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Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes

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If isolates are speciated and if a sufficient range of antibiotics is tested, underlying resistance mechanisms can often be inferred from the antibiogram data. This allows: (i) anomalous combinations of phenotype and organism to be reconsidered; (ii) prediction of further antibiotics that deserve testing; and (iii) the suppression of susceptibilities that are anomalous in the light of the inferred mechanism. This 'interpretative reading' is widely undertaken in France but is largely precluded in the UK by limited speciation and the testing of narrow ranges of antibiotics. Nevertheless, UK laboratories should be aware of: (i) grossly anomalous combinations of species and phenotype, demanding reference laboratory confirmation; (ii) useful indicator drugs, where resistance implies a mechanism conferring other resistances that may be less obvious in direct tests; and (iii) antibiotics that are prone to select resistant mutants of particular species during therapy. Details of these combinations of organism and resistance are presented. Relationships between antibiogram and mechanism are also presented to allow full interpretative reading for those testing wide panels of drugs versus speciated isolates.

Introduction

Susceptibility test results are normally recorded and categorized individually, 'susceptible to this drug'; 'resistant to this drug', etc. This strategy under-utilizes the data, since it ignores the fact that resistances to related antibiotics often depend on single mechanisms.[1,2] 'Interpretative reading' aims to analyse the susceptibility pattern, not just the results for individual antibiotics, and so to predict the underlying mechanisms. Based on this interpretation, susceptibilities that appear tentative can be identified and reviewed, and further drugs that merit testing can be identified.[1,2]

To exploit its full potential, interpretative reading requires that isolates are speciated accurately and tested with large batteries of different antibiotics. This is done in France, where panels of 16 antibiotics are routinely tested against most isolates, and in some commercial systems, such as the VITEK 2, which tests panels of up to 20 antibiotics.[1–3] Interpretative reading with such comprehensive data is discussed in the second part of this paper, where the resistance patterns associated with different mechanisms,

and their implications for antibiotic choice, are outlined. Most UK laboratories presently test too few drugs for interpretative reading to this standard. Nevertheless, susceptibility tests can and should be read with due attention to: (i) recognizing unusual results; (ii) recognizing drugs best avoided owing to their risk of selecting resistance in the particular pathogen; and (iii) using 'indicator' drugs.

Recognizing unusual resistances

New resistances of public health concern should be recognized. A list is given in Table I. Laboratories finding the organism/resistance combinations listed should re-check their result, as the most probable explanation is always an error in the speciation or susceptibility testing. If the results are reproducible, the isolate(s) should be sent to a reference or academic laboratory for independent confirmation. In England and Wales, the Public Health Laboratory Service provides this service. In most instances, the organisms should be sent to the Antibiotic Resistance Monitoring and

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Table I. Unusual resistances needing reference laboratory confirmation (see text for addresses)

Organism	Resistances requiring confirmation
S. aureus	Any of: vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin.
Coagulase-negative staphylococci	Any of: vancomycin, linezolid.
Jeikeium coryneforms	Any of: vancomycin, teicoplanin, linezolid.
S. pneumoniae	Any of: meropenem, vancomycin, teicoplanin, linezolid.
Group A, B, C, G β-haemolytic streptococci	Any of: penicillin, vancomycin, teicoplanin, linezolid.
Enterococci	Both ampicillin and quinupristin/dalfopristin.
	Linezolid.
	Teicoplanin but not vancomycin.
Enterobacteriaceae	Meropenem.
	Imipenem (except with *Proteus* spp.).
H. influenzae	Any third-generation cephalosporin, or carbapenem.
M. catarrhalis	Ciprofloxacin.
Neisseria meningitidis	Any of: penicillin (high level), ciprofloxacin.
Neisseria gonorrhoeae	Any third-generation cephalosporin.
Acinetobacter; *P. aeruginosa*	Colistin.
Anaerobes in general	Metronidazole.
Bacteroides	Any of: metronidazole, co-amoxiclav, carbapenems.
C. difficile	Any of: metronidazole, vancomycin.

Note to all tables: β-lactam groups
First generation cephalosporins: cephalexin, cephalothin, cephazolin and cephradine.
Second generation cephalosporins: cefamandole, cefaclor and cefuroxime.
Third generation cephalosporins: cefotaxime, cefpodoxime, ceftazidime and ceftriaxone.
Fourth generation cephalosporins: cefepime and cefpirome.
Oxyimino cephalosporins: cefepime, cefotaxime, cefpirome, cefpodoxime, ceftazidime, ceftriaxone and cefuroxime.
Cephamycins: cefoxitin, cefotetan.
Aminopenicillins: amoxycillin, ampicillin, mezlocillin and piperacillin.
Carboxypenicillins: carbenicillin and ticarcillin.

Reference Laboratory, CPHL, 61 Colindale Avenue, London NW9 5HT. Exceptions are that salmonellas and shigellas should be sent to the Laboratory of Enteric Pathogens, CPHL, 61 Colindale Avenue, London NW9 5HT; meningococci to the Meningococcal Reference Unit, Public Health Laboratory, Withington Hospital, Manchester M20 2LR; gonococci to the Genitourinary Infections Reference Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL; anaerobes to the Anaerobe Reference Unit, Public Health Laboratory, University Hospital of Wales, Cardiff CF4 4XW; and *Haemophilus influenzae* and other *Haemophilus* spp. to the PHLS Haemophilus Reference Unit, John Radcliffe Hospital, Oxford OX3 9DU. If there is concern about the spread of an unusually resistant strain among patients, speciation, typing and infection control advice can be provided by appropriate PHLS units: for nosocomial pathogens this is the Laboratory of Hospital Infection, CPHL, 61 Colindale Avenue, London NW9 5HT. Appropriate academic units include those with a particular research interest in the resistance type, or, for hospital infection advice, the Hospital Infection Research Laboratory, City Hospital, Birmingham.

In some cases, a report of 'susceptible' rather than resistant is anomalous and laboratories should be aware of the natural (inherent) resistance phenotypes of common pathogens. A list is provided in Table II. If any of these combinations of species and susceptibility are found, it is reasonable to be sceptical. Once again, the most probable cause of the result is an error in the speciation, and ideally both the species identification and antibiogram data should be re-checked. If this is impracticable or not considered worthwhile (e.g. because the isolate is susceptible to multiple other antibiotics), the unlikely results should not be used as a basis for prescribing.

