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Time-dependent Reduction in Bacterial Population by Antimicrobial Agents

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Abstract
The evaluation of time-dependent reduction in bacterial populations by antimicrobial agents is critical for optimizing treatment regimens and combating antimicrobial resistance. This review looks into the kinetics of bacterial killing over time in response to different classes of antimicrobial agents, including time-dependent and concentration-dependent antibiotics. By analyzing bacterial cultures exposed to varying concentrations of antimicrobials over specific time intervals, we aimed to determine the rate and extent of bacterial reduction. The standardized time-kill assays to evaluate the efficacy of agents such as beta-lactams, aminoglycosides, and fluoroquinolones against common pathogens including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were employed. Findings showed that time-dependent antimicrobials exhibited maximal bacterial suppression when concentrations remained above the minimum inhibitory concentration (MIC) for extended periods, while concentration-dependent agents achieved rapid bacterial reduction with higher peak levels. The bacterial kill curves revealed significant differences in the timing and magnitude of population decline among agents, emphasizing the importance of pharmacokinetic/pharmacodynamic (PK/PD) optimization. Additionally, prolonged exposure to sub-lethal concentrations led to bacterial regrowth in some cases, underscoring the risk of resistance development. There is necessity of aligning antimicrobial dosing strategies with the time-dependent dynamics of bacterial killing. Understanding these temporal patterns enhances therapeutic efficacy and informs clinical decisions, especially in severe or resistant infections. Overall, time-dependent bacterial reduction data provide a foundation for improving antimicrobial stewardship and advancing personalized medicine in infectious disease management.

Keywords: Time-dependent Reduction, Bacterial Population, Antimicrobial Agents, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*

Introduction
The effectiveness of antimicrobial agents in combating bacterial infections is a cornerstone of modern medicine. However, their efficacy is not merely a function of concentration; the dimension of time plays a crucial role in bacterial eradication. Time-dependent killing is a pharmacodynamic characteristic of several classes of antibiotics, where the duration the drug

concentration remains above the minimum inhibitory concentration (MIC) is more critical than the peak concentration achieved [1]. This concept is especially relevant in optimizing dosing regimens to ensure sustained bacterial suppression and minimize the development of resistance.

Antimicrobials such as β-lactams, macrolides, and glycopeptides exhibit time-dependent killing, requiring prolonged exposure to maintain concentrations above the MIC for effective bacterial inhibition [2]. The time-dependent nature of these agents underscores the importance of maintaining plasma drug levels over an extended period rather than relying on high peak levels. This pharmacokinetic/pharmacodynamic (PK/PD) relationship guides clinical decision-making and informs the design of therapeutic protocols, especially in the treatment of persistent infections [3].

Moreover, the emergence of antimicrobial resistance (AMR) further necessitates a precise understanding of time-dependent killing. Prolonged sub-inhibitory exposure due to improper dosing may promote resistance by enabling survival and adaptation of bacterial populations. Therefore, integrating time-dependent pharmacodynamics with appropriate dosing strategies enhances bacterial clearance while mitigating resistance risks.

Antiseptic

Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are generally distinguished from *antibiotics* by the latter's ability to safely destroy bacteria within the body, and from *disinfectants*, which destroy microorganisms found on non-living objects [4].

Antiseptics have a variety of uses both in and out of medical settings. In both settings, they're applied to either the skin or mucous membranes to reduce the risk of infection during surgery and other procedures.

Some antiseptics are true germicides, capable of destroying microbes (bacteriocidal), while others only prevent or inhibit their growth (bacteriostatic) [5].

Specific antiseptic uses include:

1. **Hand washing.** Antiseptics are applied to the palm or skin of the hand before sensitive activities that exposes the body to infections like eating to reduce harmful microorganisms that might be present there.
2. **Disinfecting mucous membranes.** Antiseptics can be applied to the urethra, bladder, or vagina to clean the area before inserting a catheter. They can also help to treat infections in these areas.
3. **Cleaning skin prior to surgery.** Antiseptics are applied to the skin before any kind of surgery to protect against any harmful microorganisms that might be on the skin.
4. **Treating skin infections.** You can buy OTC (over-the-counter) antiseptics to reduce the risk of infection in minor cuts, burns, and wounds. Examples include hydrogen peroxide and rubbing alcohol.
5. **Treating throat and mouth infections.** Some throat lozenges contain antiseptics to help with sore throats due to a bacterial infection.

Types of antiseptics

Antiseptics are usually categorized by their chemical structure. All types disinfect skin, but some have additional uses.

Common types with varied uses include:

- **Chlorhexidine** and other **Biguanides:** These are used on open wounds and for bladder irrigation.
- **Antibacterial dye:** These help to treat wounds and burns.
- **Peroxide** and **Permanganate:** These are often used in antiseptic mouthwashes and on open wounds.
- **Halogenated phenol derivative:** This is used in medical-grade soaps and cleaning solutions.

Are antiseptics safe?

Some strong antiseptics can cause chemical burns or severe irritation if applied to skin without being diluted with water. Even diluted antiseptics can cause irritation if they're left on skin for long periods of time. This kind of irritation is called **irritant contact dermatitis.**

If you're using an antiseptic at home, don't use it for more than a week at a time.

Avoid using OTC antiseptics for more serious wounds, such as:

- eye injuries
- human or animal bites
- deep or large wounds
- severe burns
- wounds that contain foreign objects

These are all best handled by a doctor or urgent care clinic.

