuals are able to remember them all and to apply them consistently.

There are few publications on expert rules, and these are more likely to be used as a reference source than for everyday application [1,4]. The wide range of expert rules means that they are only likely to be applied consistently and widely if they are available as a published set of rules that can be incorporated into computer systems. Rules may be incorporated into a laboratory information system (LIS), but this is limited by the capabilities of the LIS and the ability and interest of individual laboratories in incorporating rules into the LIS. Expert systems are, however, incorporated into several automated susceptibility and zone reading systems.

The purpose of the EUCAST expert rules is to provide a written description of current expert rules. The rules are a comprehensive collection that may be applied manually or incorporated into automated systems [6,7]. The rules were prepared by an expert subcommittee in consultation with European national susceptibility breakpoint committees, EUCAST national representatives, the pharmaceutical and susceptibility device-manufacturing industries, recognized experts, and others via open consultation through the

TABLE 4. Intrinsic resistance in Gram-positive bacteria; Gram-positive bacteria are also intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin, and nalidixic acid

Rule no.	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Erythromycin	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomicin	Novobiocin	Sulphonamides
4.1	<i>Staphylococcus saprophyticus</i>	R	R	-	-	-	-	-	-	-	R	R	-
4.2	<i>Staphylococcus cohnii</i> , <i>Staphylococcus xylosum</i>	-	R	-	-	-	-	-	-	-	-	R	-
4.3	<i>Staphylococcus carnis</i>	-	R	-	-	-	-	-	-	-	R	-	-
4.4	Other coagulase-negative staphylococci and <i>Staphylococcus aureus</i>	-	R	-	-	-	-	-	-	-	-	-	-
4.5	<i>Streptococcus</i> spp.	R	-	-	R ^a	-	-	-	-	-	-	-	-
4.6	<i>Enterococcus faecalis</i>	R	R	R	R ^a	R	R	R	-	-	-	-	R
4.7	<i>Enterococcus gallinarum</i> , <i>Enterococcus casseliflavus</i>	R	R	R	R ^a	R	R	R	-	-	-	-	R
4.8	<i>Enterococcus faecium</i>	R	R	R	R ^{a,b}	R	-	-	-	-	-	-	R
4.9	<i>Corynebacterium</i> spp.	-	-	-	-	-	-	-	-	-	R	-	-
4.10	<i>Listeria monocytogenes</i>	-	R	R	-	-	-	-	-	-	-	-	-
4.11	<i>Leuconostoc</i> spp., <i>Pediococcus</i> spp.	-	-	-	-	-	-	-	-	R	-	-	-
4.12	<i>Lactobacillus</i> spp. (some species)	-	-	-	-	-	-	-	-	R	-	-	-
4.13	<i>Clostridium ramosum</i> , <i>Clostridium innocuum</i>	-	-	-	-	-	-	-	R	-	-	-	-

R, resistant.

^aLow-level resistance to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides.

^bIn addition to low-level resistance to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6') enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin, arbekacin, and streptomycin) and penicillins or glycopeptides.

TABLE 5. Exceptional phenotypes of Gram-negative bacteria

Rule no.	Organisms	Exceptional phenotypes
5.1	Any <i>Enterobacteriaceae</i> (except <i>Proteae</i>)	Resistant to meropenem and/or imipenem ^a
5.2	<i>Serratia marcescens</i> and <i>Proteae</i>	Susceptible to colistin
5.3	<i>Pseudomonas aeruginosa</i> and <i>Acinetobacter</i> spp.	Resistant to colistin
5.4	<i>Haemophilus influenzae</i>	Resistant to any third-generation cephalosporin, carbapenems, and fluoroquinolones
5.5	<i>Moraxella catarrhalis</i>	Resistant to ciprofloxacin and any third-generation cephalosporin
5.6	<i>Neisseria meningitidis</i>	Resistant to any third-generation cephalosporin and fluoroquinolones
5.7	<i>Neisseria gonorrhoeae</i>	Resistant to third-generation cephalosporin and spectinomycin

^aExcept in countries in which carbapenemase-producing *Enterobacteriaceae* are not rare.

EUCAST website. Rules should not conflict with EUCAST MIC breakpoints, but it is appreciated that some antimicrobial agents are not included in EUCAST breakpoints, and many rules have developed over the years in conjunction with other breakpoint systems. Hence, rules are likely to be amended as EUCAST breakpoints are developed and in the light of experience with application of the rules and the emergence of new resistance mechanisms. This second version will undoubtedly need to be updated again in the future.

Explanatory Notes on EUCAST Expert Rules in Antimicrobial Susceptibility Testing

The EUCAST Expert Rules Subcommittee was established in 2007 with the objective of assisting clinical microbiologists in the interpretation of antimicrobial susceptibility tests beyond interpretation of *in vitro* tests for the assignment of clinical

TABLE 6. Exceptional phenotypes of Gram-positive bacteria

Rule no.	Organisms	Exceptional phenotypes
6.1	<i>Staphylococcus aureus</i>	Resistant to vancomycin, teicoplanin, linezolid, quinupristin–dalfopristin, daptomycin, and tigecycline
6.2	Coagulase-negative staphylococci	Resistant to vancomycin, linezolid ^a , quinupristin–dalfopristin ^a , daptomycin, and tigecycline
6.3	JK coryneform organisms	Resistant to vancomycin, teicoplanin, linezolid, quinupristin–dalfopristin, daptomycin, and tigecycline
6.4	<i>Streptococcus pneumoniae</i>	Resistant to imipenem, meropenem, vancomycin, teicoplanin, linezolid, quinupristin–dalfopristin, daptomycin, tigecycline, and rifampicin
6.5	Group A, B, C and G β -haemolytic streptococci	Resistant to penicillin, cephalosporins, vancomycin, teicoplanin, linezolid, quinupristin–dalfopristin, daptomycin, and tigecycline
6.6	<i>Enterococcus</i> spp.	Resistant to linezolid, daptomycin, and tigecycline. Resistant to teicoplanin but not vancomycin
6.7	<i>Enterococcus faecalis</i> , <i>Enterococcus gallinarum</i> , <i>Enterococcus casseliflavus</i> , and <i>Enterococcus avium</i>	Susceptible to quinupristin–dalfopristin. Consider likelihood of misidentification. If also resistant to ampicillin, it is almost certainly <i>E. faecium</i>
6.8	<i>Enterococcus faecium</i>	Resistant to quinupristin–dalfopristin. Consider likelihood of misidentification, especially if also susceptible to ampicillin

^aExcept in countries where linezolid-resistant or quinupristin–dalfopristin-resistant coagulase-negative staphylococci are not rare.

TABLE 7. Exceptional phenotypes of anaerobes

Rule no.	Organisms	Exceptional phenotypes
7.1	<i>Bacteroides</i> spp.	Resistant to metronidazole and carbapenems
7.2	<i>Clostridium difficile</i>	Resistant to metronidazole and vancomycin

categories of antimicrobial susceptibility. For this purpose, different rules have been produced, including those defining intrinsic resistance and exceptional phenotypes as well as interpretive rules. The latter are structured in tables (Tables 8–13 of EUCAST Expert Rules in Antimicrobial Susceptibility Testing) that group different organisms and/or classes of antimicrobial agents. They were mainly established by use of EUCAST MIC breakpoints to define the clinical categories (susceptible, intermediate, or resistant) included in the expert rule statement. These rules should be applied once the bacterial isolates have been identified to species level. Although recognition of the resistance mechanisms is an essential part of the interpretive expert rule, the final objective is to assist in the clinical use of antimicrobial agents.

Interpretive rules for β -lactam agents

β -Lactam compounds are the most widely used antimicrobial agents. They interact with the penicillin-binding proteins (PBPs), which are the enzymes involved in the terminal stages of peptidoglycan synthesis, and exert a bactericidal effect because of a subsequent imbalance of cell wall autolytic enzymes. Resistance to these compounds is mainly caused by β -lactamases, which constitute a large family of different hydrolases that disrupt and inactivate the β -lactam structure. These enzymes variably affect different β -lactam compounds, thus producing different phenotypes and/or levels of resistance, particularly in Gram-negative bacilli [8,9]. In addition, target (PBP) modification may compromise β -lactam activity. This mechanism is encountered particularly in Gram-positive cocci. The contribution of PBP modifica-

