CHEMOTHERAPEUTICS

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Structure of the Lecture

What are chemotherapeutic and antibiotic?

History of antibiotics

Mode of actions of antibiotics

Resistance to antibiotics

Determination of antibiotic sensitivity

Chemotherapeutic are antimicrobials derived from chemical substances.

Antibiotics are antimicrobials obtained from bacteria or fungi

HISTORY OF CHEMOTHERAPEUTICS

- Mercury to threat Syphilis 1495
- Natural Quinine from the bark of Cinchona three to threat Malaria in 1630.
- 19th century:

– Louis Pasteur & Robert Koch: Bacteria as causative agents

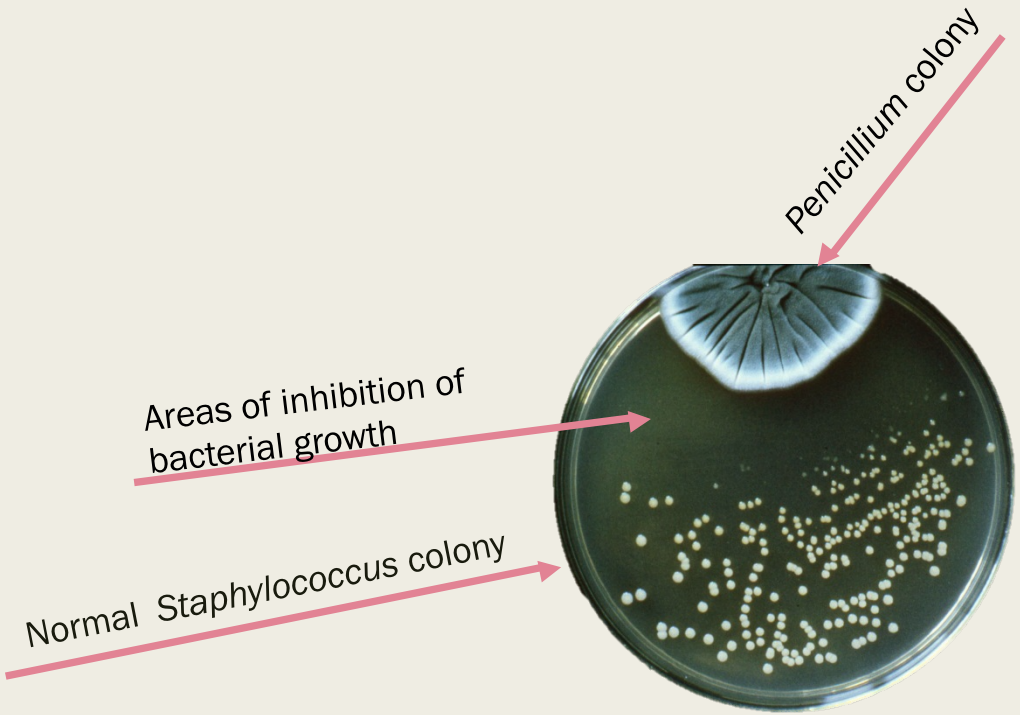
- Paul Erlich sythesized an arsenical compund salvarsan in 1910.

Paul Ehrlich

- started science of chemotherapy
- no. 606 compound = Salvarsan (1910) •
- selective toxicity!!
- Developed the Chemotherapeutic Index
- Chemotherapeutic Index = Toxic Concentration / Effective Concentration

Penicillin - the first antibiotic

- Penicillin was the first antibiotic to be discovered.
- It was discovered in 1928 by Alexander Fleming, He observed the killing of staphylococci by a fungus (*Penicillium notatum*)



- For 9 years, nobody could purify the Penicillum Notatum to get the pure penicillin.
- Finally, in 1938, a team of Oxford University Scientists, headed by Howard Florey and Ernst B. Chain helped to develop penicillin
- Florey & Chain purified penicillin by freeze- drying (1940) - Nobel prize 1945
- first used in a patient: 1942
- World War II: penicillin saved 12-15% of lives

Brief History of Antibiotics

- 1928- Penicillin discovered by Fleming
- 1932- Sulfonamide antimicrobial activity discovered {Erlich}•
- 1943- Drug companies begin mass production of penicillin
- 1948- Cephalosporins precursor sent to Oxford for synthesis
- 1952- Erythromycin derived from Streptomyces erythreus
- 1956- Vancomycin introduced for penicillin resistant staphylococcus
- 1962- Quinolone antibiotics first discovered
- 1970s- Linezolide discovered but not pursued
- 1980s- Fluorinated Quinolones introduced, making then clinically useful
- 2000- Linezolide introduced into clinical practice

- Antibiotics or chemotherapeutics are substances that have great harmful effects on microorganisms (parasitic effect) whereas the minor effects on the organism (organotropic effect) in very small quantities (treatment doses) and used with the aim of treating infectious diseases.