Disinfectants

Disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects. Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores; it is less effective than sterilization, which is an extreme physical and/or chemical process that kills all types of life. Disinfectants are different from other antimicrobial agents such as antibiotics, which destroy microorganisms within the body, and antiseptics, which destroy microorganisms on living tissue. Disinfectants are also different from biocides - the latter are intended to destroy all forms of life, not just microorganisms. Disinfectants work by destroying the cell wall of microbes or interfering with their metabolism. Disinfectants are frequently used in hospitals, dental surgeries, kitchens, and bathrooms to kill infectious organisms.

Properties of a Perfect Disinfectant

A perfect disinfectant would also offer complete and full microbiological sterilisation, without harming humans and useful form of life, be inexpensive, and noncorrosive. However, most disinfectants are also, by nature, potentially harmful (even toxic) to humans or animals. Most modern household disinfectants contain denatonium, an exceptionally bitter substance added to discourage ingestion, as a safety measure. Those that are used indoors should never be mixed with other cleaning products as chemical reactions can occur. The choice of disinfectant to be used depends on the particular situation. Some disinfectants have a wide spectrum (kill many different types of microorganisms), while others kill a smaller range of disease-causing organisms but are preferred for other properties (they may be non-corrosive, non-toxic, or inexpensive).

Active constituents in:

Dettol

Chloroxylenol: **Chloroxylenol**, also known as ***para*-chloro-*meta*-xylenol** (**PCMX**), is an antiseptic and disinfectant which is used for skin disinfection and cleaning surgical instruments. It is also used within a number of household disinfectants and wound cleaners. It is less effective than some other available agents. It is available as a liquid. Side effects are generally few but can include skin irritation. It may be used mixed with water or alcohol. Chloroxylenol is most effective against Gram-positive bacteria. It works by disruption of the cell wall and stopping the function of enzymes.

α-terpineol: **Terpineol** is a monoterpene alcohol that has been isolated from a variety of sources such as pine oil. There are four isomers, *alpha*-, *beta*-, *gamma*-terpineol, and terpinen-4-ol. *beta*- and *gamma*-terpineol differ only by the location of the double bond. Terpineol is usually a mixture of these isomers with *alpha*-terpineol as the major constituent. Terpineol has a pleasant odor similar to lilac and is a common ingredient in perfumes, cosmetics, and flavors.

Izal

Hydrogen peroxide: is a chemical compound. In its pure form, it is a very pale blue, clear liquid, slightly more viscous than water. It is used as an oxidizer, bleaching agent, and antiseptic. Concentrated hydrogen peroxide is a reactive oxygen species and has been used as a propellant in rocketry.

Hydrogen peroxide is unstable and slowly decomposes in the presence of light. Because of its instability, hydrogen peroxide is typically stored with a stabilizer in a weakly acidic solution.

Hydrogen peroxide may be used for the sterilization of various surfaces, including surgical tools, and may be deployed as a vapour for room sterilization. It demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores. In general, greater activity is seen against Gram-positive than Gram-negative bacteria; however, the presence of catalase or other peroxidases in these organisms may increase tolerance in the presence of lower concentrations. Higher concentrations of H2O2 (10 to 30%) and longer contact times are required for sporicidal activity.

Types of Disinfectants

Air Disinfectants

Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air. Disinfectants are generally assumed to be limited to use on surfaces, but that is not the case. In 1928, a study found that airborne microorganisms could be killed using mists of dilute bleach [6]. An air disinfectant must be dispersed either as an aerosol or vapour at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

In the 1940s and early 1950s, further studies showed inactivation of diverse bacteria, influenza virus, and *Penicillium chrysogenum* (previously *P. notatum*) mold fungus using various glycols, principally propylene glycol and triethylene glycol [7]. In principle, these chemical substances are ideal air disinfectants because they have both high lethality to microorganisms and low mammalian toxicity.

Although glycols are effective air disinfectants in controlled laboratory environments, it is more difficult to use them effectively in real-world environments because the disinfection of air is sensitive to continuous action.

Alcohols

Alcohol and alcohol plus quaternary ammonium cation based compounds comprise a class of proven surface sanitizers and disinfectants approved by the EPA and the Centers for Disease Control for use as a hospital grade disinfectant. Alcohols are most effective when combined with distilled water to facilitate diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins (A mixture of 70% ethanol or isopropanol diluted in water is effective against a wide spectrum of bacteria, though higher concentrations are often needed to disinfect wet surfaces.) [8]. Additionally, high-concentration mixtures (such as 80% ethanol + 5% isopropanol) are required to effectively inactivate lipid-enveloped viruses (such as HIV, hepatitis B, and hepatitis C) [9].

Aldehydes

Aldehydes, such as formaldehyde and glutaraldehyde, have a wide microbiocidal activity and are sporicidal and fungicidal. They are partly inactivated by organic matter and have slight residual activity.

Some bacteria have developed resistance to glutaraldehyde, and it has been found that glutaraldehyde can cause asthma and other health hazards, hence ortho-phthalaldehyde is replacing glutaraldehyde.

Oxidizing agents

Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.

- Electrolyzed water or "Anolyte" is an oxidizing, acidic hypochlorite solution made by electrolysis of sodium chloride into sodium hypochlorite and hypochlorous acid. Anolyte has a typical pH range of 3.5—8.5, but the most potent solution is produced at a controlled pH 5.0–6.3 where the predominant oxychlorine species is hypochlorous acid.
- Hydrogen peroxide is used in hospitals to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.
- Hydrogen peroxide vapor is used as a medical sterilant and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues, but hydrogen peroxide as with most other strong oxidants is hazardous, and solutions are a primary irritant.
- The antimicrobial action of hydrogen peroxide can be enhanced by surfactants and organic acids. The resulting chemistry is known as Accelerated Hydrogen Peroxide.