TABLE 8. Interpretive rules for β -lactam agents and Gram-positive cocci

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
8.1	<i>Staphylococcus</i> spp.	Oxacillin, cefoxitin (disk diffusion), or detection of <i>mecA</i> gene or PBP2a	All β -lactams	IF resistant to isoxazoly-penicillins (as determined with oxacillin, cefoxitin, or by detection of <i>mecA</i> -gene or of PBP2a), THEN report as resistant to all β -lactams except those specifically licensed to treat infections caused by methicillin-resistant <i>staphylococci</i> owing to low affinity for PBP2a	Production of PBP2a (encoded by <i>mecA</i>) leads to cross-resistance to β -lactams except cefbiprole and ceftaroline	A	[13,15]
8.2	<i>Staphylococcus</i> spp.	Benzylpenicillin (and β -lactamase detection)	Penicillins apart from isoxazoly-penicillins and combinations with β -lactamase inhibitors	IF resistant to benzylpenicillin or IF β -lactamase is detected, THEN report as resistant to all penicillins, regardless of MIC, except the isoxazoly-penicillins and combinations with β -lactamase inhibitors	Testing for β -lactamase production is discouraged; in most countries, the prevalence of β -lactamase producers is >90%, and testing for β -lactamase production has technical problems. In this case, it may be considered appropriate to report all isolates as resistant to benzylpenicillin, ampicillin, and amoxycillin	C	[99]
8.3	β -Haemolytic streptococci (group A, B, C, G)	Benzylpenicillin	Aminopenicillins, cephalosporins, and carbapenems	IF susceptible to benzylpenicillin, THEN report as susceptible to aminopenicillins, cephalosporins, and carbapenems	Rare isolates of group B streptococci may have diminished susceptibility to penicillins No resistance to β -lactams reported so far except in group B streptococci (MIC of benzylpenicillin up to 1 mg/L) If reduced susceptibility to penicillin, check identification and susceptibility	C	[17,18,100]
8.4	<i>Streptococcus pneumoniae</i>	Oxacillin (disk diffusion)	Benzylpenicillin, aminopenicillins, cephalosporins, and carbapenems	IF resistant by the oxacillin disk screening test, THEN determine MIC of benzylpenicillin and other relevant β -lactam agents	Production of mosaic PBPs leads to various patterns of β -lactam resistance. Report as interpreted for each of the drugs	B	[19,20]
8.5	Viridans group streptococci	Benzylpenicillin	Aminopenicillins and cefoxime or ceftriaxone	IF resistant to benzylpenicillin, THEN determine MIC of ampicillin (or amoxycillin) and cefotaxime (or ceftriaxone) and report as interpreted for each of the drugs, as results cannot be inferred from benzylpenicillin	Production of mosaic PBPs leads to various patterns of β -lactam resistance	C	[101,102]
8.6	<i>Enterococcus</i> spp.	Ampicillin	Ureidopenicillins and carbapenems	IF resistant to ampicillin, THEN report as resistant to ureidopenicillins and carbapenems	Alterations in PBP5 lead to decreased affinity for β -lactams. Rare β -lactamase-producing isolates have been reported in a few countries	C	[103,104]

PBP, penicillin-binding protein.

TABLE 9. Interpretive rules for β -lactam agents and Enterobacteriaceae, Pseudomonas spp., and Acinetobacter spp.

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
9.1	Enterobacteriaceae	Cefotaxime, ceftriaxone, cefazidime, cefepime, amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam	Amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam	IF intermediate or resistant to any third-generation (cefotaxime, ceftriaxone, cefazidime) or fourth-generation (cefepime) oxyimino-cephalosporin, AND susceptible to amoxicillin-clavulanate, ampicillin-sulbactam or piperacillin-tazobactam, THEN report as tested and enclose a warning on uncertain therapeutic outcome for infections other than urinary tract infections	ESBL producers are often categorized as susceptible to combinations of a penicillin and a β -lactamase inhibitor. With the exception of urinary tract infections and bloodstream infections secondary to this origin, the use of these combinations in infections caused by ESBL producers remains controversial, and should be approached with caution. No evidence for ticarcillin-clavulanate has been published	B	[44,45]
9.2	Enterobacter spp., Citrobacter freundii, Serratia spp., and Morganella morganii	Cefotaxime, ceftriaxone, and cefazidime	Cefotaxime, ceftriaxone, and cefazidime	IF susceptible <i>in vitro</i> to cefotaxime, ceftriaxone or cefazidime, THEN note that the use in monotherapy of cefotaxime, ceftriaxone or cefazidime should be discouraged, owing to the risk of selecting resistance, or suppress the susceptibility testing results for these agents	Selection of AmpC-derepressed cephalosporin-resistant mutants may occur during therapy. The use of a third-generation cephalosporin in combination with an aminoglycoside may also lead to failure by selection of resistant mutants. Combination with quinolones has, however, been found to be protective. The selection risk is absent or much diminished for ceftipime and ceftiofime	A (Enterobacter), B (others)	[46,47]
9.3	Enterobacteriaceae (mostly Klebsiella spp. and Escherichia coli)	Ticarcillin, piperacillin	Piperacillin	IF resistant to ticarcillin but susceptible to piperacillin, THEN edit piperacillin to resistant	Ticarcillin-hydrolysing β -lactamases also attack piperacillin, but resistance may be less obvious if expression is low-level. Does not apply to inhibitor combinations involving these penicillins	C	[23,105]

ESBL, extended-spectrum β -lactamase.

TABLE 10. Interpretive rules for β -lactam agents and other Gram-negative bacteria

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
10.1	<i>Haemophilus influenzae</i>	Ampicillin or amoxicillin (and β -lactamase detection)	Ampicillin, amoxicillin, and piperacillin	IF β -lactamase-positive, THEN report as resistant to ampicillin, amoxicillin, and piperacillin	Ampicillin is the class representative for amoxicillin. Resistance to ampicillin by production of β -lactamase may be misidentified by the disk diffusion technique. Production of β -lactamase should be examined with a chromogenic test	A	[106,107]
10.2	<i>Haemophilus influenzae</i>	Ampicillin or amoxicillin (and β -lactamase detection)	Ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin, and piperacillin-tazobactam	IF β -lactamase-negative but ampicillin-resistant (BLNAR), THEN report as resistant to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, cefaclor, cefuroxime, and cefuroxime axetil	BLNAR isolates have reduced affinity of PBPs for β -lactams. Although piperacillin and piperacillin-tazobactam appear to be less affected by the PBP-mediated resistance mechanisms, evidence regarding clinical efficacy is lacking	C	[48,49,108]
10.3	<i>Haemophilus influenzae</i>	Amoxicillin-clavulanate (and β -lactamase detection)	Ampicillin-sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin, and piperacillin-tazobactam	IF β -lactamase-positive and amoxicillin-clavulanate-resistant (BLPACR), THEN report as resistant to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, piperacillin, piperacillin-tazobactam, cefuroxime, and cefuroxime axetil	BLPACR isolates produce β -lactamase and have reduced affinity of PBPs for β -lactams. Although piperacillin and piperacillin-tazobactam appear to be less affected by the PBP-mediated resistance mechanisms, evidence regarding clinical efficacy is lacking	C	[48,108]
10.4	<i>Neisseria gonorrhoeae</i>	Benzylpenicillin, ampicillin, or amoxicillin (and β -lactamase detection)	Benzylpenicillin, ampicillin, and amoxicillin	IF positive for production of β -lactamase, THEN report as resistant to benzylpenicillin, ampicillin, and amoxicillin	Penicillin resistance can be caused by plasmid-encoded β -lactamase production (TEM-1). Chromosomal mutations affecting affinity for PBPs, decreased permeability or efflux also confer resistance to β -lactamase inhibitor combinations. Penicillin susceptibility in β -lactamase-negative isolates is indicated by the application of breakpoints	A	[55–57]

PBP, penicillin-binding protein.

tion to β -lactam resistance in Gram-negative organisms is generally less important [10]. Porin modifications and efflux pump hyperexpression in Gram-negative organisms may also compromise β -lactam compounds, but the resistance levels conferred by these mechanisms alone are commonly lower than those observed with resistance conferred by most β -lactamases [11,12]. EUCAST expert rules for β -lactams and Gram-positive cocci are focused on staphylococci, streptococci, including β -haemolytic isolates, viridans group streptococci, *Streptococcus pneumoniae*, and enterococci (Table 8).

Staphylococci. Production of penicillinase in staphylococci is very common (>90% of the *S. aureus* isolates in many countries) and leads to phenotypic resistance to all penicillins except the isoxazolyl analogues (rule 8.2). Staphylococci can also be resistant to the isoxazolyl penicillins, owing to the production of an abnormal PBP (PBP2a encoded by *mecA*), leading to cross-resistance to all β -lactams except for a few

with low affinity for PBP2a (rule 8.1) [13]. Resistance mediated by *mecA* is commonly referred to as methicillin (or oxacillin) resistance, as historically these agents have been widely used for *in vitro* testing. Detection of methicillin resistance is mandatory in *S. aureus* clinical isolates [14]. All staphylococci resistant to methicillin, oxacillin, and/or ceftiofex, or with positive test results for *mecA* or PBP2a, should be considered to be resistant to all available β -lactams [15], with the exception of those specifically licensed for the treatment of infections caused by methicillin-resistant staphylococci. Nevertheless, rare penicillinase hyperproduction may result in borderline resistance to oxacillin (but not ceftiofex) *in vitro*, owing to the lability of oxacillin, but there is no evidence that penicillinase hyperproduction is clinically relevant [16].

Streptococci. Among β -haemolytic streptococci, susceptibility to penicillins is currently the rule. No decreased susceptibility to β -lactams has been reported except in group B streptococci (MIC of benzylpenicillin up to 1 mg/L) [17]. Isolates

