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How can antibiotic resistance be reduced?

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Abstract:

Antibiotic resistance is one of the most challenges face humanity in current time and future which requires exerting more global efforts to overcome it. By 2050, the UN estimated that up to 10 million people deaths could be happened due to antimicrobial resistance, matching the annual global death toll of cancer. There is a great possibility that curable diseases cannot be treated with antibiotic which remembered us a period before discovering of antibiotic when infections were not treatable. It's very important to recognize factors that make bacteria to be more resistance to current antibiotics. Right diagnose and differentiating between viral and bacterial infections is a corner stone in fighting against this international predicament. Random using of antibiotic by people without prescription and giving them by health care provider despite there is no clinical basis which support therapeutic cases, poses a tremendous problem. This article will focus on how antibiotic resistance bacteria arise, and how unscientific prescription of antibiotics by health care provider worldwide aggravate and hinder efforts to contain this international crisis, and how can laboratory tests clinically help decrease faulty giving of unnecessary antibiotics to patients.

Introduction:

Antibiotic resistance may arise as a native to microorganism or through horizontal transfer gene. When antibiotics discovered in 1900 world thought the war against bacterial infections had been finished, but this idea was not correct when scientists showed that microbes have evolved mechanisms to avoid or escape the mechanisms of action of antibiotics which including:

1-Inhibit cell wall synthesis

2- Inhibit protein synthesis,

3- Inhibit nucleic acid synthesis,

4-Inhibit metabolic process in bacteria.

Overconsumption of antibiotics- by people without prescription with improper using, adding antibiotics to the food of animal, improper prescribing of antibiotics by health care provider and choosing broad- spectrum antibiotics as a first option, or lack of fundamental scientific basis for intended microorganism -poses main factors which will lead to increased bacterial resistant.

Consequences of antibiotics resistance are tremendous including increasing morbidity and mortality rate, increase recovery time and hospitalization, poses financial burden on health care institutes. In the united state 30% to 50% of antibiotic prescription are given for viral infections which results in 2 million people became ill each year by bacterial resistant infections, causing more than 23000 deaths 1.

As example of healthcare costs regarding resistant infections, methicillin-resistant *Staphylococcus aureus* (MRSA) infection may cost 18000 dollar per case in US, in Germany the costs are nearly 9000 dollar, in Switzerland it may cost over 100,000 Swiss francs per case. Some of health care provider prescribes antibiotics empirically to exclude possibility of any bacterial infections which contribute to the spreading of bacterial resistance.

To overcome the increase in bacterial resistance health care provider prescribes two or more drug with different mechanisms of action.

We will shed light more on the roll of national institutes of health and private medical clinics to follow scientific background in prescribing antimicrobial agents which contribute ultimately to aid in the fighting of this crisis

Bacterial resistance.

Bacterial resistance can be divided into natural and acquired resistance. Species of bacteria naturally may have intrinsic resistance or induced through endogenous genes which expressed by exposure to an antibiotic. Independent of horizontal gene transfer and previous antibiotic exposure, intrinsic resistance is a trait that is shared within bacterial species 2, 3. Reduced permeability of the outer membrane and natural activity of efflux-pump are most common mechanisms of action of intrinsic resistance 3, 4, table below show example of some bacteria with intrinsic resistance.

Examples of bacteria with intrinsic resistance.

Organism	Intrinsic resistance
Bacteroides (anaerobes)	aminoglycosides, many β-lactams, quinolones
All gram positives	Aztreonam
Enterococci	aminoglycosides, cephalosporins, lincosamides
Listeria monocytogenes	Cephalosporins
All gram negatives	glycopeptides, lipopeptides
Escherichia coli	Macrolides
Klebsiella spp.	Ampicillin
Serratia marcescens	Macrolides
Pseudomonas aeruginosa	sulfonamides, ampicillin, 1st and 2nd generation cephalosporins, chloramphenicol, tetracycline
Stenotrophomonas maltophilia	aminoglycosides, β-lactams, carbapenems, quinolones
Acinetobacter spp.	ampicillin, glycopeptides

Acquision of outside genetic material by horizontal gene transfer is the main method of acquired resistance by which bacteria become resistant to antimicrobial agent. Mutation of bacterial own chromosomal DNA through interaction with environment and internally by insertion sequences , integrins which may move genetic material outside, and internally when bacteria subject to stressor such as starvation, UV radiation, chemical, etc.

Antimicrobial groups based on mechanism of action.

Mechanism of Action	Antimicrobial Groups
Inhibit Cell Wall Synthesis	β-Lactams
	Carbapenems
	Cephalosporins
	Monobactams
	Penicillins
	Glycopeptides
Depolarize Cell Membrane	Lipopeptides
Inhibit Protein Synthesis	Bind to 30S Ribosomal Subunit
	Aminoglycosides
	Tetracyclines
	Bind to 50S Ribosomal Subunit
	Chloramphenicol
	Lincosamides

Mechanisms of bacterial resistance

There are 4 main methods bacteria use to resist antimicrobial agents:

1- Limiting uptake of a drug
2- Modifying a drug target.
3- Inactivating a drug.
4- Active drug efflux.

Antimicrobial resistance mechanisms.

