Before starting:

This sheet will start with the complement of "bacterial genetics" topic, and the second part is the rest of "disinfection and sterilization" slides.

Warning: all extra notes in this sheet were not mentioned by the doctor or brought from slides; they are just to promote understanding.

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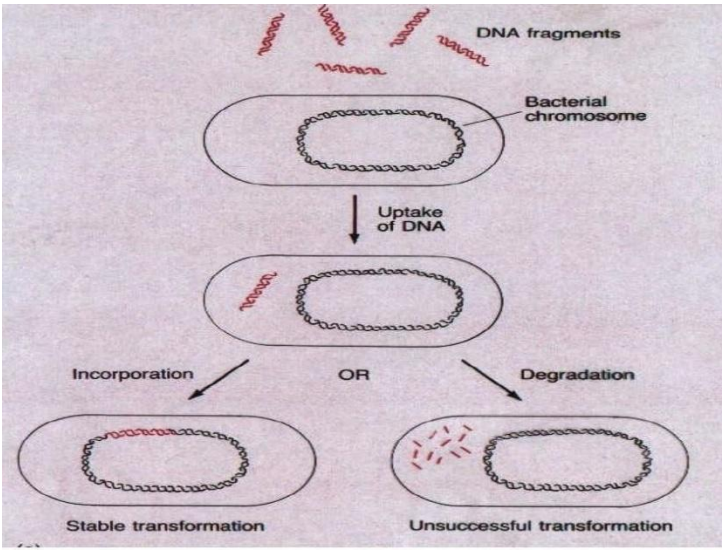
- Genes can flow from one bacterial specie to another (and might flow from a virus) ,this transfer of genes is accomplished/ followed by specific systems/mechanisms :

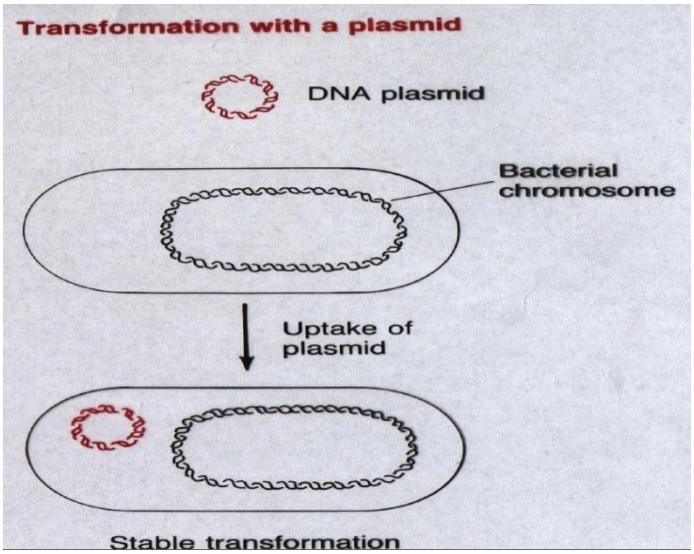
- <u>There are three mechanisms:</u>
  1- Transformation, mainly in gram+.
  2- Conjugation, mainly gram- .
  3- Transduction, gram+ and gram- equally.

The first mechanism: Transformation

***mainly among G+ve mor than G-ve bacteria.

<u>Transformation "in short":</u> *if bacterial cells can absorb small segments of DNA which originated from other cells.*





The transferred foreign DNA molecules can be found in two forms: linear double helix fragments), or in form of plasmid**.

extra note: is a small DNA molecule that is physically separate from, and can replicate independently of, chromosomal DNA within a cell. Most commonly found as small circular, double-stranded DNA molecules in bacteria

-the small segments of foreign DNA often carry number of genes (maybe 1, or more) associated with new characteristics (in relation to microbial resistance or toxicity).

- The foreign gene segments/plasmid either are:
1-able to be incorporated in the bacterial chromosome (accepted by bacterial chromosome)
2-not able (not accepted by bacterial chromosome).