TABLE 11. Interpretive rules for macrolides, lincosamides, and streptogramins

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
11.1	All	Erythromycin	Azithromycin, clarithromycin, and roxithromycin	IF susceptible, intermediate or resistant to erythromycin, THEN report the same category of susceptibility for azithromycin, clarithromycin, and roxithromycin	Erythromycin is the class representative for 14-membered and 15-membered ring macrolides. Resistance to erythromycin is generally caused by the production of a ribosomal methylase encoded by <i>erm</i> genes conferring the macrolide-lincosamide-streptogramin B (MLS _B) phenotype or by production of an efflux pump. In both cases, there is cross-resistance between erythromycin and the other 14-membered and 15-membered ring macrolides	C	[58]
11.2	<i>Staphylococcus</i> spp.	Erythromycin and clindamycin	Clindamycin	IF resistant to erythromycin but susceptible to clindamycin, THEN test for inducible MLS _B resistance. IF negative, THEN report as susceptible to clindamycin. IF positive, THEN report as resistant to clindamycin or report as susceptible with a warning that clinical failure during treatment with clindamycin may occur by selection of constitutively resistant mutants and the use of clindamycin is probably best avoided in severe infections	Staphylococci resistant to macrolides but susceptible to clindamycin produce <i>Erm</i> ribosomal methylases conferring the inducible MLS _B phenotype or express efflux pumps. In the case of inducible MLS _B resistance, constitutively resistant mutants can be selected by clindamycin. In the case of resistance by efflux, the risk for selection of mutants resistant to clindamycin is not greater than that for erythromycin-susceptible isolates. Both clinical failures and successes with clindamycin have been reported for staphylococci with inducible MLS _B resistance. With a disk diffusion test, the inducible MLS _B phenotype can be identified by the flattening of the clindamycin zone facing the erythromycin disk	B	[58,59]
11.3	<i>Streptococcus</i> spp.	Erythromycin and clindamycin	Clindamycin	IF resistant to erythromycin but susceptible to clindamycin, THEN test for inducible MLS _B resistance. IF positive, report as susceptible to clindamycin with a warning that resistance may develop during treatment	Streptococci may be resistant to macrolides by production of a ribosomal <i>erm</i> methylase gene conferring the MLS _B phenotype or by production of an efflux pump encoded by the <i>mef(A)</i> class of genes. In the case of inducible MLS _B resistance, clindamycin may or may not remain active, depending on the type and expression of the <i>erm</i> gene. In the case of resistance by efflux, the risk for selection of mutants resistant to clindamycin is not greater than that for erythromycin-susceptible isolates. With a disk diffusion test, the inducible MLS _B phenotype can be identified by the flattening of the clindamycin zone facing the erythromycin disk. However, there is no clinical evidence of treatment failures, but treatment of serious infections should be avoided	C	[58]
11.4	<i>Peptostreptococcus</i> spp. and <i>Bacteroides</i> spp.	Erythromycin and clindamycin	Clindamycin	IF erythromycin MIC is >8 mg/L for <i>Peptostreptococcus</i> spp. or MIC is >32 mg/L for <i>Bacteroides</i> spp. but susceptible to clindamycin, THEN report as resistant to clindamycin	Resistance to macrolides in <i>Peptostreptococcus</i> spp. and <i>Bacteroides</i> spp. is generally caused by the production of a ribosomal <i>Erm</i> methylase conferring the MLS _B phenotype. In the case of inducible MLS _B resistance, resistance to clindamycin is poorly expressed <i>in vitro</i> , and this agent should not be considered active	C	[62,63]
11.5	<i>Staphylococcus</i> spp.	Clindamycin	Quinupristin-dalfopristin	IF resistant to clindamycin, THEN report a warning that bactericidal activity of quinupristin-dalfopristin is reduced	Resistance to clindamycin (associated with resistance to erythromycin) is a marker of the constitutive MLS _B resistance phenotype. Cross-resistance to the streptogramin B-type factor leads to diminished bactericidal activity of the combination of quinupristin and dalfopristin. Experimental models of staphylococcal endocarditis have given conflicting results on the <i>in vivo</i> activity of quinupristin-dalfopristin for the treatment of animals infected with constitutive MLS _B resistant isolates	C	[60,61,109]

TABLE 12. Interpretive rules for aminoglycosides

Rule no.	Organisms	Agent tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
12.1	<i>Staphylococcus</i> spp.	Kanamycin	Amikacin	IF kanamycin MIC is >8 mg/L, THEN report as resistant to amikacin	Resistance to kanamycin is generally caused by the production of APH(3)-I-3, ANT(4'')-I or bifunctional APH(2)-AAC(6) enzymes that determine loss of synergism of kanamycin and amikacin with β -lactams and glycopeptides irrespective of MIC values	C	[76, 110]
12.2	<i>Staphylococcus</i> spp.	Tobramycin	Kanamycin and amikacin	IF resistant to tobramycin, THEN report as resistant to kanamycin and amikacin	Resistance to tobramycin is generally caused by the production of ANT(4'')-I or bifunctional APH(2)-AAC(6) enzymes that determine loss of synergism of kanamycin and amikacin with β -lactams and glycopeptides irrespective of MIC values	C	[110]
12.3	<i>Staphylococcus</i> spp.	Gentamicin	All aminoglycosides	IF resistant to gentamicin, THEN report as resistant to all aminoglycosides	Resistance to gentamicin is generally caused by the production of a bifunctional APH(2)-AAC(6) enzyme that determines loss of synergism of all aminoglycosides (except streptomycin and arbekacin) with β -lactams and glycopeptides irrespective of MIC values	B	[75, 111]
12.4	<i>Enterococcus</i> spp. and <i>Streptococcus</i> spp.	Streptomycin	Streptomycin	IF high level-resistance to streptomycin is detected (MIC of >512 mg/L), THEN report as high-level resistant to streptomycin	High-level resistance reflects production of ANT(6) or of other enzymes or of ribosomal mutation. There is no synergistic effect between streptomycin and β -lactam agents in enterococci with high-level resistance to streptomycin	A (Enterococcus), C (Streptococcus)	[73]
12.5	<i>Enterococcus</i> spp., <i>Streptococcus</i> spp.	Kanamycin	Amikacin	IF high-level resistance to kanamycin is detected (MIC of >512 mg/L), THEN report as having high-level resistance to amikacin	High-level resistance to kanamycin is generally caused by the production of APH(3)-I-3 or bifunctional APH(2)-AAC(6) enzymes that determine loss of synergism of kanamycin and amikacin with β -lactams and glycopeptides irrespective of MIC values	B (Enterococcus), C (Streptococcus)	[74, 76]
12.6	<i>Enterococcus</i> spp., <i>Streptococcus</i> spp.	Gentamicin	All aminoglycosides except streptomycin	IF high-level resistance to gentamicin is detected (MIC of >128 mg/L), THEN report as having high-level resistance to all aminoglycosides except streptomycin	High-level resistance to gentamicin is generally caused by the production of a bifunctional APH(2)-AAC(6) enzyme that determines loss of synergism of all aminoglycosides (except streptomycin and arbekacin) with β -lactams and glycopeptides irrespective of MIC values	A (Enterococcus), C (Streptococcus)	[73, 112]
12.7	All <i>Enterobacteriaceae</i> , <i>Pseudomonas aeruginosa</i> , and <i>Acinetobacter baumannii</i>	Tobramycin, gentamicin, and amikacin	Amikacin	IF intermediately resistant or resistant to tobramycin and susceptible to gentamicin and amikacin, THEN report amikacin as intermediate for <i>Enterobacteriaceae</i> or resistant for <i>Pseudomonas</i> spp. and <i>Acinetobacter</i> spp.	Production of acquired AAC(6'')-I enzyme may confer phenotypic resistance despite modification of amikacin	C	[77–80, 113]
12.8	All <i>Enterobacteriaceae</i>	Gentamicin and other aminoglycosides	Gentamicin	IF intermediately resistant to gentamicin and susceptible to other aminoglycosides, THEN report as resistant to gentamicin	Expression of AAC(3)-I enzyme may be low, and isolates may have decreased susceptibility to gentamicin	C	[69, 114]
12.9	All <i>Enterobacteriaceae</i>	Tobramycin, gentamicin, and amikacin	Tobramycin	IF intermediately resistant to tobramycin, resistant to gentamicin and susceptible to amikacin, THEN report as resistant to tobramycin	Expression of the ANT(2'') enzyme may be low and isolates may have decreased susceptibility to tobramycin	C	[69, 115]
12.10	All <i>Enterobacteriaceae</i>	Netilmicin and gentamicin	Netilmicin	IF intermediately resistant to netilmicin and intermediately resistant or resistant to gentamicin and tobramycin, THEN report as resistant to netilmicin	Expression of the AAC(3'')-II or AAC(3'')-IV enzyme may be low and isolates may appear with decreased susceptibility to netilmicin	C	[69, 78]

TABLE 13. Interpretive rules for quinolones

Rule no.	Organism	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
13.1	<i>Staphylococcus</i> spp.	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning of risk for development of resistance during therapy with quinolones IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least one target mutation in <i>gyrA</i>	C	[86,92]
13.2	<i>Staphylococcus</i> spp.	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning that acquisition of a first-step mutation may lead to resistance development under therapy with other quinolones	Acquisition of combined mutations in <i>gyrA</i> and <i>gyrB</i> leads to complete or partial cross-resistance to all fluoroquinolones	C	[92,116,117]
13.3	<i>Streptococcus pneumoniae</i>	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning that acquisition of a first-step mutation may lead to resistance development under therapy with other quinolones	Acquisition of at least one target mutation in, for example, <i>parC</i> (<i>parE</i>). First-step mutations can be more reliably detected in tests with norfloxacin	C	[94,118–120]
13.4	<i>Streptococcus pneumoniae</i>	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of combined mutations in, for example, <i>parC</i> and <i>gyrA</i> leads to complete or partial cross-resistance to all fluoroquinolones	B	[121]
13.5	Enterobacteriaceae	Ciprofloxacin	All fluoroquinolones	IF resistant to ciprofloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least two target mutations in either <i>gyrA</i> or <i>gyrB</i> plus <i>parC</i> . Exceptionally, production of the AAC(6)-Ib-cr enzyme may affect ciprofloxacin but not levofloxacin	B	[93]
13.6	<i>Salmonella</i> spp.	Ciprofloxacin	All fluoroquinolones	IF ciprofloxacin MIC is >0.06 mg/L, THEN report as resistant to all fluoroquinolones	Evidence for clinical failure of fluoroquinolones in cases of resistance caused by the acquisition of at least one target mutation in <i>gyrA</i>	A (<i>Salmonella typhi</i>), B (other <i>Salmonella</i> spp.)	[95,97,98]
13.7	<i>Haemophilus influenzae</i>	Nalidixic acid	All fluoroquinolones	IF resistant in nalidixic acid disk diffusion screen test, THEN determine MIC of the fluoroquinolone to be used in therapy (ofloxacin, ciprofloxacin, levofloxacin, or moxifloxacin)	Decreased susceptibility to fluoroquinolones in <i>H. influenzae</i> caused by target topoisomerase mutations can be more reliably detected in tests with nalidixic acid. High-level fluoroquinolone resistance in this organism has been rarely described. Until there is evidence of clinical significance of these isolates, they should be reported as resistant	C	[96,122]
13.8	<i>Neisseria gonorrhoeae</i>	Ciprofloxacin and ofloxacin	All fluoroquinolones	IF resistant to ciprofloxacin or ofloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least two target mutations in either <i>gyrA</i> or <i>gyrB</i> plus <i>parC</i>	C	[123]

