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A REVIEW ON GENETIC PRINCIPLES OF DRUG RESISTANCE

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ABSTRACT
Drug resistance is the reduction in efficacy of a medication to cure a disease or condition. Today, antibiotic-resistance is rising due to dangerously high levels worldwide and threatening our ability to treat even common infectious diseases. consequently, understanding the diverse molecular mechanisms underlying resistance to antibiotics and other therapeutic drugs will aid in the development of new drugs to combat rising drug resistance. Bioinformatics provide the avenue in time and cost-effectiveness to screen potential compounds, alongside rational experimentation. Next-generation, sequencing and specific databases provide a valuable resource for the detection and identification of mutations of drug resistance. These include drug resistance mechanism, methods of transfer of resistance, examples of antibiotics, anticancer and strategies to combat drug resistance.

KEYWORDS: Drug discovery, Drug resistance, Molecular mechanism, Multiple drug resistance, Next-generation sequencing, Persistence, Tolerance.

1. INTRODUCTION

Drug resistance is the reduction in the effectiveness of a medication such as an antimicrobial or an antineoplastic in treating a disease or condition.[1] A word is used in the statement of resistance which pathogens or cancers have "acquired," viz., resistance has developed. Antimicrobial resistance and antineoplastic resistance, challenge clinical care and operate research. When an organism contests more than one drug, it is said to be multidrug resistant. The evolution of antibiotic resistance in certain stems from the drugs targeting only specific bacterial molecules (proteins). Because the drug is so specific, any mutation in these molecules will constrain or deny its destructive effect, resulting in antibiotic resistance.[2]

Bacteria s capable of not only altering the enzyme targeted by antibiotics, but also by the use of enzymes to modify the antibiotic itself and thus neutralize it. Examples of target-altering pathogens are *Staphylococcus aureus*, vancomycin-resistant enterococci and macrolide-resistant *Streptococcus*, while examples of antibiotic-modifying microbes are *Pseudomonas aeruginosa* and aminoglycoside-resistant *Acinetobacter baumannii*.[3]

2. MECHANISMS OF DRUG RESISTANCE

There are several common mechanisms for drug resistance, which are summarized in figure 1.

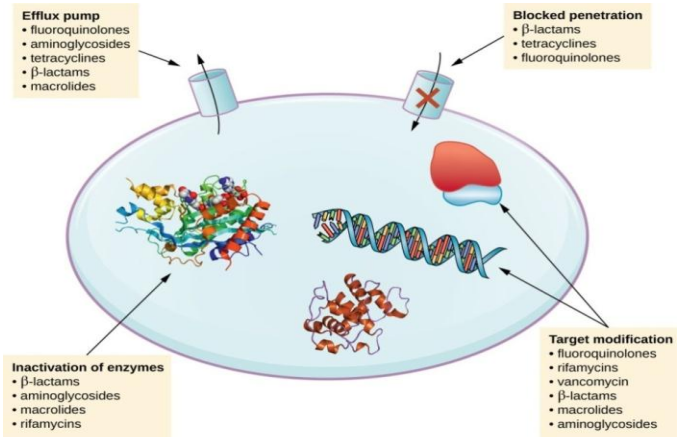

Fig. 1: Mechanisms of Drug Resistance.

➢ **Drug Modification or Inactivation**

Resistance genes may code for enzymes that chemically modify an antimicrobial, thereby inactivating it, or destroy an antimicrobial through hydrolysis. Resistance to many types of antimicrobials occurs through this mechanism.

For example, aminoglycoside resistance can obtain through an enzymatic transfer of chemical groups to the drug molecule, reducing binding the drug to its bacterial target. For β-lactams, bacterial resistance can involve the enzymatic hydrolysis of the β-lactam bond within the β-lactam ring of the drug molecule. Once the β-lactam bond is broken, the drug loses its antibacterial activity. This mechanism of resistance is mediated by β-lactamases, which are the most common mechanism of β-lactam resistance.

➢ **Prevention of Cellular Uptake or Efflux**

Microbes may develop resistance mechanisms that assume preventing the accumulation of an antimicrobial drug, which then prevents the drug from reaching its cellular target. This approach is common among gram-negative pathogens and can require changes in outer membrane lipid composition, porin channel selectivity, or porin channel concentrations.

For example, a common mechanism of carbapenem resistance among *Pseudomonas aeruginosa* is to decrease the amount of its OprD porin the primary portal of entry for carbapenems through the outer membrane of this pathogen.

