

EUCAST reading guide for broth microdilution

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EUCAST recommendations for BMD

- Perform BMD according to the ISO standard 20776-1
 - Final inoculum of 5×10^5 CFU/mL
 - Sealed panels
 - Incubation in air, at 35°C for 16-20 h
- Media
 - Un-supplemented Mueller-Hinton broth for non-fastidious organisms (ISO 20776-1)
 - MH-F broth for fastidious organisms
 - Mueller-Hinton broth with 5% lysed horse blood and 20 mg/L β -NAD

http://www.eucast.org/ast_of_bacteria/media_preparation/

EUCAST recommendations for BMD

- Guidance in Breakpoint Tables:

Enterobacterales

MIC determination (broth microdilution according to ISO standard 20776-1 except for mecillinam and fosfomycin where agar dilution is used)

Medium: Mueller-Hinton broth

Inoculum: 5×10^5 CFU/mL

Incubation: Sealed panels, air, $35 \pm 1^\circ\text{C}$, $18 \pm 2\text{h}$

Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth.

Quality control: *Escherichia coli* ATCC 25922. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor combinations, see EUCAST QC Tables.

Streptococcus pneumoniae

MIC determination (broth microdilution according to ISO standard 20776-1)

Medium: Mueller-Hinton broth + 5% lysed horse blood and 20 mg/L β -NAD (MH-F broth)

Inoculum: 5×10^5 CFU/mL

Incubation: Sealed panels, air, $35 \pm 1^\circ\text{C}$, $18 \pm 2\text{h}$

Reading: Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth.

Quality control: *Streptococcus pneumoniae* ATCC 49619. For agents not covered by this strain, see EUCAST QC Tables.

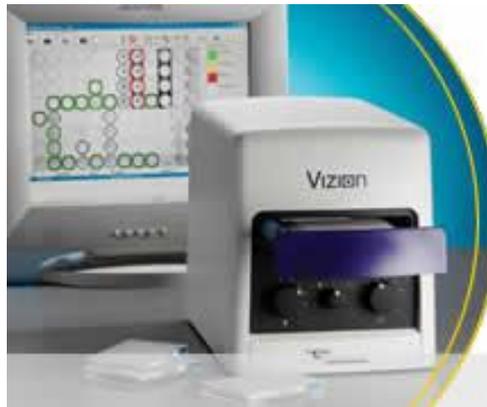
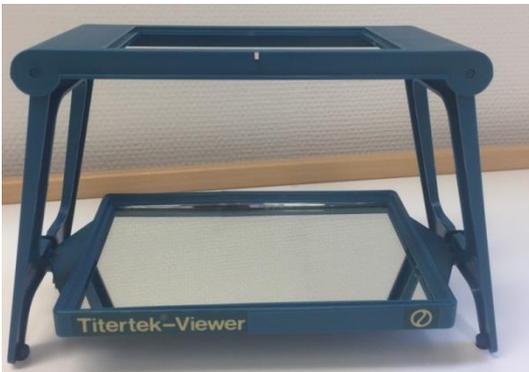
BMD reading instructions

- The revised version of ISO standard 20776-1 (to be published 2019) doesn't contain any specific reading instructions
 - Referral to CLSI and EUCAST documents
- The EUCAST BMD Reading Guide was published in January 2019