susceptible to penicillin can be reported as susceptible to aminopenicillins, cephalosporins, and carbapenems [18]. If an isolate is resistant to penicillin, identification and susceptibility should be checked (rule 8.3). Conversely, resistance to β -lactams in *Streptococcus pneumoniae* is common, owing to the production of mosaic PBPs that lead to various patterns of β -lactam resistance [19]. The oxacillin disk is traditionally used in screening tests to indicate benzylpenicillin susceptibility. Nevertheless, in addition to benzylpenicillin, when clinically needed, MICs of cephalosporins and carbapenems should be determined when the isolate is benzylpenicillin-resistant or when the oxacillin disk diffusion screening test result is interpreted as indicating resistance (rule 8.4). Among viridans group streptococci, production of mosaic PBPs also leads to various patterns of β -lactam resistance, and the oxacillin disk diffusion test developed for *Streptococcus pneumoniae* shows inadequate sensitivity in prediction of penicillin susceptibility. Moreover, susceptibility to cephalosporins and carbapenems cannot be inferred from benzylpenicillin susceptibility (rule 8.5) [20].

Enterococci. All enterococci are considered to be intrinsically resistant to cephalosporins (Table 4), but resistance to ampicillin mediated by alterations to PBP5 is increasingly recognized, particularly in *E. faecium* [21]. These alterations lead to decreased affinity for β -lactams, including all penicillins and carbapenems (rule 8.6). Penicillinase-producing *Enterococcus* isolates have been rarely detected, but have recently been described in Europe [22] (Sarti et al., 51st ICACC, 2011, Abstract C1-1785).

Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter spp.* Interpretive reading of the antibiogram is commonly based on β -lactams and β -lactamases in Gram-negative bacilli [8]. The first version of EUCAST expert rules for β -lactams and *Enterobacteriaceae* was influenced by this, particularly with isolates producing extended-spectrum β -lactamases (ESBLs) or carbapenemases. The cephalosporin and carbapenem breakpoints available when first version of EUCAST expert rules was published were later considered inappropriate, and old expert rules addressing ESBL and carbapenemase producers therefore needed to be modified in the second version of the rules.

For many years, confirmatory tests, mainly based on the synergistic effect observed between cephalosporins and β -lactamase inhibitors such as clavulanate, were applied in clinical microbiology laboratories to indicate the presence of ESBLs, mainly in *Escherichia coli* and *Klebsiella pneumoniae* isolates with reduced susceptibility to oxyimino-cephalosporins [23–25]. Following the detection of ESBL production in an

isolate, the susceptible and intermediate categories were reinterpreted as resistant on the assumption that the breakpoints were inadequate. However, some authors claimed that MIC breakpoints set at appropriate levels (decreasing their values) can detect the presence of ‘clinically significant’ resistance mechanisms, including ESBLs [26]. Animal models, pharmacokinetic (PK)/pharmacodynamic (PD) analysis, Monte Carlo simulation and new lower EUCAST breakpoints supported this approach. It is possible to avoid classification of most ESBL producers as susceptible to oxyimino-cephalosporins (mainly ceftazidime and cefepime) and aztreonam with EUCAST breakpoints as compared with CLSI breakpoints [27,28]. In addition, reduction in breakpoints so that clinically significant resistance is detected without the need for confirmatory tests avoids possible delay in reporting of susceptibility testing results for a large proportion of isolates, as the prevalence of ESBL-producing organisms has increased.

Most traditional microbiological practices have considered that all confirmed ESBL-positive organisms are resistant to all penicillins, cephalosporins, and aztreonam, thus forcing overuse of other antimicrobial classes such as carbapenems and fluoroquinolones. This, in turn, potentially exerts a selective pressure on microorganisms with other antimicrobial resistance mechanisms, including carbapenemase producers. Although clinical outcome with the use of third-generation and fourth-generation cephalosporins in the treatment of infections caused by low-MIC, ESBL-positive microorganisms remains to be fully evaluated, the new EUCAST breakpoints leave some room for the use of cefotaxime, ceftriaxone, or ceftazidime. This is supported by several clinical studies and observations, PK/PD data, Monte Carlo simulations, and animal model studies [29–34]. These studies have shown that clinical and experimental outcomes are better correlated with the MIC values than with the presence of an ESBL. With the new EUCAST breakpoints for *Enterobacteriaceae*, third-generation and fourth-generation cephalosporins should be reported as found, and the old expert rule recommending modification of reporting category for ESBL producers that appear to be susceptible is no longer necessary. This recommendation, which also applies to plasmid-mediated AmpC producers, is now included in the EUCAST breakpoint tables. Nevertheless, in many areas, ESBL detection and characterization are recommended or mandatory for infection control purposes. For consistency, and based on a similar approach, other rules, including those affecting *Klebsiella oxytoca* and *Citrobacter koseri* (old expert rule 9.3) [35] and that for isolates with carbapenemases (old expert rule 9.7), are deleted in the second version of the expert rules.

Carbapenemases, including class A, B and D enzymes, can have variable effects on carbapenems [36–38]. Moreover,

combined resistance mechanisms may also affect carbapenem susceptibility (e.g. combination of derepressed AmpC or ESBL and decreased permeability), ertapenem being particularly affected [39]. Recent data, as with ESBL producers, provide evidence to support the reporting of carbapenem susceptibility as found [40,41]. Nevertheless, more effort is required in the future to expand the evidence, particularly when a carbapenemase with low-level expression, such as with VIM enzymes, is present [42]. It is important to note that special attention should be paid to reduced susceptibility to carbapenems that may be related to true carbapenemases, not only for producers of class B (mainly VIM or IMP) or class A carbapenemases (KPC), but also for those expressing OXA-48, a class D carbapenemase that is increasingly being identified in *Enterobacteriaceae* [43].

New expert rule number 9.1 highlights the uncertain therapeutic outcome of treatment with a penicillin in combination with a β -lactamase inhibitor for *Enterobacteriaceae* isolates that are intermediate or resistant to any third-generation or fourth-generation cephalosporin in infections other than those affecting the urinary tract [44,45]. This is also the case for new expert rule number 9.2, the evidence for which is graded A for *Enterobacter* spp. and B for *Citrobacter freundii*, *Serratia* spp., and *Morganella morganii*. Rule 9.2 recommends discouraging the use of cefotaxime, ceftriaxone or ceftazidime in monotherapy or suppressing the susceptibility testing results for these agents, owing to the risk of selecting resistance in AmpC producers [46]. In some publications, it is claimed that this problem can be avoided with combination therapy, including (unlike aminoglycosides) the addition of a fluoroquinolone [47].

Other Gram-negative organisms. Other Gram-negative organisms, such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*, are considered in the EUCAST expert rules Table 10. For *H. influenzae*, resistance to ampicillin, which is considered representative of amoxicillin for susceptibility testing, is mainly attributable to β -lactamase production. Isolates producing β -lactamases, mainly TEM-1, should be considered to be resistant to both ampicillin and amoxicillin (rules 10.1) [48]. Ampicillin resistance in the absence of β -lactamase production can be conferred by mutations in the *ftsI* gene affecting PBPs and leading to reduced affinity for β -lactams [49]. These isolates, termed β -lactamase-negative and ampicillin-resistant, should be considered to be resistant to aminopenicillin- β -lactamase inhibitor combinations (amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam) and to first-generation and second-generation cephalosporins (rule 10.2) [50,51]. Although piperacillin and piperacillin-tazobactam appear less affected by the PBP-

mediated resistance mechanisms, evidence regarding clinical efficacy is lacking.

H. influenzae isolates with altered PBPs and β -lactamase production are also being increasingly found. These isolates are phenotypically resistant to amoxicillin-clavulanate and ampicillin-sulbactam (β -lactamase-positive and resistant to amoxicillin-clavulanate, and should also be considered to be resistant to piperacillin-tazobactam and to first-generation and second-generation cephalosporins (rule 10.3) [52]. ESBL-producing isolates have not yet been found in *H. influenzae*, but *bla*_{ESBL} genes have been cloned in this species, resulting in third-generation cephalosporin resistance when PBP3 is concomitantly altered [53]. Moreover, a TEM ESBL variant has also been found in *Haemophilus parainfluenzae* [54].

For *N. gonorrhoeae*, isolates that are β -lactamase-positive should be considered to be resistant to benzylpenicillin, ampicillin, and amoxicillin. Chromosomal mutations affecting affinity of PBPs, decreased permeability or efflux pumps also confer resistance to β -lactamase inhibitor combinations, and resistance will be detected by the application of EUCAST breakpoints (rule 10.4) [55-57].

Expert rules for *Moraxella catarrhalis* have been deleted in this second version of the expert rules, and the relevant points are now included in the breakpoint table.

Interpretive rules for macrolides, lincosamides, and streptogramins

Although the macrolides, lincosamides and streptogramins have different chemical structures, they share similar mechanisms of action, and can be affected by the same resistance mechanisms. EUCAST expert rules for these agents include staphylococci, streptococci, *Peptostreptococcus* spp., and *Bacteroides* spp. (Table 11, rules 11.1-11.5). Other organisms, such as *H. influenzae*, have been considered in this version of the expert rules only within the intrinsic resistance tables.