Antibiotics likely to select resistance

If a resistance emerges by high frequency mutation, there is a significant risk that it will be selected in the individual patient during therapy. Table III provides a list of high-risk combinations of organism and antibiotic. The risk is modulated by the site of infection, being increased where it is difficult to obtain high drug levels, but reduced at sites where

Table II. Natural resistances typical of common pathogens

Organisms	Natural resistances to
All Enterobacteriaceae	Penicillin G, glycopeptides, fusidic acid, macrolides, clindamycin, linezolid, streptogramins (e.g. quinupristin/dalfopristin), mupirocin.
Acinetobacter baumannii	Ampicillin, amoxycillin, first-generation cephalosporins.
P. aeruginosa	Ampicillin, amoxycillin, co-amoxiclav, first-generation cephalosporins, second-generation cephalosporins, cefotaxime, ceftriaxone, nalidixic acid, trimethoprim.
B. cepacia	Ampicillin, amoxycillin, first-generation cephalosporins, colistin, aminoglycosides.
Stenotrophomonas maltophilia	All β-lactams except ticarcillin/clavulanate, aminoglycosides.
Flavobacterium (*Chryseobacterium/Myroides*)	Ampicillin, amoxycillin, first-generation cephalosporins.
Salmonella spp.	Cefuroxime (active *in vitro*, not active *in vivo*).
Klebsiella spp., *Citrobacter diversus*	Ampicillin, amoxycillin, carbenicillin, ticarcillin.
Enterobacter spp., *C. freundii*	Ampicillin, amoxycillin, co-amoxiclav, first-generation cephalosporins, cefoxitin.
M. morganii	Ampicillin, amoxycillin, co-amoxiclav, first-generation cephalosporins, cefuroxime, colistin, nitrofurantoin.
Providenicia spp.	Ampicillin, amoxycillin, co-amoxiclav, first-generation cephalosporins, cefuroxime, gentamicin, netilmicin, tobramycin, colistin, nitrofurantoin.
Proteus mirabilis	Colistin, nitrofurantoin.
Proteus vulgaris	Ampicillin, amoxycillin, cefuroxime, colistin, nitrofurantoin.
Serratia spp.	Ampicillin, amoxycillin, co-amoxiclav, first-generation cephalosporins, cefuroxime, colistin.
Yersinia enterocolitica	Ampicillin, amoxycillin, carbenicillin, ticarcillin, first-generation cephalosporins.
Campylobacter jejuni, *Campylobacter coli*	Trimethoprim.
H. influenzae	Penicillin G, erythromycin, clindamycin.
M. catarrhalis	Trimethoprim.
All Gram-positive bacteria	Aztreonam, temocillin, colistin, nalidixic acid.
Streptococci	Fusidic acid, aminoglycosides (except as synergists).[a]
S. pneumoniae	Trimethoprim, aminoglycosides.
Methicillin-resistant *S. aureus*	All β-lactams.
Enterococci	Penicillin G, carbenicillin, ticarcillin, all cephalosporins, aminoglycosides,[a] mupirocin.
Listeria	Third-generation cephalosporins, fluoroquinolones.

See note to all tables in Table I.
[a]Low-level resistance: aminoglycosides are useful for synergy with penicillins against typical streptococci and enterococci.

the drug concentrates. In general, the antibiotic/organism combinations listed in Table III should be avoided unless there is no alternative agent or unless, as with *Pseudomonas aeruginosa* or *Burkholderia cepacia*, there is a risk of selecting resistance with virtually any antibiotic active against the species.

Indicator drugs

An indicator drug is one used to detect the presence of a mechanism that gives resistance not only to the indicator itself, but also to related agents. The indicator is chosen as the member of the drug family to which the mechanism gives the most obvious resistance. Indicator drugs are already used in several critical cases. Thus, (i) methicillin and oxacillin are used to screen staphylococci which, if found resistant, are inferred to be resistant to all β-lactams;[4] (ii) oxacillin is used to screen for penicillin resistance in pneumococci;[5] and (iii) ceftazidime and cefpodoxime are used to screen klebsiellae and *Escherichia coli* for extended-spectrum β-lactamases (ESBLs).[6] Indicator drugs can usefully be employed more widely, and examples are given in Table IV.

Table III. Antibiotic/organism combinations where mutational resistance is likely to develop

Organism	Antibiotic
Staphylococci	Fusidic acid, rifampicin, fluoroquinolones.
Erythromycin-resistant staphylococci	Clindamycin.
S. pneumoniae	Ciprofloxacin.
P. aeruginosa	All anti-pseudomonal antibiotics, except colistin and, possibly, meropenem.
B. cepacia	All relevant antibiotics.
Enterobacter, *Citrobacter*, *Serratia*, *Morganella*	All third-generation cephalosporins.
Coliforms with ESBLs	Cephamycins (via impermeability).
All coliforms	Fosfomycin, nalidixic acid (not fluoroquinolones).
Serratia marcescens	Netilmicin, tobramycin, amikacin, kanamycin.

See note to all tables in Table I.

This table excludes rarely used antibiotic/organism combinations; it also only considers the risk of resistance arising in the original pathogen, not the likelihood of overgrowth by other species (e.g. enterococci and *C. difficile*), which may also be a significant clinical hazard.

