# ATB susceptibility testing

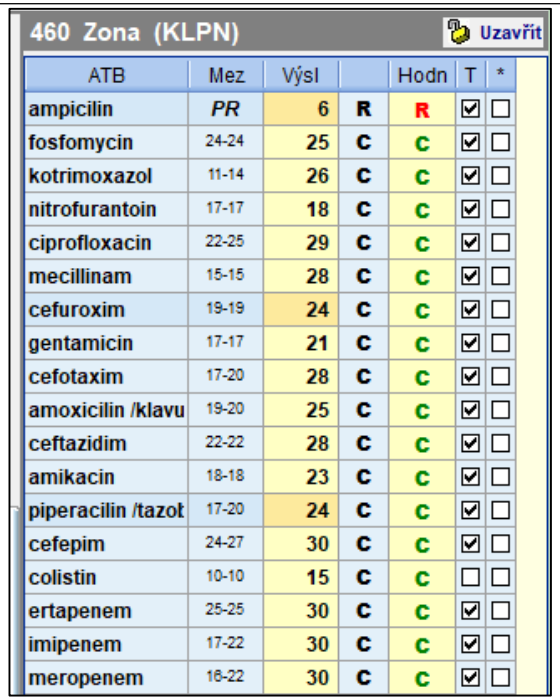
MUDr. Anežka Gryndlerová

# Content

- Brief summary of the lecture – ATBs
- Methods of ATB susceptibility testing
- Particular resistence phenotypes
- Practical tasks

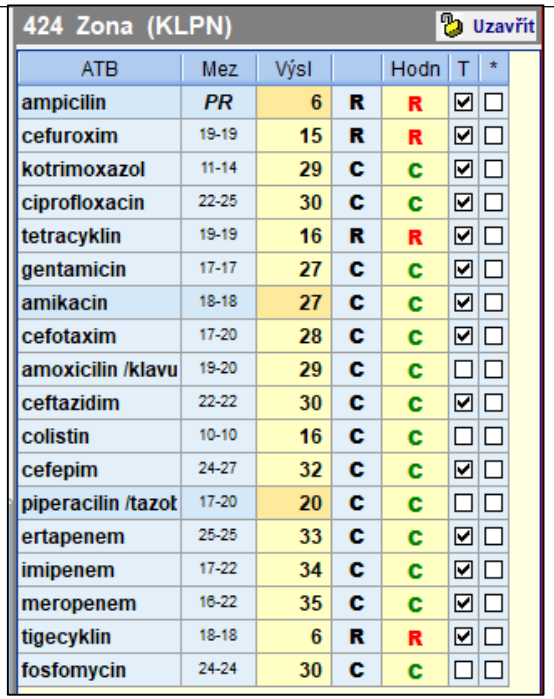
# Basic terminology

- Empirical x targeted ATB treatment
- Bactericidal X bacteriostatic ATBs
- Primary X secondary ATB resistance



460 Zona (KLPN) — Uzavřít

| ATB | Mez | Výsl | | Hodn | T | * |
|---|---|---|---|---|---|---|
| ampicilin | PR | 6 | R | R | ☑ | ☐ |
| fosfomycin | 24-24 | 25 | C | C | ☑ | ☐ |
| kotrimoxazol | 11-14 | 26 | C | C | ☑ | ☐ |
| nitrofurantoin | 17-17 | 18 | C | C | ☑ | ☐ |
| ciprofloxacin | 22-25 | 29 | C | C | ☑ | ☐ |
| mecillinam | 15-15 | 28 | C | C | ☑ | ☐ |
| cefuroxim | 19-19 | 24 | C | C | ☑ | ☐ |
| gentamicin | 17-17 | 21 | C | C | ☑ | ☐ |
| cefotaxim | 17-20 | 28 | C | C | ☑ | ☐ |
| amoxicilin /klavu | 19-20 | 25 | C | C | ☑ | ☐ |
| ceftazidim | 22-22 | 28 | C | C | ☑ | ☐ |
| amikacin | 18-18 | 23 | C | C | ☑ | ☐ |
| piperacilin /tazol | 17-20 | 24 | C | C | ☑ | ☐ |
| cefepim | 24-27 | 30 | C | C | ☑ | ☐ |
| colistin | 10-10 | 15 | C | C | ☐ | ☐ |
| ertapenem | 25-25 | 30 | C | C | ☑ | ☐ |
| imipenem | 17-22 | 30 | C | C | ☑ | ☐ |
| meropenem | 16-22 | 30 | C | C | ☑ | ☐ |



424 Zona (KLPN) — Uzavřít

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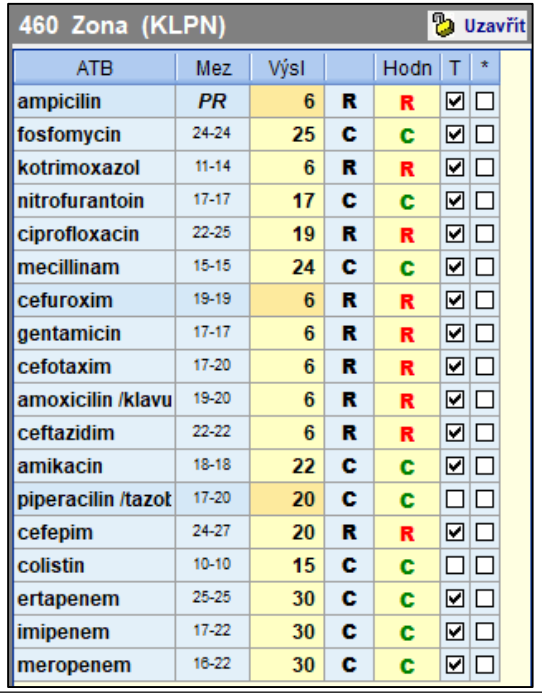
|---|---|---|---|---|---|---|
| ampicilin | PR | 6 | R | R | ☑ | ☐ |
| cefuroxim | 19-19 | 15 | R | R | ☑ | ☐ |
| kotrimoxazol | 11-14 | 29 | C | C | ☑ | ☐ |
| ciprofloxacin | 22-25 | 30 | C | C | ☑ | ☐ |
| tetracyklin | 19-19 | 16 | R | R | ☑ | ☐ |
| gentamicin | 17-17 | 27 | C | C | ☑ | ☐ |
| amikacin | 18-18 | 27 | C | C | ☑ | ☐ |
| cefotaxim | 17-20 | 28 | C | C | ☑ | ☐ |
| amoxicilin /klavu | 19-20 | 29 | C | C | ☐ | ☐ |
| ceftazidim | 22-22 | 30 | C | C | ☑ | ☐ |
| colistin | 10-10 | 16 | C | C | ☐ | ☐ |
| cefepim | 24-27 | 32 | C | C | ☑ | ☐ |
| piperacilin /tazol | 17-20 | 20 | C | C | ☐ | ☐ |
| ertapenem | 25-25 | 33 | C | C | ☑ | ☐ |
| imipenem | 17-22 | 34 | C | C | ☑ | ☐ |
| meropenem | 16-22 | 35 | C | C | ☑ | ☐ |
| tigecyklin | 18-18 | 6 | R | R | ☑ | ☐ |
| fosfomycin | 24-24 | 30 | C | C | ☐ | ☐ |



