Antibiotics I

Chemistry of antibiotics and related drugs
(Mrinal K. Bhattacharjee, Springer)
Definition of antibiotics (ATB)

- ATB, chemicals that selectively inhibits a virulent (infectious) biological agent but causes minimal damage to the host
- **anti-infectives** alternative terms for antibiotics (also contain antiviral compounds)
- **antimicrobials**, which could be divided into three groups:
  - Antibiotics – kill or inhibit the microorganisms in the body
  - Antiseptics – are applied on living tissue to prevent infection
  - Disinfectants – kill or inhibit microorganisms non-living objects

**Sterilization** – killing microorganism in liquid media or on solid object by using chemicals such as oxidizing agents or alternatively using heat or irradiation

**Sanitizing agents** – means using disinfectants, antiseptics or sterilizing agent
History of antibiotics

Ancient history: many examples........, extracts, dried plants, roots, inorganic compounds, natural compounds, etc.
Greeks – extract form male fern – to treat worm infestation
Cinchona barks – quinine to treat malaria (Peru, Bolivia)
Ipecacuanha root – diarrhea (Brasil)
And many others......

Modern history:
18th century: observation by Robert Koch and Louis Pasteur – diseases can be caused by germs (Pasteur used harmless bacteria to cure diseases caused by harmful bacteria)
1863: Antoine Bechamp – Atoxyl (arsanilic acid)
1888:
1904: Paul Ehrlich – use of chemical to kill bacteria – dyes as antibacterial agents Trypan Red, Salvarsan
1932: Gerhard Domagk – discovery of sulphonamides – Prontosil
1940s: Ian Fleming - Penicilin
Ideal antibiotic

• Selectivity
• Water solubility
• Minimal side effects
• Stability
• Low cost
• Slow resistance development
Source of antibiotics

- Majority of antibiotics used today is produced by microorganisms (bacteria, fungi)
- Chemically synthetized
- Natural sources (minimum)

Soil organisms – the best place to search for new antibiotics today (soil is a very complex ecosystem in which inhabitants developed chemical defences against each other as a response to competition for nutrients – chemical war among bacteria, and fungi)
Discovery of modern antibiotics

- 1920 – discovery of lysozyme (Fleming), „A thick milky suspension of bacteria can be quickly cleared in few seconds by the addition of a drop of human tears of egg white“
- 1928 – discovery of penicillin (Fleming), 1945 Nobel price lecture held by Fleming „My only merit is I did not neglect the observation and that I pursued the subject as bacteriologist“, *Penicillium mold*
- 1939 – discovery of gramicidin (Rene Dubos), *Bacilus brevis*
- 1940 – Howard Florey and Ernst Chain – method for purification of penicillin (Oxford)
- 1943 – discovery of streptomycin (Selman Waksman, Albert Shatz), *Streptomyces griseus*
- 1944 – Merck company, penicillin for all….
- 1947 – chloramphenicol (Paul Burkholder, Yale), 1st broad spectrum antibiotic *Streptomyces venezuela*
- Chlorotetracyclin, 2nd broad spectrum antibiotic, *Streptomyces aurofaciens*
Discovery of antibiotics

A Review on Antibiotic Resistance: Alarm Bells are Ringing
doi: 10.7759/cureus.1403
Gram positive and negative bacteria

• Difference in cell wall composition
Gram test

Crystal violet, Lugol solution, safranin

**G+ coccus:** Staphylococcus, Streptococcus, Enterococcus;

**G+ bacillus:** Corynebacterium, Clostridium, Listeria, Bacillus.

**G– coccus:** Neisseria;

**G– coccobacillus:** Haemophilus influenzae, Bordetella pertussis, Legionella, Brucella, atd.

**G– bacillus:** Klebsiella, E. coli, Enterobacter, Citrobacter, Serratia, Vibrio, Pseudomonas, Proteus, Helicobacter pylori, Yersinia, Campylobacter, Salmonella

https://www.wikiskripta.eu/w/Gramov%C3%A9_barven%C3%A9

https://www.wikiskripta.eu/w/Gramov%C3%A9_barven%C3%A9
Bacterial resistance
Development of resistance to antibiotics

• Detection of resistance – broth or agar dilution method, MIC (minimum inhibitory concentration)

• ATB resistance: *intrinsic* (natural, all bacteria is resistant, bacteria without prior exposure to ATB) and *acquired* (only a subpopulation is resistant, acquired resistance – by point mutation or by resistance gene acquisition)
Point mutation

• Natural methods: replication errors, common bacteria replication time is 20 minutes which means that every 20 min. the number of bacteria will double.