Table IV. Useful indicator antibiotics

Organism	Resistance to	Inference/action
Staphylococci	oxacillin or methicillin	Resistant to all β-lactams.
Staphylococci	erythromycin	Inducible clindamycin resistance likely; avoid clindamycin or use with caution.
Staphylococci	erythromycin and clindamycin (lincomycin may be a better indicator than clindamycin)	Constitutive MLS$_{B/c}$ resistance. Quinupristin/dalfopristin likely to be bacteriostatic, not bactericidal; dosage should be increased to thrice daily even in skin and soft tissue infection.
Pneumococci	oxacillin (zone $\leqslant$18 mm)	Probably penicillin resistant. Perform E-test for penicillin or cephalosporin to be used.
E. faecalis	ampicillin	Probably *E. faecium*, but may be less frequent species or (just possibly) may have acquired resistance: check speciation or refer.
H. influenzae	cefaclor	Likely non-β-lactamase-type resistance (better indicator than ampicillin).
N. gonorrhoeae/ *H. influenzae*	nalidixic acid	Indicates reduced susceptibility or resistance to fluoroquinolones.
Klebsiella/E. coli	ceftazidime or cefpodoxime	Likely ESBL producer.[6] Avoid all cephalosporins except cephamycins.
Any Enterobacteriaceae	any second-generation cephalosporin	Likely to have potent β-lactamase; avoid first-generation cephalosporins.
Any Enterobacteriaceae	any third-generation cephalosporin	Likely to have potent β-lactamase; avoid first- and second-generation cephalosporins except, possibly, cephamycins.
Any Enterobacteriaceae	resistant to any ureidopenicillins	Likely to have penicillinase, avoid amino- and carboxy-penicillins (e.g. piperacillin).
Any Enterobacteriaceae	resistant to any β-lactamase inhibitor combinations	Assume resistance to the corresponding unprotected penicillin.

See note to all tables in Table I.

Full interpretative reading: predicting mechanisms from resistance patterns

The strategies outlined above are only part of interpretative reading in its fuller and more sophisticated form.[1,2] If isolates are fully speciated and are tested with extended arrays of antibiotics, it is often possible to predict the underlying mechanisms from the resistance profile. This can be done manually, based on operator knowledge of phenotypes and mechanisms or, more conveniently, using 'expert rules', which increasingly feature on automated zone readers and automated 'black box' systems, such as the VITEK 2.[3] Interpretative reading at this level allows: (i) estimation of the spread of resistance mechanisms; (ii) identification of susceptibility or speciation results that appear anomalous in the light of the inferred mechanisms; and (iii) identification of little-used antibiotics that merit testing against problem isolates.[1,2,7]

To illustrate these points, a *Klebsiella* isolate might be found to be resistant to ceftazidime but susceptible to cefotaxime and ceftriaxone. In many laboratories these results would be reported without change.[8] However, interpretative reading would infer ESBL production and, since cefotaxime and ceftriaxone are substrates for ESBLs, would alter the reports for these drugs to resistant.[7] Cefotetan, carbapenems and β-lactamase inhibitor combinations would be highlighted as further drugs to test.[7] If, on the other hand, therapy is being sought, for example, for an infection caused by an *Enterobacter cloacae* interpreted to hyper-produce its AmpC enzyme, it may be worth testing mecillinam, cefpirome and temocillin as second-line drugs, but there would be little point in testing cefotetan or piperacillin/tazobactam.

For those wishing to undertake interpretative reading manually, or to program a computer themselves, Tables V–XI illustrate resistance phenotypes, the underlying mechanisms inferred and any editing of the antibiogram that should be considered. Confirmatory tests are indicated as appropriate. Note that editing a result from susceptible to resistant is sometimes advocated; editing from resistant to susceptible is never recommended, although it may be appropriate to re-check an unlikely resistance. Such rules may be included in automated zone readers and/or laboratory information systems.

Tables V–XI and the accompanying text are organized by antibiotic class and, within each class, by bacterial species. The phenotypes listed are those seen in significant numbers of isolates. Rarer phenotypes are omitted unless they are a significant potential public health concern, in which case '!!!' appears in the 'interpretation' and 'frequency' columns, and the finder is advised to refer the isolate to an appropriate Public Health or academic laboratory for confirmation (see also Table I).

β-Lactams

β-Lactams are the ideal drugs for interpretative reading since there is a wide range of resistance mechanisms, including >300 types of β-lactamase, and since different resistance mechanisms give highly different resistance phenotypes.[7,9] Relevant resistance phenotypes and interpretations are illustrated in Table V for Enterobacteriaceae, in Table VI for non-fermenters, in Table VII for fastidious Gram-negative cocci and cocco-bacilli and in Table VIII for Gram-positive cocci. Use of Table V, in particular, demands accurate speciation of Enterobacteriaceae and it is not possible to devise an all-purpose panel for 'coliforms'. No laboratory will routinely test all the β-lactam analogues listed in Table V, so the diagnostic value of particular β-lactams should be underscored.

Ceftazidime or cefpodoxime resistance is the best indicator for most of the TEM- and SHV-derived ESBL types,[6,7,10] but cefotaxime resistance is a better indicator for the CTX-M type enzymes that are prevalent in South America.[11,12] Resistance to ceftazidime, in the absence of resistance to cefoxitin (which is not a substrate for ESBLs) is strongly indicative of ESBL production, which can then be confirmed with one of the tests listed by Livermore & Brown.[6] On the other hand, cefoxitin resistance in Enterobacteriaceae is almost diagnostic of AmpC enzyme production.[7,13] Inducible AmpC, as in classical phenotypes of *Enterobacter* and *Citrobacter freundii*, gives resistance to cefoxitin without cross-resistance to oxyimino cephalosporins, and derepressed AmpC gives a resistance phenotype that includes ceftazidime and cefotaxime as well as cefoxitin.[7,10] Comparisons of results for inhibitor-protected and -unprotected penicillins are especially useful in interpretative reading. The available inhibitors (clavulanate, sulbactam and tazobactam) inhibit Class A enzymes such as TEM and SHV, but not most AmpC types (inhibition of *Morganella morganii* AmpC enzyme by tazobactam is a notable exception to this generalization).[14]

Klebsiella oxytoca isolates that hyperproduce their chromosomal K1 β-lactamase are often mistaken for ESBL producers, but are distinguished by being highly resistant to aztreonam and cefuroxime but not ceftazidime or cefotaxime.[7,15] Typically, they are resistant to inhibitor combinations, although extracted K1 enzyme is susceptible to inhibition.[7,14]

Virtually all the resistance seen to β-lactams in Enterobacteriaceae is mediated by acquired or chromosomal β-lactamases. Efflux and impermeability are more important in *P. aeruginosa* and in fastidious Gram-negative bacteria. They mostly give low-level broad-spectrum resistances, often also affecting quinolones.[16] Imipenem but not meropenem escapes the commonest form of efflux-mediated resistance in *P. aeruginosa* (upregulation of MexAB-OprM) but is more strongly compromised than meropenem by mutational loss of the OprD porin, which provides carbapenem-specific channels through the outer membrane.[17]