460 Zona (KLPN) — Uzavřít

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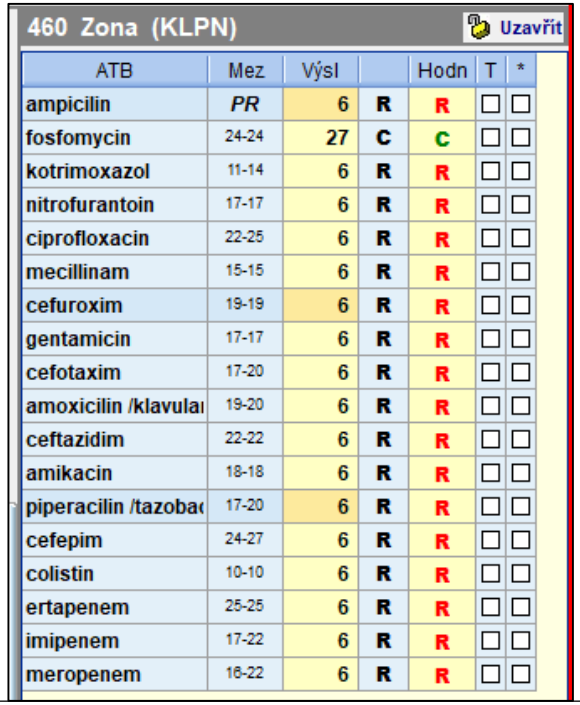
| fosfomycin | 24-24 | 25 | C | C | ☑ | ☐ |
| kotrimoxazol | 11-14 | 6 | R | R | ☑ | ☐ |
| nitrofurantoin | 17-17 | 17 | C | C | ☑ | ☐ |
| ciprofloxacin | 22-25 | 19 | R | R | ☑ | ☐ |
| mecillinam | 15-15 | 24 | C | C | ☑ | ☐ |
| cefuroxim | 19-19 | 6 | R | R | ☑ | ☐ |
| gentamicin | 17-17 | 6 | R | R | ☑ | ☐ |
| cefotaxim | 17-20 | 6 | R | R | ☑ | ☐ |
| amoxicilin /klavu | 19-20 | 6 | R | R | ☑ | ☐ |
| ceftazidim | 22-22 | 6 | R | R | ☑ | ☐ |
| amikacin | 18-18 | 22 | C | C | ☑ | ☐ |
| piperacilin /tazol | 17-20 | 20 | C | C | ☐ | ☐ |
| cefepim | 24-27 | 20 | R | R | ☑ | ☐ |
| colistin | 10-10 | 15 | C | C | ☐ | ☐ |
| ertapenem | 25-25 | 30 | C | C | ☑ | ☐ |
| imipenem | 17-22 | 30 | C | C | ☑ | ☐ |
| meropenem | 16-22 | 30 | C | C | ☑ | ☐ |



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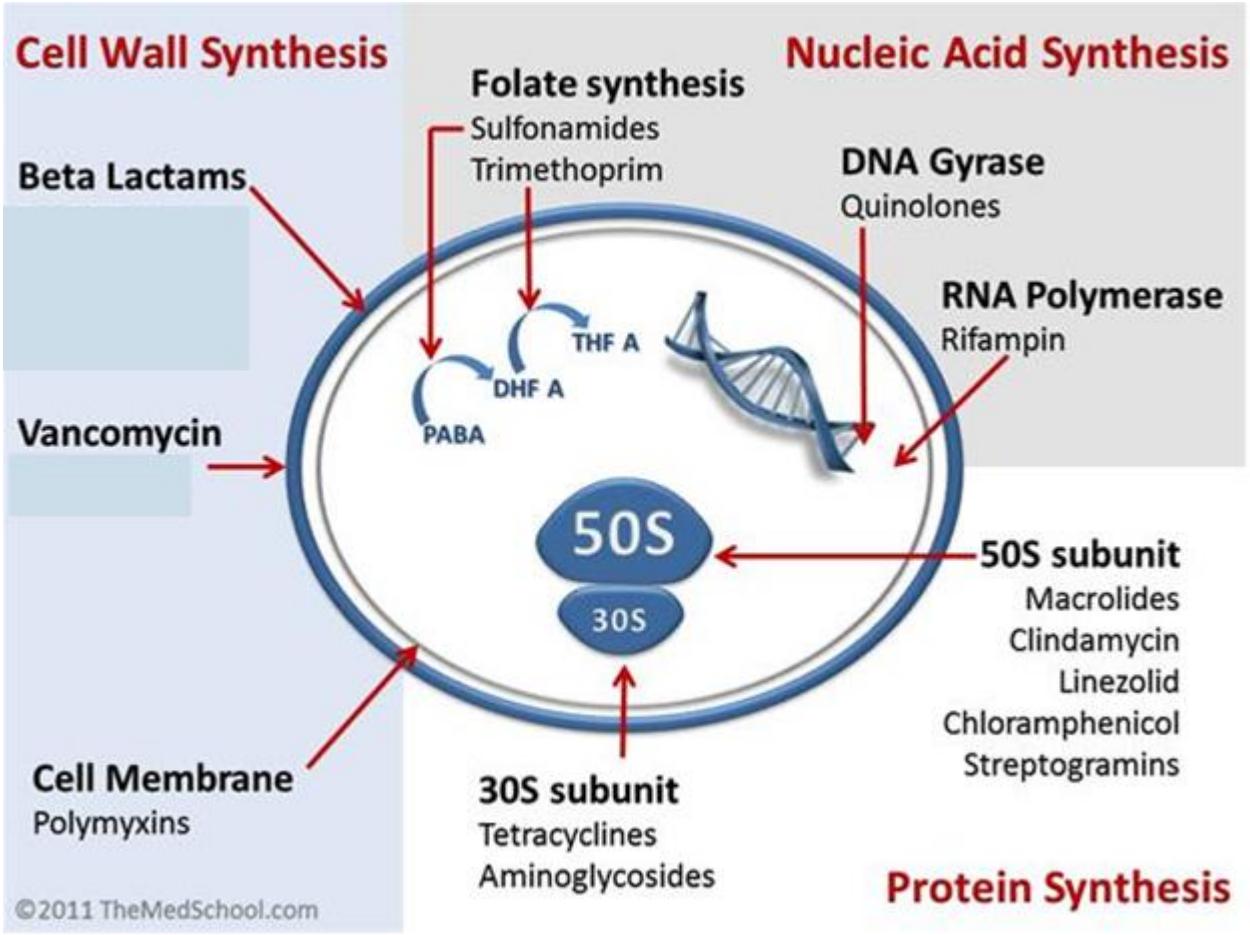
| fosfomycin | 24-24 | 27 | C | C | ☐ | ☐ |
| kotrimoxazol | 11-14 | 6 | R | R | ☐ | ☐ |
| nitrofurantoin | 17-17 | 6 | R | R | ☐ | ☐ |
| ciprofloxacin | 22-25 | 6 | R | R | ☐ | ☐ |
| mecillinam | 15-15 | 6 | R | R | ☐ | ☐ |
| cefuroxim | 19-19 | 6 | R | R | ☐ | ☐ |
| gentamicin | 17-17 | 6 | R | R | ☐ | ☐ |
| cefotaxim | 17-20 | 6 | R | R | ☐ | ☐ |
| amoxicilin /klavula | 19-20 | 6 | R | R | ☐ | ☐ |
| ceftazidim | 22-22 | 6 | R | R | ☐ | ☐ |
| amikacin | 18-18 | 6 | R | R | ☐ | ☐ |
| piperacilin /tazoba | 17-20 | 6 | R | R | ☐ | ☐ |
| cefepim | 24-27 | 6 | R | R | ☐ | ☐ |
| colistin | 10-10 | 6 | R | R | ☐ | ☐ |
| ertapenem | 25-25 | 6 | R | R | ☐ | ☐ |
| imipenem | 17-22 | 6 | R | R | ☐ | ☐ |
| meropenem | 16-22 | 6 | R | R | ☐ | ☐ |

