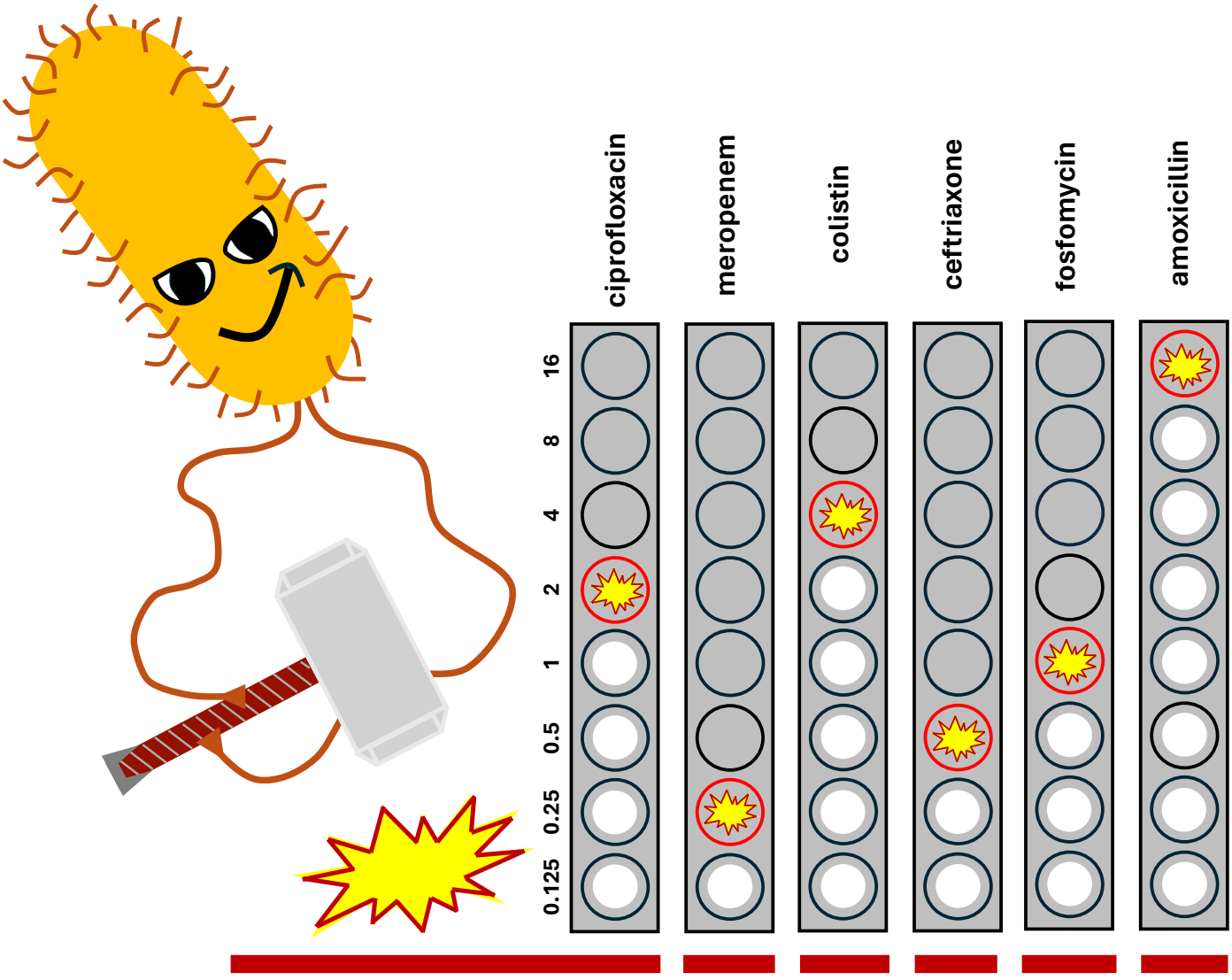
Antibiotic breakpoints and Minimum Inhibitory Concentration

Are all antibiotics useful for all bacteria?

How do we know how strong an antibiotic is?