Drug	Drug Uptake Limitation	Drug Target Modification	Drug Inactivation	Efflux Pumps
β-Lactams	Decreased numbers of porins, no outer cell wall	Gram pos—alterations in PBPs		
Carbapenems	Changed selectivity of porin		Gram pos, gram neg—β-lactamases	RND
Cephalosporins	Changed selectivity of porin			
Monobactams				
Penicillins				
Glycopeptides	Thickened cell wall, no outer cell wall	Modified peptidoglycan		
Lipopeptides		Modified net cell surface charge		
Aminoglycosides	Cell wall polarity	Ribosomal mutation,	Aminoglycoside modifying enzymes,	RND

Drug	Drug Uptake Limitation	Drug Target Modification	Drug Inactivation	Efflux Pumps
		methylation	acetylation, phosphorylation, adenylation	
Tetracyclines	Decreased numbers of porins	Ribosomal protection	Antibiotic modification, oxidation	MFS, RND
Chloramphenicol		Ribosomal methylation	Acetylation of drug	MFS, RND
Lincosamides		Gram pos—ribosomal methylation		ABC, RND
Macrolides		Ribosomal mutation, methylation		ABC, MFS, RND
Oxazolidinones		Ribosomal methylation		RND
Streptogramins				ABC
Fluoroquinolones		Gram neg—DNA gyrase modification	Acetylation of drug	MATE, MFS, RND
		Gram pos—topoisomerase IV		
Sulfonamides		DHPS reduced binding, overproduction of resistant DHPS		RND
Trimethoprim		DHFR reduced binding, overproduction of DHFR		RND

Gram negative bacteria can use all of these mechanisms, but gram positive bacteria less use limiting uptake of A drug and active drug efflux 5,6. Figure 2 can illustrate antimicrobial resistance mechanisms.

Gram negative bacteria have lipopolysaccharide cell wall layer which provide natural resistance against large molecules. Mycobacterium have high lipid content of cell wall so antimicrobial agent which is hydrophobic such as rifampicin has a good penetration to the cell wall, but hydrophilic drugs have limited access 7,8. Gram positive bacteria lack outer cell membrane and therefore this mechanism is not provided, Beta lactams and glycopeptides are example of drugs that are resistant to them because of lacking cell wall 9.

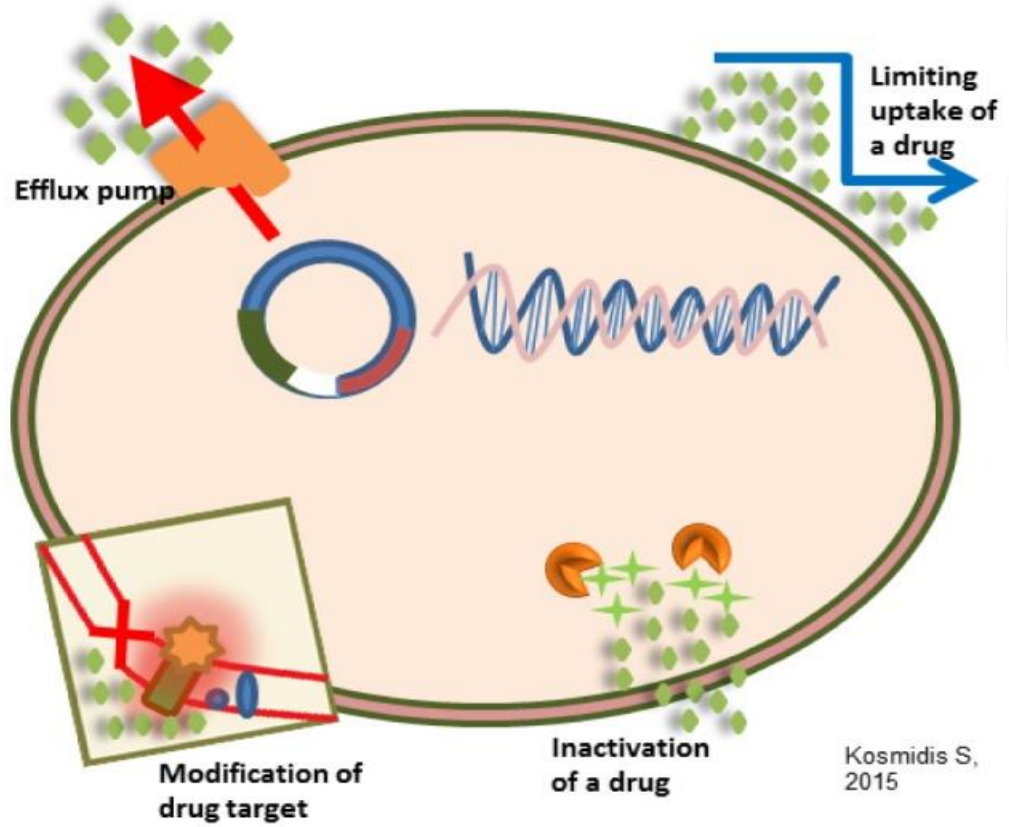

Figure 1

General mechanisms of antimicrobial resistance.