The first case (linear fragments), (the first picture):

1-if they are able:
-they are incorporated into the chromosome or plasmid of the bacteria
& it might cause certain new properties and features.
-becomes part of the bacterial genes and will be inherited to the new generations *.
*however this incorporation/integration might not stand forever-under certain conditions; if the chromosomal genes are "not happy!" with the foreign genes, they might be separated and rejected outside the chromosome (got rid of)

2-if they are not able:
-they will be eliminated by endonuclease enzymes.

_____

The second case (plasmid), (second picture):

_ under certain conditions, small bacterial plasmid might be incorporated but not necessarily within the bacterial chromosomes, but only in the cytoplasm …. The surrounded double helix of this plasmid allows it to survive in the cytoplasm
_ This foreign DNA might be at first linear and contains specific ''insertion sequences'' which produce pores in the cell wall and by that it will be able to reach the cytoplasm in a linear form, but due to the presence of specific enzymes that circulate the double helix …this DNA will be converted from linear to circular form (plasmid) and inherited just like the bacterial chromosomal genes during replication.

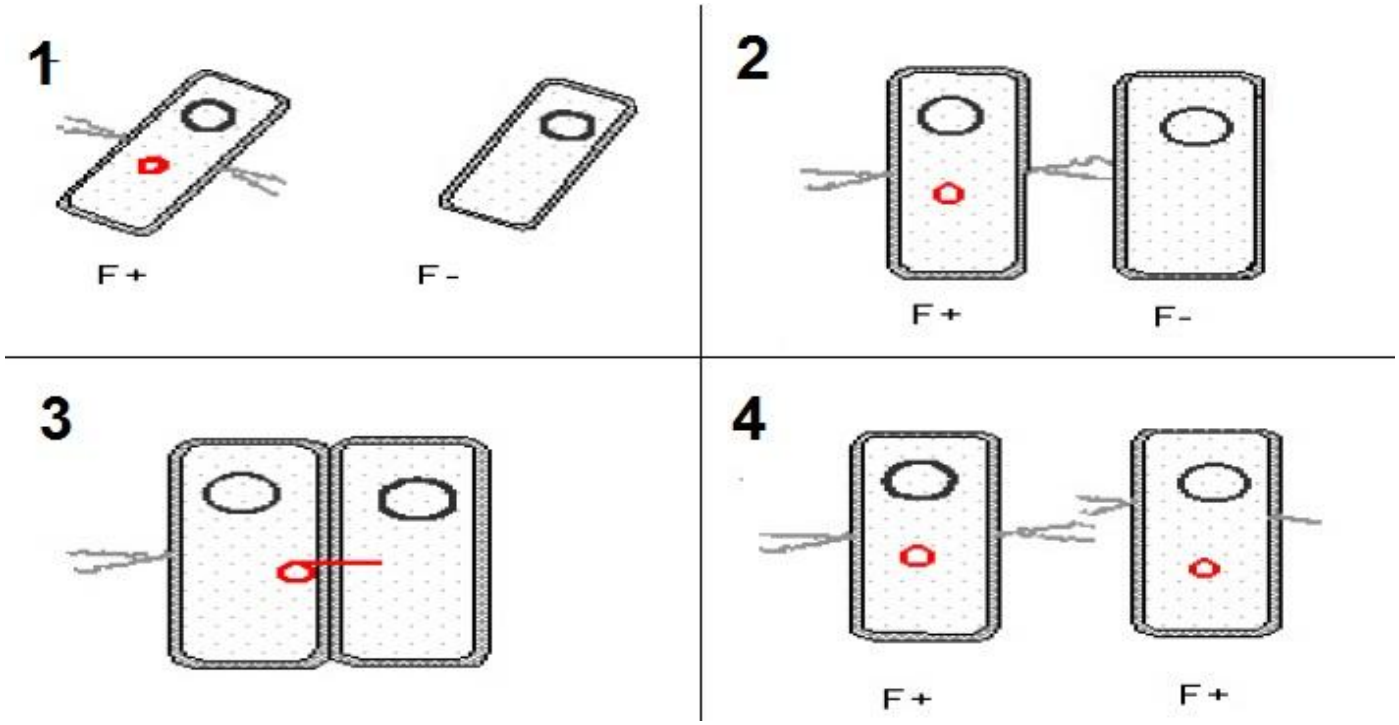
_if this plasmid carries specific genes responsible for the production of specific enzymes against antibiotics; it will convert the cell from susceptible to resistant (Against antibiotics).
-it might carry genes responsible of producing toxins, so if a bacterial cell had such a plasmid, it will become toxigenic.