# Classification – mechanisms of action



# Classification - beta-lactam ATBs

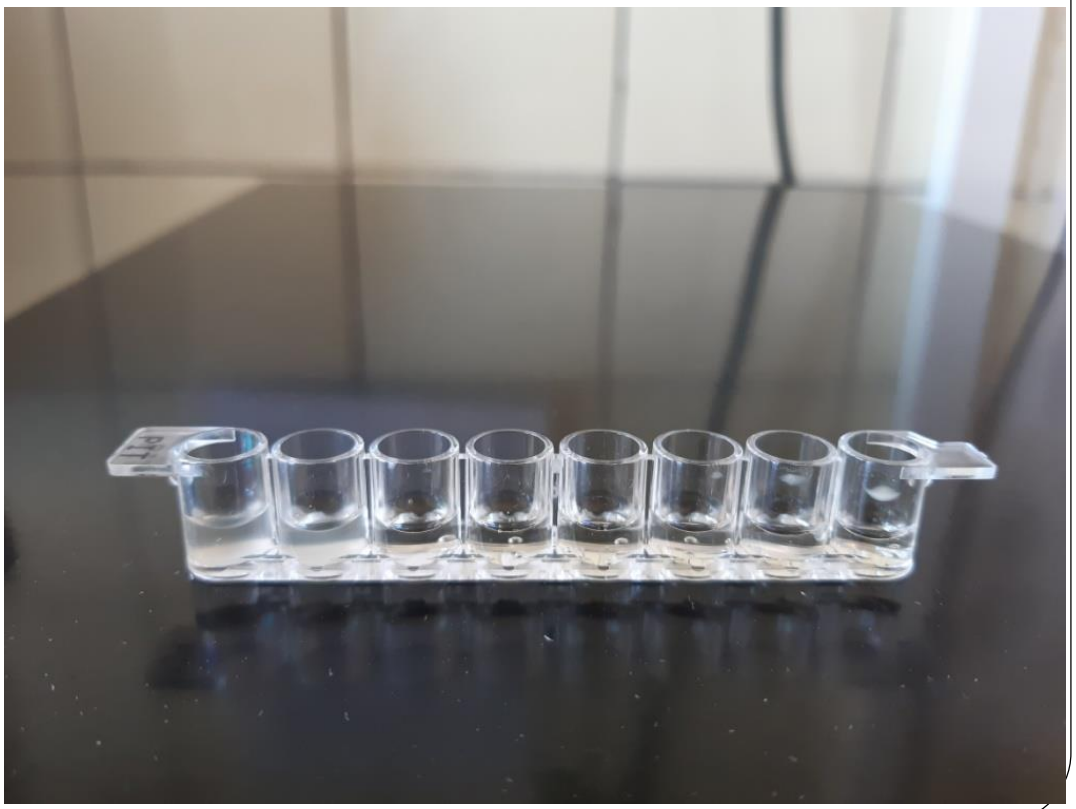
- Penicillins
- Cephalosporins - ?
  - 1.-5. gen.
- Carbapenems
- Monobactams

# ATB susceptibility testing methods

# ATB susceptibility testing

- Essential condition – grown culture
- Minimum inhibitory concentration (MIC)
- *Minimum bactericidal concentration (MBC)*

