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EUCAST Expert Rules in Antimicrobial Susceptibility Testing

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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 which 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.

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 (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 innate, rendering it clinically useless. Antimicrobial susceptibility testing is therefore unnecessary, although it may be done 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, it 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 clinically active. There are also situations where the agent appears 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 since they are rather a matter of therapeutic recommendations. Examples of intrinsic resistances 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 resistances of some bacterial species to particular antimicrobial agents which 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 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 susceptibility test results and interpretation of clinical susceptibility on the basis of the resistance mechanism [1-3]. 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, e.g. IF *S. aureus* is resistant to oxacillin or cefoxitin THEN report resistant to all β-lactams, or more complicated, e.g. IF Enterobacteriaceae are intermediate to tobramycin, resistant to gentamicin and susceptible to amikacin THEN report 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 to 13) the evidence for rules has been graded as follows:

A. There is good clinical evidence that reporting the test result as susceptible leads to clinical failures.
B. 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.
C. 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 which 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 not previously recognised and treatment is likely to fail. Comments may also be added to explain actions or warn of resistances 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 which 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 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 Laboratory Information Systems (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, recognised experts and others via open consultation through the 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 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 to assist clinical microbiologists in the interpretation of antimicrobial susceptibility tests beyond interpretation of *in vitro* tests for the assignment of clinical categories of antimicrobial susceptibility.

For this objective, different rules have been produced, including those defining intrinsic resistances 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 using 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 ß-lactam agents

ß-Lactam compounds are the most widely used antimicrobial agents. They interact with the penicillin binding proteins (PBPs) that are the enzymes involved in the terminal stages of peptidoglycan synthesis and exert a bactericidal effect due to a subsequent imbalance of cell wall autolytic enzymes. Resistance to these compounds is mainly due to ß-lactamases, which are a large family of different hydrolases that disrupt and inactivate the ß-lactam structure. These enzymes variably affect different ß-lactam compounds, thus producing different phenotypes and/or levels of resistance, particularly in Gram-negative bacilli [8,9]. In addition, target (PBP) modification may also compromise ß-lactam activity. This mechanism is particularly encountered in Gram-positive cocci. The contribution of PBP modification to ß-lactam resistance in Gram-negative organisms is generally less important [10]. Porin modifications and efflux pump hyper-expression in Gram-negative organisms may also compromise ß-lactam compounds, but resistance levels conferred by these mechanisms alone are commonly lower than those observed with resistance conferred by most ß-lactamases [11,12]. EUCAST expert rules for ß-lactams and Gram-positive cocci are focused on staphylococci, streptococci, including ß-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 due to the production of an abnormal PBP (PBP2a encoded by the *mecA* gene) leading to cross resistance to all ß-lactams except a few with low affinity to 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 cefoxitin, or with positive test for *mecA* gene or PBP 2a, should be considered resistant to all available ß-lactams [15] with the exception of those specifically licensed to treat infections caused by methicillin-resistant staphylococci. Nevertheless, rare penicillinase hyperproduction may result in borderline resistance to oxacillin (but not cefoxitin) in vitro due to lability of oxacillin but there is no evidence that penicillinase hyperproduction is clinically relevant [16].

Streptococci. Among ß-haemolytic streptococci, susceptibility to penicillins is currently the rule. No decreased susceptibility to ß-lactams has been reported except in Group B streptococci (MIC of benzylpenicillin up to 1 mg/L) [17]. Isolates susceptible to penicillin can be reported as susceptible to aminopenicillins, cephalosporins and carbapenems [18]. If resistant to penicillin, identification and susceptibility should be checked (rule 8.3). Conversely, resistance to ß-lactams in *S. pneumoniae* is common due to the production of mosaic PBPs that lead to various patterns of ß-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 is interpreted as resistant (rule 8.4). Among viridans group streptococci, production of mosaic PBPs also leads to various patterns of ß-lactam resistance and the oxacillin disk diffusion test developed for *S. 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 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 ß-lactams, including all penicillins and carbapenems (rule 8.6). Penicillinase-producing *Enterococcus* spp. isolates have been rarely detected, but have recently been described in Europe [22,23].

***Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter* spp.** Interpretive reading of the antibiogram is commonly based on ß-lactams and ß-lactamases in Gram-negative bacilli [8]. The first version of EUCAST expert rules for ß-lactams and Enterobacteriaceae was influenced by this, particularly with isolates producing extended-spectrum ß-lactamases (ESBLs) or carbapenemases. Cephalosporin and carbapenem breakpoints available when version 1 of EUCAST expert rules were published were later considered inappropriate and consequently old expert rules addressing ESBL and carbapenemase producers needed to be modified in version 2 of the rules.

For many years confirmatory tests, mainly based on the synergistic effect observed between cephalosporins and ß-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 [24-26]. 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 [27]. Animal models, Pk/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 when compared with CLSI breakpoints [28,29]. 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 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 or fluoroquinolones. This, in turn, potentially exerts a selective pressure on microorganisms with other antimicrobial resistance mechanisms, including carbapenemase producers. Although clinical outcome of the use of third 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 [30-35]. These studies showed that clinical and experimental outcomes are better correlated with the MIC values than with the presence of an ESBL enzyme. With the new EUCAST breakpoints for Enterobacteriaceae, third and fourth generation cephalosporins should be reported as found and the old expert rule recommending modification of reporting category for ESBL producers that appear susceptible is no longer necessary. This recommendation, that also applies to plasmid mediated AmpC producers, is now included in the EUCAST breakpoint tables. Nevertheless, in many areas ESBL detection and characterization is 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) [36] and that for isolates with carbapenemases (old expert rule 9.7), are deleted in version 2 of the expert rules.

Carbapenemases, including class A, B and D enzymes, can have a variable effect on carbapenems [37-39]. Moreover, combined resistance mechanisms may also affect carbapenem susceptibility (e.g. combination of derepressed AmpC or ESBL and decreased permeability), ertapenem being particularly affected [40]. Recent data, as with ESBLs producers, provide evidence to support reporting of carbapenem susceptibility as found [41,42]. 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 [43]. 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 to those expressing OXA-48, a class D carbapenemase that is increasingly identified in Enterobacteriaceae [44].

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 intermediate or resistant to any third or fourth generation cephalosporin in infections other than those affecting the urinary tract [45,46]. This is also the case for new expert rule number 9.2, which is evidence graded A for *Enterobacter* spp. and B for *Citrobacter freundii, Serratia* spp*., Morganella morganii.* Rule 9.2 recommends discouraging 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 [47]. In some publications it is claimed that this problem can be avoided with combination therapy, including (unlike aminoglycosides) the addition of a fluoroquinolone [48].

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 due to ß-lactamase production. Isolates producing ß-lactamases, mainly TEM-1, should be considered resistant to both ampicillin and amoxicillin (rules 10.1) [49]. Ampicillin resistance in the absence of ß-lactamase production can be conferred by mutations in the *ftsI* gene affecting PBPs and leading to reduced affinity for β-lactams [50]. These isolates, termed β-lactamase negative, ampicillin-resistant (BLNAR), should be considered as resistant to aminopenicillin-ß-lactamase inhibitor combinations (amoxicillin-clavulanate, ampicillin-sulbactam and piperacillin-tazobactam) and to first and second generation cephalosporins (rule 10.2) [51,52]. 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 ß-lactamase production are also increasingly found. These isolates are phenotypically resistant to amoxicillin-clavulanate and ampicillin-sulbactam (ß-lactamase positive and resistant to amoxicillin-clavulanate, BLPACR) and should also be considered resistant to piperacillin-tazobactam and to first and second generation cephalosporins (rule10.3) [53]. 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 PBP 3 is concomitantly altered [54]. Moreover, a TEM ESBL variant has also been found in *Haemophilus parainfluenzae* [55].

For *Neisseria gonorrhoeae,* isolates that are ß-lactamase positive should be considered resistant to benzylpenicillin, ampicillin and amoxicillin. Chromosomal mutations affecting affinity of PBPs, decreased permeability or efflux pumps also confer resistance to ß-lactamase inhibitor combinations and resistance will be detected by the application of EUCAST breakpoints (rule 10.4) [56-58].