Damaging effect on microorganism: → parasitic effect

Damaging effect on organism: → organotropic effect

CHARACTERISTICS OF A CHEMOTHERAPEUTIC AGENT

Destroy or prevent the activity of a microorganism without injuring the cells of the host or with minor injury to its cells

Be able to come in contact with the microorganism by penetrating the cells and tissues of the host in effective concentrations.

Leave unaltered the host's natural defense mechanisms

Selective Toxicity

- Drugs that specifically target microbial cells and not the human host cell.
- Greater parasitic effect – minor or absence organotropic effect
- The selective toxic effect is due to differences in structure and biochemical mechanisms between the microorganism cell and the mammalian cell.
- The selective toxic effect is the most important difference between chemotherapeutics and antiseptics.

Classification of Antibiotics

Chemotherapeutics are classified as;

- On the basis of spectrum activity;
- On the basis of mode of action;
- On the basis of mechanism of action;

Classification of Antibiotics

Chemotherapeutics are classified as;

- On the basis of spectrum activity;

Broad spectrum antibiotics	Short (Narrow) spectrum antibiotics
Amoxicillin	Penicillin-G
Tetracycline	Vancomycin
Cephalosporin	Bacitracin
Cholorampenicol	Cloxacillin
Erythromycin	

On the basis of mode of action;

- Bacteriostatic antibiotics
- Bactericidal antibiotics

Bacteriostatic: An antimicrobial drug that inhibits microbial growth but requires host defense mechanisms to eradicate the infection; does not kill bacteria.

Bactericidal: An antimicrobial drug that can eradicate an infection in the absence of host defense mechanisms; kills bacteria.

On the basis of mechanism of action;

- Inhibition of cell wall synthesis
- Disruption of cell membrane function
- Inhibition of protein synthesis
- Inhibition of nucleic acid synthesis
- Action as antimetabolites

1- INHIBITORS OF CELL WALL SYTHESIS

It is based on the blocking of transpeptidase and carboxypeptidase enzymes that play a role in peptidoglycan formation. (it is active during the active reproductive period of bacteria, bactericidal effect)

How do they work?

The β-lactam binds to Penicillin Binding Protein (PBP)

PBP is unable to crosslink peptidoglycan chains (Cross linking provides stability, strength)

The bacteria is unable to synthesize a stable cell wall

The bacteria is lysed

Beta-lactams kill bacterial cells only when they are actively growing and synthesizing cell wall.

- Bactericidal activity of penicillin is more against Gram positive. (Difference in organization of cell wall)

In gram positive

- Thick layer of Peptidoglycans and teichoic acid (a polyol phosphate polymer) surrounds the membrane.
- Peptidoglycans layer is easily accessible to Beta lactam antibiotics

In gram negative

- Two membranes are present. (The cytoplasmic membrane and an outer membrane with thin layer of Peptidoglycans sandwiched between the two).
- The outer membrane consists of lipopolysaccharides with narrow porin channels which function as a barrier to permeability of antibiotics

Their structure contains a beta-lactam ring.

The major subdivisions are:

(a) penicillins whose official names usually include or end in “cillin”

(b) cephalosporins which are recognized by the inclusion of “cef” or “ceph” in their official names.

(c) carbapenems (e.g. meropenem, imipenem)

(d) monobactams (e.g. aztreonam)

(e) beta-lactamase inhibitors (e.g. clavulanic acid, sulbactam).