- mg/l
- Strict adherence to the required conditions!
- 24h incubation

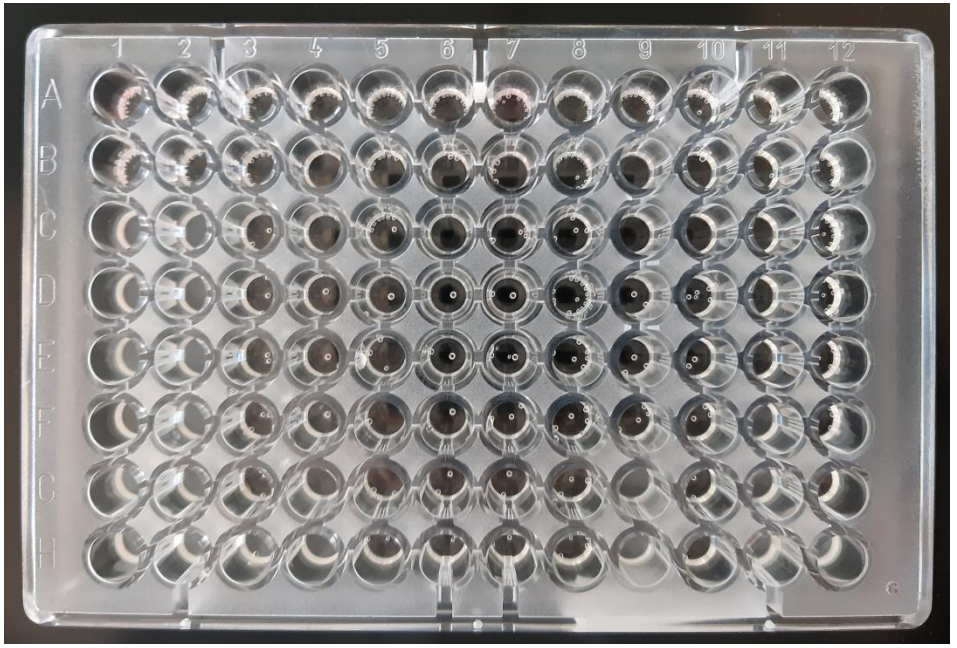


# MIC determination by broth microdilution method

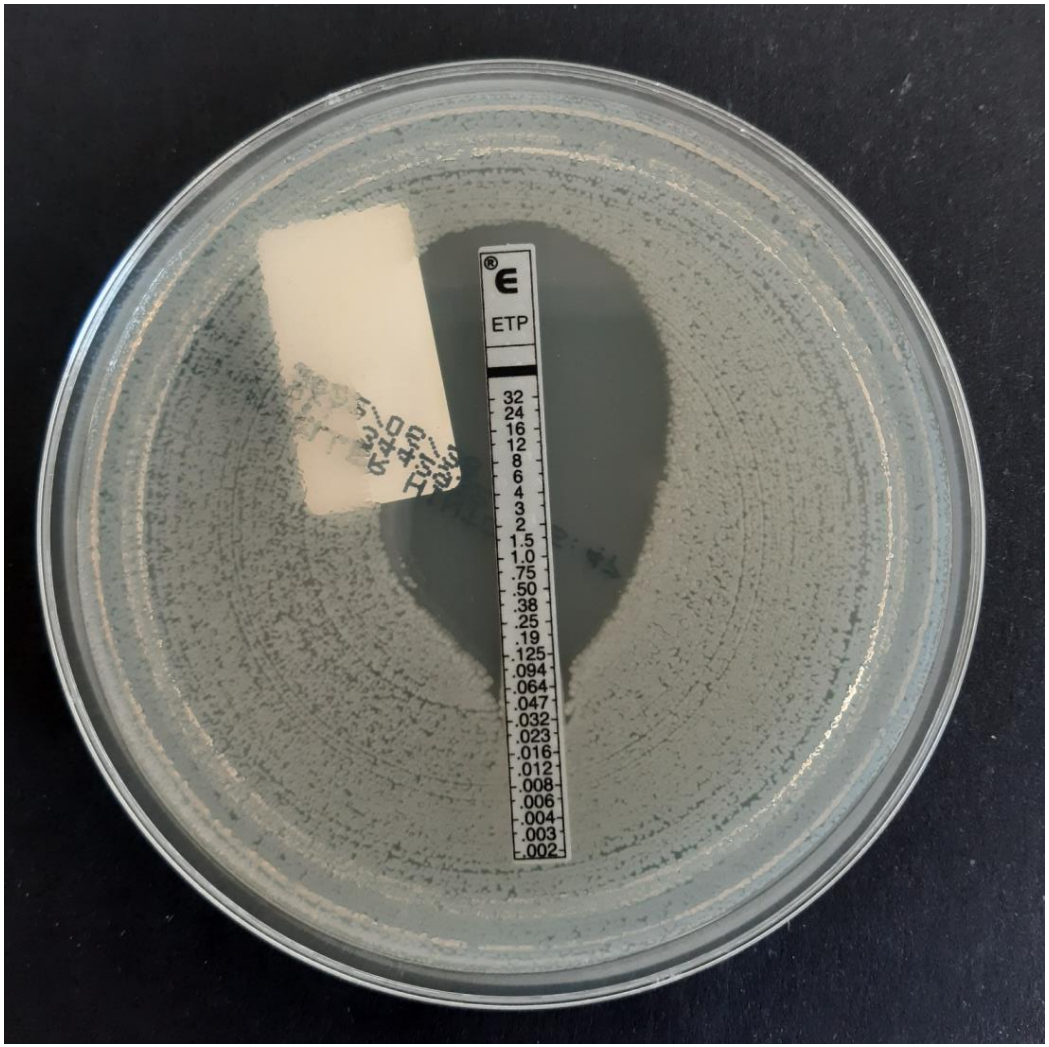
- Microtiter plates

| | 1 PEN | 2 COX | 3 ERY | 4 CLI | 5 LIZ | 6 CMP | 7 TET | 8 CIP | 9 T/S | 10 GEN | 11 VAN | 12 NFT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4 | 16 | 8 | 4 | 16 | 32 | 8 | 8 | 4/76 | 16 | 16 | 128 |
| B | 2 | 8 | 4 | 2 | 8 | 16 | 4 | 4 | 2/38 | 8 | 8 | 64 |
| C | 1 | 4 | 2 | 1 | 4 | 8 | 2 | 2 | 1/19 | 4 | 4 | 32 |
| D | 0,5 | 2 | 1 | 0,5 | 2 | 4 | 1 | 1 | 0,5/9,5 | 2 | 2 | 16 |
| E | 0,25 | 1 | 0,5 | 0,25 | 1 | 2 | 0,5 | 0,5 | 0,25/4,75 | 1 | 1 | 8 |
| F | 0,12 | 0,5 | 0,25 | 0,12 | 0,5 | 1 | 0,25 | 0,25 | 0,12/2,38 | 0,5 | 0,5 | 4 |
| G | 0,06 | 0,25 | 0,12 | 0,06 | 0,25 | 0,5 | 0,12 | 0,12 | 0,06/1,19 | 0,25 | 0,25 | 2 |
| H | 0,03 | 0,12 | 0,06 | 0,03 | 0,12 | 0,25 | 0,06 | 0,06 | 0,03/0,6 | 0,12 | 0,12 | K |

