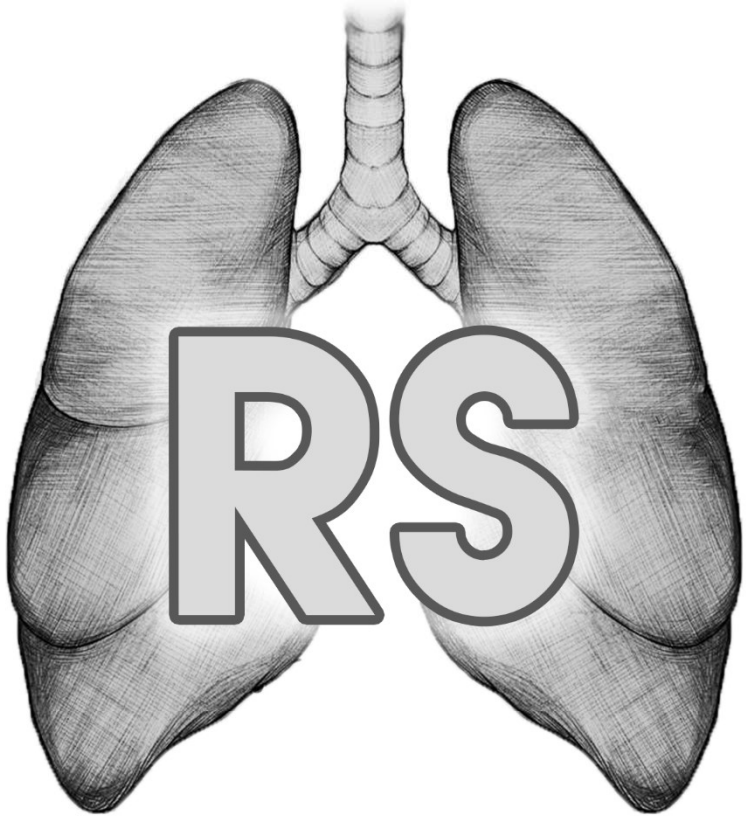

MICROBIOLOGY

☑ Sheet ○ Slide ○ Handout

Number

5

Subject

Orthomyxoviridae (Influenza virus)

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Orthomyxoviridae

When we talk about Orthomyxoviridae, we are mainly talking about **Influenza virus**

- Acute respiratory tract infection that usually occur in epidemics
- Self-limiting infection (it heals alone by itself).
- Named influenza because of the influence of cold air in winter in carrying the virus.
- Human influenza have 3 types: A, B & C classified according to different RNA antigens

	Influenza A	*Influenza B*	*Influenza C*
Number of segments	8	8	7
Host range (It could infect):	Humans & animals (birds, pigs, cattle, fish,..)	Humans	Humans & pigs
Unique structural protein	**M2 protein**	NEP/NP proteins	NEP/NP proteins
Severity of symptoms	Most severe	Intermediate	Mild / Asymptomatic (subclinical infection)
It's susceptibility to antigenic changes	Most susceptible (Unstable)	Less susceptible (Stable but can change)	Least susceptible (Most stable)

- Influenza A virus is subjected to major antigenic changes that cause major world-wide pandemics when a new subtype of Influenza A virus is found. Between these pandemics, small epidemics are scattered in different locations between 2-3 years.

  e.g.) In 1918, an antigenic shift caused for a new Influenza A subtype -(H1N1) Spanish flu- to appear, resulting in a pandemic that killed over 20,000,000 people.

Epidemic: The increase of number of affected people to more than normal ranges in a specific geographical area at a certain point of time. *e.g.) Influenza in winter in Jordan.*

Pandemic: The increase of number of affected people to more than normal ranges at a certain point of time globally (the graphical region is not confined to a certain area).

Swine = Pig

Avian = Birds

- What are these antigenic changes?
  - Antigenic drift & Antigenic shift (reassortment).

Normally, we have a level of antibodies that can fight Influenza virus

➢ *Antigenic drift*

Point mutations can occur in Influenza due to the presence of an enzyme that lacks proofreading, which is "RNA-dependent RNA polymerase" and they can happen in any part of the virus (cased, envelope, spikes, RNA polymerase, etc..).