Expert rules for *Moraxella catarrhalis* have been deleted in version 2 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 to 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 the class representative for 14-(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 clindamycin and/or streptogramins) [59]. With both mechanisms there is

cross-resistance between erythromycin and the other 14- and 15-membered ring macrolides (rule 11.1). This resistance can be with or without cross-resistance to clindamycin and lincosamides. In staphylococci and streptococci, isolates resistant to erythromycin but susceptible to clindamycin should be tested for inducible MLS_B resistance (dissociated resistance) [59].

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 disks 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, 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) [60]. In staphylococci, 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) [61,62].

In 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 positive but with a warning that resistance may develop on prolonged treatment (rule 11.3) [59]. When *Peptostreptococcus* spp. and *Bacteroides* spp. express 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) [63,64].

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: a) decreased permeability and/or accumulation of the aminoglycoside agents due

to mutations affecting passive diffusion or active transport, porin and/or lipopolysaccharide alteration (only in Gram-negatives), and efflux pump hyper-expression; b) target (ribosomal) modifications due to 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 c) aminoglycoside modifying enzymes that are acetyltransferases, phosphotransferases or nucleotidyltransferases (also known as adenylyltransferases) [65-69].

Phenotype recognition of these resistance mechanisms is generally more complex than those affecting ß-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 [69]; 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 or paramomycin), streptomycin and/or the aminocyclitol agent spectinomycin [70].

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 not used in the human clinical setting [70-73]. Other problems complicating interpretive reading of this group of antimicrobials are that the enzymatic modification 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 reading antibiograms of aminoglycosides (Table 12). In Gram-positive organisms, these rules facilitate detection of the absence of synergy between a specific aminoglycoside and ß-lactam or glycopeptide agents (rules 12.1 to 12.6). In enterococci, the evidence for these rules is graded A or B and is based on clinical data [74,75]. In staphylococci, however, the evidence for most rules is graded C due 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 [76,77].

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 to 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 [78-81].

In certain Gram-negatives, 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 as (intrinsically) resistant to these agents (Table 1, rules 1.12 and 1.14) [82-84]. *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) [85].

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 [86]. The quinolones interact with bacterial type II topoisomerases DNA gyrases encoded by genes *gyrA* and *gyrB* and topoisomerase IV encoded by *parC* and *parE* (in staphylococci *grlA* and *grlB*), being the preferential targets of Gram-negative

and Gram-positive organisms, respectively. Topoisomerase mutations in *gyrA* and *parC* genes 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 due to stepwise selection of several mutations in the same or different topoisomerase [87].

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 [88]. All of them demonstrate low expression and do not always affect all fluoroquinolone agents. Target protection mechanisms due to the Qnr proteins were the first described plasmid mediated resistance mechanisms [89]. Several families of these proteins have now been described, mainly in Enterobacteriaceae. In addition, enzymatic modification due to 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 [90]. More recently, two plasmid-mediated efflux-based mechanisms involving the QepA and OqxAB pumps related to major facilitator superfamily (MFS) transporters were described. In this case the resistance is low-level and phenotypic detection is extremely difficult [91,92].

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 due to 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 proteins, 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 reading antibiograms with quinolones, resistance to the most active fluoroquinolone *in vitro* indicates resistance to all fluoroquinolones in both Gram-negative and Gram-positive organisms [93-95]. An exception to this rule in Gram-negatives is the potential production of the AAC(6')-Ib-cr enzyme that 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 [96-98]. However, in Enterobacteriaceae this compound does not detect *qnr*- or other plasmid-mediated quinolone resistance, which is increasingly recognized all over the world. For this reason, in version 2 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 to indicate 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 *S. Typhi* but there are also case reports of poor response with other *Salmonella* species [96,98,99]. 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 possible of emergence of high level resistance to fluoroquinolones in Enterobacteriaceae with low level resistance to these compounds when using fluoroquinolones.