http://www.eucast.org/ast_of_bacteria/mic_determination/

Reading of BMD MICs

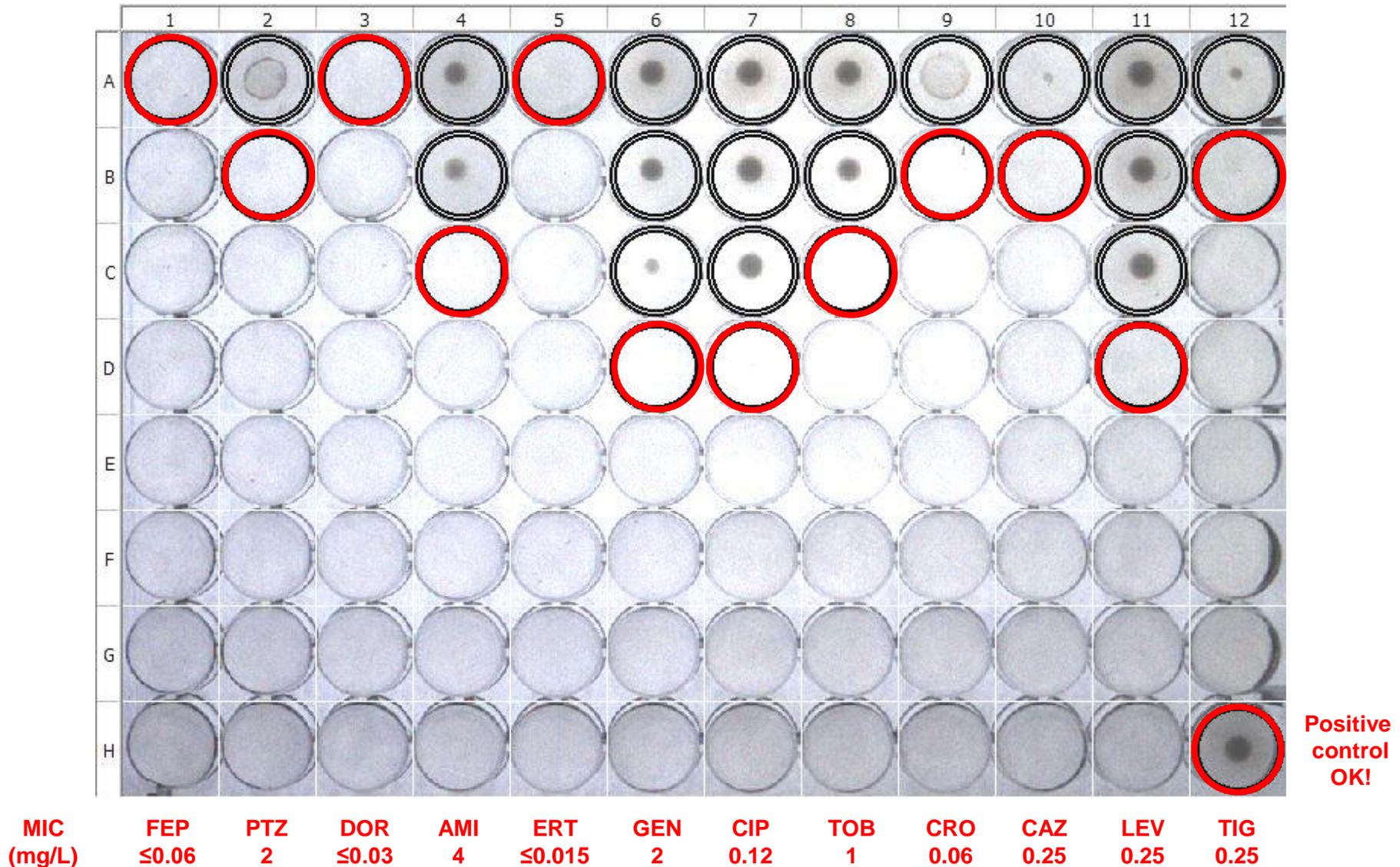
- Read MICs only for pure cultures and when there is sufficient growth (obvious button or definite turbidity) in the positive control
- Manual reading is the reference
- Automated readers or use of camera systems must be calibrated to manual reading



General reading instructions

- Read the MIC as the lowest concentration of the antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye.
 - For exceptions, see EUCAST BMD Reading Guide
- MICs are expressed as mg/L