MIC values for Staphylococci

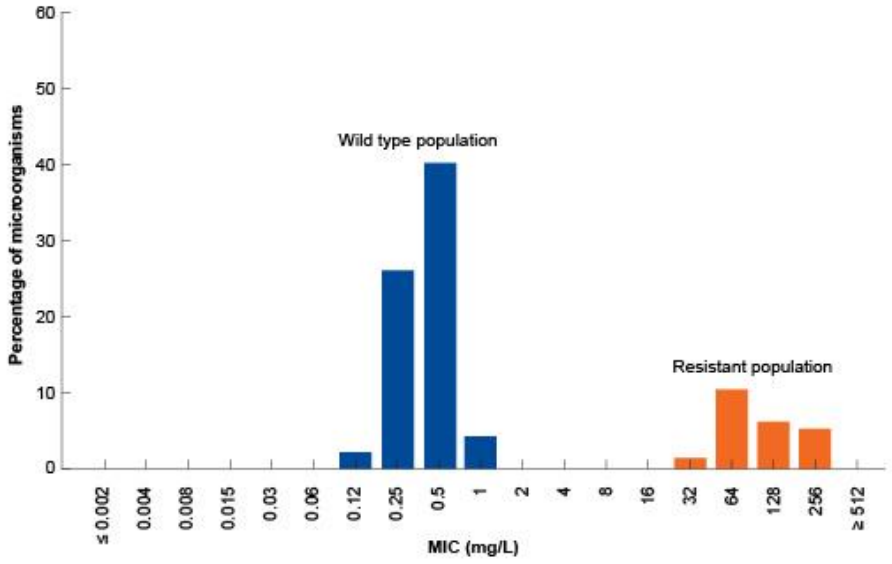


# MIC determination by E-test



- Interpretation of the measured value according to the breakpoint
  - Susceptible/susceptible - increased exposure/resistant strains
  - Clinical therapy effect

| Carbapenems[1] | MIC breakpoints (mg/L) | | |
|---|---|---|---|
| | S ≤ | R > | ATU |
| Doripenem | 1 | 2 | |
| Ertapenem | 0.5 | 0.5 | |
| Imipenem, *Enterobacterales* except *Morganellaceae* | 2 | 4 | |
| Imipenem[2], *Morganellaceae* | 0.001 | 4 | |
| Imipenem-relebactam, *Enterobacterales* except *Morganellaceae* | 2[3] | 2[3] | |
| Meropenem (indications other than meningitis) | 2 | 8 | |
| Meropenem (meningitis) | 2 | 2 | |
| Meropenem-vaborbactam | 8[4] | 8[4] | |




https://www.open.edu/openlearncreate/mod/oucontent/view.php?id=172394&section=2.2.1

# European Committee on Antimicrobial Susceptibility Testing

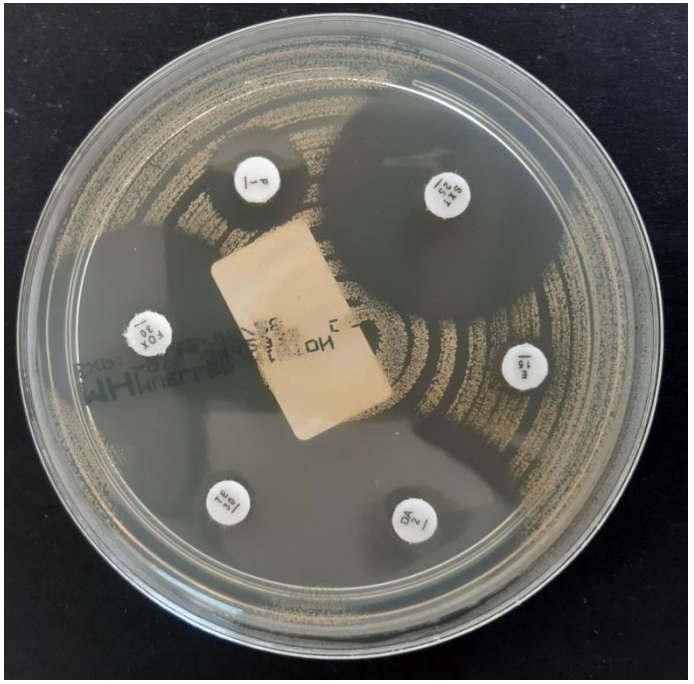
## Breakpoint tables for interpretation of MICs and zone diameters

Version 14.0, valid from 2024-01-01

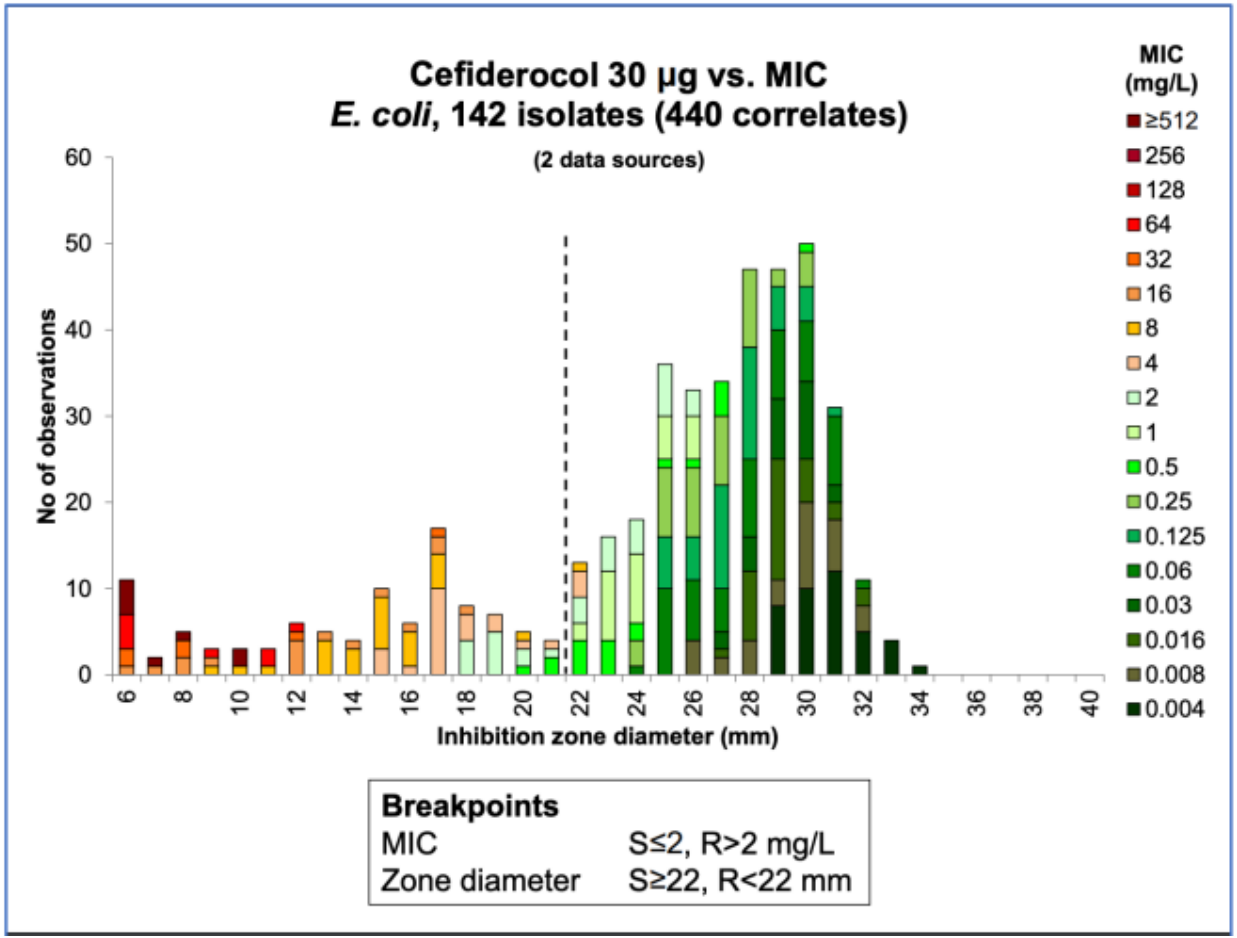
**This document should be cited as** "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, 2024. http://www.eucast.org."