A 2% solution, stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical equipment made from hard plastic, such as in endoscopes [10].

- Ozone is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid decolorization and deodorization in addition to disinfection. Ozone decomposes relatively quickly. However, due to this characteristic of ozone, tap water chlorination cannot be entirely replaced by ozonation, as the ozone would decompose already in the water piping. Instead, it is used to remove the bulk of oxidizable matter from the water, which would produce small amounts of organochlorides if treated with chlorine only. Regardless, ozone has a very wide range of applications from municipal to industrial water treatment due to its powerful reactivity.
- Potassium permanganate ($KMnO4$) is a purplish-black crystalline powder that colours everything it touches, through a strong oxidising action. This includes staining "stainless" steel, which somehow limits its use and makes it necessary to use plastic or glass containers. It is used to disinfect aquariums and is also widely used in community swimming pools to disinfect ones feet before entering the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution.

Phenolics

Phenolics are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and handwashes. Phenols are toxic to cats and newborn humans

- Phenol is probably the oldest known disinfectant as it was first used by Lister, when it was called carbolic acid. It is rather corrosive to the skin and sometimes toxic to sensitive people. Impure preparations of phenol were originally made from coal tar, and these contained low concentrations of other aromatic hydrocarbons including benzene, which is an IARC Group 1 carcinogen.
- *o*-Phenylphenol is often used instead of phenol, since it is somewhat less corrosive.
- Chloroxylenol is the principal ingredient in Dettol, a household disinfectant and antiseptic.
- Hexachlorophene is a phenolic that was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.
- Thymol, derived from the herb thyme, is the active ingredient in some "broad spectrum" disinfectants that often bear ecological claims. It is used as a stabilizer in pharmaceutic preparations. It has been used for its antiseptic, antibacterial, and antifungal actions, and was formerly used as a vermifuge.
- Although not a phenol, 2,4-dichlorobenzyl alcohol has similar effects as phenols, but it cannot inactivate viruses.

The most cost-effective home disinfectant is chlorine bleach (typically a >10% solution of sodium hypochlorite), which is effective against most common pathogens, including disinfectant-resistant organisms such as mycobacterium tuberculosis, hepatitis B and C virus, fungi, and antibiotic-resistant strains of staphylococcus and enterococcus. It has disinfectant action against some parasitic organisms (EPA's Registered Sterilizers, Tuberculocides, and Antimicrobial Products Against HIV-1, and Hepatitis B and Hepatitis C Viruses, 2015.

The benefits of chlorine bleach include its inexpensive and fast acting nature. However it is harmful to mucous membranes and skin upon contact, has a strong odour and combination with other cleaning products such as ammonia and vinegar can generate noxious gases like chlorine. The best practice is not to add anything to household bleach except water.

As with most disinfectants, the area requiring disinfection should be cleaned before the application of the chlorine bleach, as the presence of organic materials may inactivate chlorine bleach.

The use of some antimicrobials such as triclosan, is controversial because it may lead to antimicrobial resistance. The use of chlorine bleach and alcohol disinfectants does not cause antimicrobial resistance as it denatures the protein of the microbe upon contact [11].

Quaternary Ammonium Compounds

Quaternary ammonium compounds ("quats"), such as benzalkonium chloride, are a large group of related compounds. Some concentrated formulations have been shown to be effective low-level disinfectants. Quaternary Ammonia at or above 200ppm plus Alcohol solutions exhibit efficacy against difficult to kill non-enveloped viruses such as norovirus, rotavirus, or polio virus (*Guidelines Library: Infection Control. CDC).* Quats are biocides that also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth.

Non-chemical

Ultraviolet germicidal irradiation is the use of high-intensity shortwave ultraviolet light for disinfecting smooth surfaces such as dental tools, but not porous materials that are opaque to the light such as wood or foam. Ultraviolet light is also used for municipal water treatment. Ultraviolet light fixtures are often present in microbiology labs, and are activated only when there are no occupants in a room. Heat treatment can be used for disinfection and sterilization.

Testing the Effectiveness of Antiseptics and Disinfectants

Several environmental conditions influence the potency of an antimicrobial agent and its effectiveness. For example, length of exposure is particularly important, with longer exposure increasing efficacy. Similarly, the concentration of the chemical agent is also important, with higher concentrations being more effective than lower ones. Temperature, pH, and other factors can also affect the potency of a disinfecting agent.

One method to determine the effectiveness of a chemical agent includes swabbing surfaces before and after use to confirm whether a **sterile field** was maintained during use.

Additional tests are described in the sections that follow. These tests allow for the maintenance of appropriate disinfection protocols in clinical settings, controlling microbial growth to protect patients, health-care workers, and the community.

Phenol Coefficient

It is a measure of the bactericidal activity of a chemical compound in relation to phenol.

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly using the Phenol coefficient. The disinfectant to be tested is compared with phenol on a standard microbe (usually *Salmonella typhi* or *Staphylococcus aureus*). Disinfectants that are more effective than phenol have a coefficient greater than 1; those that are less effective have a coefficient less than 1.

