AUSCAST
AUSTRALIAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
Australian Society for Antimicrobials

AUSCAST Frequently Asked Questions 2025

Q1: How would you recommend reporting a *Serratia marcescens* from urine with Vitek2 meropenem MIC = 2 mg/L, ZD = 19 mm, and CIM equivocal at 27 mm. Would you report this as 'susceptible, increased exposure', or 'resistant'?

A1: This situation depends on the specimen type, clinical details and other non-beta-lactam antimicrobial options available. We would recommend the following: carefully examine the results of the other beta-lactams antimicrobials (not looking at the Vitek2 Expert interpretation categorisations), and look into other agents such as ERT, IMI, and CAZ-AVI. Given that this isolate is displaying reduced MER susceptibility, and is likely a non-wild-type strain (based on the meropenem MIC distribution of *S. marcescens*), and warrants further investigation for carbapenemase production. There are other phenotypic screening tests that could be set-up (refer to 2017 EUCAST guidelines). The CIM is only a screening test and has variable performance. There may be a number of different reasons to explain the reduced MER susceptibility, including ESBL & AmpC enzymes combined with decreased permeability and PBP changes, or a carbapenemase enzyme with reduced hydrolysis capacity (such as OXA-48, or SME-type class A beta lactamase). Strongly consider sending the isolate off to the reference lab for BMD testing and sequencing. Generally, we would avoid advising overruling the AST result to R on the final report, unless a BMD MIC result confirms categorical resistance. You could consider suppressing the MER result if it was felt that its use would be clinically inappropriate (after discussion with the treating team).

Q2: Request for some guidance in regard to testing moxifloxacin & *Proteus* species, or more specifically, why the BPs exclude *Proteus* spp.? We had a request to test moxifloxacin for a *Proteus* species, but according to the BP table we shouldn't be testing.

A2: The three species (*Morganella morganii*, *Proteus* spp. and *Serratia* spp.) excluded for moxifloxacin breakpoints have ECOFFs above the breakpoint (determined from PK-PD studies), and therefore the test result may be unreliable. Don't test, advise another quinolone, i.e. ciprofloxacin. See Note 3 in the table.

Q3: What vancomycin breakpoint would you use for coagulase-positive staphylococci? For Vitek-users, bioMérieux are suggesting using *S. aureus* vancomycin breakpoints (S ≤ 2 mg/L, R > 2 mg/L) for *S. pseudintermedius*.

A3: Coagulase-positive staphylococci, other than *S. aureus*, include *S. argenteus*, *S. schweitzeri*, S. *intermedius*, *S. pseudintermedius*, and *S. coagulans*. For *S. argenteus*, the breakpoints for *S. aureus* can be used, with the caveat that although the close relationship between these organisms suggests that this may be extrapolatable but that definitive clinical evidence is lacking.

However, for other coagulase-positive species the performance of the breakpoints is unknown (see the notes section at the top of the *Staphylococcus* tab, v.15 EUCAST Breakpoint Table). EUCAST lists vancomycin breakpoints separately for *S. aureus* (S ≤ 2 mg/L, R > 2 mg/L) and coagulase-negative staphylococci (S ≤ 4 mg/L, R > 4 mg/L), with the note that resistant isolates are rare or not yet reported. It is recommended that the identification and vancomycin result on an isolate testing resistant should be confirmed and the isolate sent to a reference laboratory.

In this example, when considering reporting vancomycin susceptibility for a *S. pseudintermedius*, an assessment can only be made when an accurate and reproducible MIC value can be obtained. The MIC should be determined using reference methods or validated surrogate methods. Disk diffusion cannot be used, and caution is advised when using gradient tests outside of the manufacturer's validated species. For example, it is known that gradient diffusion tests for vancomycin on *S. aureus* tend to read higher than those found in reference broth microdilution. Furthermore, vancomycin MIC distribution data demonstrates narrow MIC clustering for *Staphylococcus* species, therefore the impact of any methodological difficulties in testing, especially when considering surrogate testing methods, should be considered.