➢ **Target Modification**

Considering, antimicrobial drugs have very specific targets, structural changes to those targets can inhibit drug binding, provide the drug ineffective. Through spontaneous mutations in the genes encoding antibacterial drug targets, bacteria have an evolutionary advantage that allows them to develop resistance to drugs.

Genetic changes impacting the active site of penicillin-binding proteins (PBPs) can inhibit binding β-lactam drugs and provide resistance to multiple drugs within this class.

➢ **Target Overproduction or Enzymatic Bypass**

When an antimicrobial drug function as an antimetabolite, targeting a specific enzyme to inhibit its activity, there are other ways that microbial resistance may occur. Initial the microbe may overproduce the target enzyme specified that there is a sufficient amount of antimicrobial-free enzyme to carry out the proper enzymatic reaction. Subsequent, the bacterial cell may develop a bypass that avoids the need for the functional target enzyme.

➢ **Target Mimicry**

It involves the production of proteins that bind and sequester drugs, preventing the drugs from binding to their target.

For example, *Mycobacterium tuberculosis* produces a protein with efficient pentapeptide repeats that become visible to mimic the structure of DNA. This protein binds fluoroquinolones, sequestering them and owning them from binding to DNA, providing *M. tuberculosis* resistance to fluoroquinolones. Proteins that mimic the A-site of the bacterial ribosome have been found to contribute to aminoglycoside resistance as well.[4]

3. METHODS OF TRANSFER OF RESISTANCE

- **Trasformation:** In transformation, a bacterium takes in DNA from its environment, often DNA that's been shed by other bacteria. If the DNA is in the shape of a circular DNA called a plasmid, it can be imitate in the receiving cell and passed on to its descendants.
- **Transduction:** In transduction, viruses that infect bacteria move short pieces of chromosomal DNA from one bacterium to another by "accident."[5]
- **Conjugation:** In conjugation, the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells.[6]

4. EXAMPLES

Antibiotics

Antibiotic was a substance produced by one microorganism that selectively kill or inhibit the growth of another and are used to treat bacterial infections.[7]

What is antibiotic resistance in bacteria?

Antibiotic resistance is when bacteria are able to survive and grow in the existence of one or more antibiotics. When this occurs, the resistant bacteria continue to cause infection.

The development of resistance commonly occurs. However, because of the routine use of antibiotics, bacterial exposure to antibiotics is more frequent and resistance develops at a faster rate. Without effective antibiotics, common infections such as bacterial pneumonia, would become life-threatening once again. Complex procedures, such as open- heart surgery, would become much more dangerous and deaths from infection more common.

How do bacteria become resistant?

There are several ways for bacteria to become antibiotic-resistant. The main one is through selective pressure. Selective pressure develops when not all the bacteria are susceptible to the antibiotic used to treat the infection, and the surviving bacteria can sustained to multiply. This creates a bacterial population that is resistant to the antibiotic to which the bacteria was exposed.

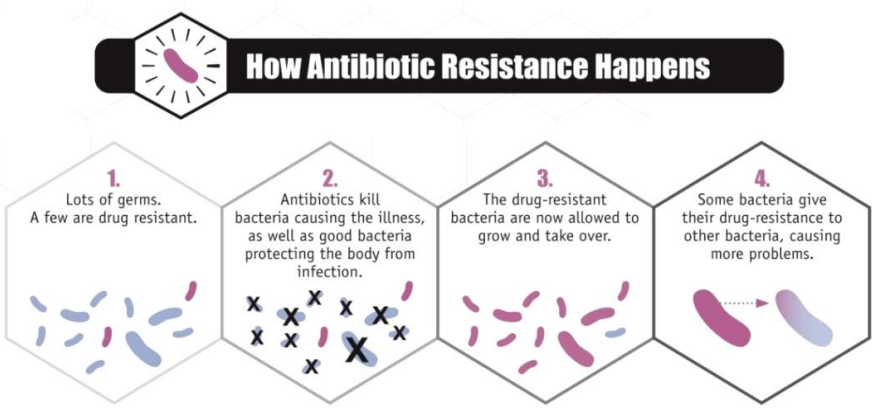

Fig. 2: How Antibiotic Resistance Happens.