An example is **formalin**, with phenol coefficients of 0.3 (*S. aureus*) and 0.7 (*S. enterica* serovar Typhi). A chemical agent with a phenol coefficient greater than 1.0 is more effective than phenol, such as chloramine, with phenol coefficients of 133 and 100, respectively.

Disk-Diffusion Method

The disk-diffusion method involves applying different chemicals to separate, sterile filter paper disks. The disks are then placed on an agar plate that has been inoculated with the targeted bacterium and the chemicals diffuse out of the disks into the agar where the bacteria have been inoculated. As the "lawn" of bacteria grows, **zones of inhibition** of microbial growth are observed as clear areas around the disks. Although there are other factors that contribute to the sizes of zones of inhibition (e.g., whether the agent is water soluble and able to diffuse in the agar), larger zones typically correlate to increased inhibition effectiveness of the chemical agent. The diameter across each zone is measured in millimeters.

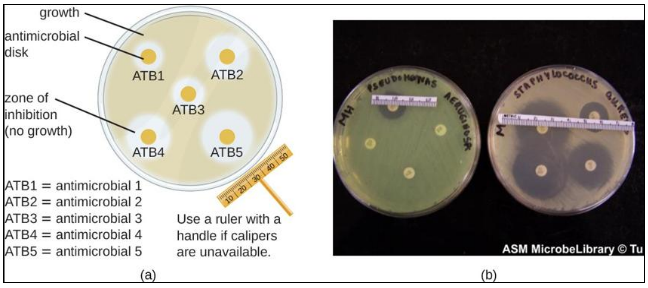


Fig 1: Disk-diffusion method

Use-Dilution Test

The **use-dilution test** is commonly used to **determine a chemical's disinfection effectiveness on an inanimate surface**. For this test, a cylinder of stainless steel is dipped in a culture of the targeted microorganism and then dried. The cylinder is then dipped in solutions of disinfectant at various concentrations for a specified amount of time. Finally, the cylinder is transferred to a new test tube containing fresh sterile medium that does not contain disinfectant, and this test tube is incubated. Bacterial survival is demonstrated by the presence of turbidity in the medium, whereas killing of the target organism on the cylinder by the disinfectant will produce no turbidity.

In-Use Test

An **in-use test** can determine whether an **actively used solution of disinfectant** in a clinical setting is microbially contaminated. A 1-mL sample of the **used disinfectant** is diluted into 9 mL of sterile broth medium that also contains a compound to inactivate the disinfectant. Ten drops, totaling approximately 0.2 mL of this mixture, are then inoculated onto each of two agar plates. One plate is incubated at 37 °C for 3 days and the other is incubated at room temperature for 7 days. The plates are monitored for growth of microbial colonies. Growth of five or more colonies on either plate suggests that viable microbial cells existed in the disinfectant solution and that it is contaminated. Such in-use tests monitor the effectiveness of disinfectants in the clinical setting.

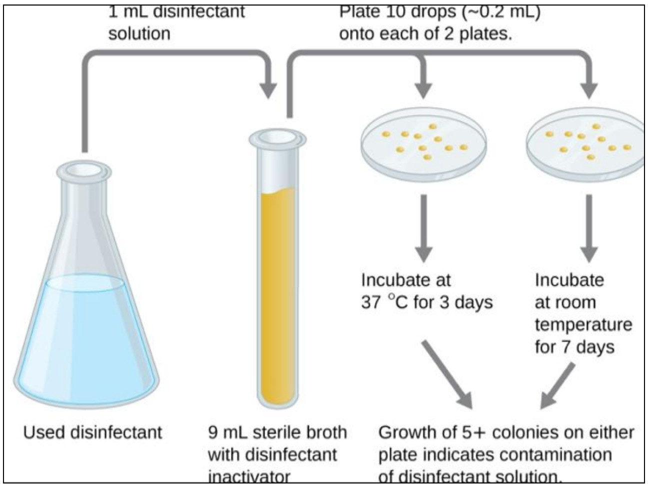


Fig 2: In-Use Test

Bacteria and their Growth

Bacteria are as interesting as they are diverse. Though tiny, these unicellular life forms make huge contributions to many systems and cycles. From helping break down food in your intestine; to making the molecular assist in all three of the carbon, phosphorus, and nitrogen cycles -- these little bacteria can accomplish big things. Unsurprisingly, bacteria are model organisms for research. Not only because of their diversity, but also because they are easily contained and reproduce quickly. When using bacteria for research, it is important to understand and track rates of bacterial growth within a sample.

Inoculant bacteria in a typical laboratory setting tend to proceed through **four distinct growth phases:**

1. Lag Phase
2. Exponential Phase
3. Stationary Phase
4. Death Phase

The lapse of these phases provides data that can be compiled into a bacterial growth curve. During the ***lag phase***, freshly cultured bacteria adjust to the media they've been placed in or on. As they get a feel for their surroundings and nutritional options, the cells increase enzyme production and cell size accordingly. The cells have entered the ***exponential phase*** when they begin to grow and divide at a constant pace. This phase is also called the **logarithmic phase** because growth occurs very rapidly and the cell count can reach into the millions and billions. **As such, the number of cells is calculated using logarithmic functions**. Metabolic processes also occur at a constant rate and are influenced by conditions such as pH, temperature, and properties of the medium. The ***stationary phase*** is marked by a plateau in growth. Cell death and proliferation are roughly equal. Nutrients are increasingly depleted, toxins accumulate, and cell viability decreases. The ***death phase*** occurs when these conditions cause a greater rate of cell mortality than cell proliferation. The population begins to decline at a once again exponential rate.