Erythromycin is considered to be the class representative for 14-membered (clarithromycin) and 15-membered (azithromycin) ring macrolides, with the exception of ketolides (telithromycin). Resistance to these compounds is generally mediated by the production of ribosomal methylases encoded by *erm* genes that confer constitutive or inducible macrolide-lincosamide-streptogramin B (MLS_B) phenotypes or by the production of an efflux pump (M phenotype, conferring resistance to erythromycin but not to clindamycin and/or streptogramins) [58]. With both mechanisms, there is cross-resistance between erythromycin and the other 14-membered and 15-membered ring macrolides (rule 11.1). This resistance can occur with or without cross-resistance to clindamycin and lincosamides. For staphylococci and streptococci, isolates resistant to erythromycin but susceptible to

clindamycin should be tested for inducible MLS_B resistance (dissociated resistance) [58].

The recommended disk diffusion test for inducible MLS_B resistance consists of an erythromycin disk in close proximity to a clindamycin disk. Flattening of the zone of inhibition around the clindamycin or lincosamide disk in the vicinity of erythromycin ('D'-shaped zone) is indicative of the inducible MLS_B phenotype, which is mediated by the presence of an *erm* gene. A negative result, i.e. no flattened zone, is associated with the presence of an efflux pump (*mef* gene). From a clinical point of view, the use of clindamycin or lincomycin is not recommended in infections caused by isolates displaying an inducible MLS_B phenotype. These isolates should be reported as resistant or with a warning indicating potential clinical failure during treatment with clindamycin or lincomycin (rule 11.2) [59]. For staphylococcal isolates that are simultaneously resistant to erythromycin and clindamycin or lincomycin, a warning of reduced susceptibility to the combination quinupristin–dalfopristin and loss of bactericidal activity should be included in the susceptibility test report (rule 11.5) [60,61].

For streptococci, less clinical evidence is available but, similarly, isolates that are resistant to erythromycin and susceptible to clindamycin should be tested for inducible MLS_B resistance and reported as clindamycin-susceptible if the result is positive but with a warning that resistance may develop on prolonged treatment (rule 11.3) [58]. When *Peptostreptococcus* spp. and *Bacteroides* spp. express an inducible MLS_B phenotype, resistance to clindamycin is difficult to detect *in vitro*, and this agent should not be considered to be clinically active (rule 11.4) [62,63].

Interpretive rules for aminoglycosides

Aminoglycoside agents have a bactericidal effect on most Gram-positive and Gram-negative organisms. They bind to 16S rRNA of the 30S bacterial ribosomal subunit and thereby inhibit protein synthesis. Several mechanisms that compromise the activity of aminoglycosides have been described: (i) decreased permeability and/or accumulation of the aminoglycoside agents because of mutations affecting passive diffusion or active transport, porin and/or lipopolysaccharide alteration (only in Gram-negative organisms), and efflux pump hyperexpression; (ii) target (ribosomal) modifications caused by mutations in ribosomal proteins (S3, S4, S5, S6, S12, S17, and L6) and as a result of the action of new methylases affecting 16S RNA; and (iii) aminoglycoside-modifying enzymes, which are acetyltransferases, phosphotransferases, or nucleotidyltransferases (also known as adenylyltransferases) [64–68].

Phenotype recognition of these resistance mechanisms is generally more complex than for those affecting β -lactam

compounds. Decreased permeability and/or resistance mechanisms involving efflux pumps usually confer a low-level resistance phenotype affecting nearly all aminoglycosides. With the exception of those described in *P. aeruginosa*, resistance mediated by efflux pumps is difficult to infer from phenotypic susceptibility [68], but cross-resistance to other antimicrobial classes, such as fluoroquinolone or tetracycline agents, might indicate their potential presence. Ribosomal mutations are extremely rare, do not confer 'class resistance', and do not always endow high-level resistance. Conversely, 16S RNA methylation confers high-level resistance, mainly affecting 4,6-disubstituted compounds (such as kanamycin, gentamicin, tobramycin, amikacin, and netilmicin), but not 4,5-disubstituted compounds (such as neomycin and paramomycin), streptomycin, and/or the aminocyclitol agent spectinomycin [69].

Aminoglycoside-modifying enzymes are the most widely distributed resistance mechanisms affecting aminoglycosides, and enzymatic modification of an aminoglycoside can be mediated by different enzymes. Modifications do not always confer phenotypic resistance, and resistance may be more clearly indicated in tests with aminoglycoside agents that are not used in the human clinical setting [69–72]. Other problems complicating interpretive reading for this group of antimicrobials are that enzymatic modifications of different aminoglycosides can be produced by a single enzyme and that unrelated enzymes can confer a similar resistance phenotype. Also, a single isolate can express different modifying enzymes, making interpretation of resistance phenotypes difficult and, in some cases, unreliable.

Despite this apparent complexity, several EUCAST interpretive rules can be applied when antibiograms of aminoglycosides are read (Table 12). For Gram-positive organisms, these rules facilitate detection of the absence of synergy between a specific aminoglycoside and β -lactam or glycopeptide agents (rules 12.1–12.6). For enterococci, the evidence for these rules is graded A or B and is based on clinical data [73,74]. For staphylococci, however, the evidence for most rules is graded C, owing to microbiological demonstration of the absence of *in vitro* synergy of the aminoglycosides with cell wall-active compounds, even with isolates that are apparently susceptible to aminoglycosides [75,76].

In Gram-negative organisms, EUCAST interpretive rules for aminoglycosides tend to change a susceptible or an intermediate result to the resistant category (rules 12.7–12.10). The evidence for all of these rules is graded C, and is mainly based on biochemical data indicating that these compounds are enzymatically affected. In most cases, the increase in MIC values or decrease in inhibition zones is very small. Modification of results to the resistant category avoids clinical use of these compounds [77–80].

In certain Gram-negative organisms, such as *Providencia stuartii* (but not *Providencia rettgerii*) and *Serratia marcescens*, the aminoglycoside-modifying enzymes are chromosomally encoded and are weakly expressed. However, as mutational events confer phenotypic resistance, these isolates should be considered to be (intrinsically) resistant to these agents (Table 1, rules 1.12 and 1.14) [81–83]. *E. faecium* intrinsically produces a chromosomal aminoglycoside-modifying enzyme that is also responsible for loss of synergy between certain aminoglycosides and cell wall-active compounds (Table 4, rule 4.8) [84].

Interpretive rules for quinolones

The quinolone agents are rapidly bactericidal within a range of concentrations, and when that range is exceeded the lethal action is diminished [85]. The quinolones interact with bacterial type II topoisomerase DNA gyrases encoded by *gyrA* and *gyrB* and topoisomerase IV encoded by *parC* and *parE* (in staphylococci, *grlA* and *grlB*), which are the preferential targets of Gram-negative and Gram-positive organisms, respectively. Topoisomerase mutations in *gyrA* and *parC* and reduction in target access, including porin modification and efflux systems, are the classical chromosomally encoded mechanisms affecting these compounds. Topoisomerase mutations can confer high-level resistance, mainly through stepwise selection of several mutations in the same or different topoisomerase [86].

Plasmid-mediated quinolone resistance mechanisms have emerged in Gram-negative bacilli during the last decades, and are now frequently observed in many parts of the world [87]. All of them demonstrate low expression, and they do not always affect all fluoroquinolone agents. Target protection mechanisms involving the Qnr proteins were the first described plasmid-mediated resistance mechanisms [88]. Several families of these proteins have now been described, mainly in *Enterobacteriaceae*. In addition, enzymatic modification involving a mutated aminoglycoside-modifying enzyme and also affecting only certain fluoroquinolones has been identified in these organisms. This enzyme (AAC(6′)-Ib-cr) affects C7-piperazinyl substituted fluoroquinolones, ciprofloxacin, and norfloxacin, but not levofloxacin [89]. More recently, two plasmid-mediated efflux-based mechanisms involving the QepA and OqxAB pumps related to major facilitator superfamily transporters have been described. In this case, the resistance is low-level and phenotypic detection is extremely difficult [90,91].

In general, older quinolones have lower activity than more recently developed agents. This is more obvious with Gram-negative organisms, and is particularly evident in *Enterobacteriaceae*. However, particularly with resistance caused by

mutations in topoisomerases, decreased susceptibility to one fluoroquinolone is reflected in reduced susceptibility to other fluoroquinolones (class resistance). With these isolates, the concomitant presence of different mutations increases the level of fluoroquinolone resistance. Nevertheless, Qnr protein, efflux and enzymatic modification resistance mechanisms may not confer resistance to all fluoroquinolones. Such low-level resistance mechanisms are difficult to detect, but they indicate the potential for selection of higher-level resistance mechanisms.