Source: own elaboration

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Antibiotic breakpoints and Minimum Inhibitory Concentration

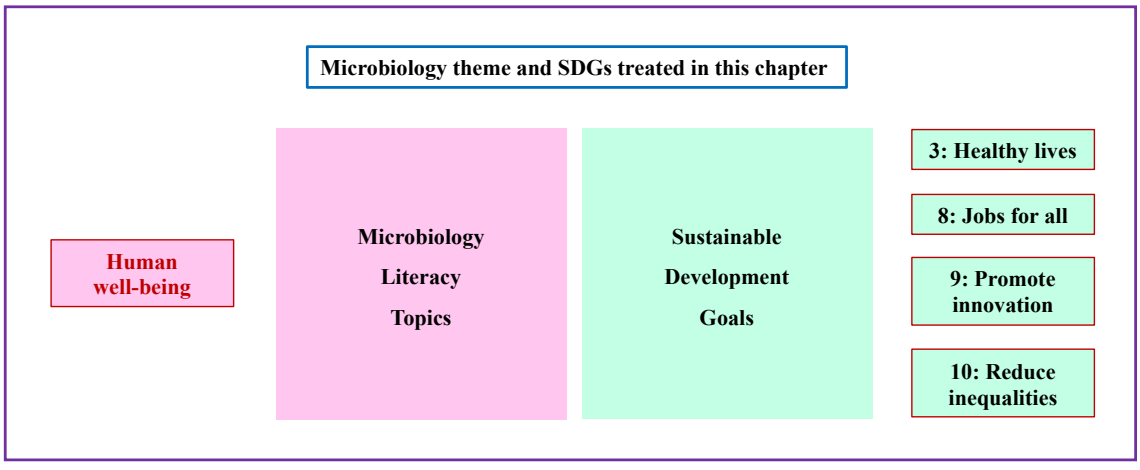
Storyline

Setting a breakpoint is essential in the management of infectious diseases. Most treatment decisions for infected patients, particularly within hospital settings, are based on **antibiotic** breakpoints. Antimicrobial therapy can be administrated empirically, when the **pathogen** or its susceptibility is still unknown and treatment targets the most likely cause of the disease. Alternatively, therapy can be directed when the causative agent and its susceptibility to antibiotics **(antibiogram)** are already known. In both cases, breakpoints are crucial to choose the most appropriate antibiotic, even though antibiotic resistance patterns differ in the diverse regions of the world. However, the complex process of establishing a breakpoint and understanding its implications is commonly overlooked. Let's dive into it!

The Microbiology and Societal Context

The microbiology: clinical diagnostics; laboratory workflow.

Sustainability issues: health; economy and employment; innovation; inequalities.



Antibiotic breakpoints and Minimum Inhibitory Concentration

1. *Are all antibiotics useful for all bacteria? How do we choose one?*

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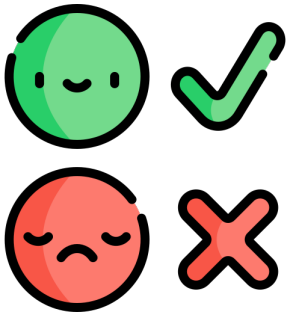
Have you ever wondered why some medicines fight illness while others don't? It's all about targeting the right enemy! Antibiotics fight infections caused by bacteria, but there are many different types of bacteria. Certain characteristics, such as the structure of the bacterial cell wall or the way bacteria take nutrients from their environment, limit the number of antibiotics that are effective against each bacterial species. Doctors choose the antibiotic that kills those bacteria more most effectively while minimizing side effects and administration-related problems.

2. *Two important concepts: susceptible and resistant*

When an antibiotic can kill a specific group of bacteria, making it suitable for treating infections caused by them, we say that those bacteria are susceptible to the antibiotic. On the other hand, if the bacteria are not affected by the antibiotic and keep growing, we say that these bacteria are resistant.



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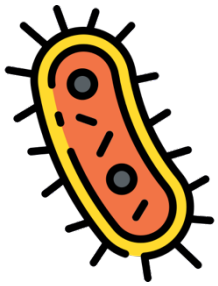
It is very important to remember that antibiotic efficacy is evaluated using doses that are approved for human use. Some antibiotics may be capable of killing bacteria at higher concentrations, but these doses could be toxic for humans. In such cases, we would not consider this antibiotic aa viable treatment option.

3. *What is a MIC?*

The term MIC stands for Minimum Inhibitory Concentration, which represents the lowest concentration of an antibiotic required to inhibit the growth of a specific bacterial strain. This value is determined in clinical microbiology laboratories through *in vitro* testing, where bacterial growth is observed in a panel of wells containing different antibiotic concentrations. The most common technique used to determine MICs is broth microdilution.