Table V. Phenotypes; interpretation of mechanisms and editing of antibiograms: β-lactams versus Enterobacteriaceae

AMP	AMX/CLAV	TIC	TIC/CLAV	PIP	PIP/TAZ	CEF	FOX	CXM	CAZ	CTX/CRO	CPR/FEP	ATM	IMP/MEM	Interpretation	Frequency	Edit/action
E. coli, P. mirabilis, Salmonella, Shigella spp.																
S	S	S	S	S	S	S	S	S	S	S	S	S	S[a]	classical	common	
R	S	R	S	r	S	r	S	S	S	S	S	S	S	penicillinase-low	common	Edit 1st gen cephs to R except in UTI.
R	r/R	R	r/R	R	r/R	R	S	S	S	S	S	S	S	penicillinase-high	common	Edit 1st gen cephs to R except in UTI.
R	R	R	R	R	R	R	R	R	R	r/R	S	r/R	S	AmpC high	rare	Consider TEMO as therapy alternative.
R	any[b]	R	any[b]	R	any[b]	R	S	R	R	R	R	R	S	ESBL-broad	rare	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	any[b]	R	any[b]	R	any[b]	R	S	r	R	r	r	r	S	ESBL-ceftazidimase	rare	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	R	r	R	R	r/R	S	S	S	S	S	S	S	S	IRT[d]	???	Refer.
any	any	any	any	any	any	any	any	any	any	any	any	any	R[1]	!!!	!!!	
Klebsiella spp., *C. diversus*																
R	S	R	S	r	S	S	S	S	S	S	S	S	S	classical-low SHV-1 or K1	common	Edit all penicillins (except TEMO) to R.
R	r/R	R	any[b]	R	r/R	R	S	S	S	S	S	S	S	penicillinase-high	common	
R	any[b]	R	any[b]	R	any[b]	R	S	R	R	R	R	R	S	ESBL-broad	scattered	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	any[b]	R	any[b]	R	any[b]	R	S	r	R	r	r	r	S	ESBL-ceftazidimase	scattered	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	R	R	R	R	r/R	S	S	S	S	S	S	S	S	IRT[d]	???	
R	R	R	R	R	R	R	S	R	S	R	S	R	S	K1 high, *K. oxytoca* only	scattered	Edit CTX to R; ??? CAZ.
R	R	R	R	R	R	R	R	R	R	R	S	r/R	S	AmpC acquired	rare	
any	any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.
Enterobacter, C. freundii																
R	R	R	S/r	S	S	R	R	S/r	S	S	S	S	S	classical AmpC inducible	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	any[b]	R	S/r	R	R	S	S	S	S	S	S	penicillinase	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	any[b]	R	S/r	R	R	R	R	R	R	R	S	ESBL-broad	rare	Edit 2/3/4 gen cephs to R.[f]
R	R	R	any[b]	R	S/r	R	R	r	R	r	R	r	S	ESBL-ceftazidimase	rare	Edit 2/3/4 gen cephs to R.[f]

													Phenotype	Frequency	Action
R	R	R	R	R	R	R	R	R	R	R	R	S	AmpC derepressed	common	Consider TEMO as therapy alternative.
any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.

M. morganii/Providencia spp.

													Phenotype	Frequency	Action
R	R	S/r	S	S	r	R	S	S	S	S	S	S	classical	common	Advise against use of 2/3 gen cephs.[e]
R	R	S	S/r	R	r	R	S	S	S	S	S	S	penicillinase	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	S	R	r	R	R	R	R	R	R	R	AmpC derepressed	scattered	Consider TEMO as therapy alternative.
any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.

P. vulgaris

												Phenotype	Frequency	Action
R	S	S	S	R	S	S	S	S	S	S	classical	common	
R	S	R	S	R	S	S	S	S	S	S	penicillinase	common	
R	S	R	S	R	S	S	R	S	S	S	Chr β-lactamase derepressed	rare	
any	any	any	any	any	any	any	any	any	any	any	!!!	!!!	Refer.

Serratia spp.

													Phenotype	Frequency	Action
R	S	S	S	R	r	R	S	S	S	S	S	S	classical	common	Advise against use of 2/3 gen cephs.[e]
R	R	any[b]	R	R	r	R	S	S	S	S	S	S	penicillinase	common	Advise against use of 2/3 gen cephs.[e]
R	R	r/R	R	R	r	R	R	S	S	R	S	R	AmpC derepressed	rare	Consider TEMO as therapy alternative.
any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.

See note to all tables in Table I.

General notes for Tables V–XI:

Classical means the historic phenotype of the species, without acquired resistance; **common** means seen in >10% of isolates; **scattered** means seen in 1–5% of isolates; and **rare** means seen in <1% of isolates. Local frequencies may be very different, especially during outbreaks and in specialist units. **Refer** and **!!!** mean send to an appropriate reference or academic laboratory for confirmation (see text); **???** means uncertain as insufficient data.

Abbreviations: AMK, amikacin; AMX, amoxycillin; AMP, ampicillin; AMX/CLAV, co-amoxiclav; ATM, aztreonam; CAZ, ceftazidime; Ceph, cephalosporin; FEP, cefepime; CLI, clindamycin; CEF, cephalothin; CPR, cefpirome; CRO, ceftriaxone; CTX, cefotaxime; CXM, cefuroxime; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; IMP, imipenem; KAN, kanamycin; MEM, meropenem; NEO, neomycin; NET, netilmicin; OXA, oxacillin/cloxacillin; PCG, penicillin G; PIP, piperacillin; TZP, piperacillin/tazobactam; Q-D, quinupristin/dalfopristin; TEMO, temocillin; TIC, ticarcillin; TIM, ticarcillin/clavulanate; TOB, tobramycin; 1st gen ceph, first generation cephalosporin; 2/3/4 gen ceph, second/third/fourth generation cephalosporin; R, resistant; r, reduced zones but likely to remain susceptible at BSAC breakpoints; B, borderline (MICs for typical strains of the species without acquired resistance, fall around the zone/MIC breakpoints); S, susceptible.