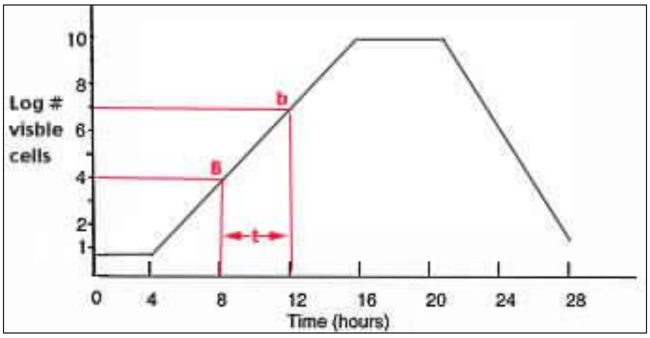
Generation time

This is the time it takes for two new cells to arise from an original cell. If starting with an inoculum with a known number of bacteria, periodic sampling can be done to calculate the generation time using the equation:

$$GT=\frac{t}{3.3\log(\frac{b}{B})}$$

- GT is generation time
- t is the time interval between measurements *b* and *B*
- *B* is the initial population
- *b* is the population after time *t*

For Example, the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth is calculated thus;



G=t
3.3 log b/B

G=240minutes
3.3 log 107/104

G = 240 minutes
3.3 x 3

G = 24 minutes

Bacterial Growth Determination

The two most common classroom methods to determine bacterial growth are the **Standard Plate Count (SPC)** technique and **turbidimetric measurement**. Examples of other methods include: microscopic count, membrane filter count, nitrogen determination, cellular weight determination, and biochemical activity measurement.

The dynamics of the bacterial growth can be studied by plotting the **cell growth (absorbance)** versus the **incubation time** or **log of cell number** versus **time**. The curve thus obtained is a sigmoid curve and is known as a **standard growth curve**. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The **Spectrophotometer** measures the **turbidity** (or **Optical density**) which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass. The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.

Plotting a Bacterial Growth Using Turbidimetric Determination

Turbidimetric determination is useful for plotting growth curves of bacteria in broth or liquid media. It is one of the simplest methods used to analyze trends in growth because it uses a spectrophotometer to track changes in the optical density (OD) over time. In other words: **as the number of cells in a sample increase, the transmission of light through the sample will decrease.** The standard OD setting is **660 nanometers** (for yellow to brown broth samples), but can be adjusted if the color is not in this range or if growth is expected to be lower or greater than average.

Turbidimetric determination is very helpful for plotting a standard growth curve; it allows us to easily track changes in growth phases without the hassle of counting plated colonies. It is simple and easy; however, other procedures often provide more detailed, quantitative information and are preferred when more precise data is necessary. Turbidimetric methods can often be used alongside these other techniques as a reinforcement to trends in the data collected. Whether it is used alone or alongside other techniques, it is a simple and efficient approach when collecting data for standard growth curves.

McFarland Standard Solution

The McFarland Standards are commonly used in Antibiotic Susceptibility Test (AST) to standardize the approximate number of bacteria in a liquid suspension or broth culture of the bacterial cell by comparing the turbidity of the cultured test suspension with that of the McFarland Standard.

Principle of McFarland Standard Solution

A McFarland Standard is a chemical solution of 1% barium chloride (BaCl2) and 1% sulfuric acid (H2SO4) Solution in appropriate proportion; the reaction between these two chemicals results in the turbid solution which is due to the production of a fine precipitate of barium sulfate (BaSO4). Thus prepared turbid solution is used as a standard solution to which the cultures bacterial suspensions are compared and standardized.

Colony-Forming Unit

In microbiology, a **colony-forming unit** (**CFU**) is a unit used to estimate the number of viable bacteria or fungal cells in a sample. Viability is defined as the ability to multiply via binary fission under the controlled conditions. Counting with **colony-forming units** requires culturing the microbes and counts only viable cells, in contrast with **microscopic examination** which counts all cells, living or dead. The visual appearance of a colony in a cell culture requires significant growth, and when counting colonies it is uncertain if the colony arose from one cell or a group of cells. Expressing results as colony-forming units reflects this uncertainty.

Automated systems

Many of the automated systems are used to counteract human error as many of the research techniques done by humans counting individual cells have a high chance of error involved.

Completely automated systems are also available from some biotechnology manufacturers. They are generally expensive and not as flexible as standalone software since the

hardware and software are designed to work together for a specific set-up.
Some of the automated systems such as the systems from MATLAB allow the cells to be counted without having to stain them. This lets the colonies to be reused for other experiments without the risk of killing the microorganisms with stains [12]. However, a disadvantage to these automated systems is that it is extremely difficult to differentiate between the microorganisms with dust or scratches on blood agar plates because both the dust and scratches can create a highly diverse combination of shapes and appearances.

Staphylococcus aureus

S. aureus *is a facultative anaerobic, Gram-positive coccal (round) bacterium also known as "golden staph".* S. aureus *is nonmotile and does not form spores. In medical literature, the bacterium is often referred to as S. aureus, Staph aureus or Staph a..*
S. aureus appears as staphylococci (grape-like clusters) when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates [13]*. S. aureus reproduces asexually by binary fission.*
Complete separation of the daughter cells is mediated by S. aureus autolysin, and in its absence or targeted inhibition, the daughter cells remain attached to one another and appear as clusters [14]*.*
S. aureus is catalase-positive (meaning it can produce the enzyme catalase). Catalase converts hydrogen peroxide (H_2O_2) *to water and oxygen. Catalase-activity tests are sometimes used to distinguish staphylococci from enterococci and streptococci. Previously, S. aureus was differentiated from other staphylococci by the coagulase test. However, not all S. aureus strains are coagulase-positive and incorrect species identification can impact effective treatment and control measures* [15]*.*
It is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. Although *S. aureus* usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin resistant.
S. aureus (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for S. aureus has been approved.
An estimated 20% to 30% of the human population are long-term carriers of *S.aureus* which can be found as part of the normal skin flora, in the nostrils, and as a normal inhabitant of the lower reproductive tract of women. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery.