Bacterial cell wall contains places used by bacteria to resist antimicrobial agents through modifying them by alteration in the number and or structure of these targets. One of the most important drug targets is penicillin binding protein PBP. By changes in number of PBP, the amount of drugs that bind to PBP decrease and bacteria become resistant to those antibiotics. Alteration in structure of PBP 2a Staphylococcus aureus by acquisition of the (*mecA* gene) may decrease the ability of the drug to bind, or totally inhibit drug binding 10,11.

Via ribosomal subunits mutation, the resistance to drugs that target these sites may occur. Tetracycline, aminoglycosides and macrolides are example of these drugs.

Interfering with nucleic acid synthesis is another method of resistance by modification in DNA gyras (gram negative bacteria—e.g. *gyrA*) or topoisomerase IV (gram positive bacteria—e.g. *grlA*). These mutations cause changes in the structure of gyrase and topoisomerase which decrease or eliminate the ability of the drug to bind to these components 12,13.

Sulfonamides and trimethoprim are drug that interfere with biosynthesis of folate which is necessary for growth of bacteria through competitive inhibition of enzyme dihydrofolate reductase. Mutation in active sites of enzyme provide changes in structure which interfere with binding ability of these drugs to enzymes 14,15.

Inactivating of drugs by hydrolyzing beta-lactam group through beta-lactamases enzymes is the most important mechanism of resistance against penicillin and cephalsporins antibiotics 16, 17.

Enzymes can hydrolyze specific site in beta- lactam ring structure which result in open-ring drugs which are unable to bind to penicillin binding protein. These enzymes are innately found on bacterial chromosome or acquired by plasmid.

Enterobacteriaceae family of gram negative bacteria possesses this beta-lactamase gene. Plasmid carried beta-lactamase gene are founded in some species of gram positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium*18,19.*Beta-lactamases inhibitors similar in*

structure with beta-lactamases, they combine with beta- lactam drug to work synergistically together, but alone they have weak antimicrobial activity.

Commonly used β-lactamase inhibitor/drug pairings include amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin/tazobactam 20,21.

Carbapenemases are also beta-lactamases enzymes against carbapenem drugs, there are two type of them: the *Klebsiella pneumoniae* carbapenemases (KPCs), and those designated as Carbapenem-Resistant Enterobacteriaceae (CRE) enzymes. To overcome this resistance, there are now new beta-lactamases inhibitors drug such as ceftolozane/tazobactam, which is mainly used against *P. aeruginosa*, and shows promise against gram negative which called extended spectrum beta-lactamase (ESBL) producing strains. Vaborbactam is another β-lactamase inhibitor which in non β-lactam structured. It was approved for use with meropenem in 2017 against gram negative bacteria causing complicated urinary tract infections (cUTIs). Unfortunately, so far none of the newer combination drugs is designed to combat the carbapenem-resistant Enterobacterales(CREs) directly 22,23.

Bacteria possess genes for pump efflux to get rid of toxic substances. Some of these genes are expressed innately, other are induced or overexpressed. There are five main families of efflux pumps in bacteria classified based on structure and energy source: the ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family.

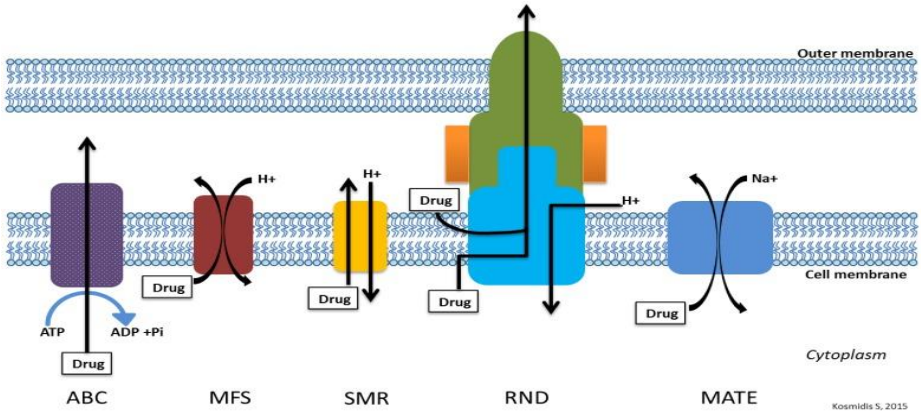

Figure 2

General structure of main efflux pump families.

Bacterial diseases:

One of the most important things when we discus antibiotic resistance is to understand pathophysiology of diseases in order to give right treatments. Living single organisms that reproduce on their own, cause bacterial infections. An organism that's not made up of cells causes viral infections. Viruses always need a host to create copies of it. Antibiotics can treat most bacterial infections, but only a few viral infections need medications that treat them. Most species of bacteria are not harmful and are often beneficial as in case of gut normal flora in digestive system, but some pathogenic species which is lower than a hundred can cause diseases. Body expose constant to bacteria which are called beneficial commensals which grow on skin and mucous membrane, but can be harmful when they could enter the body through cut, wound or when immune system become compromised. The body has ability to resist any bacterial infection of its tissue or organ which is called innate or natural defense and Infections can occur when this defense mechanism damaged by local factor or debilitating diseases such as wounding, malnutrition, fatigue, chilling and intoxication. Some species of Streptococcus and Staphylococcus are part of the normal skin microbiota and typically reside on healthy skin or in the nasopharyngeal region. Yet these species can potentially initiate skin infections. Streptococcal infections include sepsis,

pneumonia, and meningitis. These infections can become serious creating a systemic inflammatory response resulting in massive vasodilatation, shock, and death 24. Mechanism of damage includes direct effect by endotoxin which is a part of outer cell membrane. Endotoxin released after bacteria lyses, this explain why symptoms in the beginning can worsen following antibiotics treatment which kill bacteria and endotoxin secreted into the surrounding cells and damage them25. Inappropriate or an excessive immune response by infection may damage host cells.