Reading of MICs, Example *E. coli*

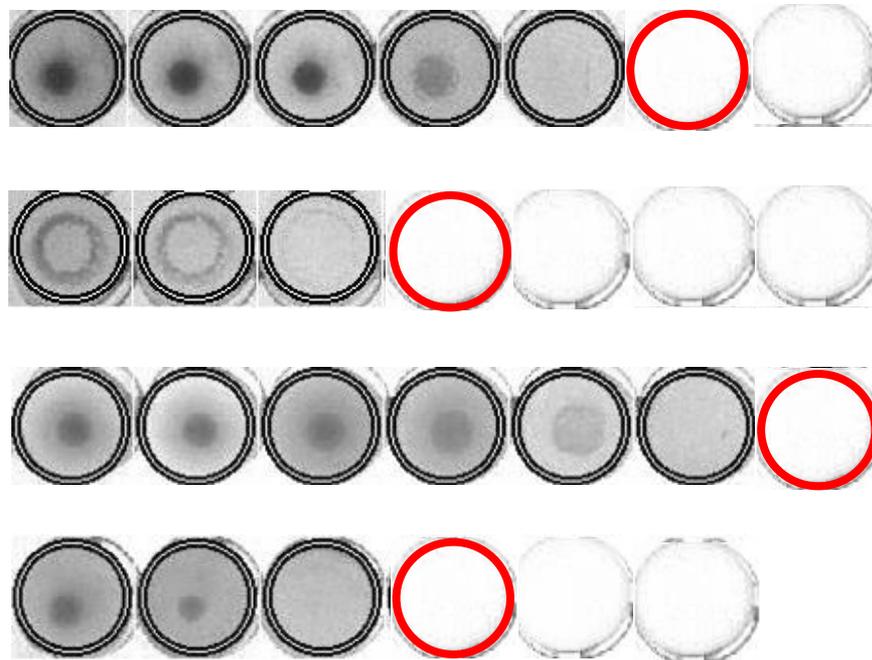


Trailing endpoints

- Most antimicrobial agent-organism combinations give distinct endpoints.
- Some agent-organism combinations may give trailing endpoints with a gradual fading of growth over 2 to 3 wells.
- Unless otherwise stated, endpoints should be read at complete inhibition of growth.

Turbidity without pellet

- Haze or turbidity without a pellet is often seen for *Pseudomonas* spp. and *Acinetobacter* spp. This should be regarded as growth and the endpoint read at the first well with complete inhibition (clear broth).



Haemolysis

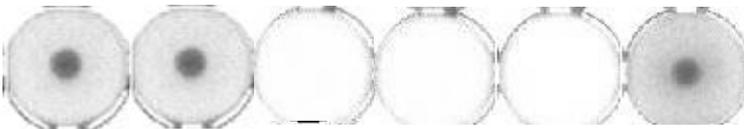
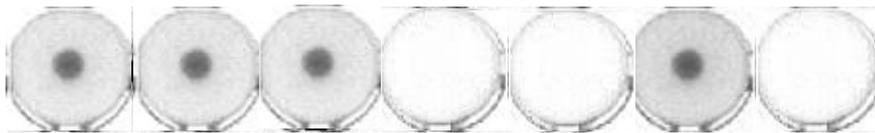
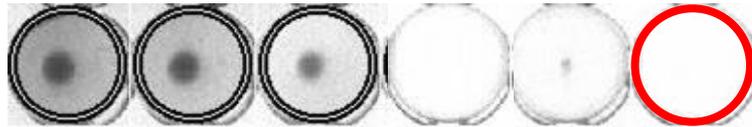
- For fastidious organisms tested in MH-F broth, haemolysis of the blood can be seen. This is often accompanied by turbidity or a deposit of growth (pellet).
- Haemolysis with turbidity or pellet should be regarded as growth when determining endpoints.



Skipped wells

- Occasionally a skip may be seen, *i.e.* a well showing no growth bordered by wells showing growth. There are several possible explanations including incorrect inoculation, contaminations, heterogenous resistance etc.
- When a single skipped well occurs, retest the isolate or read the highest MIC value to avoid reporting isolates as false susceptible.
- Do not report results for antimicrobial agents for which there is more than one skipped well.

Examples skipped wells



Retest or read the highest MIC value!

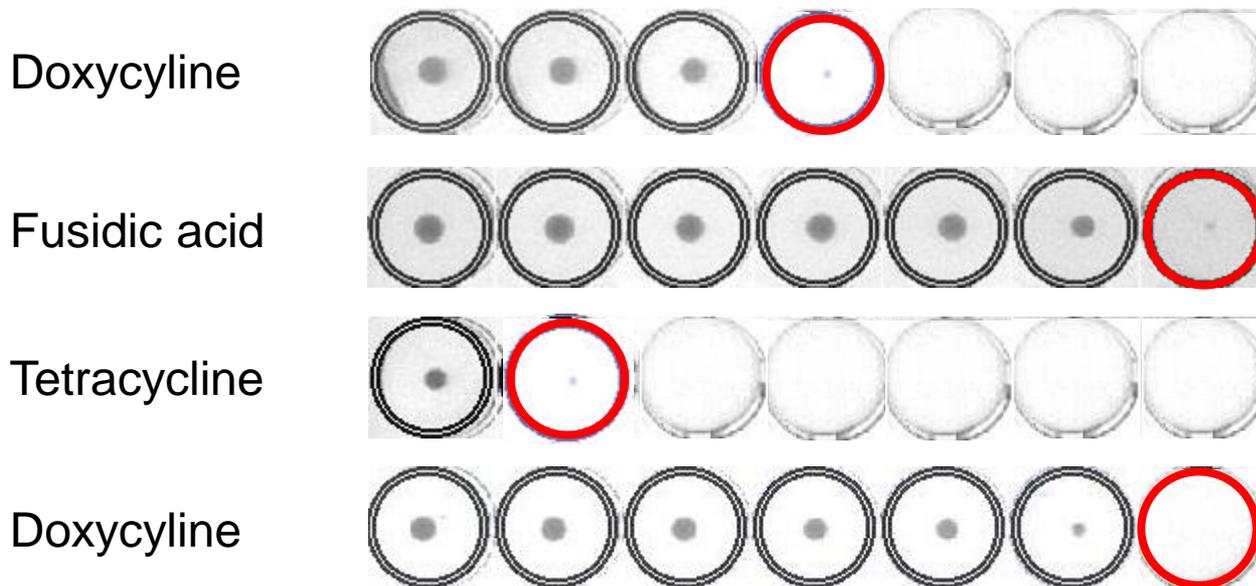
Results invalid!