PENICILLIN

6-APA (6- aminopenicillinoic acid)

1. Natural Penicillin

Penicillin V (Phenoxymethylpenicillin)

- High Gram positive effect + Less effective against Gram negative bacteria
- Used treatment for Tonsillitis • Anthrax • Rheumatic fever • Streptococcal skin infections
- Narrow spectrum • Should be given orally • susceptible to beta-lactamase

Penicillin G

- High activity against Gram positive, Low activity against Gram negative bacteria
- Used treatment for infections caused by streptococci, meningococci, enterococci, penicillin-susceptible pneumococci, non-β-lactamase-producing staphylococci, *T. pallidum* and many other spirochetes, Clostridium species, actinomyces, and other Gram-positive rods and non-β-lactamase-producing Gram-negative anaerobic organisms.
- Narrow spectrum, susceptible to beta-lactamase

2. Aminopenicillin

- Ampicillin
- EFFECTIVE AGAINST: • Gram positive + Gram negative bacteria
- TREATMENT FOR: • Ear infection • Sinusitis • Urinary tract infections • Meningitis
- CHARACTERISTICS: • Broad spectrum •
- Can be given orally and parenterally • susceptible to beta-lactamase

- Amoxicillin
- EFFECTIVE AGAINST: • Gram positive + Gram negative bacteria
- TREATMENT FOR: • Skin infection • Sinusitis • Urinary tract infections • Streptococcal pharyngitis
- CHARACTERISTICS: • Broad spectrum • Can be given orally and parenterally • Susceptible to beta-lactamase
- SIDE-EFFECTS: • Rash, diarrhea, vomiting, nausea, edema, stomatitis, and easy fatigue.

3. Anti-Staphylococcal Penicillin

Methicillin

- Effects on Gram positive bacteria
- Used treatment for: staphylococcal infections caused by methicillin-sensitive staphylococci such as osteomyelitis, endocarditis, sepsis, soft tissue infections, meningitis.
- Very narrow Spectrum • Should be given parenterally

Oxacillin

- Effects on Gram positive bacteria
- Treatment for penicillin-resistant *Staphylococcus aureus* infection
- Very narrow Spectrum • Should be given parenterally

Nafcillin

- Gram positive bacteria
- For Staphylococcal infections treatment
- Very narrow Spectrum • Should be given parenterally

Cloxacillin

- Effective against Staphylococci that produce beta-lactamase
- Very narrow Spectrum • Should be given orally

Dicloxacillin

- Effective on Gram positive bacteria + Staphylococci that produce beta- lactamase
- Very narrow Spectrum • Should be given orally

Flucloxacillin

- Effective on Gram positive bacteria + Staphylococci that produce beta- lactamase
- Very narrow Spectrum • Should be given orally

4. Anti-Pseudomonal Penicillin

Piperacillin

- Gram positive +Gram negative
- Extended Spectrum • Should be given by intravenous or intramuscular injection

*Piperacillin+Tazobactam=Zosyn

Carbenicillin

- Gram negative + Limited Gram positive
- Treatment for: • Urinary tract infections
- Highly soluble in water and acid- labile

Ticarcillin

- Effective against mainly gram negative bacteria particularly Pseudomonas aeruginosa
- Used treatment for: • Stenotrophomonas maltophilia infections

Resistance against Penicillin

Natural

- Target enzymes and PBPs are deeply located (Lipoprotein barrier in –ve)
- PBPs of organisms have low affinity for penicillin

Acquired

- Production of Penicillinase (Beta-Lactamase) enzyme, (>300 subtypes).

Organisms producing Beta-Lactamase are; Staphylococcus, *Bacillus subtilis, E. coli*

- Loss or alteration of Porin channels in gram negative
- Modification of penicillin binding proteins (PBPs)- having low affinity .
- Activation of antibiotic efflux mechanism- Some gram negative bacteria

CEPHALOSPORIN

- Chemistry: 7-ACA (7- aminosephalosporanic acid) main structure
- Classification: Cephalosporins can be classified into four major groups or generations, depending mainly on the spectrum of their antimicrobial activity.

    First-generation cephalosporins

    Second- generation cephalosporins

    Third-generation cephalosporins

    Fourth- generation cephalosporins

Common characters:

- *Activity on gram-positive bacteria: first>second>third*
- *Activity on gram-negative bacteria: first<second<third*
- *Stability to β-Lactamase produced by gram-negative rods: first<second<third*
- *Renal toxicity: first>second>third*

Gram positive

Generation	Parenteral Agents	Oral Agents
1st generation	*Cefazolin, Cephalothin*	*Cefadroxil, cephalexin,cephradine*
2nd generation	*Cefotetan, cefoxitin, cefuroxime*	*Cefaclor, cefprozil, cefuroxime axetil*
3rd generation	*Cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, Cefoperazone*	*Cefdinir, cefditoren, cefpodoxime proxetil, ceftibuten, cefixime*
4th generation	*Cefepime, cefpirome*	

Gram negative

First-generation cephalosporins are predominantly active against Gram-positive bacteria, and successive generations have increased activity against Gram-negative bacteria (with reduced activity against Gram-positive organisms).