Bacteria get into body through mouth, ear, and nose or damaged skin. Normal flora in body can become harmful when they move to places that they are not supposed to and reproduce there.

Pathogenic bacteria can spread through air by contaminated dust or droplet of water or mucus membrane. Pertussis, tuberculosis and strep throat spread by this way. Person can get bacterial infection by direct contact with another who has skin infection or indirect by contaminated surfaces. Sexual transmitted diseases are examples of direct contact. Gastrointestinal infections from *E. coli*, *Campylobacter* and *Salmonella* bacteria in contaminated food or water, can person get through this type of transmission.

Degree of dangerous of bacterial infections depend on which part of body being infected, and how deeply they penetrate organs, skin and mucus membrane. Infections are not serious but can be life-threatening when it get into blood, heart, lung, or brain.

Common bacterial infections include:

- *Campylobacter* and *Salmonella* infections, common types of food poisoning.
- Cellulitis, boils and impetigo, skin infections.
- Pneumococcal disease, including ear and sinus infections and some types of pneumonia.
- Lyme disease, a disease spread by ticks.
- Bacterial vaginosis, an overgrowth of bacteria in your vagina.
- Chlamydia and gonorrhea, sexually transmitted infections.

- Strep throat, a bacterial infection common in children that causes a sore throat.
- *C. diff,* an infection in your intestines.
- *E. coli*, a common cause of urinary tract infection (UTI).

Sign and symptoms:

Location	Symptoms
Skin.	Redness, blisters, ulcers, swollen or painful skin.
GI tract.	Diarrhea, stomach pain, nausea, vomiting.
Lungs.	Cough, shortness of breath, chest pain, phlegm (sputum).
Lining around your brain (meningitis).	Neck stiffness, nausea or vomiting, sensitivity to light, confusion.
In your bloodstream and spreading (septicemia).	High fever, weakness, sweating, low blood pressure.
Heart (endocarditis).	High fever, chest pain, night sweats, shortness of breath, cough, muscle, joint pain.
Urinary tract or genitals.	Burning or pain when you pee, discharge from your penis or vagina, increased need to pee, painful intercourse.

Inflammation

Inflammation describes as a first line of defense against inflammatory stimuli such as pathogens, irritants and damaged cells. It's part of biological process which aims to remove the primary cause of cell injury and clear out necrotic cells and repairing of damaged tissue. The sign and symptoms include redness, heat, swelling, pain and loss of function. Increased flow of blood by vasoactive mediators is responsible of redness and heat while the changes in the permeability of blood vessels which direct migration of leukocytes as well as other cells types to the site of infection by chemokines which are signaling proteins secreted by immune cells. Swelling due to accumulation of fluid in the inflamed tissue while bradykinin and histamine are responsible of pain by stimulating nerve endings. Loss of function due to multiple causes 26.

Neutrophils (primarily), basophiles (inflammatory response), and eosinophils (response to helminth worms and parasites), mononuclear cells (monocytes, macrophages) are involved in the acute phase of inflammation which occurs immediately after exposure to injury while lymphocytes, plasma cells, fibroblasts, monocytes and macrophages are major cells in chronic inflammation which is delayed and lasts up to months or years.

It's very crucial to distinguish between inflammation and infection to make right diagnose and treatment. Inflammation is not a synonym for infection. Infection describes microbial invasion which requires inflammatory response which include vascular system but inflammation describes purely immunovascular response regardless of the cause. Words ending in the suffix -itis (which refers to inflammation) are sometimes informally described as referring to infection. For example, the word urethritis strictly means only "urethral inflammation", but clinical health care providers usually discuss urethritis as a urethral infection because urethral microbial invasion is the most common cause of urethritis.

Antigen presenting cells such as(macrophages, dentric cells) contain surface receptor called pattern recognition receptors which identifies two types of molecules, pathogen associated molecular patterns(PAMPs and damage associated molecular patterns (DAMPs). PAMPs are involved with microbial agents while DAMPs are involved with host- related injury and damaged cells. Upon activation of these receptors by infection, burn and other injuries, cytokines are secreted from these cells which are implicated in the inflammatory process.

Cellular biochemical systems are activated also to spread inflammatory process which is included complement system due to bacterial infection and coagulation and fibrinolysis systems in the case of burn or trauma27. Chronic inflammation is implicated for many chronic disease such as diabetes, cardiovascular disease, COPD and allergies. There are many factors which promote chronic inflammation such as smoking, stress, obesity, physical inactivity, poverty with insufficient nutrient etc 28. Infection can be propagated when bacteria can get access to circulatory system via lymphatic system which occurs due to failure of measures during acute inflammation to contain organism. Systemic inflammatory response can occur when infection spread by blood and causes sepsis. Bacteremia is applied for bacterial sepsis and viremia is applied for viral sepsis. Septic shock may occur due to overwhelmed infection which may be fatal since it causes severe vasodilatation and organs dysfunction.