Bacteria can also acquire resistance when they pass genetic material back and forth from one bacterium to another. One way they can do this is through plasmids. Plasmids are pieces of bacterial DNA that can be transferred between bacteria. Some plasmids enable the bacteria to produce an enzyme that can make antibiotics useless. When the plasmid is inserted into other bacteria, antibiotic resistance can spread easily and quickly among bacteria.[8]

➢ EXAMPLES: PENICILLINS

Penicillins are a class of antibiotics which contain penicillin G (intravenous use), penicillin V (use by mouth), procaine penicillin, and benzathine penicillin (intramuscular use). Penicillin antibiotics were among the first medications to work against many bacterial infections caused by staphylococci and streptococci.

About 10%, of people report that they are allergic to penicillin; however, up to 90% of this group may not be allergic. Serious allergies only occur in about 0.03%.[9] Those who are allergic to penicillin are most often given cephalosporin C because of its functional groups. All penicillins are β-lactam antibiotics, which are some of the most powerful and successful achievements in modern science.[10]

Mechanism of action

Bacteria continuously mutate their peptidoglycan cell walls, at the same time building and breaking down portions of the cell wall as they grow and divide. β-lactam antibiotics inhibit the formation of peptidoglycan cross-links in the bacterial cell wall; this event is achieved through the binding of the four-membered β-lactam ring of penicillin to the enzyme DD-transpeptidase. Thus, DD-transpeptidase cannot catalyze the formation of these cross-links, and an imbalance between cell wall production and degradation develops, causing the cell to rapidly die.

The enzymes that hydrolyze, the peptidoglycan cross-links continue to function, even while those which form such cross-links do not. This event destroy the cell wall of the bacterium, and osmotic pressure becomes increasingly contributed eventually causing cell death (cytolysis).[11]

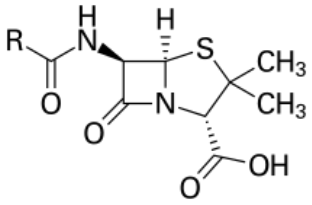

Fig. 3: Penicillins.

For example: Benzyl penicillin: also named penicillin G, is an antibiotic used to treat a number of bacterial infections. This includes pneumonia, syphilis, necrotizing enterocolitis, diphtheria, gas gangrene, leptospirosis, cellulitis, and tetanus. It is not a first-line agent for pneumococcal meningitis.[12]

Benzylpenicillin is given by injection into a vein or muscle. Two long-acting form's benzathine benzylpenicillin and procaine benzylpenicillin are available for use by injection into a muscle. Side effects include diarrhea, seizures, and allergic reactions including anaphylaxis.[12]

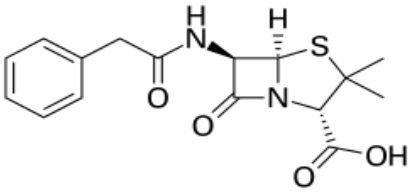

Fig. 4: Benzyl Penicillin.

As an antibiotic, benzylpenicillin is noted to posses effectiveness mainly against Gram-positive organisms. Some Gram-negative organisms such as *Neisseria gonorrhoeae* and *Leptospira weilii* are also reported to be susceptible to benzylpenicillin.[14]

➢ CEPHALOSPORINS

The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was before called "*Cephalosporium*".[15]

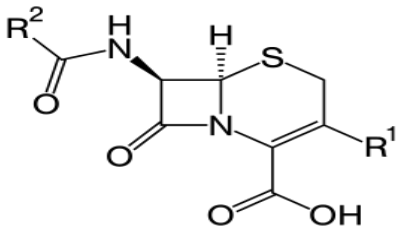

Fig. 5: Cephalosporin.

Mechanism of action

Cephalosporins are bactericidal and have the same action as other β-lactam antibiotics (such as penicillins), but are less susceptible to β-lactamases. Cephalosporins disrupt the synthesis of the peptidoglycan layer forming the bacterial cell wall.

The peptidoglycan layer is essential for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by penicillin-binding proteins (PBPs). PBPs bind to the D-Ala-D-Ala at the end of muropeptides (peptidoglycan precursors) to crosslink the peptidoglycan. Beta-lactam, antibiotics mimic the D-Ala-D-Ala site, thereby irreversibly inhibiting PBP crosslinking of peptidoglycan.

Resistance

Resistance to cephalosporin antibiotic can require either reduced affinity of existing PBP components or the acquisition of a supplementary β-lactam-insensitive PBP. Currently, some *Citrobacter freundii*, *Enterobacter cloacae*, *Neisseria gonorrhoeae*, and *Escherichia coli* strains contests cephalosporins. Some *Morganella morganii*, *Proteus vulgaris*, *Providencia rettgeri*, *Pseudomonas aeruginosa*, and *Serratia marcescens* strains have also developed resistance to cephalosporins to varying degrees.