First generation cephalosporin: cefazolin, cephalexin etc.

Cephalosporins are not active against methicillin-resistant strains of staphylococci.

Cefazolin (i.m./i.v.) may be a choice in infections for which it is the least toxic drug (eg, *K. pneumoniae*) and in persons with staphylococcal or streptococcal infections with a history of penicillin allergy.

- **Second-generation cephalosporins:** Cefamandole, cefuroxime,cefaclor
- Effective on anaerobes
- No effect on *P.aeruginosa*
- More active on gram-negative bacteria than 1st generation.
- **Clinical uses:** Gram-negative bacteria infections, Anaerobic infections
- *The oral second-generation cephalosporins are active against beta-lactamase producing H. influenzae or Moraxella catarrhalis and have been primarily used to treat sinusitis, otitis, or lower respiratory tract infections.*

- Third-generation cephalosporins: Ceftriaxone, ceftazidime

Common characters

- *Much more active on gram-negative bacteria*
- *Stable to extended β-Lactamase produced by gram-negative bacteria*
- *Effective on anaerobes and P.aeruginosa*
- *No renal toxicity*
- *Penetrating body fluids and tissues well*
- *Clinical use: a wide variety of serious infections caused by organisms that are resistant to most other drugs*

- Fourth- generation cephalosporins: Cefpirome, cefepime
- Character:
  - *Enhanced antimicrobial activity*
  - *Stable to ESBLs*
  - *More activity on gram-positive cocci*
- Clinical uses:
  - *infections caused by organisms that are resistant to third-generation cephalosporins*
  - *menengitidis: transition to cerebrospinal fluid*

OTHER B-LACTAM DRUGS

CARBAPENEMS

- Wide spectrum and high activity
- Resistant to mostβ-Lactamase(including ESBLs and cephalosporinase)
- Imipenem, Meropenem, Ertapenem

Imipenem

- EFFECTIVE AGAINST: • Aerobic and anaerobic, Gram positive and gram negative bacteria
- CHARACTERISTICS: • Broad Spectrum • Intravenous • Resistant to beta-lactamase enzymes

Meropenem

- Effectıve Against: • Aerobic and anaerobic, Gram positive and gram negative bacteria
- Ultra Broad Spectrum • Intravenous • Resistant to beta-lactamase enzymes

Ertapenem

- Effectıve Against: • Gram positive and gram negative bacteria
- Broad Spectrum • Intravenous • Resistant to beta-lactamase enzymes • Not active against MRSA

MONOBACTAM

Aztreonam

- Effectıve Against: No effect on gram-positive bacteria and anaerobes
- High activity on gram-negative bacteria
- Intravenous • Resistant to beta-lactamase enzymes • Not active against MRSA • Low toxcity • Penicillin-allergic patients tolerate well

Why selective against bacteria

- Peptidoglycans cell wall is unique to bacteria.
- In gram positive cell wall is entirely made by Peptidoglycans with extensive cross linking (More effective in Gr+)
- In gram negative cell wall consists of lipoprotein and Peptidoglycans with less cross linking (Less effective in Gr–)
- Penicillin binds with PBPs and every organism has different proteins with different affinity for penicillin.
- Gram negative bacteria have porins of specific proteins located in outer membrane. Permeability of different beta-lactam antibiotics through these channels differs leading to variable action.

BETA-LACTAMASE INHIBITORS

- Resemble β-lactam antibiotic structure
- Bind to β-lactamase and protect the antibiotic from destruction
- Most successful when they bind the β-lactamase irreversibly
- Weak antimicrobial action
- Synergism
- Three important in medicine: » Clavulanic Acid » Sulbactam » Tazobactam

Other inhibitors of cell wall synthesis

- **Bacitracin:** Topical application, against gram-positives
- **Vancomycin**: Glycopeptide, last line against resistant *S. aureus*

Beta–lactam Resistance

- Drug resistance refers to unresponsiveness of a microorganism to an antimicrobial agent.
- Drug resistance are of two types:

  Natural Resistance

  Acquired Resistance:

  **Porins*

  **Altered penicillin binding proteins*

  **beta-lactamases*

- Natural Resistance: Some microorganisms have always been resistant to certain anti-microbial agent. •They lack the metabolic process or the target side that is affected by particular drug. E.g: Gram negative bacilli are normally unaffected by Penicillin G. M. tuberculosis is insensitive to Tetracyclines. •This type of resistance does not pose significant clinical problem.
- Acquired Resistance: •It is the development of resistance by an organism which was sensitive before due to the use of antimicrobial agent over a period of time. •This can happen with any microbe and is a major clinical problem. However, the development of resistance is dependent on the microorganism as well as the drug.