Outcomes of inflammation could be divided according to morphologic pattern of ending:

1-Resolution when stimulus has been removed and inflammatory mediators are not longer to be secreted

2-Fibrosis occurs when large amount of tissue damaged by inflammation and scar which is composed primarily of collagen, replace normal tissue. Scars will not perform any function since they don't contain parenchymal cells.

3- Abscess which can defined as cavity contain puss which is composed of white blood cells, bacteria and destroyed cells.

4-Chronic inflammation occurs when inflammation continues for month or years due to persistence of stimulus that trigger immune cells such as macrophages which secret antimicrobial substance such as reactive oxygen species against invader as well as normal tissue which lead to tissue destruction.

Laboratory test which helps differentiate between viral and bacterial infections.

C- reactive protein.

C reactive protein is synthesized by liver in response to increase in the concentration of IL-6 which released by macrophages and T cells as well as

adipocytes in response to a wide range of acute and chronic inflammatory conditions such as bacterial, viral, fungal, rheumatic and other inflammatory disease, malignancy, injury tissue and necrosis.

CRP binds to phosphocholine on the surface of dead and dying cells and some bacteria to activate complement system which binding with foreign and damaged cells to enhance opsonin-mediated phagocytosis.

CRP is very important in the clinical application to assess in the differentiation cases when the using of antibiotic becomes actual. Normal range below 10 mg/ L, but some healthy adult show raised CRP at 10 mg/L. CRP can be increased with age, obesity and in chronic diseases such as cardiovascular and pulmonary disease. Half life of CRP is 19 hour and it reaches its peak at 36 to 50 hour following inflammation or injury.

Clinical significance:

CRP can help in the differentiation between bacterial and viral infection, but it must accompany by other blood test to assess every case. The range of CRP between 100-500mg predicts the bacterial infection. Viral infection may show normal CRP and higher than 40 mg so it's important not to give antibiotic directly in such cases when sign and symptoms are absent or mild. Check up CRP is necessary to see if CRP drops down or continues to increase.

Secondary infection:

Secondary infection defines as one that occurs when primary infection makes the person more susceptible to another infection, it's also called secondary infection when it develops either because or after another infection.

Antibiotics prescription may disrupt the balance of intestinal and vaginal normal flora which can lead to fungi infection of mouth or vagina. Bacteria or fungi may cause Pneumonia following upper respiratory infections caused by viruses when immune system which defends against these viruses becomes weakened.

Immune compromised and multi-illness persons are more susceptible to secondary infections. Preexisting compromised skin may develop skin infection such as cellulites. Urinary tract infection can occur after catheter use.

Some physicians explain antibiotic prescription following virus infections that bacterial secondary infection may develop during virus infection and its necessary to prevent complications in such case. They used antibiotics empirically without any scientific background and this is one of the most challenges we face to resolve this healthy global crises. To be sure that patient develops bacterial secondary infection must laboratory test such as complete blood count, CRP, blood or urine culture, be done before prescription of antibiotics. In the presence of uncertainty of pneumonia, pulmonary X ray should be performed to ensure diagnose. Under Covid-19 pandemic prescription of antibiotic was included in medical protocol in many countries on the basis that the virus weakened immune system and bacteria would seize the opportunity to invade the body. In developed countries, the prescription of antibiotic under Covid-19 was not included unless there were sign and symptoms and reliable examination tests that conformed the diagnose. We stand strictly against the use of antibiotics in the virus infections when laboratory tests exclude any possibility that obviously support the presence of bacterial infections.

Case presentation1

An 8 year-old child presented to the clinic for one week a history of cough and sore throat. She has had fever for the past 3 day and it subsided after that. She did not complain from runny nose, nasal congestion or sneezing. Her mother was very worry that the girl has dangerous disease.

Vital signs: cough, normal respirations rate, temperature 39.

Clinical examination:

HEENT: Pharynx slightly injected. No cervical adenopathy.

Pulmonary: Clear breath sounds bilateral. No wheezing or crackles.

Cardiovascular: Heart regular

Gastrointestinal: Unremarkable.

Genitourinary: Unremarkable.

Blood tests: Strep A antigen test was negative.

C-reactive protein: 65 mg/L.

Complete blood test: within normal range.

Test interpretation: we recommended repeating again C-reactive protein next day to evaluate development of symptom. C- reactive protein dropped down to 28 mg/L. Patient recovered completely without prescription of antibiotic which indicates the importance of follow up C-reactive protein. In case of continuous high CRP, NPH culture become right choice for assessment.

Case presentation 2

A 40-year-old man presented to the clinic for severe dry cough and fever 7 days ago. He has not had chest pain, dyspnea or unintentional loss weight .His chef asked health care provider to give him antibiotic since he thought that he has bacterial infection. He said that he neither has chronic diseases nor taking any medications.

Vital signs: severe cough seemed very tired, normal respiration rate, temperature 38C:

HEENT: unremarkable.