#example of transformation:
_we have a gram + ,double cocciec bacteria in our respiratory tract called : streptococcus pneumonia (from few percentage to 20%),is a very important causative agent of many types of human infections especially in relation to the lungs causing " pneumonia" ,and if reaches the blood stream
it might cause "sepsis" especially in children or might reach the meninges and cause meningitis.
_the pathogenic ability is related to the capsulated form (we might find both capsulated and non capsulated forms of it in the respiratory tract).
-During growth of the bacteria, there might be a release of all components of the cell including bacterial chromosomes which means release of certain DNA segments that if they carry genes responsible of capsule forming and they were accepted by the non capsulated streptococcus pneumonia… they will become capsulated, in other words highly pathogenic.
extra note: a photo of streptococcus pneumoniae

The second mechanism: conjugation (more complex than transformation)

***it is a very imp. Mechanism for transfer of bacterial genes among G-ve bacteria, more than G+ve.

If we have ((g- bacteria that belongs to facultative anaerobic bacteria)) it will be called: enteric bacteria. These g- bacteria are found in huge numbers in our intestines…and under certain conditions; genes might be exchanging between f+ and f- bacteria.



What are F+ bacteria? Bacterial strain which carry conjugative plasmid which can donate its DNA to f-.

Hoe does this happen?

1-an F+ bacterium has a double helix of plasmid which contains few genes responsible of "pilus" formation, while F- doesn't. pilus: is like a bridge or tube where a copy of plasmid moves from F+ to F- .
2- The two cells are brought together through pilus.
3-transfer of one strand of the double helix plasmid.
4-both will synthesize a complementary strand of the plasmid.
And the F- will becomes F+ (because they've got the genes responsible of conjugation and transferring of genes (it will be able to make a pilus).

Example:

Escherichia coli (E.coli) a gram- bacteria that lives in our intestine (also animals' intestine), there would be F+ bacteria that are resistant to antibiotics (more resistant than F-; since F+ acquire resistant genes in the plasmid) in certain number , when the antibiotics are extensively used , they will form a "selective pressure"**
**extra note: Selective pressure is any phenomena which alters the behavior and fitness of living organisms within a given environment. It is the driving force of evolution and natural selection, **

in other words : antibiotics will kill most of non-resistant E.coli ,then the remaining resistant cells F+ will replicate and increase their numbers , concurrently with increasing transferring of genes responsible of being resistant against antibiotics(in form of plasmid) from F+ to F- cells ,the percentage of resistant cells_ resulting from extensive use of antibiotics _might reach 99% of total E.coli. including resistant to common antibiotics like Ampicillin.

*Conjugation is used in bioengineering; in order to manipulate certain type of bacteria to produce useful substances… very common.

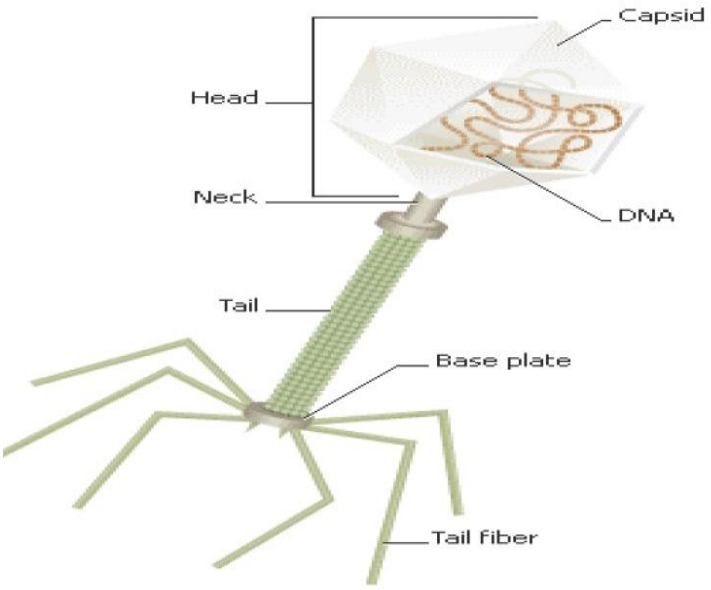
Third mechanism: Transduction

Transduction: transferring (injection) of a genetic material (DNA or RNA) from bacteriophage into bacteria cell.