- **These days, antibiotic adjuvants are also being used to overcome antibiotic resistance**

Antibiotic adjuvants are compounds that do not themselves kill bacteria instead enhance the effect of an antibiotic by inhibiting a mechanism of resistance.[17]

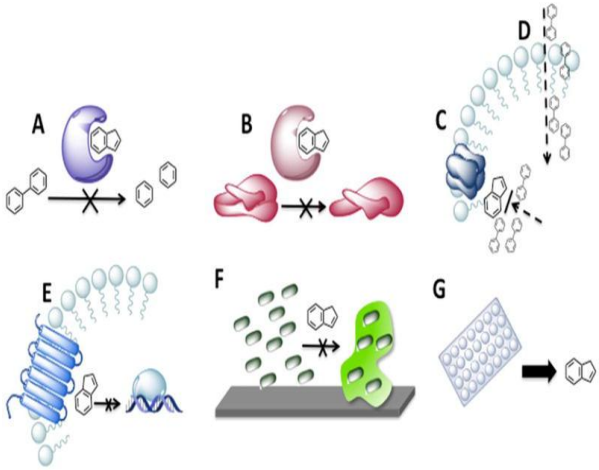

Fig.6: Examples of adjuvant mechanisms of action and discovery: (a) inhibition of antibiotic modification; (b) inhibition of target modification; (c) inhibition of efflux; (d) enhancement of antibiotic uptake; (e) inhibition of signaling pathways that mediate antibiotic resistance; (f) inhibition of biofilm formation, which leads to increased antibiotic tolerance; (g) target-blind whole cell screening of previously approved drugs for adjuvant activity.

Combination of two antibiotics are also considered adjuvants when their effect is synergistic (i.e., the coadministration of the two drugs has a significantly greater effect than that of each antibiotic alone). Antibiotic adjuvants can function either by reversing resistance mechanisms in naturally sensitive pathogens or by sensitizing intrinsic resistant strains.[18]

Examples of currently used/identified antibiotic adjuvants[19] are in table 1.

Tab.1: Antibiotic Adjuvants.

S.No	Mode of action	Adjuvant	Antibiotic
1.	Inhibition of a vital physiological pathway: • Inhibition of the synthesis and repair of the bacterial cell wall	Fosfomycin	Gentamicin, amikacin, cefepime, ciprofloxacin.
	• Oxidative stress outbreak	Tellurite	Ampicillin, cefotaxime, tetracycline, chloramphenicol.
	• Cell shape alteration	Pivmecillinam, and echinomycin	Novobiocin

2.	Inhibition of antibiotic resistance elements: • β-lactamase inhibitors • Dehydropeptidase I inhibitor	Clavulanic acid Cilastatin	Amoxicillin Imipenem
3.	Enhance the uptake of the antibiotic through the bacterial membrane: • Antibiotics that damage the cell wall improving the uptake of other antibiotics • Binds to and interferes with the integrity of the LPS-containing outer membrane layer • Damages the bacterial membrane • Permeabilizes the bacterial membrane	β-lactams, bacitracin, vancomycin, cycloserine Colistin (polymyxin E) Eugenol (from Eugenia aromatic) Phenylpropanoids loperamid	Aminoglycosides (streptomycin, gentamicin) Rifampin or vancomycin Vancomycin Amikacin, Ampicillin, Ciprofloxacin. Tetracycline
4.	Blocking of efflux pumps: • Competitive inhibition	Tetracycline analogues Fluoroquinolone analogues Aminoglycoside analogues	Tetracyclines, Macrolides, Gentamicin
5.	Change the physiology of resistant cells • Dispersal of biofilms to planktonic cells	Antibiofilm exopolysaccharides	Combined with high-spectrum antibiotics

RESISTANCE TO ANTICANCER DRUGS

Anticancer, or antineoplastic, drugs are used to treat malignancies, or cancerous growths. Drug therapy may be used alone, or in combination with other treatments such as surgery or radiation therapy.