When antibiograms with quinolones are read, resistance to the most active fluoroquinolone *in vitro* indicates resistance to all fluoroquinolones in both Gram-negative and Gram-positive organisms [92–94]. An exception to this rule in Gram-negative organisms is the potential production of the AAC(6′)-Ib-cr enzyme, which affects ciprofloxacin but not levofloxacin. EUCAST interpretive rules for fluoroquinolones (rules 13.2, 13.4, 13.5, 13.6, and 13.8) all take this approach, supported by different levels of evidence (grades B or C). In some organisms (i.e. *Enterobacteriaceae* and *H. influenzae*), nalidixic acid can be used as a predictor of resistance mechanisms affecting fluoroquinolones [95–97]. However, in *Enterobacteriaceae* this compound does not detect qnr-mediated or other plasmid-mediated quinolone resistance, which is increasingly being recognized all over the world. For this reason, in this second version of the expert rules, modification of fluoroquinolone susceptibility results on the basis of ciprofloxacin MIC values is recommended for *Salmonella* spp., as there is clear clinical evidence for ciprofloxacin indicating a poor response in systemic infections caused by *Salmonella* spp. with low-level quinolone resistance (MIC >0.064 mg/L) (rule 13.6). The available data relate mainly to *Salmonella Typhi*, but there are also case reports of poor response with other *Salmonella* species [95,97,98]. On the contrary, rule 13.6 does not apply to other *Enterobacteriaceae*, as there is a lack of such clear clinical evidence and generalization cannot be recommended. Nevertheless, laboratories might alert clinicians to the possibility of emergence of high-level resistance to fluoroquinolones in *Enterobacteriaceae* with low-level resistance to these compounds when fluoroquinolones are used.

In staphylococci and viridans group streptococci, resistance to the less active, but not to the more active, fluoroquinolones indicates that a first-step mutation may be present. In this case, a warning should be added to the susceptibility testing report, alerting clinicians to the potential for selection of a higher-level resistance mechanism involving different mutations (rules 13.1 and 13.3).

Inference of specific fluoroquinolone resistance mechanisms can be difficult in multidrug-resistant organisms, as

they may have superimposed mechanisms affecting these compounds (low-level and/or high-level resistance). Moreover, with any of the new plasmid-mediated resistance mechanisms, there is little possibility of interpretive reading. In some cases, a slight decrease in susceptibility to all quinolones is observed, but in others a greater decrease in susceptibility to fluoroquinolones than that observed with nalidixic acid can be seen [89–91].

Future of Expert Rules and Concluding Remarks

Expert rules were designed to assist clinical microbiologists in the interpretation of antimicrobial susceptibility testing results. The main objective of these rules has been to modify the clinical interpretation after application of clinical breakpoint criteria. In most instances, susceptible or intermediate clinical interpretations are modified to resistant, owing to the demonstration of the presence of a resistance mechanism that has clinical implications. These modifications are supported by clinical evidence and/or microbiological knowledge. Modifications can also imply, according to the definition of clinical breakpoints, that the breakpoints used are not optimal and thus require the support of an expert rule.

The current EUCAST process allows for revision of clinical breakpoints. Revised breakpoints can be shown to be more precise in the correlation of MIC values with expected clinical outcomes. Setting appropriate clinical breakpoints may make some of the previously defined expert rules unnecessary, as well as resulting in modification or rewording of other rules. This has been the case for the ESBL expert rule, which is no longer necessary when the revised cephalosporin breakpoints are used.

Finally, it is necessary to stress that clinical breakpoints, as defined by EUCAST, do not aim to detect all resistance mechanisms that might be present in the bacteria. Rather, they have been developed to predict the outcome of antimicrobial treatment of infected patients on the basis of microbiological, PK/PD and clinical criteria. It is also important to note that EUCAST expert rules should be used with EUCAST breakpoints, and may not be applicable if other breakpoint systems are used.

Funding

EUCAST is supported by a grant from the ECDC and by ESCMID.

Transparency Declarations

Nothing to declare.

References

1. Winstanley T, Courvalin P. Expert systems in clinical microbiology. *Clin Microbiol Rev* 2011; 24: 515–556.
2. Courvalin P. Interpretive reading of antimicrobial susceptibility tests. *ASM News* 1992; 58: 368–375.
3. Courvalin P. Interpretive reading of *in vitro* antibiotic susceptibility tests (the antibiogramme). *Clin Microbiol Infect* 1996; 2 (suppl 1): S26–S34.
4. Livermore DM, Winstanley TG, Shannon KP. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. *J Antimicrob Chemother* 2001; 48 (suppl 1): 87–102.
5. Macgowan AP, BSAC Working Parties on Resistance Surveillance. Clinical implications of antimicrobial resistance for therapy. *J Antimicrob Chemother* 2008; 62 (suppl 2): ii105–ii114.
6. Vedel G, Peyret M, Gayral JP, Millot P. Evaluation of an expert system linked to a rapid antibiotic susceptibility testing system for the detection of β -lactam resistance phenotypes. *Res Microbiol* 1996; 147: 297–309.
7. Livermore DL, Struelens M, Amorim J et al. Multicentre evaluation of the VITEK 2 advanced expert system for interpretive reading of antimicrobial resistance tests. *J Antimicrob Chemother* 2002; 49: 289–300.
8. Livermore DM. β -Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557–584.
9. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 2010; 54: 969–976.
10. Zapun A, Contreras-Martel C, Vernet T. Penicillin-binding proteins and β -lactam resistance. *FEMS Microbiol Rev* 2008; 32: 361–385.
11. Pagès JM, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nat Rev Microbiol* 2008; 6: 893–903.
12. Poole K. Efflux pumps as antimicrobial resistance mechanisms. *Ann Med* 2007; 39: 162–176.
13. Page MG. Anti-MRSA β -lactams in development. *Curr Opin Pharmacol* 2006; 5: 480–485.
14. Brown DF. Detection of methicillin/oxacillin resistance in staphylococci. *J Antimicrob Chemother* 2001; 48 (suppl 1): 65–70.
15. Chambers HF, Sachdeva M, Kennedy S. Binding affinity for penicillin-binding protein 2a correlates with *in vivo* activity of β -lactam antibiotics against methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 1990; 162: 705–710.
16. Montanari MP, Massidda O, Mingoia M, Varaldo PE. Borderline susceptibility to methicillin in *Staphylococcus aureus*: a new mechanism of resistance? *Microb Drug Resist* 1996; 2: 257–260.
17. Kimura K, Suzuki S, Wachino J et al. First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* 2008; 52: 2890–2897.
18. Casey JR, Pichichero ME. Meta-analysis of cephalosporins versus penicillin for treatment of group A streptococcal tonsillopharyngitis in adults. *Clin Infect Dis* 2004; 11: 1526–1534.
19. File TM Jr. Clinical implications and treatment of multiresistant *Streptococcus pneumoniae* pneumonia. *Clin Microbiol Infect* 2006; 12 (suppl 3): 31–41.
20. Nagai K, Davies TA, Jacobs MR, Appelbaum PC. Effects of amino acid alterations in penicillin-binding proteins (PBPs) 1a, 2b, and 2x on PBP affinities of penicillin, ampicillin, amoxicillin, cefditoren, cefuroxime,