Specific reading instructions

- The following combinations require specific reading instructions:
 - Gram-positive cocci with bacteriostatic antimicrobial agents
 - For example chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, tetracycline and tigecycline.
 - All organisms with trimethoprim and trimethoprim-sulfamethoxazole

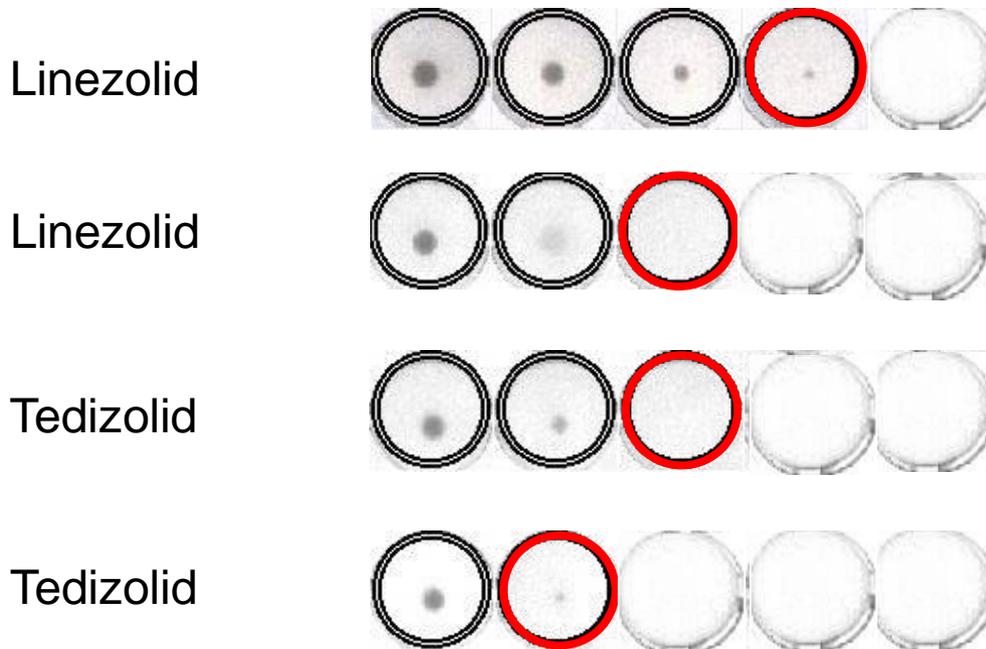
Gram-positive cocci with bacteriostatic antimicrobial agents

- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.



Gram-positive cocci with bacteriostatic antimicrobial agents

- Disregard pinpoint growth (tiny buttons) when trailing growth occurs.