(A mutation is introduced every 2,500-10,000 bps)

Upon accumulation of mutations in any part of the virus, the shape of that part will be changed so that the immune system will find it harder to fight it

- *e.g.) Changing the shape of spike due to mutations will reduce the ability for our antibodies to bind it from 80-90% to about 60-70%, and thus, leading to an epidemic.*

Large epidemics due to antigen drift occur every 3-5 years.

- ✓ Most of viral replication is defective viruses.
- ✓ If a given geographic area had an incidence of 100 infected people in a certain year, an epidemic due to antigen drift (mostly in winter) might increase the incidence up to 1000 and even more, depending on the amount of antigen drift.
  (don't memorize these numbers, this is just an example).

➢ *Antigenic shift (Reassortment)*

A single cell can be infected by more than 1 type of influenza (e.g. Human & Avian influenza, Human & Swine influenza, etc..) → Commonly occurs in pig's respiratory tract. → Each of the viruses will release their genome into the cell, replicate it and produce its structural proteins → Upon assembly; mixture of segments might assemble into a new virus

They can happen in any part of the virus (capsid, envelope, spikes, RNA polymerase, etc..) **BUT** we start to significantly feel the infection symptoms when the most antigenic parts of the virus which are the spikes (glycoproteins) are assembled.

Why are spikes the most antigenic part?

- Because they are the outermost part and most susceptible to the immune system.
- *e.g.) Antigenic shifts of spikes will cause its shape to change **completely**, so that our antibodies won't be able to bind them at all! And new antibodies will be produced, but that will take time, so you will notice that this shift hast lead to a world-wide pandemic, leading to a lot of morbidities & mortalities.*

*(If spikes are acquired from either **swine** or **birds**, it will be associated with pandemics).*

Outbreaks/Pandemics due to antigen shift occur every 5-10 years.

We conclude that antigenic shifts are more severe than antigenic drifts.
Drifts > epidemics Shifts > pandemics

More about Influenza virus

- The genome is single-stranded RNA
- Enveloped, segmented (8 segments to which protein capsomeres are attached)
- Spherical virus
- Capsid is helical
- It has 2 surface glycoproteins (spikes); (Haemagglutinin & Neuraminidase)
- Each of the segments encode a certain viral protein

Spikes (Surface glycoproteins)

Haemagglutinin (HA or H)

- Function: It binds to receptors on the target cell, facilitating the virus entry;

  It can bind specific receptors in the upper respiratory tract & receptors on RBCs.
- Derived its name from its ability to agglutinate erythrocytes.
- Encoded from segment 4
- There are 18 types of HA, the last one was discovered in 2013.
- H1, H2, H3, H5, H7 are found in humans. The rest are found in different animals.
- **H1**, **H2**, **H3** are the most important

Neuraminidase (NA or N)

- Function: It can facilitate the binding of the virus to the target cell by enhancing the fusion of the viral envelope with the host's cell membrane just like HA,

  but **main function** is to facilitate the release of the virus after the end of its life cycle by breaking the bonds between haemagglutinin & receptors of the target cell.

  It also inactivates a free mucoprotein receptor in respiratory secretions.
- Mushroom-shaped
- Encoded from segment 6
- There are 13-14 types of NA.
- **N1**, **N2** are the most important

➔ **These surface proteins can undergo antigenic variation independent of each other.**

➔ **These glycoproteins can be used in the naming of different strains of viruses**
e.g.) H1N1, H5N1, etc..

How are Influenza viruses named?