- cefprozil, and cefaclor in 18 clinical isolates of penicillin-susceptible, -intermediate, and -resistant pneumococci. *Antimicrob Agents Chemother* 2002; 5: 1273–1280.
21. Fontana R, Ligozzi M, Pitaluga F, Satta G. Intrinsic penicillin resistance in enterococci. *Microb Drug Resist* 1996; 2: 209–213.
 22. Leclercq R. Enterococci acquire new kinds of resistance. *Clin Infect Dis* 1997; 24 (suppl 1): S80–S84.
 23. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β -lactamases conferring transferable resistance to newer β -lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867–878.
 24. Livermore DM, Brown DF. Detection of β -lactamase-mediated resistance. *J Antimicrob Chemother* 2001; 48 (suppl 1): 59–64.
 25. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β -lactamase production in Enterobacteriaceae: review and bench guide. *Clin Microbiol Infect* 2008; 14 (suppl 1): 90–103.
 26. Kahlmeter G. Breakpoints for intravenously used cephalosporins in Enterobacteriaceae—EUCAST and CLSI breakpoints. *Clin Microbiol Infect* 2008; 14 (suppl 1): 169–174.
 27. MacGowan A. Breakpoints for extended-spectrum β -lactamase-producing Enterobacteriaceae: pharmacokinetic/pharmacodynamic considerations. *Clin Microbiol Infect* 2008; 14 (suppl 1): 166–168.
 28. Hawser SP, Badal RE, Bouchillon SK, Hoban DJ, Hsueh PR. Comparison of CLSI 2009, CLSI 2010 and EUCAST cephalosporin clinical breakpoints in recent clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* from the SMART Global Surveillance Study. *Int J Antimicrob Agents* 2010; 36: 293–294.
 29. Bin C, Hui W, Renyuan Z et al. Outcome of cephalosporin treatment of bacteremia due to CTX-M-type extended-spectrum β -lactamase-producing *Escherichia coli*. *Diagn Microbiol Infect Dis* 2006; 56: 351–357.
 30. Endimiani A, Paterson DL. Optimizing therapy for infections caused by enterobacteriaceae producing extended-spectrum β -lactamases. *Semin Respir Crit Care Med* 2007; 28: 646–655.
 31. Maglio D, Ong C, Banevicius MA, Geng Q, Nightingale CH, Nicolau DP. Determination of the in vivo pharmacodynamic profile of cefepime against extended-spectrum- β -lactamase-producing *Escherichia coli* at various inocula. *Antimicrob Agents Chemother* 2004; 48: 1941–1947.
 32. Wong-Beringer A, Hindler J, Loeloff M et al. Molecular correlation for the treatment outcomes in bloodstream infections caused by *Escherichia coli* and *Klebsiella pneumoniae* with reduced susceptibility to ceftazidime. *Clin Infect Dis* 2002; 34: 135–146.
 33. Paterson DL, Ko WC, Von Gottberg A et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β -lactamases: implications for the clinical microbiology laboratory. *J Clin Microbiol* 2001; 39: 2206–2212.
 34. Bhavnani SM, Ambrose PG, Craig WA, Dudley MN, Jones RN, SENTRY Antimicrobial Surveillance Program. Outcomes evaluation of patients with ESBL- and non-ESBL-producing *Escherichia coli* and *Klebsiella* species as defined by CLSI reference methods: report from the SENTRY Antimicrobial Surveillance Program. *Diagn Microbiol Infect Dis* 2006; 54: 231–236.
 35. Potz NA, Colman M, Warner M, Reynolds R, Livermore DM. False-positive extended-spectrum β -lactamase tests for *Klebsiella oxytoca* strains hyperproducing KI β -lactamase. *J Antimicrob Chemother* 2004; 53: 545–547.
 36. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo- β -lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005; 18: 306–325.
 37. Poirel L, Pitout JD, Nordmann P. Carbapenemases: molecular diversity and clinical consequences. *Future Microbiol* 2007; 2: 501–512.
 38. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004; 48: 15–22.
 39. Woodford N, Dallow J, Hill RLR et al. Mechanisms of ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the United Kingdom to a reference laboratory. *Int J Antimicrob Agents* 2007; 29: 456–459.
 40. Daikos GL, Petrikkos P, Psychogiou M et al. Prospective observational study of the impact of VIM-1 metallo- β -lactamase on the outcome of patients with *Klebsiella pneumoniae* bloodstream infections. *Antimicrob Agents Chemother* 2009; 53: 1868–1873.
 41. Daikos GL, Markogiannakis A. Carbapenemase-producing *Klebsiella pneumoniae*: (when) might we still consider treating with carbapenems? *Clin Microbiol Infect* 2011; 17: 1135–1141.
 42. Tato M, Morosini M, García L, Albertí S, Coque MT, Cantón R. Carbapenem heteroresistance in VIM-1-producing *Klebsiella pneumoniae* isolates belonging to the same clone: consequences for routine susceptibility testing. *J Clin Microbiol* 2010; 48: 4089–4093.
 43. Livermore DM. Has the era of untreatable infections arrived? *J Antimicrob Chemother* 2009; 64 (suppl 1): i29–i36.
 44. Gavin PJ, Suseno MT, Thomson RB Jr et al. Clinical correlation of the CLSI susceptibility breakpoint for piperacillin–tazobactam against extended-spectrum- β -lactamase-producing *Escherichia coli* and *Klebsiella* species. *Antimicrob Agents Chemother* 2006; 50: 2244–2247.
 45. Rodríguez-Baño J, Navarro MD, Romero L et al. Bacteremia due to extended spectrum β -lactamase-producing *Escherichia coli* in the CTX-M era: a new clinical challenge. *Clin Infect Dis*, 2006; 43: 1407–1414.
 46. Chow JW, Fine MJ, Shlaes DM et al. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med* 1991; 115: 585–590.
 47. Schwaber MJ, Graham CS, Sands BE, Gold HS, Carmeli Y. Treatment with a broad-spectrum cephalosporin versus piperacillin–tazobactam and the risk for isolation of broad-spectrum cephalosporin-resistant *Enterobacter* species. *Antimicrob Agents Chemother* 2003; 47: 1882–1886.
 48. Tristram S, Jacobs MR, Appelbaum PC. Antimicrobial resistance in *Haemophilus influenzae*. *Clin Microbiol Rev* 2007; 20: 368–389.
 49. Ubukata K, Shibasaki Y, Yamamoto K et al. Association of amino acid substitutions in penicillin-binding protein 3 with β -lactam resistance in β -lactamase-negative ampicillin-resistant *Haemophilus influenzae*. *Antimicrob Agents Chemother* 2001; 45: 1693–1699.
 50. Morikawa Y, Kitazato M, Mitsuyama J, Mizunaga S, Minami S, Watanabe Y. In vitro activities of piperacillin against β -lactamase-negative ampicillin-resistant *Haemophilus influenzae*. *Antimicrob Agents Chemother* 2004; 48: 1229–1234.
 51. Kubota T, Higa F, Kusano N et al. Genetic analyses of β -lactamase negative ampicillin-resistant strains of *Haemophilus influenzae* isolated in Okinawa, Japan. *Jpn J Infect Dis* 2006; 59: 36–41.
 52. Matic V, Bozdogan B, Jacobs MR, Ubukata K, Appelbaum PC. Contribution of β -lactamase and PBP amino acid substitutions to amoxicillin/clavulanate resistance in β -lactamase-positive, amoxicillin/clavulanate-resistant *Haemophilus influenzae*. *J Antimicrob Chemother* 2003; 52: 1018–1021.
 53. Bozdogan B, Tristram S, Appelbaum PC. Combination of altered PBPs and expression of cloned extended-spectrum β -lactamases confers cefotaxime resistance in *Haemophilus influenzae*. *J Antimicrob Chemother* 2006; 57: 747–749.
 54. Tristram SG, Pitout MJ, Forward K, Campbell S, Nichols S, Davidson RJ. Characterization of extended-spectrum β -lactamase-producing isolates of *Haemophilus parainfluenzae*. *J Antimicrob Chemother* 2008; 61: 509–514.
 55. Dillon JA, Yeung KH. β -Lactamase plasmids and chromosomally mediated antibiotic resistance in pathogenic *Neisseria* species. *Clin Microbiol Rev* 1989; 2: S125–S133.
 56. Ropp PA, Hu M, Olesky M, Nicholas RA. Mutations in *ponA*, the gene encoding penicillin-binding protein I, and a novel locus, *penC*, are

- required for high-level chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2002; 46: 769–777.
57. Olesky M, Zhao S, Rosenberg RL, Nicholas RA. Porin-mediated antibiotic resistance in *Neisseria gonorrhoeae*: ion, solute, and antibiotic permeation through PIB proteins with penB mutations. *J Bacteriol* 2006; 188: 2300–2308.
58. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis* 2002; 34: 482–492.
59. Lewis JS, Jorgensen JH. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned? *Clin Infect Dis* 2005; 40: 280–285.
60. Entenza JM, Drugeon H, Glauser MP, Moreillon P. Treatment of experimental endocarditis due to erythromycin-susceptible or -resistant methicillin-resistant *Staphylococcus aureus* with RP 59500. *Antimicrob Agents Chemother* 1995; 39: 1419–1424.
61. Batard E, Jacqueline C, Boutoille D et al. Combination of quinupristin–dalfopristin and gentamicin against methicillin-resistant *Staphylococcus aureus*: experimental rabbit endocarditis study. *Antimicrob Agents Chemother* 2002; 46: 2174–2178.
62. Reig M, Fernández MC, Ballesta JP, Baquero F. Inducible expression of ribosomal clindamycin resistance in *Bacteroides vulgatus*. *Antimicrob Agents Chemother* 1992; 36: 639–642.
63. Reig M, Moreno A, Baquero F. Resistance of *Peptostreptococcus* spp. to macrolides and lincosamides: inducible and constitutive phenotypes. *Antimicrob Agents Chemother* 1992; 36: 662–664.
64. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob Agents Chemother* 2000; 44: 3249–3256.
65. Jana S, Deb JK. Molecular understanding of aminoglycoside action and resistance. *Appl Microbiol Biotechnol* 2006; 70: 140–150.
66. Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. *Clin Microbiol Rev* 2003; 16: 430–450.
67. Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. *Clin Infect Dis* 2007; 45: 88–94.
68. Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2006; 43 (suppl 2): S49–S56.
69. Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* 1993; 57: 138–163.
70. Davies J, Wright GD. Bacterial resistance to aminoglycoside antibiotics. *Trends Microbiol* 1997; 5: 234–240.
71. Wright GD. Aminoglycoside-modifying enzymes. *Curr Opin Microbiol* 1999; 2: 499–503.
72. Azucena E, Mobashery S. Aminoglycoside-modifying enzymes: mechanisms of catalytic processes and inhibition. *Drug Resist Updat* 2001; 4: 106–117.
73. Chow JW. Aminoglycoside resistance in enterococci. *Clin Infect Dis* 2000; 31: 586–589.
74. Thauvin C, Eliopoulos, GM, Wennersten, C, Moellering, RC Jr. Antagonistic effect of penicillin–amikacin combinations against enterococci. *Antimicrob Agents Chemother* 1985; 28: 78–83.
75. Martel A, Moreau N, Capmau ML, Soussy CJ, Duval J. 2'-O-phosphorylation of gentamicin components by a *Staphylococcus aureus* strain carrying a plasmid. *Antimicrob Agents Chemother* 1977; 12: 26–30.
76. Courvalin P, Davies J. Plasmid-mediated aminoglycoside phosphotransferase of broad substrate range that phosphorylates amikacin. *Antimicrob Agents Chemother* 1977; 11: 619–624.
77. Benveniste R, Davies J. Enzymatic acetylation of aminoglycoside antibiotics by *Escherichia coli* carrying an R factor. *Biochemistry* 1971; 10: 1787–1796.
78. Le Goffic F, Martel A, Witchitz J. 3-N enzymatic acetylation of gentamicin, tobramycin, and kanamycin by *Escherichia coli* carrying an R factor. *Antimicrob Agents Chemother* 1974; 6: 680–684.
79. Martin P, Jullien E, Courvalin P. Nucleotide sequence of *Acinetobacter baumannii* aphA-6 gene: evolutionary and functional implications of sequence homologies with nucleotide-binding proteins, kinases and other aminoglycoside-modifying enzymes. *Mol Microbiol* 1988; 2: 615–625.
80. Shaw KJ, Hare RS, Sabatelli FJ et al. Correlation between aminoglycoside resistance profiles and DNA hybridization of clinical isolates. *Antimicrob Agents Chemother* 1991; 35: 2253–2261.
81. Knothe H, Kettner M, Krcmery V. R-plasmids in *Providencia* and *Proteus rettgeri* strains from Frankfurt University Hospital. In: Mituhashi S, Roswal L, Krcmery V, eds. *Plasmids. Medical and theoretical aspects*. Berlin: Springer-Verlag, 1977; 435–439.
82. Widermann B, Klopfer-Kaul I, Tetzloff G. Untersuchungen über das Aminoglykosid-Antibiotika inaktivierende Enzym AAC(6'). *Infection* 1979; 7: S192–S196.
83. Macinga DR, Rather PN. The chromosomal 2'-N-acetyltransferase of *Providencia stuartii*: physiological functions and genetic regulation. *Front Biosci* 1999; 4: D132–D140.
84. Chen HY, Williams JD. Transferable resistance and aminoglycoside-modifying enzymes in enterococci. *J Med Microbiol* 1985; 20: 187–196.
85. Smith JT. The mode of action of 4-quinolones and possible mechanisms of resistance. *J Antimicrob Chemother* 1986; 18 (suppl D): 21–29.
86. Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis* 2005; 41 (suppl 2): S120–S126.
87. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis* 2006; 6: 629–640.
88. Martínez-Martínez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; 351: 797–799.
89. Robicsek A, Strahilevitz J, Jacoby GA et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* 2006; 12: 83–88.
90. Martínez-Martínez L, Eliecer Cano M, Manuel Rodríguez-Martínez J, Calvo J, Pascual A. Plasmid-mediated quinolone resistance. *Expert Rev Anti Infect Ther* 2008; 6: 685–711.
91. Rodríguez-Martínez JM, Cano ME, Velasco C, Martínez-Martínez L, Pascual A. Plasmid-mediated quinolone resistance: an update. *J Infect Chemother* 2011; 17: 149–182.
92. Jones ME, Visser MR, Klootwijk M, Heisig P, Verhoef J, Schmitz FJ. Comparative activities of clinafloxacin, grepafloxacin, levofloxacin, moxifloxacin, ofloxacin, sparfloxacin, and trovafloxacin and nonquinolones linezolid, quinupristin–dalfopristin, gentamicin, and vancomycin against clinical isolates of ciprofloxacin-resistant and -susceptible *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 1999; 43: 421–423.
93. Komp Lindgren P, Karlsson A, Hughes D. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract infections. *Antimicrob Agents Chemother* 2003; 47: 3222–3232.
94. Montanari MP, Tili E, Cochetti I, Mingoa M, Manzin A, Varaldo PE. Molecular characterization of clinical *Streptococcus pneumoniae* isolates with reduced susceptibility to fluoroquinolones emerging in Italy. *Microb Drug Resist* 2004; 10: 209–217.
95. Helms M, Vastrup P, Gerner-Smidt P, Molbak K. Excess mortality associated with antimicrobial drug-resistant *Salmonella typhimurium*. *Emerg Infect Dis* 2002; 8: 490–495.
96. Rodríguez-Martínez JM, López L, García I, Pascual A. Characterization of a clinical isolate of *Haemophilus influenzae* with a high level of fluoroquinolone resistance. *Antimicrob Chemother* 2006; 57: 577–578.
97. Kadiravan T, Wig N, Kapil A, Kabra SK, Renuka K, Misra A. Clinical outcomes in typhoid fever: adverse impact of infection with nalidixic acid-resistant *Salmonella typhi*. *BMC Infect Dis* 2005; 5: 37.