Anticancer drugs are used to control the growth of cancerous cells. Cancer is commonly defined as the uncontrolled growth of cells, with a loss of differentiation and frequently, with metastasis, spread of the cancer to other tissue's and organs. Cancers are malignant growths. However, benign growths remain encapsulated and grow within a well defined area. While benign tumors may be fatal if untreated, due to pressure on essential organs, as for a benign brain tumor, surgery, or radiation are the preferred methods of treating growths which have a well defined location. Drug therapy is used when the tumor has spread, or may spread, to all areas of the body.[20]

Cancer Cell Mechanisms

Cancer cells can become resistant to many drugs by altered membrane transport, enhanced DNA repair, apoptotic pathway defects, alteration of target molecules, protein and pathway mechanisms, such as enzymatic deactivation.[21]

➢ ***Altered membrane transport***

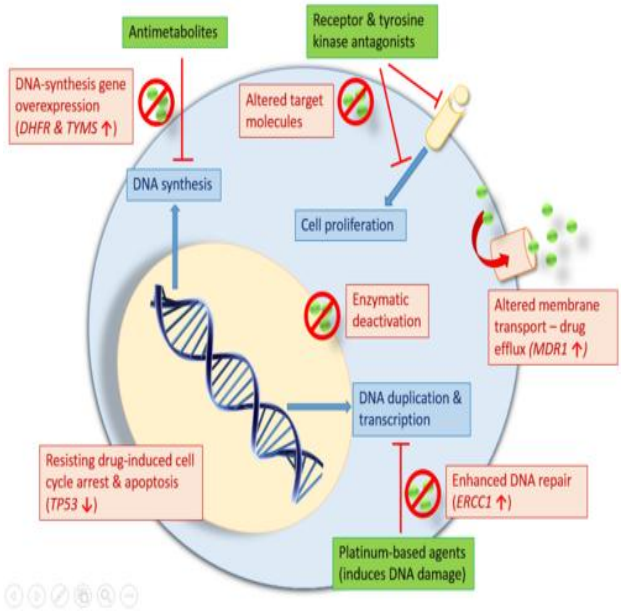

Fig.7: Altered Membrane Transport.

Many classes of antineoplastic drug act on intracellular components and pathways, like DNA, nuclear components, meaning that they need to enter the cancer cells. The p-glycoprotein (P-gp), or the several drug resistance protein, is a phosphorylated and glycosylated membrane transporter which can shuttle drugs out of the cell, thereby decreasing or ablating drug efficacy. This transporter protein is encoded by the *MDR1* gene and is also called the ATP-binding cassette (ABC) protein. *MDR1* has promiscuous substrate specificity, allowing it to transport many structurally diverse compounds across the cell membrane, mainly hydrophobic compounds. Studies have found that the *MDR1* gene can be activated and overexpressed for pharmaceutical drugs, thus forming the basis for resistance to many drugs.[22]

➢ ***Enhanced DNA repair***

Enhanced DNA repair contributes to the ability for cancer cells to overcome drug-induced DNA damages. Platinum-based, chemotherapies such as cisplatin, target tumor cells by cross-linking their DNA strands, causing mutations and damage.[22] such damage will trigger programmed cell death (e.g., apoptosis) in cancer cells. Cisplatin resistance occurs when cancer cells develop an enhanced ability to reverse such damage by removing the cisplatin from DNA and repairing any damage done.[22][21] The cisplatin-resistant cells upregulate expression of the excision repair cross-complementing (ERCC1) gene and protein.[22]

➢ ***Apoptotic pathway defects***

TP53 is a tumor suppressor gene encoding the p53 protein, which responds to DNA damage either by DNA repair, cell cycle arrest, or apoptosis. Losing *TP53* via gene deletion can allow cells to continuously replicate despite DNA damage. The tolerance of DNA damage can grant cancer cells a method of resistance to those drugs that normally induce apoptosis through DNA damage.[21][22]

➢ ***Altered target molecules***

During targeted therapy, often times the target has modified itself and decreased its expression to the point that therapy is no longer effective. One example of this is the loss of estrogen receptor (ER) and progesterone receptor (PR) upon anti-estrogen treatment of breast cancer. Tumors with loss of ER and PR no longer respond to tamoxifen or other anti-estrogen treatments, and while cancer cells remain somewhat responsive to estrogen synthesis inhibitors, they eventually become unresponsive to endocrine manipulation and no longer dependent on estrogen for growth.[23]

➢ **Altered metabolism**

One of the mechanisms of antineoplastic resistance is over-expression of drug-metabolizing enzymes or carrier molecules.[22] By increasing expression of metabolic enzymes, drugs are more rapidly converted to drug conjugates or inactive forms that can then be excreted. For example, increased expression of glutathione promotes drug resistance, as the electrophilic properties of glutathione allow it to react with cytotoxic agents, inactivating them.[24] Sometimes, decreased expression or loss of expression of drug-metabolizing enzymes confers resistance, as the enzymes are needed to process a drug from an inactive form to an active form.