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- and/or high-level resistance). Moreover, with any of the new plasmid mediated resistance

mechanisms there is little possibility for 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 [90-92].

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 applying clinical breakpoint criteria. In most instances, susceptible or intermediate clinical interpretations are modified to resistant due 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 with 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 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 using the revised cephalosporin breakpoints.

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 based on 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.

Transparency declarations

Nothing to declare

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Table 1. Intrinsic resistance in Enterobacteriaceae. Enterobacteriaceae are also intrinsically resistant to benzylpenicillin, glycopeptides, fusidic acid, macrolides (with some exceptions[1]), lincosamides, streptogramins, rifampicin, daptomycin and linezolid.

Rule no.	Organisms	Ampicillin	Amoxicilin-clavulanate	Ticarcillin	Piperacillin	Cefazolin	Cefoxitin	Cefamandole	Cefuroxime	Aminoglycosides	Tetracyclines/ tigecycline	Polymyxin B/ Colistin	Nitrofurantoin
1.1	*Citrobacter koseri*	R		R	R								
1.2	*Citrobacter freundii*	R	R			R	R						
1.3	*Enterobacter cloacae*	R	R			R	R						
1.4	*Enterobacter aerogenes*	R	R			R	R						
1.5	*Escherichia hermannii*	R		R									
1.6	*Hafnia alvei*	R	R			R							
1.7	*Klebsiella* spp.	R		R									
1.8	*Morganella morganii*	R	R			R			R		R	R	R
1.9	*Proteus mirabilis*										R	R	R
1.10	*Proteus vulgaris*	R				R		R	R		R	R	R
1.11	*Proteus penneri*	R				R		R	R		R	R	R
1.12	*Providencia rettgeri*	R	R			R					R	R	R
1.13	*Providencia stuartii*	R	R			R				Note[2]	R	R	R
1.14	*Serratia marcescens*	R	R			R		R	R	Note[3]		R	R
1.15	*Yersinia enterocolitica*	R	R	R		R	R	R					
1.16	*Yersinia pseudotuberculosis*											R	

R = resistant

[1] Azithromycin is effective *in vivo* for the treatment of typhoid fever and erythromycin may be used to treat travellers' diarrhoea.

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

[3] All *Serratia marcescens* 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	Ceftriaxone	Ceftazidime	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim	Trimethoprim-sulfamethoxazole	Fosfomycin	Tetracyclines/ Tigecycline	Polymyxin B/ Colistin
2.1	*Acinetobacter baumannii, Acinetobacter calcoaceticus*	R[1]	R[1]					R	R	R		R						R		R		
2.2	*Achromobacter xylosoxydans*	R						R	R	R		R										
2.3	*Burkholderia cepacia* complex[2]	R	R	R	R			R				R	R		R	R	R[3]	R		R		R
2.4	*Elizabethkingia meningoseptica*	R		R	R			R	R	R	R	R	R	R								R
2.5	*Ochrobactrum anthropi*	R	R	R	R	R	R	R	R	R	R	R										
2.6	*Pseudomonas aeruginosa*	R	R					R	R	R		R				R	Note[4]	R[5]	R[5]		R	
2.7	*Stenotrophomonas maltophilia*	R	R	R		R	R	R	R	R	R[6]	R	R	R			R[3]	R[7]		R		

R = resistant

[1] *Acinetobacter baumannii* may appear susceptible to ampicillin-sulbactam due to activity of sulbactam against this species.

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

[3] *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* produce the AAC(6')Iz enzyme.

[4] *Pseudomonas aeruginosa* is intrinsically resistant to kanamycin and neomycin due to low level APH(3')-IIb activity.

[5] *Pseudomonas aeruginosa* typically is resistant to trimethoprim and moderately susceptible to sulfonamides. Although it may appear susceptible *in vitro* to trimethoprim-sulfamethoxazole, it should be considered resistant.

[6] *Stenotrophomonas maltophilia* may show low ceftazidime MIC values but should be considered resistant.

