Antimicrobial therapy
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Antibiotics are substances against bacteria

Other groups:
Antivirotics – against viruses
Antituberculotics - against mycobacteria
Antiparasitics – against parasites
Antibiotics are divided due to mechanism of efficacy into 4 groups:

1. Inhibition of cell wall synthesis (beta-lactamases, glycopeptides)
2. Cell membrane destruction (polypeptides)
3. Inhibition of NA synthesis (quinolones, imidazoles)
4. Inhibition of proteosynthesis (tetracyclines, chloramphenicol, macrolides, lincosamides, aminoglycosides)

**Attack against bacterial metabolism (sulfonamides)**
Betalactames

- Baktericidal, only for growing bacteria
- Often causes allergy

- Penicillins (PNC, oxacillin, ampicillin, piperacillin)
- Cefalosporines (1.- 4. generation)
- Monobactams (aztreonam)
- Carbapenems (imipenem, meropenem)
Glycopeptides

- Reserved for G+ bacteria
- Vancomycin and less toxic, but more expensive
teinoplanin

Polypeptides

- Ototoxic and nefrotoxic
- Polymyxin B only local as part of ear drops - Otosporin
- Polymyxin E – colistin rare used
- Primary resistance: all G+ bacteria, proteus,providencia, morganella, serratia etc.
Aminoglycosides

- Bactericidal, ototoxicity and nefrotoxicity
- Synergy with betalactames – decrease of toxicity
- Preparates: Streptomycin only against tuberculosis, gentamicin, netilmicin, amikacin, neomycin with bacitracin = framykoin (neomycin is too toxic, only for local using)
Tetracyklines

- Broad spectrum
- Don‘t use until 10 years (teeth development)
- Less used

Chloramphenicol

- Broad spectrum
- Good penetration to liquor, Hematotoxicity
Macrolides

I. generation: erythromycin, rare used

II. generation: roxithromycin

III. generation: clarithromycin, azithromycin – good intracellular penetration and longlasting effect, for G+ bacteria

Lincosamides

Lincomycin and clindamycin

Reserved for surgery, good effect to G+ bacteria and anaerobes in addition to Clostridium difficile – risc of pseudomembranous enterocolitis
Quinolones

- Bactericidal
- Don’t use until 15 years (growth cartilages)
- I. generation (oxolin acid), II. generation (norfloxacin) only for urinary infection
- III. generation: ofloxacin, ciprofloxacin – also for systemic infection – often used
Analogs of folate acid

- Sulfametoxazol in combination with trimetoprim form co-trimoxazol known as BISEPTOL
- Bacteriostatic, worse penetrate into tissues

  Nitrofurantoin (and nifuratel)

- Effectivity on sugar metabolism. Bacteriostatic, broad spectrum
- For urinary tract infection. Weighty undesirable effect: GIT disorder etc.

Other antibiotics

Linezolid (zyvoxid) – against resistant staphylococci
Nitroimidazols

- For anaerobes, for protozoas (T. vaginalis etc.)
- Metronidazol, Ornidazol

Antituberculotics

- HRZS, HRZE - starting therapy (INH, rifampicin, pyrazinamid, streptomycin, etambutol) + other
- HRZ, HRE – sequenced therapy
Antivirotics

- Against herpes – acyclovir...
- CMV – gancyclovir, foscarnet
- Influenza – amantadine, rimantadine, tamiflu
- Antiretrovirus therapy – inhibitors of reverse transcriptase (nucleosid+nonucleosid), inhibitors of protease – in combination

Preparates: zidovudin, didanosin ...
Antimycotics

- Fluconazol, itraconazol, ketoconazol etc. – local (vaginal, skin infection)
- Amphotericin B – i.v. (in sepsis)

Antiparasitics

- Against protozoa, helmintes, ectoparasites (moore in parasite capitol)

Other preparates

- Antimalarial: primachin, chlorochin, meflochin...
- Leprosity: dapson
Disc diffusion test

- MH agar is inoculated with suspension of bacteria
- Antibiotic discs (paper impregnated with antibiotics) are applied at MH – atb diffuse from disc through agar
- Concentration of atb decrease with distance from disc
- If microb grow to disc/if there is little zone - is resistant (not susceptible)
- Big zone (higher than defined size) means susceptibility.
The effect of therapy does not correspond with susceptibility testing in many cases.