Food poisoning

S. aureus is also responsible for food poisoning. It is capable of generating toxins that produce food poisoning in the human body. Its incubation period lasts one to six hours, with the illness itself lasting from 30 minutes to 3 days. Preventive measures one can take to help prevent the spread of the disease include washing hands thoroughly with soap and water before preparing food. Stay away from any food if ill, and wear gloves if any open wounds occur on hands or wrists while preparing food. If storing food for longer than 2 hours, keep the food above 140 or below 40 °F.

Virulence factors

Enzymes

S. aureus produces various enzymes such as **coagulase** which clots plasma and coats the bacterial cell, probably to prevent phagocytosis.

Hyaluronidase (also known as spreading factor) breaks down hyaluronic acid and helps in spreading it.
S. aureus also produces **deoxyribonuclease**, which breaks down the DNA, **lipase** to digest lipids, **staphylokinase** to dissolve fibrin and aid in spread, and **beta-lactamase** for drug resistance (Medical Laboratory Manual For Tropical Countries vol two).

Toxins

Depending on the strain, S. aureus is capable of secreting several exotoxins, which can be categorized into three groups. Many of these toxins are associated with specific diseases [16]. Antigens known as **superantigens** can induce **toxic shock syndrome (TSS)**.
This group includes the toxins TSST-1, and enterotoxin type B, which causes TSS associated with tampon use.
Toxic shock syndrome is characterized by fever, erythematous rash, low blood pressure, shock, multiple organ failure, and skin peeling.

Exfoliative toxins

Exfoliative toxins are exotoxins implicated in the disease staphylococcal scalded skin syndrome (SSSS), which occurs most commonly in infants and young children.The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS [17].
Other Staphylococcal toxins that act on cell membranes include alpha toxin, beta toxin, delta toxin, and several bicomponent toxins. The bicomponent toxin PVL is associated with severe necrotizing pneumonia in children.

Biofilm

Bioflims are groups of microorganisms, such as bacteria, that attach to each other and grow on wet surfaces. S. aureus biofilm has high resistance **to host immune response [18].** Though the exact mechanism of resistance is unknown, *S. aureus* biofilms have increased growth under the presence of cytokines produced by the host immune response. And **to antibiotic treatments**, One hypothesis for explaining this is that the biofilm matrix protects the embedded cells by acting as a barrier to prevent antibiotic penetration [19].

Other immunosuppressive strategies

Protein A

Protein A is anchored to staphylococcal peptidoglycan pentaglycine bridges by the transpeptidase sortase A. Protein A (an IgG-binding protein), binds to the Fc region of an antibody. In fact, studies involving mutation of genes coding for protein A resulted in a lowered virulence of S. aureus as measured by survival in blood, which has led to speculation that protein A-contributed virulence requires binding of antibody Fc regions.

Protein A in various recombinant forms has been used for decades to bind and purify a wide range of antibodies by immunoaffinity chromatography. Transpeptidases, such as the sortases responsible for anchoring factors like protein A to the staphylococcal peptidoglycan, are being studied in hopes of developing new antibiotics to target MRSA infections [20].

Staphylococcal pigments

Some strains of S. aureus are capable of producing staphyloxanthin (a golden-coloured carotenoid) pigment.

This pigment acts as a virulence factor, primarily by being a bacterial antioxidant which helps the microbe evade the reactive oxygen species which the host immune system uses to kill pathogens [21]**.**

Mutant strains of *S.aureus* modified to lackstaphyloxanthin are less likely to survive incubation with an oxidizing chemical, such as hydrogen peroxide, than pigmented strains. Mutant colonies are quickly killed when exposed to human neutrophils, while many of the pigmented colonies survive [22]. In mice, the pigmented strains cause lingering abscesses when inoculated into wounds, whereas wounds infected with the unpigmented strains quickly heal.

These tests suggest the Staphylococcus strains use staphyloxanthin as a defence against the normal human immune system. Drugs designed to inhibit the production of staphyloxanthin may weaken the bacterium and renew its susceptibility to antibiotics.

Diagnosis

Depending upon the type of infection present, an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification by using biochemical or enzyme-based tests. A **Gram stain** is first performed to guide the way, which should show typical **Gram- positive bacteria, cocci, in clusters**. Second, the isolate is cultured on **mannitol salt agar**, which is a selective medium with **7–9% NaCl** that allows S. aureus to grow, producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH.

Furthermore, for differentiation on the species level, **catalase** (positive for all Staphylococcus species), **coagulase** (fibrin clot formation, positive for S. aureus), **DNAse** (zone of clearance on DNase agar), **lipase** (a yellow color and rancid odor smell), and **phosphatase** (a pink color) tests are all done.