98. Slinger R, Desjardins M, McCarthy AE et al. Suboptimal clinical response to ciprofloxacin in patients with enteric fever due to *Salmonella* spp. with reduced fluoroquinolone susceptibility: a case series. *BMC Infect Dis*, 2004; 4: 36.
99. Nathwani D, Wood MJ. Penicillins. A current review of their clinical pharmacology and therapeutic use. *Drugs*, 1993; 6: 866–894.
100. Karlowsky JA, Jones ME, Mayfield DC, Thornsberry C, Sahn DF. Ceftriaxone activity against Gram-positive and Gram-negative pathogens isolated in US clinical microbiology laboratories from 1996 to 2000: results from The Surveillance Network (TSN) Database-USA. *Int J Antimicrob Agents*, 2002; 5: 413–426.
101. Kuriyama T, Karasawa T, Nakagawa K, Nakamura S, Yamamoto E. Antimicrobial susceptibility of major pathogens of orofacial odontogenic infections to 11 β -lactam antibiotics. *Oral Microbiol Immunol*, 2002; 5: 285–289.
102. Jones ME, Draghi DC, Karlowsky JA, Sahn DF, Bradley JS. Prevalence of antimicrobial resistance in bacteria isolated from central nervous system specimens as reported by US hospital laboratories from 2000 to 2002. *Ann Clin Microbiol Antimicrob*, 2004; 3: 3.
103. Weinstein MP, Mirrett S, Kannagara S et al. Multicenter evaluation of use of penicillin and ampicillin as surrogates for *in vitro* testing of susceptibility of enterococci to imipenem. *J Clin Microbiol*, 2004; 8: 3747–3751.
104. Ono S, Muratani T, Matsumoto T. Mechanisms of resistance to imipenem and ampicillin in *Enterococcus faecalis*. *Antimicrob Agents Chemother*, 2005; 7: 2954–2958.
105. Jarlier V, Soussy CJ, Chanal M et al. *In vitro* effect of piperacillin on aerobic bacteria. Variations according to the phenotypes of resistance to β -lactam antibiotics. *Press Med* 1986; 15: 2272–2278.
106. Thomas WJ, McReynolds JW, Mock CR, Bailey DW. Ampicillin-resistant *Haemophilus influenzae* meningitis. *Lancet*, 1974; 7852: 313.
107. Medeiros AA, O'Brien TF. Ampicillin-resistant *Haemophilus influenzae* type B possessing a TEM-type β -lactamase but little permeability barrier to ampicillin. *Lancet*, 1975; 7909: 716–719.
108. Kim IS, Ki CS, Kim S et al. Diversity of ampicillin resistance genes and antimicrobial susceptibility patterns in *Haemophilus influenzae* strains isolated in Korea. *Antimicrob Agents Chemother*, 2007; 51: 453–460.
109. Fantin B, Leclercq R, Garry L, Carbon C. Influence of inducible cross-resistance to macrolides, lincosamides, and streptogramin B-type antibiotics in *Enterococcus faecium* on activity of quinupristin–dalbopristin *in vitro* and in rabbits with experimental endocarditis. *Antimicrob Agents Chemother*, 1997; 41: 931–935.
110. Le Goffic F, Baca B, Soussy CJ, Dublanche A, Duval J. ANT(4⁺): a new aminoglycoside nucleotidyltransferase found in *Staphylococcus aureus*. *Ann Microbiol (Paris)*, 1976; 127: 391–399.
111. Asseray N, Caillon J, Roux N et al. Different aminoglycoside-resistant phenotypes in a rabbit *Staphylococcus aureus* endocarditis infection model. *Antimicrob Agents Chemother*, 2002; 46: 1591–1593.
112. Mederski-Samoraj BD, Murray BE. High-level resistance to gentamicin in clinical isolates of enterococci. *J Infect Dis*, 1983; 147: 751–757.
113. Galimand M, Lambert T, Gerbaud G, Courvalin P. Characterization of the *aac(6')-Ib* gene encoding an aminoglycoside 6'-N-acetyltransferase in *Pseudomonas aeruginosa* BM2656. *Antimicrob Agents Chemother*, 1993; 7: 1456–1462.
114. Witichit J. Plasmid-mediated gentamicin resistance not associated with kanamycin resistance in Enterobacteriaceae. *J Antibiot*, 1972; 25: 622–624.
115. Benveniste R, Davies J. R-factor mediated gentamicin resistance: a new enzyme which modifies aminoglycoside antibiotics. *FEBS Lett*, 1971; 14: 293–296.
116. Stein GE, Schooley S, Tyrrell KL, Citron DM, Goldstein EJ. Bactericidal activities of methoxyfluoroquinolones gatifloxacin and moxifloxacin against aerobic and anaerobic respiratory pathogens in serum. *Antimicrob Agents Chemother*, 2003; 47: 1308–1312.
117. Santos Sanches I, Mato R, de Lencastre H, Tomasz A, CEM/NET Collaborators and the International Collaborators. Patterns of multidrug resistance among methicillin-resistant hospital isolates of coagulase-positive and coagulase-negative staphylococci collected in the international multicenter study RESIST in 1997 and 1998. *Microb Drug Resist* 2000; 6: 199–211.
118. Perez-Trallero E, Marimon JM, Gonzalez A, Ercibengoa M, Larruskain J. *In vivo* development of high-level fluoroquinolone resistance in *Streptococcus pneumoniae* in chronic obstructive pulmonary disease. *Clin Infect Dis*, 2005; 41: 560–564.
119. Urban C, Rahman N, Zhao X et al. Fluoroquinolone-resistant *Streptococcus pneumoniae* associated with levofloxacin therapy. *J Infect Dis*, 2001; 184: 794–798.
120. Varon E, Houssaye S, Grondin S, Gutmann L, Groupe des Observatoires de la Resistance du Pneumocoque. Nonmolecular test for detection of low-level resistance to fluoroquinolones in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother*, 2006; 50: 572–579.
121. Davidson R, Cavalcanti R, Brunton JL et al. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. *N Engl J Med*, 2002; 346: 747–750.
122. Pérez-Vázquez M, Román F, Aracil B, Cantón R, Campos J. Laboratory detection of *Haemophilus influenzae* with decreased susceptibility to nalidixic acid, ciprofloxacin, levofloxacin, and moxifloxacin due to GyrA and ParC mutations. *J Clin Microbiol* 2004; 42: 1185–1191.
123. Knapp JS, Fox KK, Trees DL, Whittington WL. Fluoroquinolone resistance in *Neisseria gonorrhoeae*. *Emerg Infect Dis*, 1997; 3: 33–39.