Type of influenza (A,B, C) → If it's human or animal influenza (we don't mention if it's human) → Geographical location (where it was first isolated) → Strain number → Year of isolation → Haemagglutinin & Neuraminidase numbers.

e.g.) A/ Hong Kong/ 1/68 (H3, N2). ***(Notice here that the word "human" was not mentioned)***

A/Swine/New Jersey /8/76 (H1, N1). ***("Swine" is mentioned because it's an animal virus)***

***Don't memorize these examples, just understand the idea* :D**

❖Avian influenza A virus (H5N1) – إنفلونزا الطيور

- The first documented infection of humans by this virus occurred in: 1997 in Hong Kong.
- The source was domestic poultry (جاج)

  Back then when the pandemic has appeared, all the poultry were killed in order to eradicate the virus and stop the pandemic.
- All the 8 segments of this virus are from avian origin (no human backbone)
  So, it cannot be transmitted from human to human; it jumped across different species.
- When a strain of virus jumps across different species, it will cause a pandemic of a more pathogenic infection causing a lot of mortalities.

✓ The general rule says that most of these viral strains are a result of antigenic shifts (reassortments), BUT Avian influenza A is transmitted across different species, so it's **NOT** a result of an antigenic shift.
✓ All human pandemic strains have been reassortments between avian and human influenza viruses. ***(except** this outbreak of Avian influenza A in 1968 in Hong Kong)*.

Other viruses that jumped across species:

- SARS coronavirus – from cats to humans.
- MERS coronavirus – from camels & bats to humans.

Back to talk generally about Influenza…

Pathogenesis

- Transmission: by aerosols/droplets as a result of sneezing or coughing in a confined space
- Replicates in the RS tract, leads to desquamation (shedding) of mucus-secreting & ciliated cells
- The symptoms include: high-grade fever, runny nose (rhinorrhea & rhinitis), sneezing, coughing, general fatigue, arthralgia, myalgia, ocular symptoms.

  Note: Conjunctivitis (red eyes) is associated with adenovirus more than influenza virus.
- As a general rule, viral infections can cause a transient drop in immunity; therefore Influenza can lead to superimposed infections with bacteria **or** infect the lower RS tract (pneumonia).

The doctor talked about some simple information that we all know about the immune system:

- Innate immune system

  It's the first line of defence; it prevents the spread of the virus and it consists of: Macrophages, NK cells, Cytokines, **Interferons**, Cilia & IgA in mucus of the respiratory tract, etc..
- Adaptive immune system
  - Cell-mediated: Cytotoxic T-cell kills the cell b producing perforins & granzymes
  - Humoral: B-cell → Plasma cells (that produces antibodies) & Memory B-cells

- Cytotoxic T-cells recognize intracellular antigens presented by MHC I (which is present in all our body cells) through the binding of CD8.
- MHC II is not present in our cells; it's just present on the surfaces of APCs. APCs engulf extracellular antigens and present them on MHC II and then recognized by CD4 of helper T-cells.
- Upon binding of Th0 to MHC II, it will be differentiated into either:
  - Th1 cell → further activates cytotoxic T-cells (cell-mediated immunity).
  - Th2 cell → further activates plasma cells to produce more antibodies (humoral immunity).

Virulence factors

- Ability to infect lower respiratory tract
- A strong induction of pro-inflammatory cytokines & chemokines (cytokine storm)
- Apoptosis induction
- Systemic infection
- Evasion of innate immune response (IFN)

Figure 1 shows normal respiratory tract with cilia.

Figure 2 shows the respiratory tract after 3 days, acute lytic infection caused the death of cilia.

➔ The cilia require 6-8 months in order to grow back to normal.

Clinical findings

- It's more severe in immunocompromised patients (very young & elderly people) and in people with heart or lung diseases.
- ***Pulmonary complications:***
    1) Croup (Acute laryngotracheobrochitis)
    2) Primary pneumonia
    3) Secondary bacterial infection (S. pneumonia, Staph. aureus or H. influenza)

Croup is mainly caused by parainfluenza, but it can be caused by other viruses such as influenza.

- ***Non-pulmonary complications:***
    1) Myositis (rare)
    2) Cardiac complications (rare)
    3) Encephalopathy (rare)
    4) Reye's syndrome (Liver & CNS)
    5) Guillian-Barré syndrome (PNS)

Reye's syndrome

Hepatoencephaloathy; fatty depositions in the liver & brain

Characterized by: brain edema, vomiting, lethargy, coma.

Seen mostly in children due to treating fever by Aspirin.