# Disc diffusion method

- Mueller-Hinton agar
- Inhibition zone (mm) around ATB discs



| Carbapenems[1] | MIC breakpoints (mg/L) | | | Disk content (µg) | Zone diameter breakpoints (mm) | | |
|---|---|---|---|---|---|---|---|
| | S ≤ | R > | ATU | | S ≥ | R < | ATU |
| Doripenem | 1 | 2 | | 10 | 24 | 21 | |
| Ertapenem | 0.5 | 0.5 | | 10 | 23 | 23 | |
| Imipenem, *Enterobacterales* except *Morganellaceae* | 2 | 4 | | 10 | 22 | 19 | |
| Imipenem[2], *Morganellaceae* | 0.001 | 4 | | 10 | 50 | 19 | |
| Imipenem-relebactam, *Enterobacterales* except *Morganellaceae* | 2[3] | 2[3] | | 10-25 | 22 | 22 | 20-22 |
| Meropenem (indications other than meningitis) | 2 | 8 | | 10 | 22 | 16 | |
| Meropenem (meningitis) | 2 | 2 | | 10 | 22 | 22 | |
| Meropenem-vaborbactam | 8[4] | 8[4] | | 20-10 | 20 | 20 | 15-19[A] |



# ATB susceptibility testing - conclusion

- From grown culture
- Minimum inhibitory concentration (MIC)
  - Broth microdilution method
  - E-test strip
- Disc diffusion method

- Interpretation according to breakpoints (MIC and DD)
  - Susceptible/resistant strain (+ susceptible - increased exposure)
  - MIC value doesn´t correlate with dosage

- ATBs tested
  - According to the agent, to the infection site

# Particular resistance phenotypes

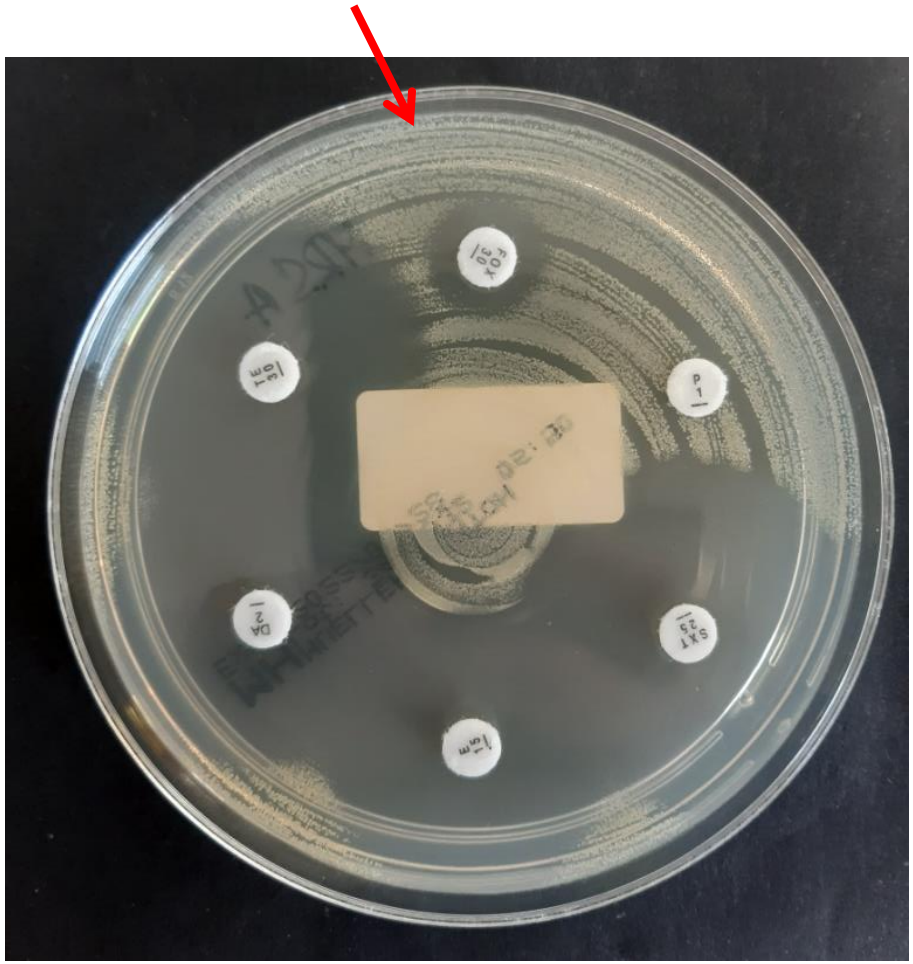
# Resistance mechanisms?