"bacteriophage" (Informally "phage") : a virus (DNA or RNA) infects bacteria.

_the phage nucleic acid is packaged with phage-encoded proteins that determine phage structure and interacts with specific receptors on the bacterial cell surface to initiate infection.
-specificity : each phage requires the presence of a particular receptor on the bacterial surface, so bacteria cells that lack the specific receptor to a specific bacteriophage is immune to infection by that

bacteriophage.



_the structure of *E.coli bacteriophage:

1-head: there is a capsid containing nucleic material (DNA or RNA), (E.coli phages contain DNA)
2-a tail and tail fibers provid attachment to bacteria)

*not all bacteriophages have this structure, some are composed of only head without tail and tail fibers and some are composed of head and 1or 2 tail fibers .

In transduction there are two methods of viral reproduction:
1-lytic cycle (virulent phages).
2-lysogenic cycle (temperate phages).
The key difference between the lytic cycle and the lysogenic cycle is that the lysogenic cycle does not lyse the host cell.

Phages that replicate only via the lytic cycle are known as virulent phages while phages that replicate using both lytic and lysogenic cycles are known as temperate phages.

1-lytic cycle: responsible for killing the cell and production of new generation of viruses.

-in brief: attachment >injection>replication>lysing of bacteria and huge number of bacteriophages produced.

Steps:

A-Attachment (in tens & hundreds) through fiber tails (attachment is specific)

B- Production of specific enzymes to allow the injection of the N.A into the cytoplasm of these bacteria, only the nucleic material will enter ,the remaining of its structure will stay outside the bacteria.

To infect a cell, a virus must first enter the cell through the plasma membrane and (if present) the cell wall. Viruses do so by attaching to a receptor on the cell's surface. The virus then releases its genetic material (either single- or double-stranded RNA or DNA) into the cell. In doing this, the cell is infected.*