Please refer to the EUCAST Guidance document: What to do when there are no breakpoints. In this instance, given the absence of a species-specific breakpoint, no wild-type MIC distribution or ECOFF value, a conservative approach would be to consider the *S. aureus* breakpoints and PK/PD cut-off values. For MIC-values above 2 mg/L, therapy with vancomycin should be discouraged. In general, we

recommend refraining from categorical reporting (especially "S" and "I") for MIC-values less than or equal to 2 mg/L. Reporting should instead be in the form of guidance. For laboratories applying LIS rules in their AST systems, one approach could be to assign *S. aureus* breakpoints, but suppress the result from routinely reporting, and use the MIC > 2 mg/L as a trigger for further testing.

Q4: How should clinical laboratories approach the testing and detection of inducible clindamycin resistance (ICR) in *Corynebacterium* spp.?

A4: There are currently no recommendations for testing *Corynebacterium* spp. for inducible clindamycin resistance (ICR; antagonism of clindamycin activity by a macrolide agent). This can be extended to all organisms other than staphylococci and streptococci because clinical evidence of treatment failure associated with ICR is lacking and there are no validated tests.

Clinical trial evidence that demonstrates the importance of ICR has only been found in situations of serious and deep-seated infections caused by staphylococci and streptococci. More studies are needed to characterize the clinical importance of multidrug-resistant corynebacteria, such as *C. jeikeium*. As such, we do not recommend the routine use of ICR testing in these instances. If the laboratory does perform this test, this is a non-validated test and the result should be treated with caution.

Q5: How should the laboratory approach the situation when an *Enterobacterales* is cultured from a urine sample, such as *E. coli*, that has discrepant antimicrobial susceptibility test results among third generation cephalosporin agents, namely "susceptible" or "susceptible, increased exposure" to either ceftriaxone or ceftazidime (neither testing resistant), with concurrent cefalexin and cefazolin resistance, cefoxitin screen negative, and cefepime susceptible? What is the chance that an isolate falling within this category harbours an ESBL gene? Regardless of whether the laboratory investigates for the presence/expression of an ESBL gene, is the reporting of "susceptible increased exposure" and the recommendation to use use high dose therapy (e.g., ceftriaxone 2 g 12-hourly, or ceftazidime 2 g 8-hourly) appropriate?

A5: There are two separate questions to address, namely the determination of susceptibility to inform patient treatment (S/I/R), and the screening for specific resistance mechanisms, such as ESBLs and carbapenemases.

In the EUCAST breakpoint table (v15.0) for Enterobacterales, the susceptible, increased exposure category applies for ceftriaxone at a MIC 2 mg/L and disk diffusion zone 24-26 mm, and for ceftazidime at a MIC 2-4 mg/L and disk diffusion zone 19-21 mm. A screening breakpoint for the detection of ESBLs is MIC > 1 mg/L for either agent, or disk diffusion zone < 23 mm for ceftriaxone and < 22 mm for ceftazidime, usually followed by phenotypic (and in some cases genotypic) confirmation tests. The detection of ESBLs may be indicated

for infection control practices, however, the result should not impact on the susceptibility category reported.

EUCAST guidelines for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (v2, July 2017) outlines that some resistance mechanisms, particularly for ESBLs and carbapenemases in Gram-negative bacilli, do not always confer clinical resistance. This could be due to mechanisms not being expressed, or expressed only at a low-level, or relate to the properties of the enzyme, which will not give rise to phenotypic resistance. There are a number of non-ESBL beta-lactamases that give some unusual results with perceived "discrepancies" between narrow and extended-spectrum cephalosporins. In addition, the co-presence of other resistance mechanisms (other β-lactamases, active efflux, altered permeability) result in the large variety of resistance phenotype observed among ESBL-positive isolates. Therefore, the detection of the mechanism of resistance does not in itself lead to classification as clinically resistant.

In this instance, if the initial disk diffusion test was performed directly from the urine specimen, some caution should be taken when interpreting the result. Disk testing directly from urine specimens for susceptibility is not endorsed by EUCAST and may contribute to conflicting results. AUSCAST hopes to lead a multi-laboratory study examining direct susceptibility testing from urine to help clarify best practice in this area. Laboratories should also ensure their disk QC values and media depth are within the acceptable range, and that the disk diffusion methodology (15-15-15 minute rule, incubation time and reading instructions) are followed accurately.