• One bacteria, in 10 hours, will double 30x, .......2^{30} ca. one bilion of bacteria

• Induced methods: harsh environmental conditions, UV, oxidation agents, alkylation agents

• Effect of point mutation – change in protein sequence
Gene Acquisition

- E.g. Beta-lactamases
- **Plasmids** – are small (up to a 1000x smaller than the chromosome) piece of extrachromosomal DNA, usually circular
  - Can contain **more than one resistance genes**
  - Does not contain any usefull function for the cells and may be lost in daughter cell during replication, the daughter cell who do not receive the copy is killed by ATBs – **selection**
- **Plasmid maintenance system** – specific genes – each daughter cell always receive a copy of resistance gene
Gene Acquisition

- **Transposons** (or **insertion sequence (IS) elements**) – small pieces of DNA that can insert into the chromosome (randomly or specifically, jumping genes). Can also be excised and inserted somewhere else.

- Requirements: IS sequence contains direct or inverted repeat sequence at the two ends. And IS sequence is preceded or followed by the sequence for Transponase enzyme.

- Transposon sequence can have 1) **gene for ATB resistance** or 2) can be inserted into the **gene which is responsible for proper functioning of an ATB**
Gene Acquisition

- **Integrons**: similar to transposons – mobile genetic element with possibility of multiple ATB resistance. They do not have repeated sequence at the two ends and they contain an integrase enzyme sequence needed for insertion process.

- **Transfer of resistance gene between bacteria:**
  1. **bacterial conjugation** – conjugative plasmids – capable of being transferred, and mobilizable plasmids – contain some, not all information for conjugation – can be transferred only with conjugative plasmid.
  2. **bacterial transformation** – bacteria takes DNA from outside, usually released from dead bacteria.
  3. **bacterial transduction/transfection** – DNA is transferred via bacteriophage.
ATB resistance pool

- Taking insufficient dose
- Not completing the full ATB course
- Taking wrong ATBs, or using it against viral infections
- Other misuse: ATBs in animals – therapeutic and subtherapeutic used
- Mechanisms of antimicrobial resistance: 1) altering the target of the ATB (ATB no longer works), 2) decreasing the concentration of ATB to the level lower than MIC – a) preventing entry, b) pumping out the ATB after it enters the cell, c) degrading ATB

<table>
<thead>
<tr>
<th>ATB</th>
<th>Year introduced</th>
<th>Year resistance reported</th>
<th>Years taken for resistance development</th>
</tr>
</thead>
<tbody>
<tr>
<td>penicillin</td>
<td>1943</td>
<td>1940</td>
<td>-3</td>
</tr>
<tr>
<td>tetracycline</td>
<td>1950</td>
<td>1959</td>
<td>9</td>
</tr>
<tr>
<td>methicillin</td>
<td>1960</td>
<td>1962</td>
<td>2</td>
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<tr>
<td>vancomycin</td>
<td>1972</td>
<td>1988</td>
<td>16</td>
</tr>
<tr>
<td>levofloxacin</td>
<td>1996</td>
<td>1996</td>
<td>0</td>
</tr>
</tbody>
</table>
Multidrug resistant (MDR) microorganisms

• Microorganism which are resistant to at least 3 of the 4 antibiotic classes – ATBs which affect cell membrane, cell wall, nucleic acid synthesis, and protein synthesis.

• Great concern because most of the ATBs does not work against them.