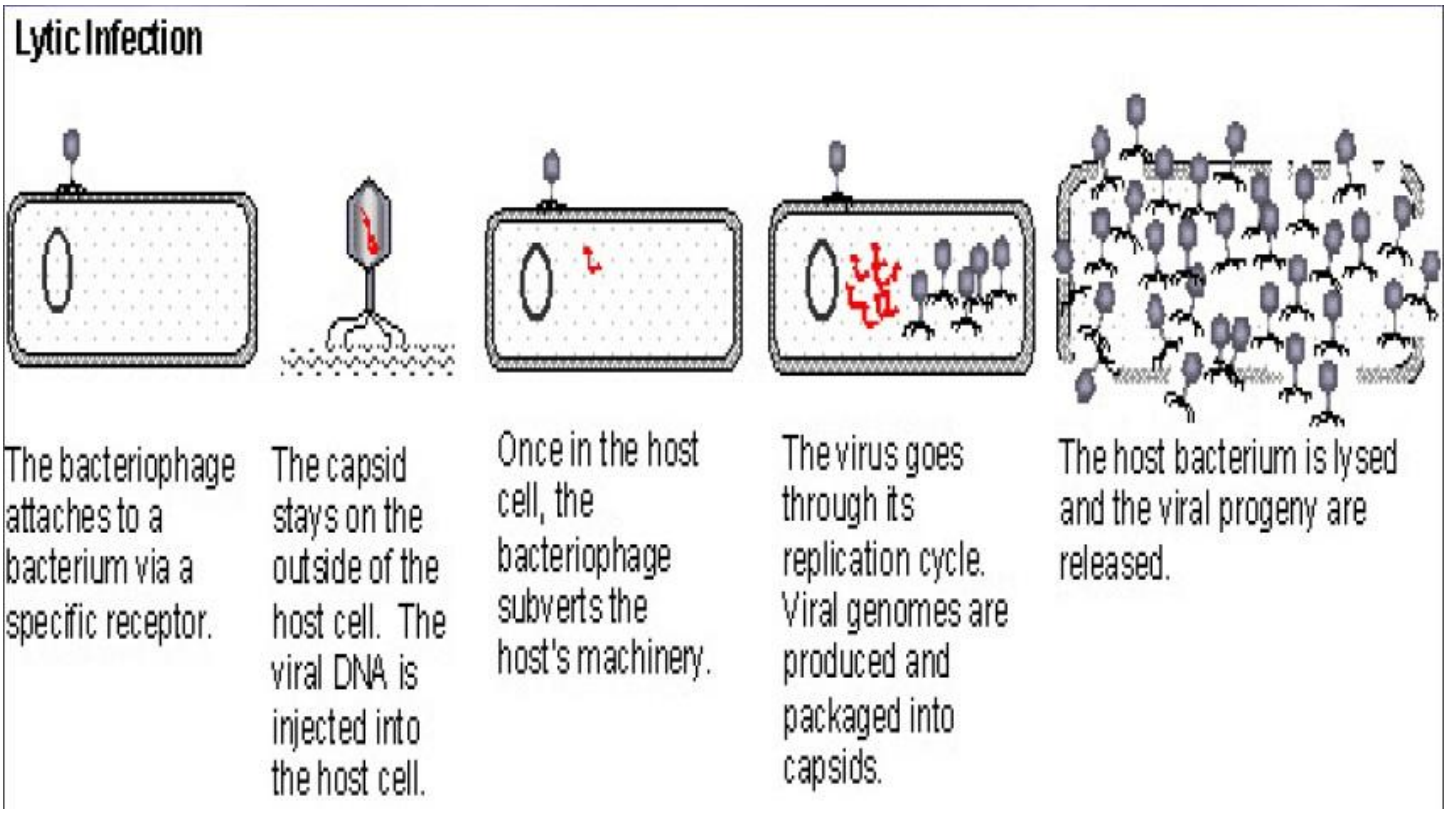
*remember that either gram+ or gram- undergoes transduction equally.

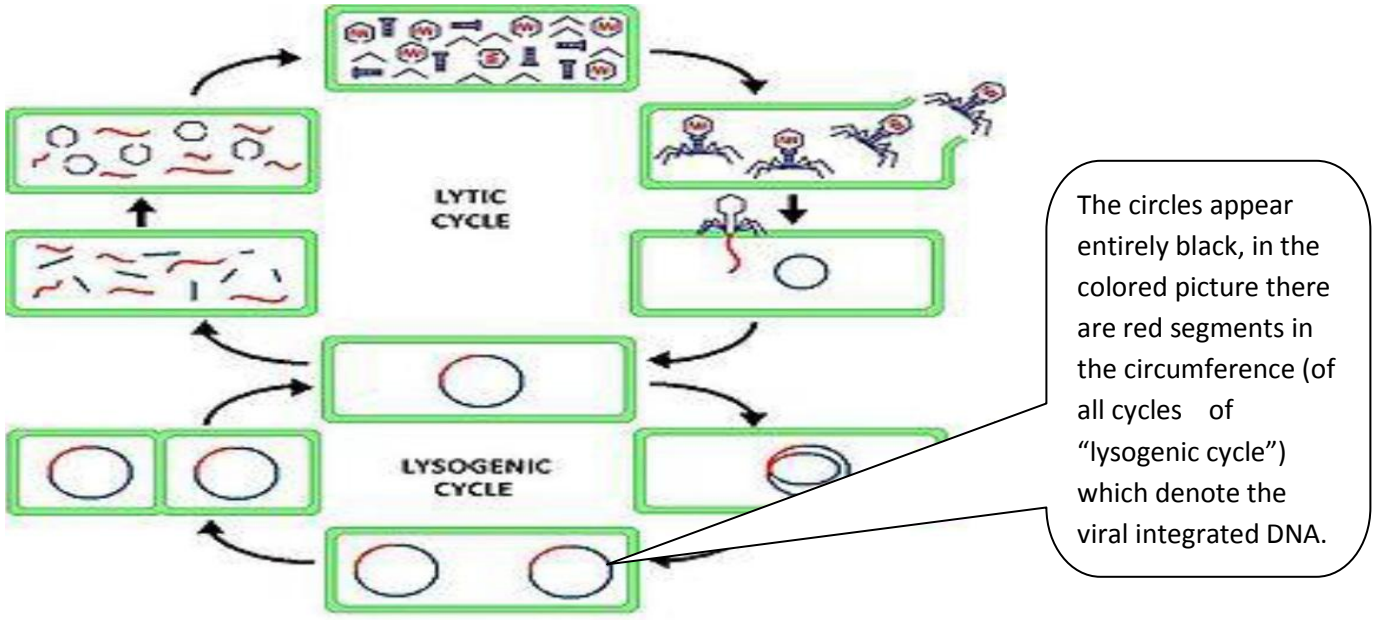
C- Once the DNA -4 example- enters the cell, it begins to produce different necessary enzymes which attack directly bacterial chromosome(making it in segments) and begin to control all metabolic activities inside bacterial cells; so instead of producing components for the production of new bacterial cells; the cell will produce all components necessary for production of new bacteriophage.