Treatment

The treatment of choice for S. aureus infection is penicillin. An antibiotic derived from some Penicillium fungal species, penicillin inhibits the formation of peptidoglycan cross-linkages that provide the rigidity and strength in a bacterial cell wall. The four-membered β-lactam ring of penicillin is bound to enzyme DD-transpeptidase, an enzyme that when functional, cross-links chains of peptidoglycan that form bacterial cell walls. The binding of β-lactam to DD-transpeptidase inhibits the enzyme's functionality and it can no longer catalyze the formation of the cross-links. As a result, cell wall formation and degradation are imbalanced, thus resulting in cell death. In most countries, however, penicillin resistance is extremely common, and first- line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (for example, oxacillin or flucloxacillin, both of which have the same mechanism of action as penicillin).

Combination therapy with gentamicin may be used to treat serious infections, such as endocarditis, but its use is controversial because of the high risk of damage to the kidneys [23]. The duration of treatment depends on the site of infection and on severity.

MRSA, is one of a number of greatly feared strains of *S. aureus* which have become resistant to most β-lactam antibiotics. For this reason, vancomycin, a glycopeptide antibiotic, is commonly used to combat MRSA. Vancomycin inhibits the synthesis of peptidoglycan, but unlike β-lactam antibiotics, glycopeptide antibiotics target and bind to amino acids in the cell wall, preventing peptidoglycan cross-linkages from forming.

MRSA strains are most often found associated with institutions such as hospitals, but are becoming increasingly prevalent in community-acquired infections. Minor skin infections can be treated with triple antibiotic ointment [24].

Escherichia coli

Escherichia coli is a Gram-negative, facultative anaerobic (It uses oxygen when it is present and available. It can, however, continue to grow in the absence of oxygen using fermentation or anaerobic respiration. The ability to continue growing in the absence of oxygen is an advantage to bacteria because their survival is increased in environments where water predominates.), rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms [25].

The outer membrane surrounding the cell wall provides a barrier to certain antibiotics such that E. coli is not damaged by penicillin. Strains that possess flagella are motile. The flagella have a peritrichous arrangement. It also attaches and effaces to the microvilli of the intestines via an adhesion molecule known as intimin. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (CDC National Center for Emerging and Zoonotic Infectious Diseases).

The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K2 and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship[26]. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards [27].

E. coli and other facultative anaerobes constitute about 0.1% of gut microbiota, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them Synonyms potential indicator organisms to test environmental samples for fecal contamination.

Culture growth

Optimum growth of E. coli occurs at 37 °C (98.6 °F), but some laboratory strains can multiply at temperatures up to 49 °C (120 °F). E. coli grows in a variety of defined laboratory media, such as lysogeny broth, or any medium that contains glucose, ammonium phosphate monobasic, sodium chloride, magnesium sulfate, potassium phosphate dibasic, and water.

Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen, and amino acids, and the reduction of trimethylamine substrates such N-oxide. as oxygen, nitrate, fumarate, dimethyl sulfoxide, and E. coli is classified as a facultative anaerobe. It uses oxygen when it is present and available. It can, however, continue to grow in the absence of oxygen using fermentation or anaerobic respiration. The ability to continue growing in the absence of oxygen is an advantage to bacteria because their survival is increased in environments where water predominates [28].

Role in disease

Most E. coli strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease.

Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever.

In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, sepsis, and Gram-negative pneumonia. Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome, however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with E. coli.

Some strains of *E. coli* for example O157:H7, can produce Shiga toxin, This toxin causes premature destruction of the red blood cells, which then clog the body's filtering system, the kidneys, causing hemolytic-uremic syndrome (HUS). Unlike most *E. coli* that naturally live in the gut, the Shiga toxin that causes inflammatory responses in target cells of the gut (the lesions the toxin leaves behind are the reason why bloody diarrhea is a symptom of an Shiga toxin producing E. coli infection). Signs of hemolytic uremic syndrome, include decreased frequency of urination, lethargy, and paleness of cheeks and inside the lower eyelids. In addition, this strain causes the buildup of fluid (since the kidneys do not work), leading to edema around the lungs and legs and arms. This increase in fluid buildup especially around the lungs impedes the functioning of the heart, causing an increase in blood pressure.

Treatment

The mainstay of treatment is the assessment of dehydration and replacement of fluid and electrolytes. Administration of antibiotics has been shown to shorten the course of illness and duration of excretion of enterotoxigenic E. coli (ETEC) in adults in endemic areas and in traveller's diarrhea, though the rate of resistance to commonly used antibiotics is increasing and they are generally not recommended (US Centers for Disease Control and Prevention). The antibiotic used depends upon susceptibility patterns in the particular geographical region. Currently, the antibiotics of choice are fluoroquinolones or azithromycin, with an emerging role for rifaximin. Oral rifaximin, a semisynthetic rifamycin derivative, is an effective and well-tolerated antibacterial for the management of adults with non-invasive traveller's diarrhea.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans.

It's a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes.

It generally affects the immunocompromised but can also infect the immunocompetent as in hot folliculitis. Treatment of P. aeruginosa infections can be difficult due to its natural resistance to antibiotics. It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonization occurs in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal [29]. Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is also able to decompose hydrocarbons and has been used to break down tarballs and oil from oilspills [30].

P. aeruginosa is not extremely virulent in comparison with other major pathogenic bacterial species, for example *Staphylococcus aureus* and *Streptococcus pyogenes* – though *P. aeruginosa* is capable of extensive colonization, and can aggregate into enduring biofilms [31].