Pulmonary: Normal breath sound, no crepitation, no wheezing.

Heart: Normal heart sound.

Gastrointestinal tract: Unremarkable.

C-reactive protein 50.

Assessment:

No antibiotics was given and patient came next day to follow up C-reactive protein which dropped down to 25. Antitussive was prescribed and patient recovered completely.

Interpretations:

This case give us indication that high CRP is not always required prescription of antibiotic and giving it without any benefit would increase likelihood of bacterial resistance in future.

Case presentation 3

An 75-year- old women presented to other clinic with shortness of breath, severe cough and fever. Covid-19 was positive with C-reactive protein 101 mg/L. Doctor prescribed antibiotic meropenam 1gm i.m, desloratadin 5mg and antitussive drug. After 5 days from treatment her health condition had been deteriorated with oxygen saturation below 60%. Her relatives contacted us to explain the condition. We asked them to take new CRP and IL-6 which showed 108mg/L and 31pg/Ml respectively. Patient had been given dexamethasone 6 mg daily orally for 10 days. After 3 days oxygen saturation raised to 95% with much better breathing. CRP checked up and it was 11mg/L.

Interpretation:

This case give us a good example that elevation of CRP is not related to bacterial infection since meropenam antibiotic did not help patient and did not decrease CRP, but its increased. IL6 elevation attributed to the high CRP which indicated that inflammation but not bacterial infection was corner stone in this case. Corticosteroid played a big role in decreasing inflammation and saving life of patient.

Case presentation 4

An 77-year-old women has been admitted at emergency unit in Baghdad for persistent dry cough which led to sleep disturbance. She did not have asthma, chronic obstructive pulmonary disease or heart failure in the medical history. She denies Shortness of breath or high fever. No chest pain with breathing or at rest. There was no sneezing or runny eye. Doctor there ordered complete blood test, CRP and X-ray for lung, all were normal, but despite of that he prescribed Imipenem antibiotic as fist choice of treatment. She had never been improved and cough episodes became worsening. Relatives contacted us, we reviewed all

medical report and we prescribed betamethasone 0,5 mg 6 tablets once daily for 5 days. From first day patient improved and she became better after completion of course. She left hospital after that and practiced her normal activities.

Interpretation of case:

This case give us a good indication that patient had not shown sign of bacterial infection and giving her antibiotic was without any scientific background. Allergy of unknown cause or hyper reactive mucous membranes in the airways can be the potential cause. Corticosteroid was the first choice of treatment in this case and it actually helped patient very good.

Complete blood count with Differential blood count.

Complete blood count is ordered to show if blood cells fall within normal range or not. If WBC are high, then doctors can order differential white blood cells count to check percentage, number of white blood cells in order to determine if there is infection disease or other diseases.

Normal white blood cell count range and function:

Total leukocytes(5000-10000 cells per cubic millimeter)

Neutrophils,(2,500-6000 cells per cubic millimeter) 40% -60%. They are responsible for destroying bacteria in injured or infected tissue.

Lymphocytes, (1000-4000 cells per cubic millimeter) 20%-40%. There are three types of lymphocytes. B lymphocytes produce antibodies to attack specific viruses, bacteria, and other foreign invaders. T lymphocytes help to recognize cells that require an immune response and kill them. Natural killer cells, the third type, destroy cancer cells and viruses.

Monocytes, (200-800 cells per cubic millimeter) 4%-8%. Monocytes are similar to neutrophils. They destroy bacteria, but usually those causing chronic infections. Monocytes also play a role in repairing damaged tissues.

Eosinophils, 50-300 cells per cubic millimeter 1%-3%. These are responsible for fighting infections caused by parasites. Eosinophils also control the immune system's response to allergic reactions.

Basophils, (0-100 cells per cubic millimeter) 0 -1%. Basophils are the least common type of white blood cell. Their function is still unclear, but they may play a role in allergic reactions.

Leukocytosis.

Refers to elevated white blood cell count, typically above 11,000 cells per cubic millimeter . There is variation between laboratories regarding exact value of WBC elevation.

Causes:

- Low-grade elevation: (11000–30000 cells per cubic millimeter). Can be seen in smoking, stress, splenectomy, cortisone, lithium or NSAID-treatment.
- Moderate-grade elevation: (30000–50000 cells per cubic millimeter) . can be seen in bacterial infection, inflammation and blood malignancy.
- High-grade elevation: Above (50000 cells per cubic millimeter). Can be seen in acute or chronic leukemia or bacterial infection and can be above this range.

It's very important to check up thrombocytes, hemoglobin, CRP, SR to differentiate between infections and malignancy.

Leucopenia.

Can be defined as the number of white blood cells <3000 cells per cubic millimeter. Granulocytes < 500 cells per cubic millimeter, means severe infection tendency.

Causes:

- Infection: Bacterial sepsis, malaria, HIV, influenza,
- Drugs such as cytostatics and thyrostatic medication.
- Disease of bone marrow.
- Splenomegaly.
- Vitamin B12 and or folic acid deficiency.

Diagnosis of leucopenia depends on physical examination, anamnesis and order complete blood tests such as complete blood count and differential white blood count, CRP, SR,T and B cells(immunophenotyping).