D- Within short period (usually few hours), the infected cell will be full of bacteriophages.

E- Death of the cell and releasing thousands of phages.

Why does the cell undergo lysing /bursting??





1- bacteriophages will increase the osmotic pressure inside bacterial cell.
2-bacteriophages produce specific enzymes that can cause lysis to cell membrane and cell wall.

2-lysogenig cycle:
incorporation of the bacteriophage nucleic acid into the host bacterium's genome(chromosome)/ become part of the infected bacteria.

Only DNA/RNA segments (RNA segment will be transcribed and converted into DNA in the lysogenic cycle), so only DNA segments will enter the circular bacterial chromosome & become part of it.

Information:
-prophage: is a viral genome inserted and integrated into a circular bacterial DNA >
-the integrated viral DNA will be inherited to the new generations.
-DNA of the infected bacteria has a specific region that accepts the foreign DNA to be incorporated.
-lysogenic cycle may stand for along period (weeks or months).
-Under certain conditions, the bacterial cells might get rid from these incorporated viral DNA/get excluded to the cytoplasm; which means that the lysogenic cycle becomes lytic resulting in lytic reactions.
- Since the prophage contains genes, it can confer new properties to the bacteria, including new end products.

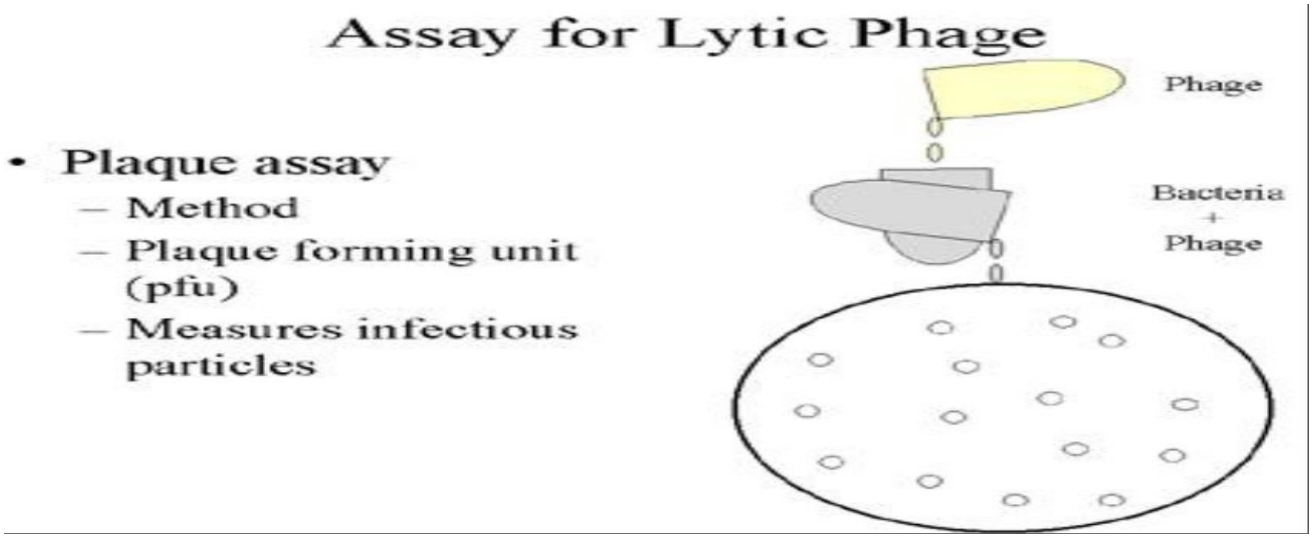
?what is the significance of "temperate phage" concerning human diseases?
(The answer was given as an example).
#example:
we have in our respiratory tract a type of bacteria called corynebacterium diphtheriae (gram+) which is a causative agent of diphtheria ,corynebacterium diphtheria are found in 2 forms:

1-toxigenic (pathogenic) , which is the lysogenic form carrying a specific bacteriophage (which has genes responsible for the production of the toxins of the bacteria).

2-non-toxigenic –lack exotoxins-

-The toxigenic type produces exotoxins (bacterial toxin), the toxins will be absorbed by the mucosa, reach blood stream and finally when it reaches the heart it will cause damage to heart muscles called "myocarditis", then more complications will happen, at the end it will cause death.

-if the non-toxigenic and the toxigenic met at the respiratory tract, the non-toxigenic will be converted to toxigenic type, due to gene transfer; since the toxigenic bacteria are infected with the temperate phages causing their toxicity.
-as mentioned before there will be new characteristics and end products, due to presence of the viral genes incorporated/integrated inside bacterial genome.



Assay for *lytic* phage :

How can we detect the presence of lytic phages (lytic bacteriophages) ?
The method of detecting Escherichia coli (E.coli) bacteriophages (gram-):

1-isolation then culturing of E.coli until we get a heavy growth on the surface of the agar.
2-we obtain polluted water (sewage), we apply water to feltration (to obtain viruses) in order to detect the presence of bacteriophages specific for E.coli.
3-we put few dose of the viruses in the culture media, incubation 37o 24-48 hours.

after 1-2 days ,we see the results>> if we find holes (as appear in the picture above) that indicates that the lytic specific bacteriopghages are present, and the holes are due to killing E.coli by the specific bacteriophages (the holes are free of E coli cells; lysed)

In relation to temperate phage; we have to use molecular technique to recognize if the bacterial chromosome has acquired the prophage (the part of the phage which has been integrated into the bacterial chromosome)…The principle fact that is used in the method:
is that the prophage is not highly stable, and the bacteria tries to get rid of any foreign DNA to conserve its own only, but sometimes it has to co with the DNA for a while till it get rid of, phages can be found present in the culture due to spontaneous lysis of a small number of cells (foreign DNA rejected to the cytoplasm…lysogenic is converted into lytic cycle)

Back to sterilization and disinfection:

there are 2 methods to control microbial growth:
1-Physical control/ methods, related to heat, radiation, etc…

2-Chemical control/ methods

Both are used to get rid of majority of pathogens.

_ **Aim of disinfection** : to kill pathogens up to high percentage, ie, we cover the majority of pathogens except very few numbers particularly A)Those of the spore forming bacteria; which are imp. to cause imp. diseases. B) Certain viruses, like hepatitis virus & HIV. C) Certain fungi… All these previous categories cant be killed by chemical methods, we have to use the physical methods.

-In order to apply any successful disinfection methods, you have to apply on clean surfaces or skin…
Don't begin to use alcohol/ iodine without at **first** washing the hands/ surface with water: Why?
- Water ensure high effectiveness in reducing the number of pathogens to the safe level.

Second: there are disinfectant agents used only for inanimate objects & others only for animate obj.

Some Disinfectant/antiseptic agents:

(some of them can be used as antiseptic and also as disinfectant)
1-alcohol,ethyl alcohol (ethanol)

2- (aqueous iodine, tincture iodine, betadine (commercial organic iodine)

3-chlorhexidine ,cetrimide ,savlon
4-chlorine gas, Na-hypochlorate.

5-oxidizing agents : ozon O_3 and hydrogen peroxide H_2O_2.

6-phenol compounds:hexochlorphene,detol.

