Chloramphenicol
Chloramphenicol

- Chloramphenicol is a broad spectrum antibiotic isolated from *Salmonella venezuelae* (found in Venezuela) by Ehrlich in 1947.
- It has similar activity to tetracyclines except it has lower activity on Gram +ve bacteria.
- It contains chlorine and isolated from actinomycete (filamentous bacteria) thus named chloromycetin.
- Usually used for serous infections such as *H. influenza*, *S. typhi*, *S. pneumonia* and *N. meningitis*.
- It can penetrate into CNS and used for meningitis.
Chloramphenicol - Chemistry

• It is white-greyish-yellow crystalline powder.
• Slightly soluble in water, soluble in alcohol and propylene glycol.
• Due to its simple structure, it was the first antibiotic to be easily produced synthetically.
• The total synthesis produces a mixture of 4 diastereomers, thus the other inactive isomers need to be removed.
• It contains nitrobenzene moiety and is derived from dichloroacetic acid.
• It has 2 chiral centers and 4 possible isomers. The R,R-isomer is the biologically active isomer.
Chloramphenicol is very stable as solid but undergoes slow hydrolysis in solution. The rates of these reactions depend on pH, heat, and light. Hydrolytic reactions include:

1. General acid–base-catalyzed hydrolysis of the amide to give $1-(p$-nitrophenyl)$-2$-aminopropan-$1,3$-diol and dichloroacetic acid.

2. Alkaline hydrolysis (above pH 7) of the -chloro groups to form the corresponding,-dihydroxy derivative.
• It is bacteriostatic and binds to 50S subunit of ribosome and inhibit the movement of ribosome along mRNA (probably by inhibiting peptidyl transferase reaction).

• Chloramphenicol may bind at ribosome at similar sites of macrolides, thus the two cannot be used as combination.
SAR of p-nitrophenyl group

1. The replacement of nitro group by other electron withdrawing groups gives active compounds as CH$_3$SO$_2^-$ (Thiamphenicol) or CH$_3$C(=O)- (Cetophenicol)

2. Replacement of p-nitrophenyl by other aryl structures $\rightarrow$ active compounds

3. Shifting nitro from para position to other positions $\rightarrow$ reduces the activity

4. Replacement of phenyl group by alicyclic (saturated rings) $\rightarrow$ reduces the activity

Thiamphenicol
1.7x more active than chloramphenicol
Low incidence of aplastic anemia

Chloramphenicol
SAR for Chloramphenicol (Cont.)

SAR of dichloracetamide side chain
1. Other dihaloderivatives of the side chain → slightly less active
2. Trihaloderivatives (e.g. NHCOCF₃) → slightly more active
3. The dichloracetamide group can be replaced with other electronegative groups → no appreciable loss of activity
SAR of 1,3-propanediol side chain

1. The primary alcohol at C1 and C2 is important for activity
2. Conversion of C1 and C2-OH to C=O causes loss of activity

Chloramphenicol
large number of structural analogues of chloramphenicol have been prepared on the basis of the following themes:

1. removal of the chlorine atom, transference of chlorine atom to the aromatic nucleus
2. transference of the nitro moiety to the ortho- or meta-position,
3. esterification of the hydroxyl function(s),
4. replacement of the phenyl ring with furyl, naphthyl and xenyl rings respectively,
5. addition of alkyl or alkoxy substituents to the aryl ring
6. replacement of the inherent nitro group by a halogen atom.

However, none of these provide analogue of activity even similar to chloramphenicol towards *Shigella paradysenteriae*
Bacterial resistance

- Reduction of nitro group
- Hydrolysis of amide linkage by acetyltransferase
- Acetylation of secondary and to some extent the primary hydroxyl groups by chloramphenicol acetyltransferase. Both 3-acetoxy and 1,3-diacetoxy metabolites no longer bind to the ribosome.
Metabolism

- Rapidly and completely absorption after oral administration
- It has short half-life due to metabolism to inactive products by:
  1. O-glucuronidation at C1 and C3
  2. and to lesser extent by:
     - Reduction of the $p$-nitro group to the aromatic amine.
     - Hydrolysis of the amide
     - Hydrolysis of the $\alpha$-chloracetamido group, followed by reduction to give the corresponding $\alpha$-hydroxyacetetyl derivative
     - Dehalogenation
- Not recommended for UTI (why?)… only 5-10% of the unmetabolized chloramphenicol is excreted in urine.
- It inhibits liver enzymes thus activates (i.e. reduces metabolism of) the co-administered drugs such as anticoagulant coumarins, sulfonamides, oral hypoglycemic and phenytoin.
Nitro reduction to aromatic amine

O-glucuronide conjugate (major)

Amide hydrolysis

Oxidation

[O]

Proteins

Nucleic acids

Systemic Toxicity
Drugs forms

- Prodrugs of chloramphenicol have better physicochemical properties than Chloramphenicol which has both bitter taste and bad water solubility.
- It can be given as prodrug of C3 palmitate to mask the bitter taste for oral use. It is prepared as suspension (insoluble in water). The prodrug is cleaved in the duodenum to liberate chloramphenicol.
- A prodrug of C3 hemisuccinoyl ester is also used as prodrug to improve water solubility which is cleaved by estrases.