• ESKAPE group of bacteria: *Eterococcus faecium* (vancomycin resistant), *Staphylococcus aureus* (methicilin or vancomycin resistant), *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.* (carbapenem resistant).
Antibiotics
ATBs that inhibit cell wall synthesis

• Cell wall composition: mostly peptidoglycans („carbohydrate polymers (glycan) with some peptides linked to it“)
  glycan consist: N-acetylglucosamine (NAG), N-acetylmuramic acids (NAM) linked by β(1→4) glycosidic bond
Bacterial cell wall – peptidoglycan cross-linking
Beta lactams

- Peptidoglycan biosynthesis – transglycosidases (cross linking the glycan strands with sugar bound peptide chain)
- Beta lactam mimics D-alanyl alanine, interaction with penicillin binding protein
Beta-lactams
Avoiding beta-lactamase resistance

- Beta-lactamase inhibitors

![Chemical structures of clavulanic acid, sulbactam, tazobactam, and avibactam]
Glycopeptides

- Glycopeptides bind to D-alanyl alanine part of peptidoglycan subunit
- Vancomycin

Peripheral modifications of $[\Psi[CH2NH]Tpg4]vancomycin$ with added synergistic mechanisms of action provide durable and potent antibiotics

www.pnas.org/cgi/doi/10.1073/pnas.1704125114
Inhibitors of protein biosynthesis

• DNA → RNA (mRNA) transcription
• Protein synthesis on ribosome
• Bacterial S70 ribosome: 2 subunits, S30 and S50
Inhibitors of S30 subunits

- **Aminoglycosides**
- Positively charged molecules (low cell penetration, active transport, oxygene and H\(^+\) force)
- Synergism with beta-lactams and glycopeptides

Inhibitors of S30 subunits

- **Tetracyclines** (tetracycline, chlortetracycline, doxycycline, minocycline)
- Inhibitions of t-RNA binding to A site of S30
Tetracycline binding on ribosome
Inhibitors of S50 subunits

- **Chloramphenicol**
- Inhibitions of t-RNA binding to A site of S50 (peptidyl transferase cavity of the 23S r-RNA)
- used to treat meningitis, plague, and cholera
- Isolated from *Streptomyces venezuelae* in 1947


Inhibitors of S50 subunits

- **Macrolides**
- Inhibit protein translocation (peptidyl transferase center of the 23S r-RNA of the 50S ribosomal subunit)

A platform for the discovery of new macrolide antibiotics, nature.com, doi:10.1038/nature17967
Novel macrolides

A platform for the discovery of new macrolide antibiotics, nature.com, doi:10.1038/nature17967
Inhibitors of S50 subunits

- **Oxazolidinones** (Linezolid)

- 1) Inhibition of 23Sr RNA of the 50S subunit and 2) suppression of 70S inhibition and interact with peptidyl-t-RNA

Post linezolid oxazolidinones

Inhibitors of DNA replication

- **Quinolones**
- Inhibition of bacterial gyrase (nicking of double stranded DNA – by formation of negative supercoils, and resealing the DNA ends) – separation of DNA strands to permit replication or transcription.
- Nadixilic acid isolated during the synthesis of chloroquine

Chinolone antibiotics generation I-IV.

![Chemical structures of Nalidixic Acid, Ciprofloxacin, Moxifloxacin, and Trovafloxacin](image)

*Brazilian Journal of Pharmaceutical Sciences vol. 47, n. 4, oct./dec., 2011*
Folic acid metabolism inhibitors

- **Sulfonamides and trimetoprim**
- Inhibition of folate synthesis

**Trimethoprim**

https://en.wikipedia.org/wiki/Trimethoprim

Pharmacy 2019, 7, 132; doi:10.3390/pharmacy7030132
Nature derived antibiotics (produced by microorganisms)
Most ATBs are derived from nature

Actinomycetes
- Lincomamides
- Chloramphenicol
- Lipoglycopeptides
- Carbapenems
- Lipopeptides
- Macrolides
- Ansamycins
- Aminoglycosides
- Streptogramins
- Tubercactinomycins
- Glycopeptides
- Tetracyclines

Other bacteria
- Mupirocin
- Monobactams
- Polypeptides
- Polymyxins

Fungal
- Penicillins
- Cephalosporins
- Pleuromutilins
- Fusidic acid

Synthetic
- Azoles*
- Nitrofurans
- Oxazolidinones
- Quinolones
- Sulfonamides
Clinically used ATBs (from actinomycetes)