2- INHIBITORS OF PLASMA MEMBRANE

They disrupt the selective permeability of the cytoplasmic membrane or cause lysis of the membrane.

It reacts with phospholipids in the cytoplasmic membrane to increase cell permeability and disrupts osmotic integrity (substance exchange breaks down). they show bactericidal effect.

- Polymyxin B (Gram negatives)
- Topical
- Combined with bacitracin and neomycin (broad spectrum)

3- INHIBITORS OF PROTEIN SYNTHESIS

- Chemotherapeutic agents of this type are mostly broad spectrum and show bacteriostatic effect. Their effect is by disrupting m-RNA.
- toxicity problems

30S Ribosome inhibitors

- Aminoglycosides: Streptomycin, neomycin, gentamycin (GN aerobic bacilli)
- Tetracyclines: Bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site of ribosome. Not used in children, staining and impairment of the structure of bone and teeth

doxycycline, tetracycline (GP, Rickettsia, Mycoplasma)

50S ribosome inhibitors

- **Macrolides:** Binds to 50 S ribosomal subunit inhibiting protein synthesis. Erythromycin, clarithromycin, azithromycin

GP, Haemophilus and atypical bacteria(Legionella, Mycoplasma)

- **Lincosamides:** clindamycin (most GP, GN anaerobes),

chloramphenicol (broad spectrum), Linezolid (for resistant GP infection)

inhibits peptide bond formation at 50S, inhibit peptidyl trasferase action of tRNA at 50S

4- INHIBITORS OF NUCLEIC ACID SYNTHESIS

- Quinolones and Fluoroquinolones: inhibit DNA gyrase, poor GP activity, GN activity

Ciprofloxacin, levofloxacin, ofloxacin

- Rifampin: inhibits RNA polymerase, good adjunct for treating prosthetic device infection (bacterial biofilm)
- Metronidazole: Forms toxic metabolites in bacterial cell which damage microbial DNA, Anaerobes activity (C. difficile, Trichomonas, Entamoeba)

5- Anti-metabolites

- The ability of enzymes to provide DNA synthesis and protein synthesis in a cell and to synthesize purines and pyrimidines depends on the presence of folic acid. Human cells take this from the outside. But bacteria synthesize. Because they can not absorb folic acid from the environment.

- The folic acid precursor para amino benzoic acid (PABA) is converted to folic acid by a series of reactions with enzymes.
- The chemical structures of PABA and sulfonamides are very similar. With this similarity, sulfonamides replace PABA in the bacterial cell. The dihydropteroate synthetase enzyme that acts in the first step of folic acid synthesis is not attached to PABA but to sulfonamide and stops folic acid synthesis.