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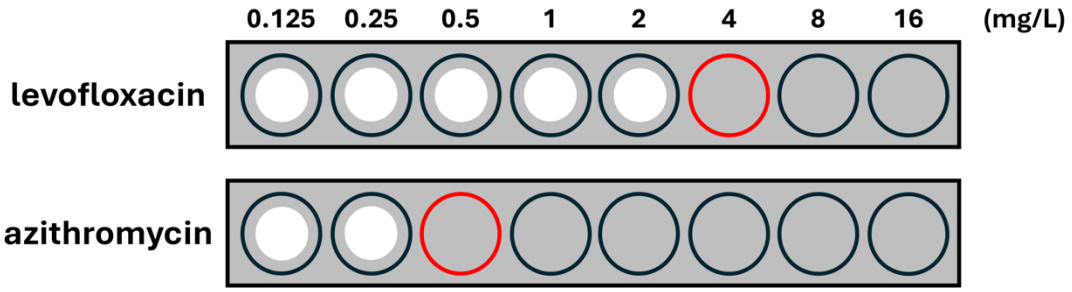
Example 1: there is an *Escherichia coli* isolate with a MIC of 8 mg/L. This means that an antibiotic concentration of 1 mg/L will not prevent its growth, while concentrations of 8 mg/L or 32 mg/L will.

We can compare two isolates of the same bacterial species: the lower the MIC, the more susceptible the isolate is to the antibiotic. In other words, a lower concentration of antibiotic is required to inhibit its growth.

Example 2: consider two *Escherichia coli*, isolate 1 has an amoxicillin MIC of 32 mg/L and isolate 2 has a MIC of 1 mg/L. In this case, isolate 2 is more susceptible to amoxicillin than isolate 1. That is to say isolate 1 is more resistant than isolate 2 because it needs a higher antibiotic concentration to inhibit its growth.



Source: own elaboration. This figure represents how a broth microdilution plate can be interpreted. Black circles represent wells containing increasing antibiotic concentrations; wells showing bacterial growth contain a filled white circle, and the MIC concentration well is highlighted red. Levofloxacin MIC is 4 mg/L and for azithromycin one 0.5 mg/L.

Source: adapted from EUCAST guidance document on cefiderocol BMD (page 2). The image shows a real broth microdilution plate lecture, with the MIC well highlighted in red. Available in: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Guidance_documents/Cefiderocol_MIC_testing_EUCAST_guidance_document_201217.pdf

4. What is a clinical breakpoint?

We have learned that the MIC indicates how active an antibiotic is against bacteria under laboratory conditions. But this is only part of the picture. In the real-life clinical situations, the interaction between the antibiotic and the human body must also be considered.

Clinical breakpoints are like cut-off points that consider how well the antibiotic works inside the body, to predict if a bacterial isolate is likely to be treatable in the patient. Think of breakpoints as guidelines for doctors:

- MIC less than or equal to the breakpoint: the antibiotic is likely to achieve enough activity at a safe dosage to successfully treat the infection.
- MIC greater than the breakpoint: the antibiotic is unlikely to be effective at a safe dose and may not successfully treat the infection.

Breakpoints are specific for bacterial species-antibiotic combinations. Let´s consider some examples:

		S	**R**
Staphylococcus aureus			
	Azithromycin	<= 2	> 2
	Doxycycline	<= 1	> 1
Streptococcus pneumoniae			
	Azithromycin	<= 0.25	> 0.25
	Doxycycline	<= 1	> 1

Source: EUCAST Clinical Breakpoint Tables v 14.0

In these examples, the breakpoint for azithromycin is different in *S. aureus* (2 mg/L) and *S. pneumoniae* (0.25 mg/L). This indicates that higher concentrations of azithromycin are required to inhibit *S. aureus* growth than S. *pneumoniae*. However, the breakpoint for doxycycline is the same for both species; if an isolate has a MIC of 0.5, we can use doxycycline for treatment of either microorganism.

5. How are breakpoints established?

Several organizations establish and regularly update clinical breakpoints. Two of the most widely used are: EUCAST (The European Committee on Antimicrobial Susceptibility Testing), used primarily in Europe and CLSI (Clinical &

Laboratory Standards Institute,) widely used in f the United States of America and many other countries.

. Setting a breakpoint is a very complex process that involves clinical, pharmacological, and microbiological evidence; including pharmacokinetics (how the body absorbs, distributes, metabolizes and eliminates the antibiotic) and pharmacodynamics studies (how the antibiotic acts against bacteria within the body). Clinical outcome data from treated patients and microbiological data describing bacterial susceptibility patterns. By combining these data, experts determine the MIC value most closely associated with therapeutic success when the antibiotic is administered as its approved dosage.