Treatment is focusing on background causes but it is often required antibiotic treatment to prevent complication.

Interleukin6.

Interleukin 6 is a cytokine that secreted in response to infectious and non-infectious inflammation to help body clear harmful pathogen and molecules from tissue and star healing process. Both pathogen- associated molecular patterns (PAMPs) and damage- associated molecular patterns (DAMPs) play a key role in the emergence of inflammation by binding to Toll-like receptor which belongs to the family of pattern recognition receptors. These receptors located on cell surface and intracellular compartments which are upon activation induce intracellular signal cascades which ultimately lead to inflammatory cytokine secretion. It's very important to note that this cytokine is not related just to microbial infection, but to any stimuli that trigger immune e response. Interleukin- 6 is connected with inducing production of acute phase proteins by lever such as C-reactive protein to help innate immune system to fight any invasion. C- reactive protein will continue to appear in serum as long as interleukin-6 exists. Interleukin-6 is secreted by macrophage, T-lymphocytes, B-Lymphocytes, endothelial and epithelial cells which promote hematopoiesis and differentiation of B-cells into immunoglobulin- secreting cells which is very important in the humoral immunity. Healthcare provider can order this test to show presence of inflammation and to distinguish between infectious

inflammatory from non-infectious since it has been raised during chronic inflammation.

It's worth to note that obese individuals show high level of Interleukin- 6 which is secreted by adipocytes in the absence of any infection which promote production of C-reactive protein 29. This may explain the connection between obesity with chronic disease such as diabetes and atherosclerosis.

Procalcitonin:

Procalcitonin is protein produced by many types of cells in the body. The secretion can be induced often in response to bacterial infection but also in response to tissue damage. A high level of procalcitonin may be a sign of serious infection or sepsis.

When can health care provider order the test?

This test is conducted in current time at emergency department and intensive care unit to show if the critically ill person has the sign and symptoms that suggest severe bacterial infection and/or sepsis.

The test may be ordered to distinguish between bacterial infection from non-bacterial in clinical conditions such as pneumonia or meningitis.

The test may be ordered when doctors suspect if the kidney is involved with a child who has sign and symptoms of urinary tract infection.

The health care practitioner may order this test at intervals when he or she wants to monitor antibiotic treatment and/ or decides whether treatment can be safely discontinued.

The test may occasionally be ordered when health care practitioner suspects that a patient who has tissue damage from trauma, surgery or a burn, may develop a secondary bacterial infection.

How does health care provider interpret the procalcitonin test?

Procalcitonin results are interpreted when health care provider ordered other laboratory tests such as CRP, complete blood count, differential white blood count, blood culture, urine culture, etc beside full clinical examination.

Low level of procalcitonin in critically ill patient may show a low risk of progressing to severe sepsis and/ or septic shock, but there is no guaranty to exclude it. Low level may explain that patient has a cause other than bacterial infection, such as viral infection. It is better to repeat the test to be ensured that the disease has not progressed to systemic infection if it started not more than 6 hour.

High level of the test may indicate sepsis or progression to severe sepsis and septic shock in addition it may be seen in patients with serious bacterial infection such as meningitis.

It is north to note that health care provider ordered procalcitonin test for severely ill patients with Covid-19 in order to show if they developed secondary bacterial infection.

Moderate level may indicate non-infectious condition, or may be due to early infection in conjunction with other findings and clinical examination. It also may be seen in children with kidney infections.

When person has been treated with antibiotics and procalcitonin continues to decrease so it means a sign of response to therapy, but stable level or when the level increases over time, it may indicate the need to continue treatment.

It’s very important to order procalcitonin test to ensure right diagnose in order to give antibiotic in right time to avoid serious complication in high risk disease such as meningitis or pneumonia. Giving antibiotic in non-bacterial infection can lead to antibiotic-resistant bacteria.

Procalcitonin can be used beyond critically ill ICU patients as more studies are collected for further using of this test in clinical application.

Interpretation of result:

Procalcitonin level < or =0.1 ng/mL: No systematic inflammatory response

Procalcitonin level 0.10 – 0.49 ng/mL: Minor or no significant inflammatory response. Local inflammation and local infection are possible.

Procalcitonin level 0.50 – 1.99 ng/mL: Moderate risk for progression to severe systemic infection (Severe Sepsis). Patient should be closely monitored clinically, and retested if indicated.

Note: Increased PCT levels are not always related to infection. High level may also be seen in:

- First days after major trauma, major surgery, severe burns, treatment with drugs that stimulate release of pro-inflammatory cytokines.
- Patients with invasive fungal infections and acute infection with plasmodium falciparum malaria.
- Prolonged or severe cardiogenic shock, prolonged severe organ perfusion anomalies, small cell lung cancer, and medullary C-cell carcinoma of the thyroid.

Procalcitonin level 2.00 – 9.99 ng/mL: Severe systemic inflammatory response, most likely due to sepsis, unless other causes are known. High risk for progression to severe systemic infection.

Procalcitonin level0 > or = 10.00 ng/mL: High likelihood of severe sepsis or septic shock

Procalcitonin levels >10 ng/ml are almost exclusively due to severe bacterial sepsis or septic shock.