- Sulfonamides (Trimethoprim –sulfamethoxazole) : inhibits folic acid pathway

GP, esp. *S.aureus*, GN bacilli, broad spectrum

- Nitrofurantoin: reactive metabolites inhibit ribosomal protein synthesis

GN bacilli, Enterococcus

MECHANISMS OF ANTIBIOTIC RESISTANCE

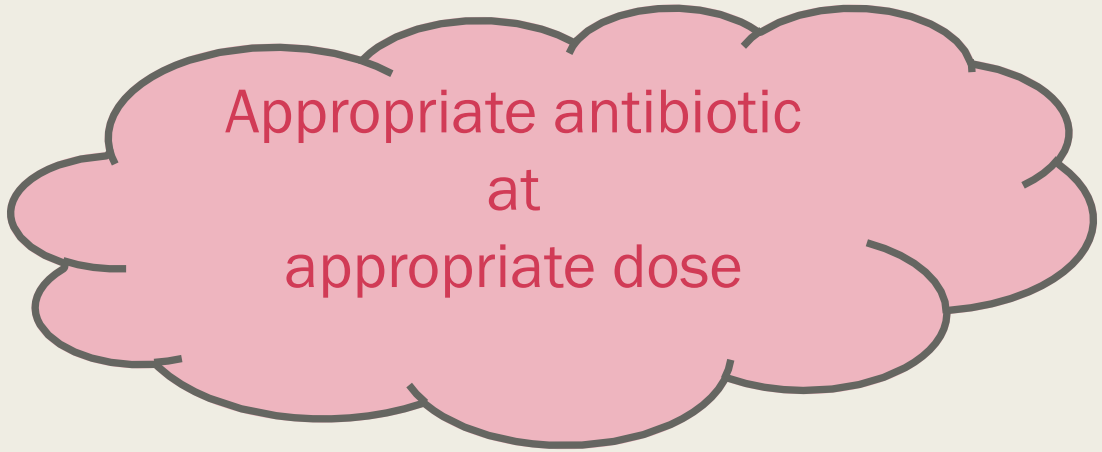
- Enzymatic destruction of drug: beta lactamase enzymes that inactivate Beta lactam antibiotics. Beta lactamases hydrolyze a bond in the beta-lactam ring and causing the ring to break down and the antibiotic to become ineffective.

- **Prevention of penetration of drug**: In Gram negative bacteria, the penetration of beta lactam antibiotics into the cytoplasmic membrane occurs due to porin proteins present in the cell wall. The change in the genes encoding the porin proteins causes the structure of these proteins to change. As a result, beta-lactam antibiotics can not reach the target molecules in the cell and gain bacterial resistance.

- **Alteration of drug's target site:** When the structure of the molecule that the antibiotic binds in the cell changes with the chromosomal mutation, the molecular suitability of the antibiotic decreases or disappears.
- **Rapid ejection of the drug:** In this type of resistance encoded by chromosomes or plasmids, antimicrobial agents are excreted by the bacteria into the cell.

- Exposure to sub-optimal levels of antimicrobial
- Exposure to microbes carrying resistance genes

These factors promote antimicrobial resistant



Historical Aspects

- 1941 Albert Alexander first recepient of penicillin
- 1942 first resistant isolates of Staph aureus reported
- 1960 Methicillin introduced
- 1964 first MRSA reported
- 1980s MRSA became major nosocomial infection

Historical aspects

- 1980s –ESBL producing GN bacteria
- 1990 Vancomycin resistant Enterococci emerged
- 2000 VISA (intermediate level resistance)
- 2002-VRSA (high level resistance)
- 2002- Linezolid resistant enterococci and Staphylococci reported

Antimicrobial susceptibility tests

- Disc diffusion test
- Agar dilution test
- Microdilution test

Disc diffusion method

- For this purpose, isolates are cultured at 37◦C for 24 h in Mueller Hinton Agar (MHA) and bacterial suspensions are prepared with Mueller Hinton Broth(MHB) to match McFarland standard No. 0.5 turbidity. Bacterial suspension is spread on a MHA plate. Sterile paper discs (6 mm diameter) impregnated with antimicrobial agents placed on the surface of the MHA plates. These plates were subsequently incubated at the appropriate temperature for 24 h and after incubation period the diameter (mm) of the zone of inhibitions are measured.

Agar dilution method

- Agar plates are prepared as containing different concentration of antimicrobial agents. For this, firstly different concentrations of antibiotics are prepared, and then MHA melt and warm to approximately at 45-50 ^{o}C. The different concentrations of EO are poured into agar at a ratio 1/10.
- Bacterial suspensions are adjusted to Mc Farland 0.5 with turbidity with nephelometer and diluted at ratio 1/10 and added the inoculum suspension to the multi-point inoculator wells.
- The suspension is transfer to the surface of agar plates. The agar plates are incubated at 37^{o}C for 16-20 h.
- After incubation MIC values are determined as the lowest concentration without visible growth. The test for each isolates is repeated three times.

Microdilution method

- Firstly 100 µL Mueller-Hinton Broth (MHB) is dispensed into 96-well micro titer plates.
- Then 100 µL antimicrobial is added to the first well and serially diluted by two-fold dilution.
- 100 µL of bacterial suspension (final concentartion ($5x10^5$ cfu/mL) was added to each well. Agar plates were incubated at 37 ^oC for 16-20 h. The lowest concentration that inhibits growth is determined as MIC value.

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