[7] *Stenotrophomonas maltophilia* typically is susceptible to trimethoprim-sulfamethoxazole, 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	*Haemophilus influenzae*	I	R			
3.2	*Moraxella catarrhalis*				R	
3.3	*Neisseria* spp.				R	
3.4	*Campylobacter fetus*		R	R	R	R
3.5	*Campylobacter jejuni, Campylobacter coli*		R	R	R	

R = resistant; I = intermediate

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	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii, Staphylococcus xylosus*		R									R	
4.3	*Staphylococcus capitis*		R								R		
4.4	Other coagulase-negative staphylococci and *Staphylococcus aureus*		R										
4.5	*Streptococcus* spp.	R			R[1]								
4.6	*Enterococcus faecalis*	R	R	R	R[1]	R	R	R					R
4.7	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[1]	R	R	R	R				R
4.8	*Enterococcus faecium*	R	R	R	R[1,2]	R							R
4.9	*Corynebacterium* spp.										R		
4.10	*Listeria monocytogenes*		R	R									
4.11	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.12	*Lactobacillus* spp. (some species)								R	R			
4.13	*Clostridium ramosum*, *Clostridium innocuum*								R				

R = resistant;

[1] Low-level resistance (LLR) 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.

[2] In addition to LLR 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 Enterobacteriaceae (except Proteae)	Resistant to meropenem and/or imipenem[1].
5.2	*Serratia marcescens,* Proteae	Susceptible to colistin.
5.3	*Pseudomonas aeruginosa* and *Acinetobacter* spp.	Resistant to colistin.
5.4	*Haemophilus influenza*	Resistant to any third-generation cephalosporin, carbapenems, fluoroquinolones.
5.5	*Moraxella catarrhalis*	Resistant to ciprofloxacin, any third-generation cephalosporin.
5.6	*Neisseria meningitides*	Resistant to any third generation cephalosporins, fluoroquinolones.
5.7	*Neisseria gonorrhoeae*	Resistant to third-generation cephalosporins, spectinomycin.

[1]Except in countries in which carbapenemase-producing Enterobacteriaceae are not rare.

Table 6. Exceptional phenotypes of Gram-positive bacteria

Rule no.	Organisms	Exceptional phenotypes
6.1	*Staphylococcus aureus*	Resistant to vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, daptomycin, tigecycline.
6.2	Coagulase-negative staphylococci	Resistant to vancomycin, linezolid[1], quinupristin-dalfopristin[1], daptomycin, tigecycline.
6.3	JK coryneform organisms	Resistant to vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, daptomycin, tigecycline.
6.4	*Streptococcus pneumoniae*	Resistant to imipenem, meropenem, vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, daptomycin, tigecycline, rifampicin.
6.5	Group A, B, C and G β-haemolytic streptococci	Resistant to penicillin, cephalosporins, vancomycin, teicoplanin, linezolid, quinupristin-dalfopristin, daptomycin, tigecycline.
6.6	*Enterococcus* spp.	Resistant to linezolid, daptomycin, tigecycline. Resistant to teicoplanin but not vancomycin.
6.7	*Enterococcus faecalis, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus avium*	Susceptible to quinupristin-dalfopristin. Consider likelihood of misidentification. If also resistant to ampicillin it is almost certainly *E. faecium*.
6.8	*Enterococcus faecium*	Resistant to quinupristin-dalfopristin. Consider likelihood of misidentification, especially if also susceptible to ampicillin.

[1]Except in countries where linezolid or quinupristin-dalfopristin resistant coagulase-negative staphylococci are not rare.

Table 7. Exceptional phenotypes of anaerobes

Rule no.	Organisms	Exceptional phenotypes
7.1	*Bacteroides* spp.	Resistant to metronidazole, carbapenems.
7.2	*Clostridium difficile*	Resistant to metronidazole, vancomycin.