Case presentation 5:

A 80-year- old man presented to the hospital in October 2020 for severe cough, shortness of breath, fever and generalized fatigue. He had prostate hypertrophy. Laboratory test showed the following:

Covid19: positive

C-reactive protein:	Normal range
222 mg/L	<5.0
D-Dimer:	Normal range
8,60 ng/ml	0-0.5
Ferritin:	Normal range
>2000 ng/ml	20-250
SGPT (ALT):	Normal range
323 U/L	0-40
SGOT(ASTL):	Normal range
343 U/L	0-40
Albumin:	Normal range
187 g/dl	3.4-4.8
Bilirubin direct:	Normal range
0.581mg/dl	0-0.2
Blood urea:	Normal range
73.2 mg/dl	16.60-48.50
Procalcitonin:	Normal range
1.03 ng/ml	see above.
IL-6:	Normal range
235.9 pg/ml	<7 pg/ml

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Treatment:

The doctor has prescribed the following medication:

Piperacillin/tazobactam vial 4.5G

Vancomycin vial 1G

Remdisivir vial infusion.

Decadrone amp 4mg

Montiluksat 10mg

IV fluid NS.

Terazocin tablet 5 mg.

Anti DVT.

CA gluconate.

Assessment of laboratory tests and treatment.

Covid-19 infection confirmed and patient showed inflammatory response as IL-6 was very high which in turn induced extreme lever production of C-reactive protein. High ferritin value support presence of inflammation due to Covid-19. It was very important to distinguish between bacterial infections from viral infection and in this case procalcitonin test was important. 1.03 ng/ml indicated according to the reference range that patient had Moderate risk for progression to severe systemic infection, but it was not indicating true bacterial infection. Repeated procalcitonin test was necessary when doctor decided to order antibiotic, but doctor had prescribed two types of antibiotics despite the procalcitonin had not reached the value which indicated completely that patient suffered from bacterial infection or sepsis. It's necessary to monitor procalcitonin test to be ensured that patient was in need to antibiotic and to know the efficacy of antibiotic therapy and when doctor can discontinue therapy. Covid-19 can induce systemic

inflammatory response syndrome without secondary bacterial infection. Corticosteroid was very important in this case to reduce mortality in hospitalized patients with COVID-19 who require supplemental oxygen. It works by limiting the COVID-19-induced systemic inflammatory response that can lead to lung injury and multisystem organ dysfunction. Anti DVT was important since patient showed high risk of developing DVT as complication of Covid-19.

Conclusion:

Antibiotic-resistance is becoming a global problem and international community must face it with more efforts to overcome this crisis. Health institutions and health care provider bear a big responsibility to combat this challenge through following scientific knowledge regarding prescription of antibiotic. In Many countries worldwide can people buy antibiotics from pharmacy without prescription which is one of the most obstacles to combat it. Media may play a key role in current time and in the future to shed light on the risks of antibiotic resistance. Our responsibility as health care providers require giving concrete scientific knowledge about how can doctors avoids prescription of unnecessary antibiotic in clinical applications. Distinguish between viral and bacterial infection is the corner stone in the combating bacterial resistance. Common cold and flu are contagious respiratory illnesses. Colds caused by more than 200 different types of viruses which are not treated by antibiotics. Clinical examination and laboratory tests are very important in determining the cause of infection. If a patient presented to clinic with sign and symptoms such as sneezing, cough, fever, sore throat so the patient with high confidence does not need antibiotic. C-reactive protein, complete blood count and differential blood count may be sufficient to determine it. Even high level of CRP in conjunction with sign and symptoms of common cold, just needs to be retested after 2 days to monitor level of CRP. According to our experience, the extreme majority of patients did not require any antibiotic. Patients who seek doctor with sore throat and fever

without cold symptoms with positive strep test indicate bacterial infection which requires antibiotics, similar in urinary tract infections with positive culture. Lower respiratory infection with abrupt sever cough, high fever, rapid respiratory rate, dyspnea, general fatigue and high CRP and leukocytes, can indicate bacterial infection, in order to be sure can patient be referred to do x-ray lung.

Most of gastrointestinal infections are virus-related which are self-limited and resolve within a few days. Antibiotics are not usually recommended since they have no effect on viral infection, in addition they may cause side effects and overuse can increase the risk of antibiotic resistance. Antibiotics may be recommended in particular cases of gastroenteritis or a specific bacterium has been identified as a cause such as Helicobacter pylori.

Procalcitonin test can play a key role in the distinguishing between bacterial and viral infections. This test has been used at emergency department and intensive care units to guide health care provider if the patient has bacterial sepsis or septic shock, or to monitor antibiotic therapy. Author recommends health care provider to use this test in the outpatient clinics to give them good evidence in differentiating between viral and bacterial infections. This test can take one hour in the laboratory to be ready. Polymerase chain reaction PCR requires more efforts and cost to perform it. Result of blood or other body fluid culture may take many days, despite that culture is still necessary in the assessment of pathological diseases.

Some health care provider prefer to prescribe antibiotics empirically as a request from patient , or when they worry that condition may progress to secondary infection, or as evidence that doctors were not confident in the diagnose. All of these are not correct and why we here to share our experience to our colleagues so that they prescribe only antibiotics when it is necessary.

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