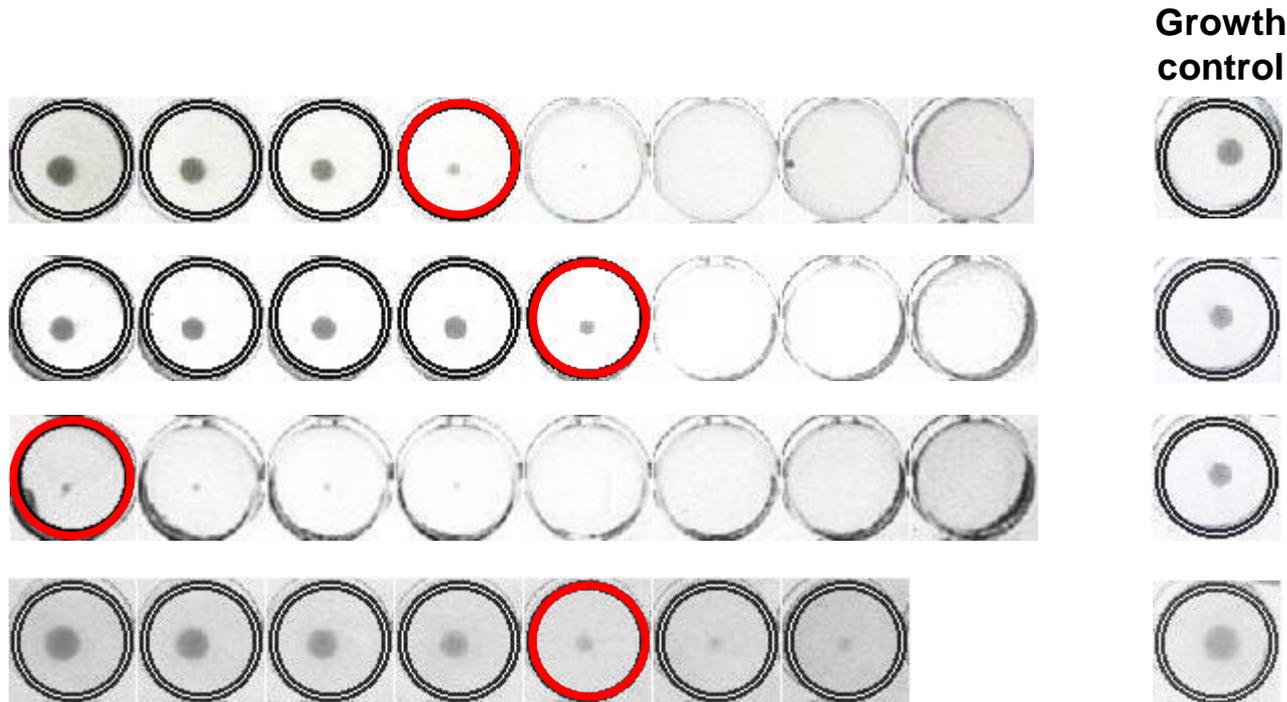


This is also valid for Gram-negative bacteria and tigecycline.

To be added to next version of BMD Reading Guide.

Trimethoprim and trimethoprim-sulfamethoxazole

Read the MIC at the lowest concentration that inhibits $\geq 80\%$ of growth as compared to the growth control.



Interpretation of results

- Make sure that MIC values for relevant Quality Control strains are within acceptable ranges before reporting results for clinical isolates.
 - See quality control criteria in EUCAST QC Tables (www.eucast.org).
- Interpret MIC values into susceptibility categories (S, I and R) according to the current EUCAST Breakpoint Tables (www.eucast.org).



AST of bacteria

- Organization
- EUCAST News
- New definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- Consultations - New!
- MIC and zone distributions and ECOFFs

AST of bacteria

- Media preparation
- MIC determination**
- Disk diffusion methodology
- Disk diffusion implementation
- Breakpoint tables
- QC Tables
- Calibration and validation
- Warnings!
- Guidance documents
- Projects and data submission
- MIC testing services from EUCAST
- Previous versions of documents



MIC determination of non-fastidious and fastidious organisms

The EUCAST recommendations for MIC determination for non-fastidious organisms are in complete agreement with the recommendations from the International Standards Organisation (↔ [ISO](#)).

For fastidious organisms (streptococci including *S. pneumoniae*, *H. influenzae*, *Moraxella catharrhalis*, *Listeria monocytogenes*, *Pasteurella* spp, *Kingella kingae*, *Aerococcus* spp, *Campylobacter* spp, and others), EUCAST recommends the same methodology but with the use of MH-F broth (MH broth with lysed horse blood and beta-NAD), see EUCAST media preparation file.

[Media preparation](#) (agar and broth for non-fastidious and fastidious organisms)
[Broth microdilution - EUCAST reading guide](#) (v 1.0, 19 January, 2019)

There are a number of commercially available surrogate MIC determination methods, such as commercial broth microdilution methods, gradient tests, semi-automated devices, etc. It is the responsibility of the manufacturer to guarantee the accuracy of their systems but it is the responsibility of the user to quality control the results obtained with these systems. We also refer the users to the [EUCAST Warnings page](#) where some of these products are criticized.



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