Source: https://clsi.org

Source: https://www.eucast.org

6. *Is it necessary to set a clinical breakpoint?*

Absolutely. Whenever an antibiotic is used to treat a human infection caused by a specific bacteria strain, a clinical breakpoint is necessary. Without a defined breakpoint, the MIC value obtained in the laboratory cannot be evaluated against a reference standard. Consequently, we would not know if the bacterial isolate should be classified as susceptible or resistant to the antibiotic, so we cannot determine the appropriateness of the antibiotic for treating that infection.

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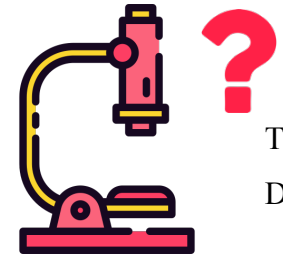
7. *Is it necessary to determine the MIC in the laboratory?*

Not always. In many cases, bacteria very rarely develop resistance to a particular antibiotic, allowing clinicians to prescribe that antibiotic without performing susceptibility testing. In such cases, knowing the MIC value is not essential as it can reasonably be inferred to be very low.



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The most common example is the use of a penicillin for tonsillitis. Doctors can prescribe directly a penicillin because tonsillitis is

often caused by a type *Streptococcus* that very infrequently develop resistance to penicillin.

Take-home messages

- MIC values indicate the lowest concentration of an antibiotic that inhibits bacterial growth in a laboratory setting. It is determined in microbiology laboratories for each specific bacterial isolate.
- Clinical breakpoints provide information about the likelihood of treatment success, by evaluating if an isolate MIC falls below or above a predetermined threshold. Unlike MICs, breakpoints consider the interaction antibiotic-body, and are established by international institution rather than by individual microbiology laboratories.
- Although determining MIC values is not be always mandatory, having a defined clinical breakpoint is essential whenever an antibiotic is used to treat an infection.

Relevance for Sustainable Development Goals and Grand Challenges

The establishment of clinical breakpoints and the determination of MICs contribute to several SDGs, including:

- **Goal 3: Ensure healthy lives and promote well-being for all at all ages.**

The more information that is available about breakpoints, and antimicrobial susceptibility the better-informed treatment decisions can be. It is well known that the correct antibiotic in the first stages of the infection is linked to a better prognosis, particularly in severe infections as sepsis. Appropriate antimicrobial therapy, including the correct choice of antibiotic and treatment duration, can shorten hospital stays and accelerate recovery and prevents the development of complications.

- **Goal 8: Promote sustained, inclusive, and sustainable economic growth, full and productive employment, and decent work for all.**

Clinical microbiologists are responsible for the determination of the patients' isolates MICs for hospitals and primary healthcare centres. Depending on the country, testing may be performed in hospital-based laboratories or centralised laboratories that receive isolates from multiple health centres. In either case, the availability of well-trained staff in every country is crucial to ensure a correct interpretation of the isolate's susceptibility. Specialised training should be encouraged for professional involved in these activities.

- **Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation.**

Determining MIC values is not always an easy task. Sometimes, and mainly with new antibiotics, having this information can be challenging and thus new techniques are currently being developed. Innovation of faster and more accurate assays, as well as automatizing the process as much as possible, is essential to ensure rapid and reliable information to make decisions. For this reason, many companies are developing different fast automated MIC-based methods, reducing turnaround times while maintaining accuracy.

- **Goal 10: Reduce inequality within and among countries.**

Access to susceptibility testing technologies varies considerable between countries. This leads to inequality in access to accurate microbiological information, and consequently in the quality of health care. Linked with Goal 9, techniques to determine MICs in development not only need to be fast and precise, but also affordable. All countries should have access to basic antimicrobial information within a clinically relevant frame time.

The complete list of the Sustainable Development Goals indicators, agreed upon by the UN Statistical Commission in March 2016, is available on the UN website: https://unstats.un.org/sdgs/report/2016/Overview/

These indicators continue to be refined and improved as methods evolve and data became available.

Potential implications for decisions

1. ***Community policies***
    a. Access to clinical Microbiology Services. Ensure that all healthcare centres are either provided with or have their samples referred to a clinical microbiology laboratory with capacity of determining MICs within a clinically relevant timeframe.
2. ***National policies***
    a. Harmonization of breakpoints. Promote that all the centres in the country follow the same breakpoints, so that information can be shared and distributed with the same interpretation in the whole territory.