[a] Discount low-level imipenem resistance in *P. mirabilis*.

[b] Varies with amount of β-lactamase produced.

[c] See Livermore and Brown[6] for ESBL tests for these species.

[d] IRT: inhibitor-resistant TEM mutant.

[e] If second- or third-generation cephalosporins are used, there is substantial risk of selection of derepressed, mutants during therapy. See Table III.

[f] ESBL tests[6] are of dubious value for AmpC-inducible species since clavulanate-induced AmpC enzymes (which evade the action of clavulanate) are prone to attack the indicator cephalosporin. The pattern of cefotetan-susceptible, ceftazidime-resistant would imply ESBL production, but this principle has not been evaluated critically.[6]

Table VI. Phenotypes, interpretation of mechanism and editing of antibiograms: β-lactams versus non-fermenters

TIC	TIC/CLAV	PIP	PIP/TAZ	CAZ	CPR/FEP	ATM	IMP	MEM	Interpretation	Frequency	Edit/action
P. aeruginosa[a]											
S	S/r	S	S	S	S	S	S	S	classical	common	beware mutational resistance; see Table III
R	any	R	any	S	S	S	S	S	penicillinase	rare	
r	R	r	r	r	r	R	S	S	AmpC part derepressed	common	
r/R	r/R	R	R	R	S/r	R	S	S	AmpC fully derepressed	rare	
R	R	r/R	r/R	r/R	R	r/R	S	r	increased efflux[b]	common	
S	S/r	S	S	S	S	S	R	r	loss of OprD porin	scattered	
Acinetobacter spp.[c]											
S. maltophilia[d]											

See note to all tables in Table I and general notes for Tables V–XI in Table V.

[a]Isolates may have multiple mechanisms, with profiles superimposed on each other.

[b]Isolates typically also have r/R to quinolones.

[c]Relationships between antibiogram and mechanisms poorly defined. Carbapenems have the most consistent activity against the genus: refer carbapenem-resistant *Acinetobacter* isolates.

[d]May appear susceptible to penicillins and cephalosporins on IsoSensitest agar, but is generally resistant on Mueller–Hinton agar. Among β-lactams, ticarcillin/clavulanate has best provenance, although co-trimoxazole (not trimethoprim alone) is the usual drug of choice.

The role of *mec*A in giving resistance to all β-lactams in methicillin-resistant staphylococci is discussed elsewhere[4] and no comment is needed here. In the case of pneumococci, β-lactam resistance accrues stepwise and affects all members of the antibiotic class.[18] Oxacillin resistance can be taken as an indicator of penicillin-binding protein changes.[19] These changes usually reduce susceptibility to penicillin, cefotaxime, ceftriaxone and meropenem.[18] The two cephalosporins generally remain more active than penicillin against strains with the mechanism, but the position is reversed for a few isolates.[20] Rare pneumococci are resistant to oxacillin, but not penicillin.[18]

Glycopeptides

At present, transferable glycopeptide resistance is exclusive to enterococci. *Enterococcus faecalis* and *Enterococcus faecium* strains with the classical VanA phenotype show resistance to vancomycin and resistance, or markedly reduced susceptibility, to teicoplanin; those with classical VanB are resistant to vancomycin but remain susceptible to teicoplanin.[21] Teicoplanin remains acceptable therapy against strains inferred, on this basis, to have VanB. VanC is exclusive to rarer enterococci, specifically *Enterococcus casseliflavus* and *Enterococcus gallinarum*. It gives low-level resistance to vancomycin, but not teicoplanin.[18] Intermediate glycopeptide resistance remains very rare in *S. aureus*, although teicoplanin resistance is frequent in coagulase-negative staphylococci. MIC tests are required to detect these mechanisms.

Aminoglycosides

In contrast to the β-lactamases, aminoglycoside-modifying enzymes modify their substrate compounds at various different positions, variously acetylating, nucleotidylating or phosphorylating amino or hydroxyl groups. There are different forms of some modifying enzymes, often with markedly different substrate specificities. This variation is particularly evident in the AAC(3) and AAC(6′) families.[22,23] On the other hand, unrelated enzymes can confer the same resistance phenotype to one another.

Most laboratory susceptibility test results with aminoglycosides can be accepted without editing. Nevertheless, the enzymes produced by isolates can often be predicted from the antibiogram data, as illustrated in Tables IX and X. Because few organisms have chromosomally encoded aminoglycoside-modifying enzymes, it is not necessary to split bacteria into as many groups as for β-lactamases. With a few exceptions, Enterobacteriaceae can be treated as a single group (Table IX). However, *Klebsiella* spp. are shown separately, because resistance is more frequent than in most other genera.[24] *Serratia* is also shown separately because of its chromosomally encoded AAC(6′) enzyme.[25] This is usually expressed weakly and the organism remains susceptible to the aminoglycosides, but mutational hyper-

Table VII. Phenotypes; interpretation of mechanism and editing of antibiograms: β-lactams versus fastidious Gram-negative bacteria and *M. catarrhalis*

PCG	AMP/ AMX	AMX/ CLAV	CCL	CTX/CRO/ CFIX	MEM	IMP	Interpretation	Frequency	Edit/action
H. influenzae									
R	S	S	S	S	S	S	classical	common	
R	R	S	S	S	S	S	β-lactamase +ve	common	Confirm with β-lactamase test.[a]
R	r/R	r/R	R	r	S	S/R[b]	intrinsic resistance-altered PBPs; impermeability or efflux	rare	
any	any	any	any	any	R	any	!!!	!!!	Refer.
any	any	any	any	R	any	any	!!!	!!!	Refer.
N. gonorrhoeae									
S	S	S	–	S	–	–	classical	common	
R	R	S	–	S	–	–	β-lactamase +ve	common	Confirm with β-lactamase test.[a]
r/R	r/R	r/R	–	S	–	–	intrinsic impermeability or efflux	common	
any	any	any	any	R	–	any	!!!	!!!	Refer.
N. meningitidis									
S	S	S	–	S	–	–	classical	common	
r	R	r	–	S	–	–	impermeability or efflux	common	
Substantial R to any β-lactam					–	–	!!!	!!!	Refer.
M. catarrhalis									
R	S	S	S	S	S	S	classical	common	Confirm β-lactamase negative by direct test; if +ve, report as ampicillin/amoxycillin-resistant.
R	R	S	S	S	S	S	BRO-1/2 β-lactamase +ve	common	

See note to all tables in Table I and general notes for Tables V–XI in Table V.