Recommendations in this case are:
(1) Report the susceptibility category as tested.
(2) Perform follow-on phenotypic testing for β-lactamase production if indicated by infection control practices, standard laboratory workflow practices, or concerns regarding the clinical severity of infection.

We do not recommend overriding S/I/R results even if a resistance mechanism is found. Depending on the clinical scenario, laboratories could consider the use of additional comments and recommendations on the final report, and in some instances may choose to suppress selected antimicrobials in the susceptibility test results.

Q6: Does AUSCAST have any guidance on how the laboratory should be approaching Measurement of Uncertainty (MU) in the antimicrobial susceptibility testing results? In recent laboratory audits, MU has been requested for the automated disc reader and Vitek2 AST results.

A6: Whilst the "Requirements for the estimation of measurement of uncertainty (2007 Edition)" provides general guidance for pathology laboratories, there is currently no EUCAST or AUSCAST guidance on how this might be applied to antimicrobial susceptibility testing. AUSCAST hopes to publish a guidance document in the future. In the

meanwhile some useful information can be found in the two SOPs developed together with CLSI (SOPs 11 and 13). BSAC recommendations are available on their website.

Q7: In the EUCAST breakpoint table (v15.0), the viridans group streptococci has a dash against ceftazidime, i.e. do not test as the agent is considered unsuitable. In contrast, ceftriaxone has breakpoints (S ≤ 0.5 mg/L; R > 0.5 mg/L). Is the activity of ceftazidime against viridans group streptococci significantly different from the other third generation cephalosporin agents (e.g. ceftriaxone) such that testing and use is discouraged?

A7: Ceftazidime is a semisynthetic third-generation cephalosporin with notable activity against *Pseudomonas aeruginosa*, but lacks activity against Gram-positive bacteria. No interpretative criteria for antimicrobial susceptibility tests have been established by EUCAST and CLSI regarding ceftazidime and Gram-positive bacteria. Data regarding wild-type Gram-positive isolates are limited to a few species of streptococci. However, MICs for ceftazidime for wild-type and clinical isolates of viridans group streptococci are consistently higher than those of ceftriaxone, cefotaxime, and cefepime (Kucers', Chapter 30). The same is found for *S. pneumoniae* and *S. pyogenes*.

Viridans group streptococci:

Ceftazidime	MIC_{90} 4 to >16 mg/L
Ceftriaxone	MIC_{90} 0.5 - 1 mg/L

Therefore, we do not recommend the testing and reporting of ceftazidime against viridans group streptococci. Laboratories should apply caution when using gradient strips to determine MIC measurements. Note that a significant bias between BMD and gradient Etest was observed for ceftriaxone and non-beta-haemolytic *Streptococcus* invasive strains, for which MICs were lower with Etest in 67% of the isolates, respectively (Plainvert et al.).

Q8: For enterococci, the EUCAST breakpoint table has a dash next to benzylpenicillin, which would suggest that the "microbe can be reported resistant without further testing". However, treating teams often request susceptibility testing when wanting to switch from ampicillin to benzylpenicillin to support OPAT management. How should laboratories approach requests to perform benzylpenicillin susceptibility testing in *E. faecalis* isolates? If testing is performed, and the isolate is shown to be wild type, what dose of benzylpenicillin should be recommended (i.e., as per the dosages tab: standard dose = 0.6 g (1 MU) x4, high dose = 1.2 g (2 MU) x6, meningitis dose = 2.4 g (4 MU) x6; or other common dosing schedules such as 1.8 g (3 MU) x6)?

A8: The PK/PD target for benzylpenicillin (penicillin G) and enterococci is not clearly established. If assuming that it's the same as for streptococci, namely *f*T>MIC of 40% (see public consultation on *S. pneumoniae* and viridans group streptococci, and

MIC- and zone diameter distributions), then even at a benzylpenicillin dose of 3 g iv 4-hourly, it isn't possible to reach 95% target attainment for MICs of 8 mg/L (the *E. faecalis* ECOFF)

The ESC Guidelines states that for cases of beta-lactam susceptible enterococcal infective endocarditis, penicillin-susceptible strains can be treated with benzylpenicillin or ampicillin combined with gentamicin (or ampicillin plus ceftriaxone), with ampicillin the preferred agent since the MIC is two to four times lower than that of benzylpenicillin. However, the use of ampicillin can be limited via the OPAT service due to drug stability concerns. In comparison, benzylpenicillin is stable, and can be given via continuous iv infusion, with the recommended dose (as per UpToDate) of 18 to 30 million units per 24 hours (combined with gentamicin for the first 2-weeks of treatment).