Quantitative tests (MIC, E-tests) – in relevant patients

Qualitative tests (disc diffusion method) – enough for common cases (susceptible - resistant)
Disc diffusion test

MH medium inoculated with bacteria + via dispenser applied 6 papers impregnated with antibiotics

Cultivation + (37°C/18-24h)

ATB diffuses from paper

Interpretation:
High zone = susceptible bacteria (S)
Small/Any zone = resistant bacteria (R)
Microdilution test (MIC)

- MIC is the lowest concentration, which inhibits growth (first clear row)
- On paper stencil is assigned breakpoint. If MIC is lower than breakpoint, bacteria are susceptible. If MIC is higher, bacteria will be resistant.
- 1 plastic plate is used for 1 bacteria, for 12 antibiotics, in 8 various (decreasing) concentration (12th only in 7, because corner row upper right is growth control)
MIC – Material and methods

- **bacterial suspension (0.5-1 CFU)**
- **inoculation of plate with pipette**
- **microtiter plate with 96 rows**
- **cultivation (37°C/18-24h)**
- **reading**

1 row is a growth control
### Interpretation

<table>
<thead>
<tr>
<th>Break-point 4</th>
<th>Model šablony mikrodit. testu</th>
<th>Case - susceptible</th>
<th>Case - resistant</th>
</tr>
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<tbody>
<tr>
<td>64</td>
<td>Kmen jasně citlivý MIC je mnohem nižší než break-point</td>
<td>64 Kmen jasně rezistentní</td>
<td>32</td>
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</table>
### Interpretation of MIC - antibiogram – goes to clinician!

**PEN (penicillin)....4......resistant**

**AMS (unasyn)......2.....susceptible**
E-tests (quantitative)

- Similar to disc diffusion test, but strip is used.
- An increasing concentration of atb is used. Zone is egg like.
- There is a scale on strip – simply reading

MIC value is 0,75 mg/l (where borderline of zone cross the scale)
Resistance of microbes to antibiotics

- **Primary resistance:** all strains of bacteria are resistant.
- **Secondary resistance:** arises unsensitive mutants, by selective antibiotics pressure became dominant

* MBC (minimum bactericidal concentration) is the lowest concentration, which kills bacteria

**Primary bactericid:** atb, where MIC and MBC are almost equal

**Primary bacteriostatic:** atb, where MBC is X-fold higher than MIC - unreal baktericidal effect in human body
Resistance factors detection

Special detection methods for resistance factors (for ex. betalactamase). It can be diagnostic strips (chemical detection of specific ensym) or other tests (ESBL)

1. Betalactamase testing

In neisseria, *M. catarrhalis, H. influenzae*

destroys betalactams

For therapy we use ATB with inhibitors of betalactamase like clavulanate, sulbactam...
Detection of betalactamase

Petri dish with bacteria

Strain produces betalactamase

Paper with substrate + moisturing solution

Touch

Colour change (red)
2. ESBL (extended spectrum betalactamase)

*E. coli, K. pneumoniae* etc. produces ESBL, which destroys cheap betalactams. For therapy we use expensive carbapenems, aminoglycosides (toxicity), problem of ICU, big hospitals

ESBL – screening

- Inhibition of growth between discs – owing to a synergism of 2-3 antibiotics such as aztreonam, AMC, ceftriaxon
ESBL detection (CLSI)

4 discs: Cefotaxim (1) and ceftazidim (2), cefotaxim with clavulanate (3) and ceftazidim with clavulanate (4)

Difference between cefalosporines (1,2) and cefalosporines with clavulanate (3,4) is higher than 5mm

Compare 1 with 3 and 2 with 4
ESBL and AmpC detection set (A,B,C,D)

Susceptible strain

A cefpodoxime (CPD)
B CPD+ESBL inhibitor
C CPD+AmpC inhibitor
D CPD+both inhibitors

AmpC+

ESBL+,AMPc+

Interpretation: compare inhibition zones, if differences are higher than 5 mm