- Aminoglycosides – Kanamycin A (*Streptomyces kanamyceticus*)
- Tetracyclines – Tetracycline (*Streptomyces aureofaciens*)
- Amphenicols – Chloramphenicol (*Streptomyces venezuelae*)
- Macrolides – Erythromycin (*Saccharopolyspora erythraea*)
- Glycopeptides – Vancomycin (*Amycolatopsis oirientalis*)
- Cycloserines – Seromycin (*Streptomyces orchidaceus*)
- Streptogramins – Pristinamycin (*Streptomyces pristinaespiralis*)
- Lipopetides – Daptomycin (*Streptomyces roseosporus*)
Clinically used ATBs (from other bacteria)

• Polypeptides – Gramicidin A (*Bacillus brevis*)
• Bacitracin – Bacitracin A (*Bacillus subtilis*)
• Polymyxins – Colistin (*Paenibacillus polymyxa*)
• Mupirocin – Mupirocin (*Pseudomonas fluorescens*)
• Monobactams – Aztreonam (semi synthetic, *Chromobacterium violaceum*)
Clinically used ATBs (from fungi)

• Penicillins – Amoxicillin (semi synthetic, *Penicillinum chrysogenum*)
• Fusidic acid – (*Fusidium coccienum*)
• Enniatins – Fusafungine (*Fusarium lateritium*)
• Cefalosporins – Cefacebrile (*Acremonium chrysogenum*, semi syntetic)
• Pleuromutilins – Retapamulin (*Pleuritus mutilius*, semi syntetic)
2018 approvals

**Omadacycline**, brand name **Nuzyra**, is a broad spectrum antibiotic medication belonging to the aminomethylcycline subclass of tetracycline antibiotics. ([bacterial pneumonia](https://en.wikipedia.org/wiki/Bacterial_pneumonia), acute skin infections)

https://en.wikipedia.org/wiki/Omadacycline

**Tafenoquine**, sold under the brand name **Krintafel**, is a medication used to prevent and to treat **malaria**. It may be used to prevent all types of malaria. Oral administration.

https://en.wikipedia.org/wiki/Tafenoquine

2019 approvals

[Chemical structures and details for each drug]

https://drughunter.com/resource/2019newdrugs/
2020 approvals

**Fostemsavir (Rukobia)**
- Oral HIV-1 gp120 attachment inhibitor
- 600 mg BID
- Decline in HIV-1 RNA from d1–8 vs. placebo
- NCT02362503/BRIGHTEN (371 pts)
- Infectious Disease – HIV

**Remdesivir (Veklury)**
- IV nucleotide RNA polymerase inhibitor
- 200 mg loading dose, 100 mg QD
- Time to recovery, d11/d14
- Clinical status category
  - 04280705, 04292899/2730 (1062, 397, 584)
- Infectious Disease – COVID-19
2021 approvals

- **cabotegravir + rilpivirine (Cabenuva)**
  - HIV1 integrase strand transfer inhibitor (INSTI)
  - HIV1 infection
  - IM: 400 + 600 mg Q1M*

- **maribavir (Livtency)**
  - CMV pUL97 kinase inhibitor
  - post–transplant CMV infection
  - oral: 400 mg BID

- **ibrexafungerp (Brexafemme)**
  - β-1,3-glucan synthesis inhibitor
  - vulvovaginal candidiasis
  - oral: 300 mg BID

- **fexinidazole (Fixinidazole)**
  - nitroimidazole antimicrobial
  - African trypanosomiasis
  - oral: up to 1200 mg QD
2022 approvals

- **Sunlenca**, HIV-1 capsid inhibitor (lenacapavir) for multidrug resistant HIV-1 infection
  
  - 927 mg SC Q6M after loading period
  
  - *Gilead*  

- **Vivjoa**, antifungal (otezoline) (CYP51 inhibitor)
  
  - for RVVC in non-reproductive females
  
  - 600-150 mg oral 11 week regimen
  
  - *Mycovia*  

- **Voquezna**, K+/H+-ATPase potassium channel blocker
  
  - (vonoprazan, amoxicillin, and clarithromycin) for H. pylori infection
  
  - 20 mg BID for 14 days in combo
  
  - *Takeda*