Although a dash does appear in the breakpoint table, given that the benzylpenicillin ECOFF for *E. faecalis* is 8 mg/L (compared with 4 mg/L for ampicillin), in some clinical circumstances, laboratories may choose to perform a benzylpenicillin MIC test to determine if the isolate belongs to the wild type.

1. If wild-type, the report should still refrain from categorical reporting of "S", given that therapy is given in combination with gentamicin, or is being used as follow-on therapy after initial active therapy or other measure.
2. If non-wild type, the report should include a comment to discourage the use of benzylpenicillin.

The dosing recommendation depends on the clinical scenario and baseline renal function. Based on the UpToDate guidelines, assuming normal renal function, the dosing should be at least to 1.8 g 4-hourly (= 18 MU/24 h), and may benefit from higher dosing with 2.4 g 4-hourly (= 24 MU/24 h). This coincides with the highest dose with stability data for administration in an infuser (60 mg/mL in 240 mL; Australian Injectable Drugs Handbook). If a continuous infusion of benzylpenicillin is used, it is recommended to perform TDM for the free benzylpenicillin level given that continuous infusion can magnify intersubject PK variation (Visser *et al.*; Walton *et al.*).

Given that the probability of infective endocarditis in enterococcal bacteraemia is high, presumably the main indication for OPAT referrals, and the treatment outcomes when using benzylpenicillin, either alone or in combinations, are inferior to ampicillin, we would suggest caution in recommending the use of benzylpenicillin. Furthermore, work presented at ASA'25 highlighted that fewer isolates demonstrate penicillin-ceftriaxone synergy compared to amoxicillin-ceftriaxone synergy. Following on from the momentum of the SNAP trial, Australian investigators are planning for a platform trial investigating optimal treatment approaches for enterococcal bacteraemia and endocarditis. The benefit of combination therapy over monotherapy has also been recently questioned in the literature (Prosty C. *et al.*).

Q9: Our laboratory applies an old 'EUCAST expert rule' in the aminoglycoside reporting for *S. marcescens*. When an isolate tests resistant to tobramycin (TOB), while amikacin (AMI) and gentamicin (GEN) test susceptible, the interpretation of AMI is changed to resistance (i.e., report both TOB and AMI as R), and leave GEN as susceptible. If all the aminoglycosides test susceptible, no changes are made and all are reported as susceptible. It doesn't seem to happen that often. Should special rules be applied to aminoglycoside reporting for *S. marcescens*?

A9: The 2011 "EUCAST expert rules in antimicrobial susceptibility testing" document (Leclercq R. et al.) included the footnote to table 1, intrinsic resistance in *Enterobacteriaceae*, "All *Serratia marcescens* isolates produce a chromosomal AAC(6')-Ic enzyme that affects the activity of clinically available aminoglycosides, except streptomycin, gentamicin, and arbekacin".

S. marcescens harbor a chromosomal aminoglycoside-modifying enzyme of the AAC(6') family, AAC(6')-Ic that may increase MICs to amikacin and tobramycin, but not gentamicin. However, this chromosomally encoded AAC(6')-Ic enzyme is normally expressed weakly or at low levels. There are reports that treatment with amikacin or tobramycin may result in the selection of a hyperproducing mutant of the chromosomal enzyme (Mahlen SD.). The clinical relevance of this observation is uncertain.

In a AAC(6')-Ic-hyperproducing strain, that isolate would test phenotypically resistant to amikacin and tobramycin. Therefore, hypothetically a *S. marcescens* isolate could test GEN susceptible, while TOB and/or AMI test resistant. This phenotype would be unusual among more commonly encountered Enterobacterales, for example, AMI resistance in an *E. coli* that is susceptible to both GEN and TOB would normally warrant additional confirmation of the identification of the bacteria and AST results.