[a]See Livermore & Brown[b] for β-lactamase tests.

[b]*H. influenzae* with intrinsic resistance to penicillins and cephalosporins are either fully susceptible to imipenem, or show a high level of resistance, implying that the group encompasses at least two different genotypes.

Table VIII. Phenotypes; interpretation of mechanism and editing of antibiograms: β-lactams versus Gram-positive cocci

PCG	AMP/ AMX	AMX/ CLAV	OXA	Any cephalosporin	IMP/ MEM	Interpretation	Frequency	Edit/action
Staphylococci								
S	S	S	S	S	S	classical, now uncommon	scattered	
R	R	S	S	S	S	β-lactamase +ve	common	Edit all penicillins except oxacillin and methicillin to R.
any	any	any	R	any	any	methicillin/oxacillin resistant	common	Edit all β-lactams to R.
S. pyogenes								
S	S	S	S	S	S	classical	common	
R	any	any	any	any	any	!!!	!!!	Refer.
S. pneumoniae								
S	S	S	S	S	S	classical	common	Determine MICs of drugs intended for use. Cefotaxime and ceftriaxone, also meropenem, often remain active, with oral cephalosporins mostly less active than amoxycillin.
any	any	any	R	any	any	PenR pneumococcus		
E. faecalis								
r	S	S	R	R	S	classical	common	
R	R	S	R	R	S	β-lactamase +ve	!!!	Refer.
R	R	R	R	R	R	probably *E. faecium*	error	Check speciation.
E. faecium								
R	S	S	R	R	S	classical, now rare	scattered	
R	R	R	R	R	R	uses PBP-5 to cross-link peptidoglycan	common	

See note to all tables in Table I and general notes for Tables V–XI in Table V.

Table IX. Phenotypes; interpretation of mechanism and editing of antibiograms: aminoglycosides versus Gram-negative bacteria

GEN	NET	TOB	AMK	KAN	NEO	Interpretation	Frequency	Edit/action and comments
E. coli and other Enterobacteriaceae <u>not</u> shown separately								
S	S	S	S	S	S	classical	common	
R	S	S	S	S	S	AAC(3)I	rare	Also R to fortimicin.
R	R	R	S	R	S	AAC(3)II	rare	Greater R to GEN than to TOB or NET.
R	R	R	S	r	R	AAC(3)IV	rare	Also R to apramycin (used in veterinary practice). Mostly in *E. coli.*
S/r	R	R	R	R	R	AAC(6′)	rare	One component of GEN remains active but *in vivo* use best avoided.
R	S	R	S	R	S	ANT(2′)	rare	Equal R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	common	Usually more R to KAN than NEO. Was common, now rarely seen.
r/R	r/R	r/R	r/R	r/R	r/R	'impermeability'	rare	Low level R to all aminoglycosides.
Klebsiella spp.								
S	S	S	S	S	S	classical	common	
R	S	S	S	S	S	AAC(3)I	rare	Also R to fortimicin.
R	R	R	S	r	S	AAC(3)II	scattered/rare	Greater R to GEN than to TOB or NET.
S/r	R	R	R	R	R	AAC(6′)	rare	One component of GEN remains active, but *in vivo* best avoided.
R	S	S	S	R	S	ANT(2′)	scattered/rare	Equal R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	common?	Usually more R to KAN than NEO. Was common, probably remains so.
r/R	r/R	r/R	r/R	r/R	r/R	'impermeability'	rare	Low-level R to all aminoglycosides.
Serratia spp.								
S	S	S	S	S	S	classical	common	Chromosomal AAC(6′) expressed weakly: risk of selection of over-producers in therapy with AMK, TOB, NET.
R	S	S	S	S	S	AAC(3)I	rare	Also R to fortimicin.
R	R	R	S	r	S	AAC(3)III	rare	Greater R to GEN than TOB or NET.
S/r	R	R	R	R	R	AAC(6′)	common	Mutation causes over-production of chromosomal AAC(6′).
R	S	R	S	R	S	ANT(2′)	rare	Equal R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	rare	Usually greater R to KAN than to NEO.
r/R	r/R	r/R	r/R	r/R	r/R	'impermeability'	rare	Low-level resistance to all aminoglycosides.
Providencia stuartii								
R	r	R	S	S	R	AAC(2′)	classical	Chromosomal. AAC(2′); poorly expressed.
R	R	R	S	S	R	AAC(2′)	common	Mutation causes overproduction of AAC(2′).
P. aeruginosa								
S	S	S	S	R	R	classical	common	
R	S	S	S	R	R	AAC(3)I	rare	Also R to fortimicin.
R	S	R	S	R	R	AAC(3)III	rare	
S/r	R	R	R	R	R	AAC(6′)	rare	One component of GEN remains active, but *in vivo* use best avoided.
R	R	R	S	R	R	AAC(6′)II	rare	R pattern not obviously predictable from enzyme activity.
R	S	R	S	R	R	ANT(2′)	rare	Equal levels of R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	common	Usually more R to KAN than to NEO.
r/R	r/R	R/R	r/R	r/R	r/R	'impermeability'	rare	Low-level R to all aminoglycosides.

See note to all tables in Table I and general notes for Tables V–XI in Table V.