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	*Staphylococcus* spp.	Oxacillin, cefoxitin (disk diffusion) or detection of *mecA* gene or PBP2a	All β-lactams	IF resistant to isoxazolyl-penicillins (as determined with oxacillin, cefoxitin, or by detection of *mecA*-gene or of PBP2a) THEN report as resistant to all β-lactams except those specifically licensed to treat infections caused by methicillin-resistant staphylococci due to low affinity for PBP2a	Production of PBP2a (encoded by *mecA*) leads to cross resistance to β-lactams except ceftobiprole and ceftaroline.	A	[13, 15]
8.2	*Staphylococcus* spp.	Benzylpenicillin (and β-lactamase detection)	Penicillins apart from isoxazolyl-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 isoxazolyl-penicillins and combinations with β-lactamase inhibitors.	Testing of β-lactamase production is discouraged, in most countries the prevalence of β-lactamase producers is more than 90% and testing β-lactamase production has technical problems. In this case it may be considered appropriate to report all isolates resistant to benzylpenicillin, ampicillin and amoxicillin.	C	[100]
8.3	β-Haemolytic streptococci (Group A, B, C, G)	Benzylpenicillin	Aminopenicillins, cephalosporins and carbapenems	IF susceptible to benzylpenicillin THEN report 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	[16,17,101]
8.4	*Streptococcus*	Oxacillin (disk	Benzylpenicillin,	IF resistant by the oxacillin disk	Production of mosaic PBPs leads to	B	[19,20]

	pneumoniae	diffusion)	aminopenicillins, cephalosporins, carbapenems	screening test, THEN determine MIC of benzylpenicillin and other relevant ß-lactam agents.	various patterns of β-lactam resistance. Report as interpreted for each of the drugs.		
8.5	Viridans group streptococci	Benzylpenicillin	Aminopenicillins and cefotaxime or ceftriaxone	IF resistant to benzylpenicillin THEN determine MIC of ampicillin (or amoxicillin) 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	[102,103]
8.6	*Enterococcus* 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	[104,105]

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, ceftazidime, cefepime, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam	Amoxicillin-clavulanate, ampicillin-sulbactam and piperacillin-tazobactam	IF intermediate or resistant to any 3rd generation (cefotaxime, ceftriaxone, ceftazidime) or 4 th 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 plus a β-lactamase inhibitor. With the exception of urinary tract infections and blood stream 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 with ticarcillin-clavulanate has been published.	B	[45,46]
9.2	*Enterobacter* spp., *Citrobacter freundii, Serratia* spp., *Morganella morganii*	Cefotaxime, ceftriaxone, ceftazidime	Cefotaxime, ceftriaxone and ceftazidime	IF susceptible *in vitro* to cefotaxime, ceftriaxone or ceftazidime, THEN note that the use in monotherapy of cefotaxime, ceftriaxone or ceftazidime should be discouraged owing to 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 3rd 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 cefepime and cefpirome.	A (*Entero-bacter*) B (others)	[46,47]
9.3	Enterobacteriaceae (mostly *Klebsiella* spp. and *Escherichia*	Ticarcillin, piperacillin	Piperacillin	IF resistant to ticarcillin but susceptible to piperacillin, THEN edit piperacillin to resistant.	Ticarcillin-hydrolyzing β-lactamases also attack piperacillin, but resistance may be less obvious if expression is low-level.	C	[24,106]

	coli)				Does not apply to inhibitor combinations involving these penicillins.		

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	*Haemophilus influenzae*	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	[107,108]
10.2	*Haemophilus influenzae*	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 less affected by the PBP-mediated resistance mechanisms evidence regarding clinical efficacy is lacking.	C	[49,50,109]
10.3	*Haemophilus influenzae*	Amoxicillin-clavulanate (and β-lactamase detection)	Ampicillin-sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin and	IF β-lactamase positive and amoxicillin-clavulanate resistant (BLPACR) THEN report as resistant to ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, piperacillin, piperacillin-	BLPACR isolates produce β-lactamase and have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin-tazobactam appear less affected by the PBP-mediated resistance mechanisms evidence regarding clinical	C	[49,109]

			piperacillin-tazobactam.	tazobactam. cefuroxime and cefuroxime axetil.	efficacy is lacking.		
10.4	*Neisseria gonorrhoeae*	Benzylpenicillin, ampicillin or amoxicillin (and β-lactamase detection)	Benzylpenicillin, ampicillin and amoxicillin	IF positive for production of β-lactamase, THEN report resistant to benzylpenicillin, ampicillin and amoxicillin.	Penicillin resistance can be caused by plasmid encoded β-lactamase production (TEM-1). Chromosomal mutations affecting affinity to 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	[56-58]