The breakpoints for aminoglycosides were reviewed by EUCAST in 2019. It was noted that many of the old expert rules for aminoglycosides were based on very few laboratory experiments only and not on clinical data. Therefore, expert rules for aminoglycosides were removed for Enterobacterales. EUCAST also introduced the concept of "breakpoints in brackets" to warn against the use of specific agents, such as aminoglycosides, without the use of additional therapeutic measures. For these agents, clinical evidence as monotherapy is usually lacking, but they may still be used for a specific indication or in combination with another active agent or measure.

Breakpoints in brackets are in essence ECOFFs that distinguish between isolates with and without acquired resistance. The ECOFFs sometimes serve more than a single species so this represents a “best fit” ECOFF. When in doubt as to the validity of the bracketed breakpoint, go to www.mic.eucast.org to find the precise ECOFF for a specific species.

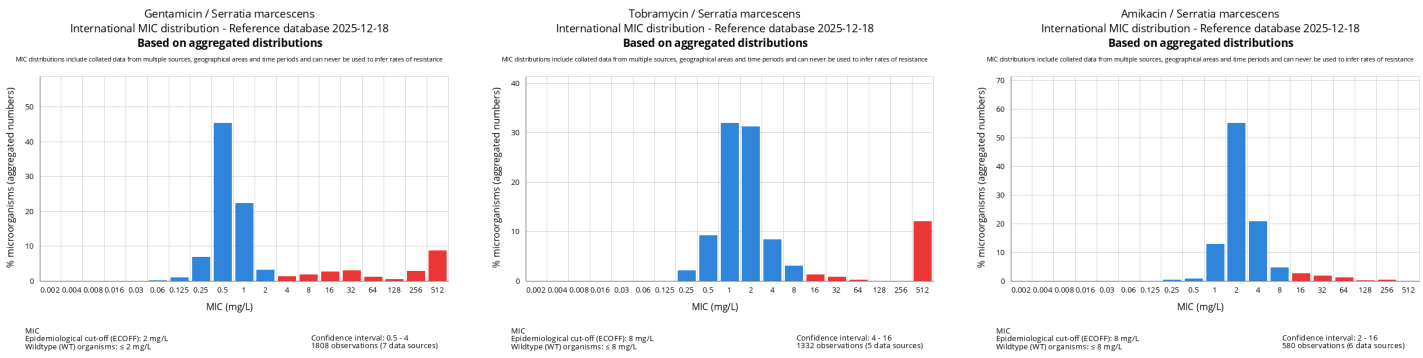
AUSCAST acknowledges that hospital sepsis treatment guidelines now often include tobramycin or amikacin as the aminoglycoside of choice, rather than gentamicin. However, given that aminoglycosides should be given with “other active therapy” for infections outside of the urinary tract, monotherapy with TOB or AMI for a *S. marcescens* infection is not recommended, so the theoretical inducible upregulation of resistance enzymes leading to treatment failure is likely to be less of an issue.

In general, we recommend that AST results should not be routinely overruled. It is preferable that categorical results are suppressed from the final report rather than manually changed. Guided by situations covered by the EUCAST Expected Phenotypes, susceptibility testing is best avoided, and a result which goes against the expected phenotype should be viewed with suspicion.

In the example provided, after confirming the identification and ensuring no errors have been made in the AST methodology/reporting, a S. *marcescens* isolate that tests GEN-(S), TOB-(R), AMI-(S) could be reported as such, after considering how your laboratory reports “breakpoints in brackets”. We recommend the laboratories routinely include a comment on their final report cautioning against the use of aminoglycosides as monotherapy. Where laboratories are relying on automated AST methods (e.g., Vitek2), results can be confirmed using a disk diffusion method for uncommon phenotypes. Further, manufacturers may provide instructions to laboratories to enable system rules which prompt testing by alternative methods or suppression of antimicrobials if validation studies have insufficient evidence for reliable results. In such cases, manufacturer guidance should be followed unless independently reviewed and validated by the user.

Q10: In contrast to the EUCAST Clinical Breakpoint table v 15.0, why does the Blood Culture RAST Breakpoint table not provide details regarding the amoxicillin-clavulanate drug formulation, i.e., oral versus intravenous, when listing zone diameter breakpoints?

A10: Blood culture RAST breakpoints apply to intravenous use of the agent tested; this will be explained by EUCAST in an update to the RAST tables in the near future.



Q9 Aminoglycoside MIC distribution and ECOFFs for Serratia marcescens