Table X. Phenotypes; interpretation of mechanism and editing of antibiograms: aminoglycosides versus Gram-positive bacteria

GEN	NET	TOB	AMK	KAN	NEO	Interpretation	Frequency	Edit/action and comments
Staphylococci								
S	S	S	S	S	S	classical	common	
S	S	R	R	R	S	ANT(4′) (4″)I	rare	Unlike 'Gram-negative' ANT(4′), also modifies dibekacin at 4″.
R	r	R	r	R	r	APH(2′) AAC(6′)	rare scattered	Greater R to TOB.
S	S	S	S	R	R	APH(3′)	common	Usually more R to KAN than NEO.
S	S	S	R	R	R	APH(3′)III	rare	Rare.
r/R	r/R	R/R	r/R	r/R	r/R	'impermeability'	rare	Low-level R to all aminoglycosides.
E. faecalis								
R	R	R	R	R	R	classical	common	Intrinsic low-level resistance.
R	R	HLR	HLR	HLR	R	ANT(4′) (4″)I	rare	
HLR	R	HLR	R	HLR	R	APH(2″)/ AAC(6′)	scattered	Greater R to GEN than TOB.
R	R	R	R	HLR	HLR	APH(3′)	common	Usually more R to KAN than NEO.
R	R	R	HLR	HLR	HLR	APH(3′)III	rare	Rare.
E. faecium								
R	R	R	R	R	R	AAC(6′)I	classical	Chromosomal AAC(6′), intrinsic to *E. faecium*.
R	R	HLR	HLR	HLR	R	ANT(4′) (4′)	rare	
R	R	R	R	HLR	HLR	APH(3′)	common	Usually greater R to KAN than NEO.
R	R	R	HLR	HLR	HLR	APH(3′)III	rare	

See note to all tables in Table I and general notes for Tables V–XI in Table V.
HLR = high-level resistance in enterococci.

production gives a characteristically resistant phenotype.[26] *Providencia stuartii* possesses a chromosomal AAC(2′) enzyme, which usually is expressed weakly but nevertheless confers low-level resistance to its substrates.[27] This enzyme is virtually unknown outside *Providencia* spp. Many of the plasmid-encoded enzymes seen in Enterobacteriaceae also occur in *P. aeruginosa* (Table IX), but AAC(3)II is very rare whereas AAC(3)III and AAC(6′)II are more frequent.[28] Broad-spectrum resistance, normally low level, is frequent in pseudomonads and is presumed to reflect poor uptake,[28,29] although efflux-mediated resistance has recently been observed in some organisms.[30] *P. aeruginosa* is inherently resistant to kanamycin and neomycin, (kanamycin MICs around 64 mg/L) owing to low-level APH(3′) activity.[29] Isolates with acquired mechanisms, e.g. plasmid-encoded APH(3′), are more resistant.

Gram-positive organisms have different aminoglycoside-modifying enzymes to Gram-negative ones (Table X). Bi-functional AAC(6′)/APH(2′) is by far the most important and frequent enzyme,[28,31] giving resistance to all analogues except streptomycin. Enterococci characteristically have low-level resistance to all aminoglycosides, but detection of high-level resistance (MICs > 1000 mg/L) is significant, since it contra-indicates synergy with cell wall active agents.

Streptomycin is omitted from Table IX since it is seldom tested or used, and because there is no cross-resistance with other aminoglycosides, except when resistance is caused by impermeability.[29,32] Streptomycin resistance in Enterobacteriaceae mostly depends on ANT(3′)I or APH(3′).[33] High-level resistance in enterococci mostly reflects ANT(6)[31] which, like other streptomycin-modifying enzymes, does not give cross-resistance to other aminoglycosides. Production of multiple enzymes is an even greater problem with aminoglycoside-modifying enzymes than with β-lactamases.[28,32] The simultaneous production of APH(3′) plus a gentamicin-modifying enzyme can often be inferred from the resistance pattern. However, it is difficult to more than guess at the identity of combinations of enzymes that modify gentamicin or tobramycin, e.g. AAC(3)II + AAC(6′), without resorting to use of experimental compounds such as the 2′ and 6′-*N*-ethyl derivatives of netilmicin. Such experimental drugs can serve as a powerful tool in the prediction of aminoglycoside modifying enzyme types,[32,34] but are beyond the scope of this review.

Quinolones

Quinolones differ in their activity against bacterial species, doubtless reflecting differences in their ability to permeate, evade efflux and bind to different topoisomerases. Resistance, however, is a class effect, and isolates with resistance to one analogue invariably show reduced susceptibility or resistance to other members of the family. In these circumstances there is little scope for interpretative reading, but a few general principles can be proposed.

Firstly, based on recent literature,[35,36] the most active analogues against different groups are:

Enterobacteriaceae: ciprofloxacin
Non-fermenters: ciprofloxacin
Pneumococci: moxifloxacin, gemifloxacin
Enterococci: no available analogue has adequate activity
Staphylococci: high risk of mutational resistance to all analogues

Secondly, the differentials in activity between ciprofloxacin, ofloxacin, norfloxacin, levofloxacin and moxifloxacin against Enterobacteriaceae are small (four-fold MIC variation).[35,36] If an isolate is resistant to one of these drugs, susceptibility to the others is likely to be marginal at best. In these circumstances, quinolones should only be used if there are no alternatives in other therapeutic classes. If an isolate appears highly susceptible to one fluoroquinolone but highly resistant to others, a testing problem is likely.

Thirdly, non-fermenters and Gram-positive cocci have lower inherent susceptibility to quinolones than Enterobacteriaceae. Isolates (even of classical phenotypes) may be susceptible to some analogues but marginally resistant to others. The most active analogues should be recommended for therapy, since it is hardest for resistance to develop.

Fourthly, the value of using nalidixic acid as an indicator for reduced susceptibility or resistance to fluoroquinolones in fastidious Gram-negative bacteria (Table IV) should be re-emphasized.

MLS drugs (macrolides, lincosamides and streptogramins)

Table XI gives interpretative reading guidelines for these agents. The most important source of resistance is the macrolide, lincosamides, streptogramin B (MLS$_B$) system encoded by the *erm* genes, which may be constitutive or inducible in expression.[37,38] Expression is regulated further by the sequences upstream of this gene, which vary among the host elements prevalent in different species.