Chloramphenicol sod. succinate
much more water soluble than chloramphenicol
Given IV

Chloramphenicol palmitate
tastless.
normally given orally
This is cleaved in the body to produce active chloramphenicol. Because cleavage in muscles is too slow, this product is used intravenously rather than intramuscularly.

Chloramphenicol succinate ester → Chloramphenicol
• Aromatic amines are so toxic and carcinogenic
• Chloramphenicol nitro group is reduced to a hydroxylamine which caused methaemoglobin in babies in the 1950s (grey baby syndrome).

1. NO2 is reduced by NADPH-dependent reductase → NH2
2. NH2 can be oxidized by CYP of liver to:
   - hydroxylamine which binds to DNA → carcinogenic
   - Nitrosoarene which binds to tissue → cytotoxic

   Since hepatocytes and erythrocytes contain large amounts of NADPH and NADH reductases, respectively, they will be the most affected type of cells

• Blood dyscrasias such as pancytopenia of the blood caused by reduction of aromatic nitro group of chloramphenicol
Present in hepatocytes and erythrocytes

Figure 8.7 Stages of oxidation and reduction of aromatic amines
C-3 glucuronide conjugate

Major metabolite

Chloramphenicol

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2-2008
• Foyes 1110
• Wilson 308
• Graham 455
• Alagar 323
Macrolides
Macrolides

- **Macrolides** are macro lactone rings made up of 12 or more atoms, and usually contain one or more sugars.
- They are bacteriostatic agents that inhibit protein synthesis.
- The best-known example of this class is **erythromycin** (a metabolite isolated in 1952 from the soil microorganism *Streptomyces erythreus* found in the Philippines. It is one of the safest antibiotics in clinical use.
- The structure is composed from 14-membered macrocyclic lactone ring with attached sugars and amino sugars (the sugar residues are important for activity).
- Erythromycin contains a **14-membered lactone ring** and two sugars, **desosamine** (attached at C$_5$) and **cladinose** (attached at C$_3$).
 Mechanism of action

• The macrolide binding site is located on the large ribosomal subunit inside the nascent peptide exit tunnel near the peptidyl transferase center.

• Its proximity to the peptidyl transferase center explains the inhibitory effect of some macrolides on peptide bond formation.

• The sugar residues attached at the C5 position of the lactone ring protrude towards the peptidyl transferase center.
The macrolides inhibit bacterial protein biosynthesis by binding to the 23S rRNA in the polypeptide exit tunnel adjacent to the peptidyl transferase center in the 50S ribosomal subunit.

This prevents the growing peptide from becoming longer than a few residues, resulting in the dissociation of peptidyl tRNA molecules.

Macrolides, clindamycin, lincomycin, and chloramphenicol bind in the same vicinity, leading to extensive crossresistance between them.
Macrolides isolated from microorganisms are generally having the following molecular characteristics:

✓ Large lactone ring (12, 14, 15, or 16-membered rings)
✓ A highly substituted macrocyclic lactone: aglycone moiety.
✓ A ketone group.
✓ One or two amino sugars glycosidically linked to the aglycone moiety.
✓ The presence of dimethylamino moiety on the sugar residue → increase basicity → aid in salt formation.
✓ Being highly lipophilic (although glycosidic linkages are hydrophilic)
✓ Being basic
The lactone ring usually has 12, 14, or 16 atoms and is often unsaturated.

Macrolide antibiotics are weak bases and different salts with pKa range of 6.0-9.0 can be formed on the amino group.

Macrolides are water-insoluble molecules. Salts prepared by glucoheptonic and lactobionic salts are water soluble, whereas stearic acid and laurylsulfuric acid salts are water-insoluble.

Macrolides are stable in aqueous solutions at or below room temperature. They are unstable in acidic or basic conditions or at high temperatures.
Chemistry (Cont.)

Aglycone Or Erythronolide moiety

Lactone

Erythromycin; \( X = \text{OH} \)
Claritromycin; \( X = \text{OMe} \)

Aminosugar (desosamine)

Glycone

Sugar (cladinose)
Erythromycin A: $R = \text{OH}, \ R' = \text{CH}_3, \ R'' = \text{H}$
Erythromycin B: $R = R'' = \text{H}, \ R' = \text{CH}_3$
Erythromycin C: $R = \text{OH}, \ R' = R'' = \text{H}$
Erythromycin D: $R = R' = R'' = \text{H}$
Erythromycin F: $R = R'' = \text{OH}, \ R' = \text{CH}_3$
Chemistry (Cont.)