    National coordination. Establish a national coordinating body responsible for adapting breakpoints recommendations to the local setting, updating changes to healthcare institutions and laboratories

Pupil participation

Class discussion of clinical cases:

1. Tonsillitis caused by *Streptococcus pyogenes*. Treatment: penicillin (amoxicillin).

    In this case, we know that *S. pyogenes* very rarely develop resistance to penicillin, which are the antibiotics f most used to treat this infection. Therefore, no MIC determination is usually required, and the antibiotic can be given directly when the bacterial infection is suspected. However, there is a breakpoint because it is an antibiotic used in humans.

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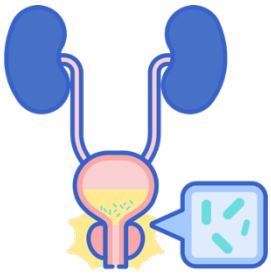
Streptococcus groups A, B, C and G
Expert Rules and Expected Phenotypes
For abbreviations and explanations of breakpoints, see the Notes sheet

This group of bacteria includes many species, which can be grouped as follows: **Group A:** *S. pyogenes* **Group B:** *S. agalactiae* **Group C:** *S. dysgalactiae* (plus the more rarely isolated *S. equi*) **Group G:** *S. dysgalactiae* and *S. canis* *S. dysgalactiae* includes the subspecies *equisimilis* and *dysgalactiae*, *S. equi* includes the subspecies *equi* and *zooepidemicus*.

Penicillins[1]	MIC breakpoints (mg/L)		
	S ≤	R >	ATU
Benzylpenicillin (indications other than meningitis)[2]	0.25	0.25	
Benzylpenicillin (meningitis)[2], *S. agalactiae* (group B streptococci)	0.125	0.125	

Source: EUCAST Clinical Breakpoint Tables v 14.0.

2. Urinary tract infection (UTI) caused by *Escherichia coli*. Treatment: fosfomycin.



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In this patient, the causative microorganism is *E. coli*, the most common agent of UTI. *E. coli* can develop resistance to Fosfomycin: however, in this case we are considering a region where more than 80% of *E. coli* are susceptible to fosfomycin. Therefore, no MIC determination in needed and the antibiotic treatment can be given directly.

Note: it may still be appropriate to determine the MIC under certain circumstances, such as: if we were in an area with higher resistance rates (we couldn't assume that the isolate is susceptible) or if there had been a previous failure with Fosfomycin treatment (we would suspect that the isolate could be resistant).

As with the previous example, a clinical breakpoint exists because this antibiotic is used in clinical practice.

Enterobacterales*
Expert Rules and Expected Phenotypes
For abbreviations and explanations of breakpoints, see the Note

Miscellaneous agents	MIC breakpoints (mg/L)		
	S ≤	R >	ATU
Chloramphenicol	Note[1]	Note[1]	
Colistin[2]	(2)[3]	(2)[3]	
Daptomycin	-	-	
Fosfomycin iv (infections originating from the urinary tract), *E. coli*	8[4]	8[4]	
Fosfomycin iv (other indications), *E. coli*	Note[5]	Note[5]	
Fosfomycin iv, other *Enterobacterales*	Note[6]	Note[6]	
Fosfomycin oral (uncomplicated UTI only), *E. coli*	8[4]	8[4]	

Source: EUCAST Clinical Breakpoint Tables v 14.0.

3. Wound infection caused by *Pseudomonas aeruginosa*.

Initial treatment option: meropenem

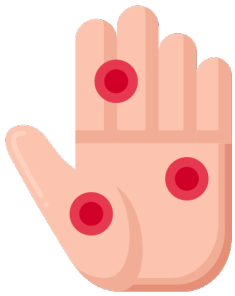
Alternative treatment: ceftazidime-avibactam.

In this example, it is necessary to treat a *P. aeruginosa* infection. MIC testing is necessary because 1) it is a serious infection and 2) we cannot t assume that the isolate would be susceptible to the first-line option: meropenem.

We obtain a MIC value of 32 for meropenem (it means that the concentration of antibiotic must be at least of 32 mg/L to inhibit the growth of this *P. aeruginosa*). Checking the breakpoint, we see that this isolate is resistant to meropenem (the value of the MIC, 32, is over 2 mg/L, the breakpoint). We cannot treat this *P. aeruginosa* infection with meropenem since it will probably lead to a therapeutic failure.