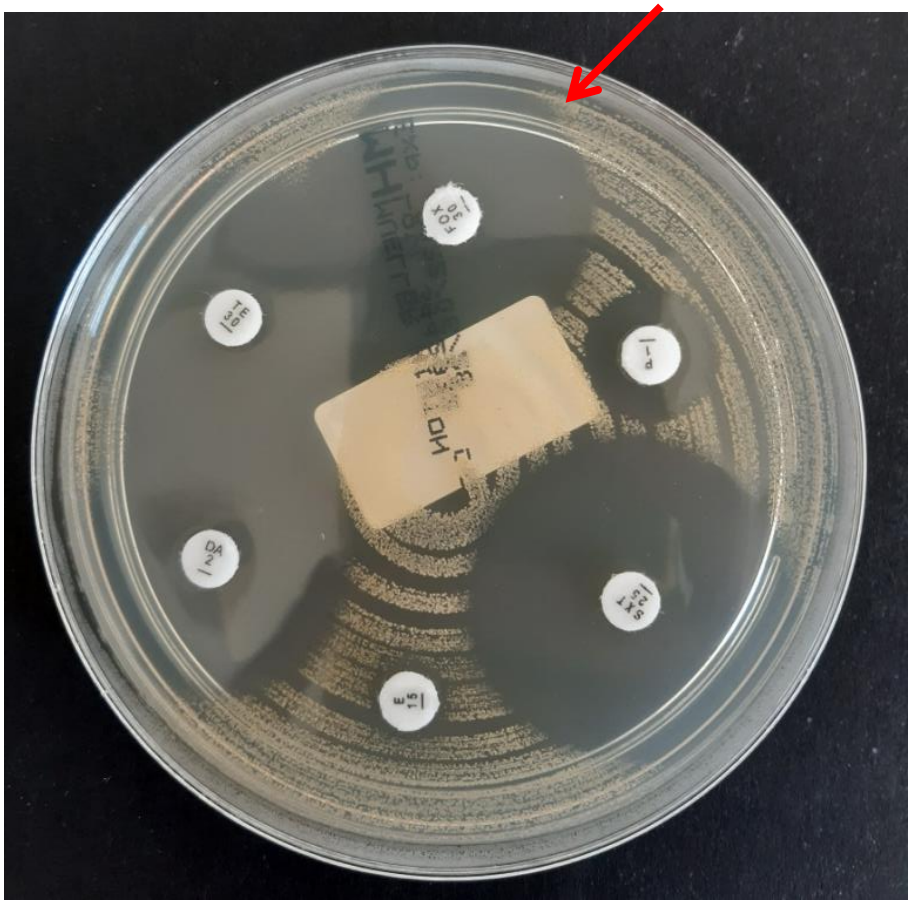
- Target molecule change
- Enzymatic degradation
- Efflux
- Cell entry restriction (porins)
- Alternative metabolic pathway

# Particular resistence phenotypes

- MRSA (methicillin resistant *Staphylococcus aureus*)

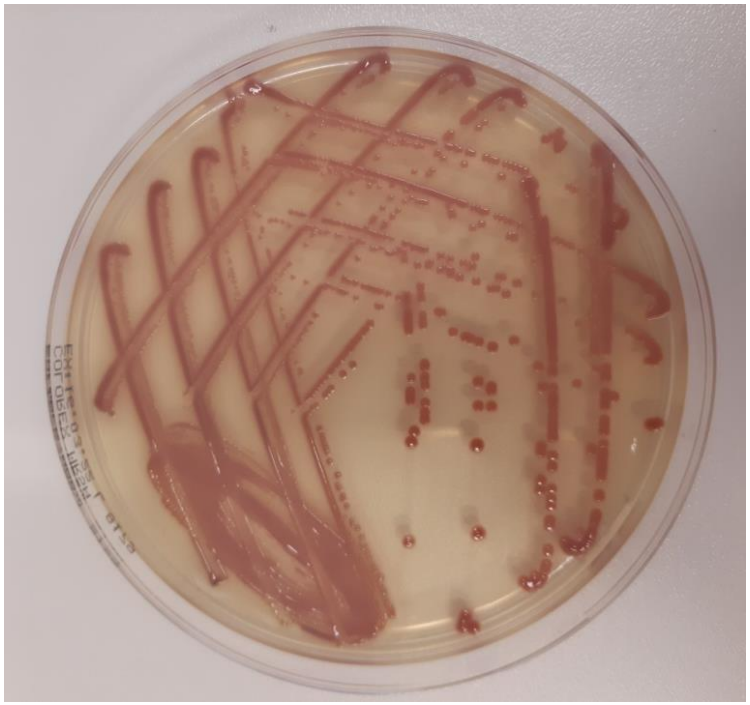
x Methicilin susc. SA





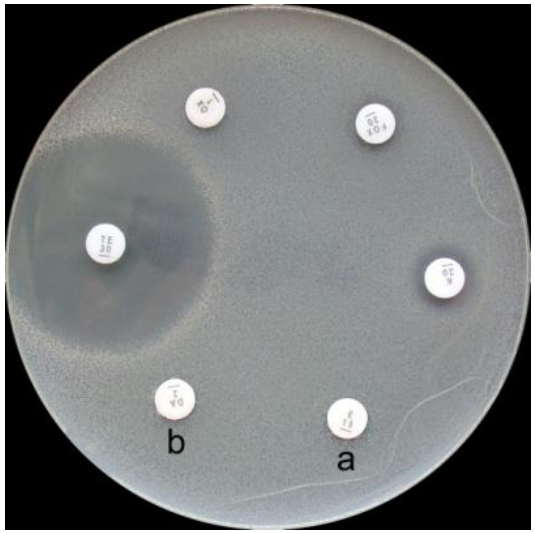
# Selective agar for MRSA

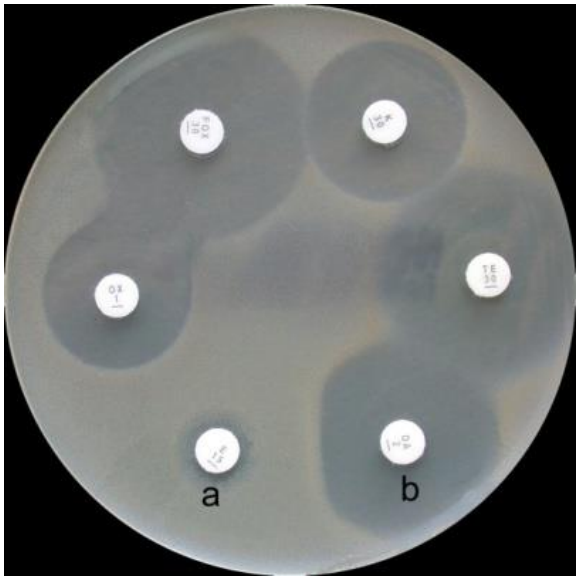
- Selective-diagnostic?