Diagnosis

Depending on the nature of infection, an appropriate specimen is collected and sent to a bacteriology laboratory for identification. As with most bacteriological specimens, a Gram stain is performed, which may show Gram-negative rods and/or white blood cells. P. aeruginosa produces colonies with a characteristic "grape-like" or "fresh-tortilla" odor on bacteriological media. In mixed cultures, it can be isolated as clear colonies on MacConkey agar (as it does not ferment lactose) which will test positive for oxidase. Confirmatory tests include production of the blue-green pigment pyocyanin on cetrimide agar and growth at 42 °C.A TSI slant is often used to distinguish nonfermenting Pseudomonas species from enteric pathogens in faecal specimens. When P. aeruginosa is isolated from a normally sterile site (blood, bone, deep collections), it is generally considered dangerous, and almost always requires treatment. However, *P. aeruginosa* is frequently isolated from nonsterile sites (mouth swabs, sputum, etc.), and, under these circumstances, it may represent colonization and not infection. The isolation of *P. aeruginosa* from nonsterile specimens should, therefore, be interpreted cautiously, and the advice of a microbiologist or infectious diseases physician/pharmacist should be sought prior to starting treatment. Often, no treatment is needed.

Treatment

Many *P. aeruginosa* isolates are resistant to a large range of antibiotics and may demonstrate additional resistance after unsuccessful treatment. It should usually be possible to guide treatment according to laboratory sensitivities, rather than choosing an antibiotic empirically. If antibiotics are started empirically, then every effort should be made to obtain cultures (before administering first dose of antibiotic), and the choice of antibiotic used should be reviewed when the culture results are available.

Due to widespread resistance to many common first-line antibiotics, carbapenems, polymyxins, and more recently tigecycline were considered to be the drugs of choice; however, resistance to these drugs has also been reported. Despite this, they are still being used in areas where resistance has not yet been reported. Use of β-lactamase inhibitors such as sulbactam has been advised in combination with antibiotics to enhance antimicrobial action even in the presence of a certain level of resistance. Combination therapy after rigorous antimicrobial susceptibility testing has been found to be the best course of action in the treatment of multidrug resistant P. aeruginosa. Some next-generation antibiotics that are reported as being active against P. aeruginosa include doripenem, ceftobiprole, and ceftaroline. However, these require more clinical trials for standardization. Therefore, research for the discovery of new antibiotics and drugs against P. aeruginosa is very much needed. Antibiotics that may haveactivity against P. aeruginosa include:

- aminoglycosides (gentamicin, amikacin, tobramycin, but not kanamycin)
- quinolones (ciprofloxacin, levofloxacin, but not moxifloxacin)
- cephalosporins (ceftazidime, cefepime, cefoperazone, cefpirome, ceftobiprole, but not cefuroxime, cefotaxime, or ceftriaxone)
- antipseudomonal penicillins: carboxypenicillins (carbenicillin and ticarcillin), and ureidopenicillins (mezlocillin, azlocillin, and piperacillin). P. aeruginosa is intrinsically resistant to all other penicillins.
- carbapenems (meropenem, imipenem, doripenem, but not ertapenem)
- polymyxins (polymyxin B and colistin)
- monobactams (aztreonam)

As fluoroquinolones are one of the few antibiotic classes widely effective against P. aeruginosa, in some hospitals, their use is severely restricted to avoid the development of resistant strains.

On the rare occasions where infection is superficial and limited (for example, ear infections or nail infections), topical gentamicin or colistin may be used.

For pseudomonal wound infections, acetic acid with concentrations from 0.5% to 5% can be an effective bacteriostatic agent in eliminating the bacteria from the wound. Usually a sterile gauze soaked with acetic acid is placed on the wound after irrigation with normal saline.

Dressing would be done once per day. Pseudomonas is usually eliminated in 90% of the cases after 10 to 14 days of treatment [32].

In the year 2008, the effect of Dettol on viability of some microorganisms associated with nosocomial infections was evaluated by EL Mahmood, A. M. and Doughari, J. H.

The efficacy of the liquid disinfectant **Dettol** against nosocomial Staphylococcus aureus, Escherichia coli e.t.c was investigated. Use-dilutions of the disinfectant were not immediately lethal to the microorganisms [33].

Viable counting techniques was used to determine the number of cells that survived the effects of use-dilutions of Dettol.

For all the organisms there was an overall similarity in the shapes of the curves. There were some initial shoulders before the exponential phases of death depending on which use-dilution of Dettol and type of organism considered. **For each of the organisms, there was little or no decline in the number of cells after 5min of exposure to Dettol in both Sterile Deionized Water(SDW) and Sterile Tap Water(STW).**

The loss of viability was more in SDW than in STW.

However, there was rapid decline in the cell count after 10 min of treatment*, the viability dropped to* ***12.0%*** *for the* ***1: 20*** *use-dilution of Dettol in SDW. For the higher use-dilution* ***3: 400****, after 10 min of contact with the disinfectant, the population of cells decreased to* ***42.0%*** *and to fewer than* ***0.40%*** *after 30 min of contact in SDW for S. aureus (SA1). A similar trend was recorded with E.coli.*

Conclusion

Antimicrobial agents play a critical role in reducing bacterial populations by targeting essential cellular functions and inhibiting bacterial growth. Their effectiveness varies based on the agent's spectrum, concentration, and mode of action. Proper usage is vital to prevent resistance development and ensure sustained efficacy. Continued research and responsible application are necessary to optimize antimicrobial strategies, safeguard public health, and combat the growing threat of antimicrobial resistance in both clinical and environmental settings.

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