7- organic acids

1-Ethyl alcohol: (often used as antiseptic (applied on skin 4 example), but also can be used as disinfectant)
in order to have an effective alcoholic agent it must be mixed with water:
>water makes alcohol more chemically active.
>water reduce the rate of evaporation : 100% ethyl alcohol(ethanol) applied on a surface will evaporate within 1 minute, on the other hand if we applied (70-80) % ethyl alcohol the evaporation will take 2 minutes, one more minute is important to have more effective killing of pathogens.
>water will enhance penetration of (OH) group of alcohol into the cell wall of bacteria, resulting in oxidizing process.

2-aquous iodine (used like ethanol alcohol), 3-5 % mixed with water, if the percentage of iodine is higher, it will stain the skin. (Antiseptic)

-Tincture iodine: very useful iodine form, due to mixing with alcohol, 3 gm of iodine in 100 ml of alcohol, also called: Alcohol-Iodine solution… more effective.

A small percentage of the population develops an allergic reaction (in skin or oral cavity) if treated with aqueous iodine, so they created an:

_ Organic iodine solution called betadine (I associated with a chemical compound) having the same efficiency but less associated with allergic reaction & damage in the mucosa.

Applying iodine antiseptic: you have to apply at least for 2 minutes before beginning with any invasive technique, so you cannot inject in a surface which iodine has just been applied on it, you have to wait 2-3 minutes.

3-cholrohexidene,cetrimide,salvon.(the Dr talked about them all together)
used as disinfectant agents >walls, floors.. etc
also can be used as a skin antiseptic with adults (low concentration) to give quick result, mustn't be used with babies; there will be an allergic reaction .

4- chlorine gas, Na-hypochlorate.
Preface:

-tap water is considered to be free of pathogens (specially those that cause GI diseases).

-in order to have healthy tap water you must get rid of any type of enteric pathogens and it is not easy to test the presence of all types of enteric pathogens in a sample of water (there are many: salmonella, cholera ,parasites, viruses…) , instead, you might have only 1 type of bacteria called indicator type of bacteria : (E coli)(lives in the intestine of humans and animals).

-One single cell of E-coli per 100ml means that water is not fit for consumption, because it's a bacterium that is originated from feces of human or animal, which may reach water by direct animal contamination, so it indicates (might be) an increased number of harmful pathogens in the water.

-chlorine gas and Na-hypochlorite are used in treatment of water(to kill most common pathogens in water)(the only cheap way), but: not as a first step, there are stages that precede applying these agents such like: filtration—sedimentation—then chlorination is the last step (disinfection step).
When we start chlorination… Na-hypochlorite is added 2-3part/million (ppm) which equals 2 gm of Na-hypochlorite in m^3 of water

Na-hypochlorite once reaches water; it will release free active Cl that will interact with bacterial cell wall & produce killing effects.

-the conc. of chlorine in water that reaches our houses should be less than 0.5 ppm ,or also it will have a taste,(changing coffee taste and other …),change hair color and cause an allergy in the eyes.

5-oxidizing agents: ozone and hydrogen peroxide.
O_3: very effective/ active O, can be used in water, kills almost all types of bacteria & parasites.

H_2O_2: 3% (accepted in low conc.) solution is used as antiseptic for skin and wounds in the epidermis and dermis, not deeper, mustn't reach subcutaneous tissue(even if there was a deep wound); because it's very strong oxidizing agent then it will cause damage,(only surface injuries).

6-phenol compounds.
Like dettol ,a commercial disinfectant agent, it's used as a household cleaner, mustn't be used in relation to our oral cavity or skin ,and mustn't be applied for disinfections on food (ex. Vegetables),to disinfect vegetable >>by water but also lactic acid and citric acid can be used.

7- Organic acids.
Sorbic acid and benzoic acid: used in conservation of food.

_Washing detergents (like: soap, shampoo, washing powder) work with the same mechanism:
-they contain phosphate group ($PO4^{-3}$) associated with cationic/anionic compound, the ions interact with all type of proteins and carbohydrates >>produce a larger molecule that can be washed by water.
((This is the principle for washing machines))

-Water:
-hand washing is a simplest way to prevent spread of infection and it's safe.