- Naturally occurring macrolides (erythromycin) are acid-labile, have short $t_{1/2}$ (1.5h) and narrow spectrum (Gram-positives, *Staphylococci, Streptococci, Bacilli*).

- Semi-synthetic derivatives (clarithromycin, $t_{1/2}$=3-7h), azalides (azithromycin, $t_{1/2}$>35h!), have improved stability, pharmacokinetic properties and spectrum (Gram-negatives, *Haemophilus influenzae*, atypical bacteria: *Legionella, Chlamydia, Mycoplasma*).
The structure consists of a 12, 14, 15 or 16-membered macrolactone ring with a sugar (e.g. cladinose) and an amino sugar (e.g. desosamine) attached. The sugars are important for activity.

Due to the amino sugar the molecule is weakly basic (pKa=8)

It is acid sensitive, thus given orally as coated tablets.

The acid sensitivity of erythromycin is due to the presence of a ketone and two alcohol groups which form intramolecular ketal in presence of acids.

**FIGURE 19.68** Intramolecular ketal formation in erythromycin.
FIGURE 33.30 Acid-catalyzed intramolecular ketal formation with erythromycin.
Since neither the hemiketal nor the spiroketal exhibits significant antibacterial activity, unprotected erythromycin is inactivated substantially in the stomach.

Furthermore, evidence suggests that the hemiketal may be largely responsible for the GI (prokinetic) adverse effects associated with oral erythromycin.
• Methods for protecting erythromycin from acid-catalyzed ketal formation:
  1. the hydroxyl groups are changed to methoxy groups as in clarithromycin which has improved acid-stability and oral absorption.
2. Increasing the member atoms of the macrolide (e.g. 15-membered ring of azithromycin).

3. Formation of salts with fatty acids
• The free bases of erythromycins are moderately soluble in water
• Water solubility can be improved by salt formation with some organic acids such as glucoheptonic and lactobionic acids to be used for parenteral administrations
Water solubility can be decreased if salts are prepared with fatty acids as stearate, estolate and laurylsulfate salts.

Erythromycin stearate is a very insoluble salt form of erythromycin. The water insolubility helps:

1. To increase stability toward acids
2. To increase oral absorption
3. To mask bitter taste
FIGURE 25 - Conversion of erythromycin stearate and erythromycin estolate into erythromycin.
Erythromycin ethyl succinate is a mixed double-ester prodrug in which one carboxyl of succinic acid esterifies the C-2′ hydroxyl of erythromycin and the other ethanol.

This prodrug is frequently used in an oral suspension for pediatric use largely to mask the bitter taste of the drug.

This prodrug is acid-sensitive, slightly soluble in water, and slowly hydrolyzed after absorption to free erythromycin.
Erythromycin estolate, erythromycin propionate lauryl sulfate: is the lauryl sulfate salt of the 2-propionate ester of erythromycin.

It is acid stable and absorbed as the propionate ester. The ester undergoes slow hydrolysis in vivo. Only the free base binds to bacterial ribosomes.
The 14-membered ring macrolides are synthesized in bacteria from propionic acid units so that every second carbon of erythromycin, for example, bears a methyl group and the rest of the carbons are oxygen bearing (with one exception [H]).

Two carbons bear so-called “extra” oxygen atoms [O] introduced later in the biosynthesis (not present in a propionic acid unit), and two hydroxyls are glycosylated.

**FIGURE 33.29** Biosynthetic pathway to erythromycins from propionic acid units. → refers to modifications to the basic ring skeleton.
**Formulations of macrolides**

- **ETHYLSUCCINATE**: Erythromycin ethyl succinate is a mixed double-ester prodrug in which one carboxyl of succinic acid esterifies the C-2′ hydroxyl of erythromycin and the other ethanol.

- This prodrug is frequently used in an oral suspension for pediatric use largely to mask the bitter taste of the drug.
Most naturally occurred macrolides have undesirable chemical and biological characteristics.

Modifications has been made to design macrolides with improved: potency, spectrum, stability, pharmacokinetics and toxicity.