It used to be common, but it's rare now due to the presence of other drugs that can treat fever.

Diagnosis

It's a clinical diagnosis (based on the clinical picture of the patient);
This has to do with the season (more in winter), symptoms & the signs that the physicians notice.

Procedures like (Isolation, Serology by ELISA, complement fixation, haemagglutinin inhibition) are used only in cases of pandemics/outbreaks. *(Once the virus is identified, these procedures are not needed anymore).*

Treatment of Influenza

Influenza is a self-limiting disease; it can heal alone.

Symptomatic treatments are routinely made such as: Vitamin C, Chamomile (بابونج), Antipyretics (for fever), Antitussives (for cough), Decongestants (for runny nose), and the most important one is bed rest.

Only in severe cases, we use anti-viral drugs such as:

- Amantidine
- Rimantidine
- Oseltamavir (Tamiflu)
- Zanamavir

➔ **Amantidine** & **Rimantidine** are used in treating Influenza A, because they work on the uncoating step, specifically at the M2 protein.

➔ **Oseltamavir** & **Zanamavir** are *Neuraminidase Inhibitors (NAIs)*; They are used against Influenzas A & B, they reduce number of viruses that are released by the infected cell (counteract the action of neuraminidase).

> Just like bacteria, also viruses can develop resistive strains against anti-viral drugs!
>
> So, always take the right doses and in sufficient amounts (it must maintain its therapeutic index) to prevent development of resistant viruses.
>
> ✓ There is 90% resistance against Amantidine & Rimantidine.

➢ Prophylactic treatment: it's given to family members in case one of the family is infected & in case of an outbreak/pandemic.

Prevention

How can we prevent the influenza virus from spreading?

- Prophylaxis with anti-viral drugs as we mentioned.
- Vaccines (3 types of human vaccine; A, B, C)
  - If you take the vaccine, you can still be infected with influenza in the same year! Why is that? It's because of the antigenic drifts & shifts that lead to development of new strains of influenza virus yearly.
  - These vaccines are produced according to our previous knowledge, depending on the geographical region; some countries kept track and studied strains that caused pandemics in previous years. There are WHO recommendations for the northern hemisphere & southern hemisphere for influenza vaccine.
  - Trivalent vaccines (2 A strains, 1 B strain) were usually used
  - Nowadays, Quadrivalent vaccines (2 A strains, 2 B strains) are being used.
  - Today's vaccine has the strains of the pandemics of 2013, 2011, 2009 (H1N1).
  - 2 forms of vaccine: **Intranasal** (Live-attenuated)
    & **Intramuscular (IM)** that is given in the deltoid muscle.

➔ **How is the live-attenuated (intranasal) vaccine prepared?**

1) We bring glycoprotein segments from the 2 A viruses & 1 segment from the B virus
2) We mix all these 3 in a new virus (once it replicates it will express glycoproteins of those 3 viruses)
3) We allow this virus to replicate in a chicken's egg at a temperature of 34-35 °C incubator
4) We reduce the temperature into optimal temperature (24 °C), this will weaken the virus, although it will stay alive to replicate & infect but NOT cause illness.

(You should ask the patient if he has allergy from eggs before giving him the vaccine because they might get an anaphylactic shock. Some patients have a little allergy that they will only develop hives (طفح جلدي) these are given the vaccine but with caution).

- ✓ Live-attenuated vaccines are contraindicated in immunocompromised patients.
- ✓ **WHO recommendation (2016/17): Never give the live-attenuated vaccine, because there are big question marks on its efficacy.**

➔ **How is the injectable vaccine prepared?**

They might use the same procedure if live-attenuated vaccine or they allow the A viruses alone & the B virus to grow alone, then they combine them together and form a lean treat (dead) virus.

- Once the virus is injected, the virus will break and release all its antigens, although our body will mainly produce antibodies against the glycoprotein antigens.

When is the vaccine given?

A new influenza vaccine is developed every year, so once it's released it can be given.

In the USA, they start giving it in July, but the best time to give it to get the maximum benefit is in (August, September, October).

 End of text, good luck! :D