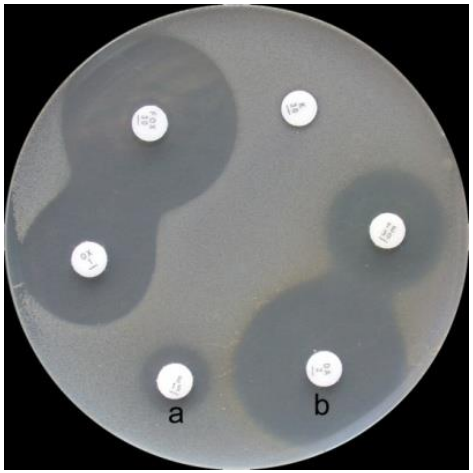


# Particular resistence phenotypes

- MLSB resistance (macrolides, lincosamides, …) – ribosome methylation
- 1 - Consitutive
- 2 - Inducible
- 3 – Other mechanism (efflux)




http://www.szu.cz/stafylokoky-indukovana-rezistence-ke-klindamycinu

# Beta-lactamases

| Type | Ambler Molecular Class | Characteristics | Examples of Enzymes |
|---|---|---|---|
| Narrow-spectrum β-lactamases[12,18,19] | A | Hydrolyze penicillin; produced primarily by *Enterobacteriaceae* | Staphylococcal penicillinase, TEM-1, TEM-2, SHV-1 |
| Extended-spectrum β-lactamases[20] | A | Hydrolyze narrow and extended-spectrum β-lactam antibiotics | SHV-2, CTX-M-15, PER-1, VEB- |
| Serine carbapenemases[20] | A | Hydrolyze carbapenems | KPC-1, IMI-1, SME-1 |
| Metallo-β-lactamases[21,22] | B | Hydrolyze carbapenems | VIM-1, IMP-1, NDM-1 |
| Cephalosporinases[10,23,24] | C | Hydrolyze cephamycins and some oxyimino β-lactams; inducible; chromosomally mediated | AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1 |
| OXA-type enzymes[25-27] | D | Hydrolyze oxacillin, oxyimino β-lactams, and carbapenems; produced by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* | OXA enzymes |

ESBL

https://www.researchgate.net/figure/b-Lactamases-Ambler-Classification_tbl1_267735062

- TEM-1

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| colistin | 10-10 | 15 | C | C | ☐ | ☐ |
| ertapenem | 25-25 | 30 | C | C | ☑ | ☐ |
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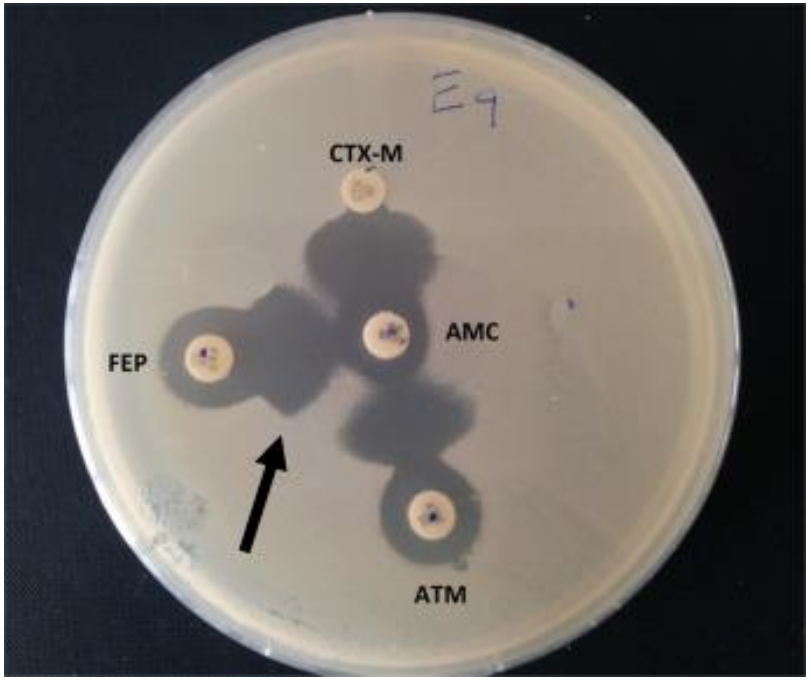
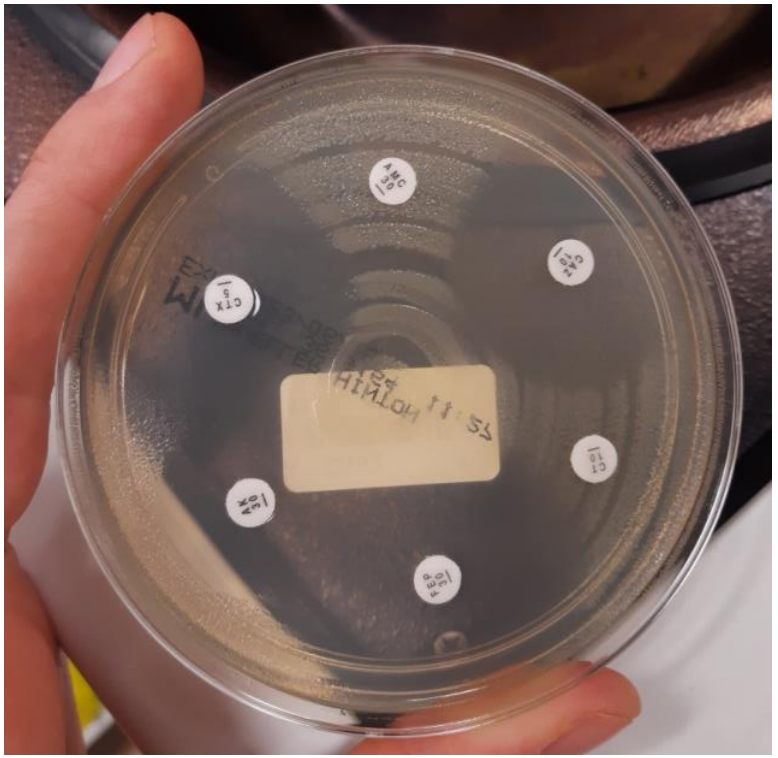
| meropenem | 16-22 | 30 | C | C | ☑ | ☐ |

## ESBL




Cefepime

## AmpC



Cefepime

https://www.researchgate.net/figure/b-Lactamases-Ambler-Classification_tbl1_267735062

# Carbapenemases

- imunochromatography
- MALDI-TOF


https://www.cureus.com/articles/140093-oxa-48-carbapenemase-producing-salmonella-typhimurium-nosocomial-bacteremia-in-the-intensive-care-unit-a-case-report-and-review-of-the-literature#!/

# Beta-lactamase inhibitors?

- Clavulanic acid (amoxicillin/clavulanate)
- Sulbactam (ampicillin/sulbactam)
- Tazobactam (piperacillin/tazobactam)

- + more – lecture

# Practical lesson

- 1. Disc diffusion method preparation
- 2. ATB susceptibility results interpretation

# Disc diffusion method

- https://www.youtube.com/watch?v=iRveNVZ-xxk&list=PLQU_kWRWBld4X9Acg59iNKlj4QwNRJShJ&index=2
- 1:37
- 2:37