***Pseudomonas* spp.**
Expert Rules and Expected Phenotypes
For abbreviations and explanations of breakpoints, see the Notes sheet

Carbapenems	MIC breakpoints (mg/L)		
	S ≤	R >	ATU
Doripenem	0.001	2	
Ertapenem	-	-	
Imipenem	0.001	4	
Imipenem-relebactam, *P. aeruginosa*	2[1]	2[1]	
Meropenem (indications other than meningitis), *P. aeruginosa*	2	8	
Meropenem (indications other than meningitis), Pseudomonas other than *P. aeruginosa*	2	8	
Meropenem (meningitis), *P. aeruginosa*	2	2	
Meropenem-vaborbactam, *P. aeruginosa*	8[2]	8[2]	



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Source: EUCAST Clinical Breakpoint Tables v 14.0.

Then, we consider an alternative treatment: ceftazidime-avibactam. The MIC value calculated in the laboratory was found to be 0.125 mg/L . This means that the growth of this *P. aeruginosa*, is inhibited at a concentration below or equal to 0.125 mg/L. When compared with the clinical breakpoint, we see that this isolate is susceptible to ceftazidime-avibactam (the value of the MIC, 0.125, is below 8 mg/L, the breakpoint). Then, we can use ceftazidime-avibactam to treat this *P. aeruginosa* infection because it will probably lead to a therapeutic success.

***Pseudomonas* spp.**
Expert Rules and Expected Phenotypes
For abbreviations and explanations of breakpoints, see the Notes sheet

Cephalosporins	MIC breakpoints (mg/L)		
	S ≤	R >	ATU
Cefaclor	-	-	
Cefadroxil	-	-	
Cefalexin	-	-	
Cefazolin	-	-	
Cefepime	0.001	8	
Cefiderocol, *P. aeruginosa*	2[1]	2[1]	
Cefixime	-	-	
Cefotaxime	-	-	
Cefoxitin	-	-	
Cefpodoxime	-	-	
Ceftaroline	-	-	
Ceftazidime	0.001	8	
Ceftazidime-avibactam, *P. aeruginosa*	8[2]	8[2]	

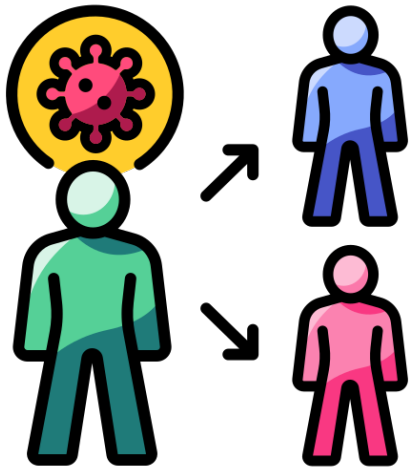
Source: EUCAST Clinical Breakpoint Tables v 14.0.

Further Reading

Clinical breakpoints resources (free access):

- https://www.eucast.org/clinical_breakpoints (in the top of the page you can find the latest version).
- https://em100.edaptivedocs.net/dashboard.aspx (once logged in with your email address, in "Document Updates" you can find the latest version and search within the document – usually M100).

Glossary



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antibiogram: information of antibiotic susceptibility data regarding one isolate, including different antibiotics that are commonly used to kill this type of strain, to inform which ones could be used and which ones should be avoided.

antibiotic: a drug used to treat infections caused by bacteria. Some of them are bactericidal (killing bacteria), and others are bacteriostatic (preventing the growth of bacteria).

bacteria: a group of unicellular prokaryotic microorganisms (without a nucleus, having their genetic material free in the cell). They were one of the first life forms to be present on Earth.

broth microdilution: method used to determine MICs consisting of plastic plates with twofold dilutions of multiple antibiotics, usually with 96 wells. They are filled with broth containing different concentrations of the antibiotic.

infection: a condition in which a pathogenic microorganism (capable of causing disease) begins to multiply in the body and may cause disease.

microorganism: an organism that can be observed only using a microscope. Microorganisms can be pathogenic (causing diseases), generally classified as bacteria, viruses, fungi, and some parasites (such as protozoa).

pathogen: a microorganism causing an infectious disease.

well: each one of the small compartments in a microdilution plate in which bacteria are exposed to a known concentration of an antibiotic. Bacterial growth or inhibition can be observed within each well.