In staphylococci, 14- and 15-membered macrolides, including erythromycin, clarithromycin and azithromycin, are inducers whereas clindamycin and 16-membered macrolides do not induce. MLS$_B$-inducible strains consequently express resistance to erythromycin but not clindamycin, whereas resistance to both drugs is expressed by MLS$_B$-constitutive (MLS$_{B/c}$) organisms. For MLS$_B$-inducible isolates, erythromycin antagonizes clindamycin, a phenomenon easily demonstrated in double disc tests. Distinguishing MLS$_{B/c}$ resistance in staphylococci is important since the dosage frequency for quinupristin/dalfopristin is changed from twice to thrice daily in MRSA skin and soft tissue infections when this mechanism is inferred.[39] Whether to report MLS$_B$-inducible staphylococci (erythromycin-resistant, clindamycin-susceptible) as clindamycin-resistant remains debatable. Some authors[40] support this approach, since MLS$_B$-inducible strains segregate clindamycin resistant MLS$_{B/c}$ mutants, which may be selected in therapy.[41] Nevertheless, one of the most-cited examples[42] of resistance emerging during clindamycin treatment concerns a staphylococcal strain that was susceptible to erythromycin and so was unlikely to have harboured an *erm* gene. Lincosamide inactivation is an occasional source of resistance to clindamycin (not macrolides) in coagulase-negative staphylococci, but is very rare in *Staphylococcus aureus*.[38] MLS resistance occurs in streptococci as well as staphylococci and, once again, can be inducible or constitutive. However, clindamycin as well as erythromycin often acts as an inducer. Thus, cross-resistance to both erythromycin and clindamycin is indicative of MLS$_B$ but does not prove constitutive expression. Resistance to erythromycin but not clindamycin may indicate an MLS$_B$-inducible phenotype, but may also be contingent on efflux mediated by the products of *mef* genes. In the case of enterococci, the critical point is that *E. faecalis* is resistant to quinupristin/dalfopristin whereas almost all *E. faecium* isolates are susceptible—a pattern that is the perfect mirror image of that seen for ampicillin. Thus, microbiologists should be sceptical of any isolate that is resistant or susceptible to both of these drugs.

Tetracyclines

No interpretative reading table for tetracyclines is provided, since multiple analogues are virtually never tested. Nevertheless, not all the analogues are equally affected by the prevalent efflux [*tet*(A)-*tet*(F), *tet*(K) and *tet*(L)] or ribosomal protection [*tet*(M) and *tet*(O)] mechanisms, and a system of interpretative reading could be devised. Minocycline, unlike other analogues, retains activity against staphylococci with *tet*(K), but is compromised against those with *tet*(M) and is often worth testing against multi-resistant strains of these and other species.[43] More generally, *tet*(B) and *tet*(M) confer high-level resistance to all tetracycline derivatives whereas *tet*(A), *tet*(C), *tet*(D), *tet*(K) and *tet*(L) provide poor protection against doxycycline as well as minocycline.[44] The development of new tetracyclines (specifically glycylcycline)[43] may provide increased potential and value for interpretative reading in the future.

Table XI. Phenotypes; interpretation of mechanisms and editing of antibiograms: MLS drugs versus Gram-positive cocci

ERY[a]	CLI	Q-D	Interpretation	Frequency	Edit/action
Staphylococci					
S	S	S	classical	common	
R	S	S	may be MLS$_B$ inducible may have macrolide efflux	common	Check if erythromycin antagonizes clindamycin; if antagonism seen, isolate has MLS$_B$, and clindamycin should be used with caution (if at all).
R	R	S	MLS$_B$ constitutive	common	Note Specification of Product Characteristics recommendation that Q-D should be given thrice daily even in skin and soft tissue infection.
any	any	R	!!!	refer	
Streptococci, including *S. pneumoniae*					
S	S	S	classical	common	
R	R	S	MLS$_B$ constitutive/inducible	common	NB-inducible resistance usually affects clindamycin as well as erythromycin in streptococci.
R	S	S	efflux; MLS$_B$ inducible	common	
any	any	R	!!!	refer	
E. faecalis					
S	S	R	classical	common	
R	S	R	may be MLS$_B$ inducible may have macrolide efflux	common	Check if erythromycin antagonizes clindamycin, e.g. with a double disc test. If antagonism is seen, the isolate has MLS$_B$, and clindamycin should be used with caution, if at all.
R	R	R	MLS$_B$ constitutive	common	
any	any	S	probable mis-speciation		If also AMP resistant, almost certainly *E. faecium*, not *E. faecalis*. Refer if confirmed as *E. faecalis*.
E. faecium					
S	S	S	classical	common	
R	S	S	may be MLS$_B$ inducible may have macrolide efflux	common	Check if erythromycin antagonizes clindamycin; if antagonism seen, isolate has MLS$_B$, and clindamycin should be used with caution (if at all).
R	R	S	MLS$_B$ constitutive	common	
any	any	R	probable mis-speciation; possible quinupristin efflux or modification		If also AMP susceptible, almost certainly *E. faecalis*, not *E. faecium*. Refer if confirmed as *E. faecium*.

See note to all tables in Table I and general notes for Tables V–XI in Table V.
[a]Other macrolides, e.g. clarithromycin and azithromycin behave similarly to erythromycin.

Other antibiotics

Other antibiotics are either the sole analogues within a class (e.g. chloramphenicol, fosfomycin; nitrofurantoin, trimethoprim) or belong to classes with little differentiation in microbiological activity (sulphonamides); hence there is little or no scope for useful interpretative reading. Nevertheless, interpretation is possible to the extent of recognizing inherently unlikely combinations of organisms and antibiotic susceptibility or resistance (Tables I and II), and the microbiologist should be alert to the likelihood of resistance emerging (Table III).

The limits of interpretative reading

Interpretative reading is an advance on current diagnostic laboratory practice, but is no substitute for identifying

resistance mechanisms by genetic and biochemical investigation. Even in diagnostic microbiology, its constraints should be recognized. First, and most importantly, bacteria with multiple resistance determinants affecting the same class(es) of antibiotics are increasingly frequent. Shaw *et al.*[32] found multiple determinants in >70% of 4088 aminoglycoside-resistant enterobacteria examined, and Essack *et al.*[45] found 84 TEM and SHV β-lactamase genes in a collection of 25 *Klebsiella pneumoniae* isolates, only 20 of which had phenotypes indicating ESBL production. The resistance patterns given by isolates with multiple mechanisms may be confusing or misleading. For example, there is little to reliably distinguish the resistance pattern of a *Klebsiella* with an AmpC enzyme from that of a strain with both an ESBL and a permeability lesion. Secondly, interpretative reading cannot identify new resistance mechanisms if these give a resistance profile identical to that given by a known mechanism.[1,3]

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