The macrolides can be classified into generations:

- 1\textsuperscript{st} generation: Picromycin and Erythromycin
- 2\textsuperscript{nd} generation:
  - 14-membered ring: Clarithromycin, Roxithromycin, Dirithromycin, and Flurithromycin
  - 15-membered ring: Azithromycin
- 3\textsuperscript{rd} generation: 16-membered ring: Tylosin, Carbomycin A and Spiramycin (This gen has no 3-\textit{O}-cladinose)
The 2nd Generation of macrolides

- Structural modifications have been made to erythromycin to solve acid-instability and to mask its bitter taste.
- Semisynthetic macrolides such as
  1. **Clarithromycin**: is similar to erythromycin with the C₆ hydroxyl in erythromycin has been converted to an methoxy. Due to this modification, clarithromycin has
     - The spectrum is slightly improved compared to erythromycin
     - greater acid stability than erythromycin.
     - Does not cause cramp in GIT
     - Higher blood concentrations.
     - More lipophylicity
     - Longer half-life
2. **Roxithromycin**: The addition of hydroxylamine to the ketone to form oxime

- increased acid stability by reducing intermolecular ketalization
- diminished antimicrobial activity compared to erythromycin
- 9-oxime decreased its affinity for P-450, which reflects reduced interaction with hepatic mono-oxygenases and reduces interaction with metabolism of other drugs
- higher tissue distribution and a longer half-life
3. **Azithromycin**: nitrogen atom has been introduced to expand a 14-membered ring to 15-membered azalide ring. Removal of the 9-keto group coupled with incorporation of a weakly basic tertiary amine nitrogen function into the macrolide ring increases the stability. Which offers:

- Spectrum is slightly different than erythromycin and clarithromycin. It is more active vs Gram-ve and less active vs Gram+ve bacteria.
- Stability toward acids (no ketal formation → no abdominal cramp)
- Longer half life (once daily dosage)
- Increased lipophilicity
- Greater tissue penetration
The 2nd Generation of macrolides

• Erythromycin 9-oxime is currently the intermediate to synthesize 2nd generation erythromycin analogues
  - Azithromycin (erythromycin 9-oxime derivative)
  - Roxithromycin (erythromycin 9-oxime ether)
  - Clarithromycin (6-O-methylerythromycin)

• The hemi-ketal erythromycin is used as intermediate to synthesize flurithromycin
clarithromycin

azithromycin

erythromycin
9-oxime

roxithromycin

dirithromycin
**Scheme 1.** The degradation of erythromycin A under acidic condition.

- **1a** erythromycin A
- **3** 8,9-anhydro-6,9-hemiketal
- **4** 6,9;9,12-spiroketal

**8,9-anhydro-6,9-hemiketal**

**flurithromycin**
The main product of liver metabolism of erythromycin is the N-demethylated analogues.

Macrolides inhibit CYP3A4 isoform of the cytochrome P450 oxidase family and lead to potentiation of co-administered drugs.
Ketolides are erythromycin derivatives. They have improved antibacterial activity and they are structurally different from macrolides in three ways:

1. The presence of 3-keto group in place of L-cladinose moiety: the sugar is essential for antimicrobial activity of macrolides, however, the removal of the sugar is compensated by change in macro-lacton ring (C11/C12). 3-keto group improves activity against erythromycin resistant strains

2. The C6-OH is changed to methoxy to prevent ketal formation (similar to clarithromycin)

3. Large aromatic N-substituted carbamate is extended at C11/C12 which improves interaction to ribosome, thus activity against resistant strains.

ketolides

3-keto function
- Avoids MLS$_B$ resistance induction

Methoxy group at C6
- Improves acid stability

C11,12 carbamate side chain
- Increases affinity for the ribosomes
New ketolides

- Telithromycin ➔ Solithromycin
- Pyridine ring ➔ Aniline ring
- Imidazole ring ➔ triazole ring
  - C2-H ➔ C2-F
The SAR for Macrolids

- 3-O-caldinose is necessary for antibacterial activity. Removal of caldinose forms 3-descladinosyl erythromycin derivatives which are all inactive analogues such as:
  - 3-OH
  - 3-\(O\)-acetyl
  - 3-\(O\)-(substituted)benzoyl
  - 3-keto derivatives

- Picomycin was the earliest discovered macrolide and showed low antibacterial activity

- Note ketolides have 3-keto but with extra C11, C12-carbamate side chain that increases binding to ribosome
The spectrum of antibacterial activity for erythromycin is similar to penicillin.

1. Active against Gram +ve cocci and bacilli
2. Active against Gram –ve cocci (especially Neisseria spp)
3. Low activity against Gram-ve bacilli

Unlike penicillins, macrolides are also active against *Mycoplasma, Chlamydia, Campylobacter* and *Legionella* spp.
Bacterial resistance to macrolides

- Lower binding to bacterial ribosome due to:
  1. Methylation of a specific guanine residue of rRNA \( \rightarrow \) reduce affinity to erythromycin but not telithromycin
  2. Adenine to guanine mutation in rRNA \( \rightarrow \) 1000 fold reduction in affinity of erythromycin and clarithromycin to 23S rRNA

- Active efflux (expel) of macrolide from the cell
- Lack of penetration mechanism as in some Gram-ve bacteria