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, roxithromycin	IF susceptible, intermediate or resistant to erythromycin, THEN report the same category of susceptibility for azithromycin, clarithromycin, roxithromycin .	Erythromycin is the class representative for 14- and 15-membered ring macrolides. Resistance to erythromycin is generally due to the production of a ribosomal methylase encoded by *erm* 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- and 15-membered ring macrolides.	C	[59]
11.2	*Staphylococcus* spp.	Erythromycin, clindamycin	Clindamycin	IF resistant to erythromycin but susceptible to clindamycin, THEN test for inducible MLS_B resistance. IF negative, THEN report 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 Erm ribosomal methylases conferring the inducible MLS_B phenotype or express efflux pumps. In 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	B	[59,60]

					staphylococci with inducible MLS_B resistance. By a disk diffusion test, the inducible MLS_B phenotype can be identified by the flattening of the clindamycin zone facing the erythromycin disk.		
11.3	*Streptococcus* spp.	Erythromycin, clindamycin	Clindamycin	IF resistant to erythromycin but susceptible to clindamycin, THEN test for inducible MLS_B resistance. If positive, report susceptible to clindamycin with a warning that resistance may develop during treatment.	Streptococci may be resistant to macrolides by production of a ribosomal *erm* methylase gene conferring the MLS_B phenotype or by production of an efflux pump encoded by the *mef*(A) class of genes. In the case of inducible MLS_B resistance, clindamycin may remain active or not depending on the type and expression of *erm* 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. By 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	[59]
11.4	*Peptostreptococcus* spp., *Bacteroides*	Erythromycin, Clindamycin	Clindamycin	IF erythromycin MIC >8 mg/L for *Peptostreptococcus* spp. or MIC >32	Resistance to macrolides in *Peptostreptococcus* spp. and *Bacteroides*	C	[63,64]

	spp.			mg/L for *Bacteroides* spp. but susceptible to clindamycin, THEN report resistant to clindamycin.	spp. is generally due to the production of a ribosomal Erm methylase conferring the MLS_B phenotype. In the case of inducible MLS_B resistance, resistance to clindamycin is poorly expressed *in vitro* and this agent should not be considered as active.		
11.5	*Staphylococcus* 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 macrolide-lincosamide-streptogramin B (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 lead to conflicting results on the *in vivo* activity of quinupristin-dalfopristin for the treatment of animals infected with constitutive MLS_B resistant isolates.	C	[61,62,110]

Table 12. Interpretive rules for aminoglycosides

Rule no.	Organisms	Agent tested	Agents affected	Rule	Exceptions, scientific basis and comments	Evidence grade	References
12.1	*Staphylococcus* spp.	Kanamycin	Amikacin	IF kanamycin MIC >8 mg/L, THEN report resistant to amikacin.	Resistance to kanamycin is generally due to the production of APH(3')I-3, ANT(4')(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	[77,111]
12.2	*Staphylococcus* spp.	Tobramycin	Kanamycin, Amikacin	IF resistant to tobramycin, THEN report as resistant to kanamycin and amikacin.	Resistance to tobramycin is generally due to the production of ANT(4') (4'')I or bifunctional APH(2')-AAC(6) enzymes that determine loss of synergism of kanamycin, tobramycin and amikacin with β-lactams and glycopeptides irrespective of MIC values.	C	[111]
12.3	*Staphylococcus* spp.	Gentamicin	All aminoglycosides	IF resistant to gentamicin, THEN report as resistant to all aminoglycosides.	Resistance to gentamicin is generally due to the production of 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	[77,112]
12.4	*Enterococcus* spp., *Streptococcus* spp.	Streptomycin	Streptomycin	IF high level-resistance to streptomycin is detected (MIC >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	A (*Entero-coccus*) C (*Strepto-*	[74]

					and β -lactam agents in enterococci with high-level resistance to streptomycin.	*coccus*)	
12.5	*Enterococcus* spp., *Streptococcus* spp.	Kanamycin	Amikacin	IF high level-resistance to kanamycin is detected (MIC >512 mg/L), THEN report as high-level resistant to amikacin.	High-level resistance to kanamycin is generally due to 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 (*Entero-coccus*) C (*Strepto-coccus*)	[75,77]
12.6	*Enterococcus* spp., *Streptococcus* spp.	Gentamicin	All aminoglycosides except streptomycin	IF high level-resistance to gentamicin is detected (MIC >128 mg/L), THEN report as high-level resistant to all aminoglycosides except streptomycin.	High-level resistance to gentamicin is generally due to the production of 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 (*Entero-coccus*) C (*Strepto-coccus*)	[74,113]
12.7	All Enterobacteriaceae *Pseudomonas aeruginosa, Acinetobacter baumannii*	Tobramycin, gentamicin, amikacin	Amikacin	IF intermediate or resistant to tobramycin and susceptible to gentamicin and amikacin, THEN report amikacin as intermediate for Enterobacteriaceae or resistant for *Pseudomonas* spp. and *Acinetobacter* spp.	Production of acquired AAC(6')I may not confer phenotypic resistance despite modification of amikacin.	C	[78-81, 114]
12.8	All Enterobacteriaceae	Gentamicin and other aminoglycosides	Gentamicin	IF intermediate 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	[70,115]
12.9	All	Tobramycin	Tobramycin	IF intermediate to tobramycin,	Expression of ANT(2") enzyme may be	C	[70,116]

	Enterobacteriaceae	gentamicin amikacin		resistant to gentamicin and susceptible to amikacin, THEN report as resistant to tobramycin.	low and isolates may have decreased susceptibility to tobramycin.		
12.10	All Enterobacteriaceae	Netilmicin, gentamicin	Netilmicin	IF intermediate to netilmicin and intermediate or resistant to gentamicin and tobramycin, THEN report as resistant to netilmicin.	Expression of AAC(3")II or AAC(3")IV may be low and isolates may appear with decreased susceptibility to netilmicin.	C	[70,79]

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Table 13. Interpretive rules for quinolones

Rule no.	Organism	Agents tested	Agents affected	Rule	Exceptions, scientific basis and comments	Evidence grade	References
13.1	*Staphylococcus* spp.	Ofloxacin, ciprofloxacin, levofloxacin, 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.	Acquisition of at least one target mutation in *grlA.*	C	[87,93]
13.2	*Staphylococcus* spp.	Levofloxacin, moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones.	Acquisition of combined mutations in *grlA* and *gyrA* leads to complete or partial cross resistance to all fluoroquinolones.	C	[93,117,118]
13.3	*Streptococcus pneumoniae*	Ofloxacin, ciprofloxacin, levofloxacin, 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 e.g. *parC (parE)*. First step mutations can be more reliably detected in tests with norfloxacin.	C	[95,119-121]
13.4	*Streptococcus pneumoniae*	Levofloxacin, moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones.	Acquisition of combined mutations in e.g. *parC* and *gyrA* leads to complete or partial cross resistance to all fluoroquinolones.	B	[122]
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 *gyrA* or *gyrA* plus *parC.* Exceptionally, the production of the	B	[94]

					AAC(6')-Ib-cr enzyme may affects ciprofloxacin but not levofloxacin		
13.6	*Salmonella* spp.	Ciprofloxacin	All fluoroquinolones	IF ciprofloxacin MIC >0.06 mg/L, THEN report as resistant to all fluoroquinolones.	Evidence for clinical failure of fluoroquinolones in case of resistance due to the acquisition of at least one target mutation in *gyrA.*	A (*Salmonella typhi*) B (other *Salmonella* spp.)	[96,98,99]
13.7	*Haemophilus influenzae*	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 *H. influenzae* due to 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	[97,123]
13.8	*Neisseria gonorrhoeae*	Ciprofloxacin, 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 *gyrA* or *gyrA* plus *parC.*	C	[124]