5. STRATEGIES TO OVERCOME DRUG RESISTANCE

Chemotherapy is commonly used in cancer treatment. So far, chemotherapy agents can be categorized into three types: classical chemotherapeutic drugs, molecular target agents and cellular machineries target drugs.[25] Although the action mechanisms of these three classes of drugs are different, these drugs are facing the same challenge of drug resistance.

Several approaches are now available to counteract drug resistance. Certain drugs have been found to have an increased effect on tumor cells resistant to specific agents. This event is called collateral sensitivity. Recently, biochemical studies of resistant cell lines have led to rational approaches to the design of new agents that can exploit metabolic changes in the resistant cells.[26] By use of alternating non-cross-resistant chemotherapy, one may be able to maintain a population of tumor cells with sensitivity to both therapies.[27][28]

Another approach is to directly alter the expression of the genes related to the drug resistance by the design of agents that target to specific sequences of DNA or RNA, by the design of drugs that interact with the gene products causing drug resistance, or by pharmacologically producing changes in the methylation patterns of DNA. This chapter will summarize current research in collateral sensitivity, and these other approaches concentrating on modification of DNA

methylation that shows promise in counteracting resistance to cytosine arabinoside (ara-C).

Recently, Cheng and Brockman[26] have reviewed research designed to rationally exploit collateral sensitivity. Promising approaches include:

1. Use of lipophilic antifols in cells lacking a transport system for methotrexate and reduced folates;
2. Increased conversion of 2'-fluoro-2'-deoxy-S-iodo-1-~-D-arabino-furanosylcytosine to an active uracil compound in cells with high activity of deoxycytidine deaminase, an enzyme that degrades ara-C;
3. Increased sensitivity to the inhibitors of de novo synthesis in cells lacking salvage pathways for purines and pyrimidines;
4. Increased sensitivity of human KB cells with overexpression of ribonucleotide reductase to thioguanine that converted by this enzyme to its deoxynucleotide; S.design of drug analogues that exploit differences in drug binding affinity to altered targeted enzymes.

6. CASE STUDIES

A case report of a multi-drug resistant bacterial infection in a diabetic patient treated in northeast Brazil:

According to the American Diabetes Association, diabetes mellitus (DM) means as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. This chronic hyperglycemia is associated, after long periods, with injury and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[29]

The diabetic population is currently increasing worldwide, especially in developing countries like Brazil. In 1985, there were approximately 30 million adults suffering from diabetes worldwide. In 1995, this figure amounted to 135 million, while in 2002 the number of diabetics was 173 million and is expected to reach a total of 300 million in 2025. About two-thirds of diabetics live in developing countries where the epidemic is most intense with an increasing proportion in younger age groups.[30]

World population data indicate that about 82,000 people have diabetes-related amputations of feet and lower extremities each year.[31] It is also estimated that 14-20% of diabetic patients with foot ulcers undergo an amputation, while 85% of amputations are preceded by ulcers.[32] The ulcers result from multiple pathophysiological mechanisms, and in diabetic patients, this is mainly due to a complication of such a critical illness.[33]

The multi-drug resistant organisms of greater importance in the hospital environment include oxacillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* species (VRE), multi-resistant *Streptococcus pneumonia*, Gram-negative organisms, and multi-resistant Gram negative bacteria including *Pseudomonas* species, *Acinetobacter* species, *Klebsiella pneumonia*, *Enterobacter* species and other organisms.[34]

Given this resistance's panorama, it is necessary to determine the bacteriological profile of admitted patients in a hospital setting, and also determine the bacteriological profile of hospitalized patients in order to reduce the high rates of amputation and in-hospital mortality rate in people with diabetes and foot ulcers.[35] Patients with diabetes have a 12-25% lifetime risk of developing a foot ulcer. Diabetic foot ulcers have increased the public health care awareness and its associated morbidities, patient's quality life impairment, and the implied costs for management have attracted the attention of numerous health care providers.[36]

CASE REPORT

Diabetic patient with an infected neuro-ischemic ulceration and osteomyelitis

An 86-year-old male presented to the emergency department with a chief complaint of an ankle injury and a foot non-healing ulcer sustained from a drill accident 2 months prior to his visit in our hospital. The patient also related that he had diabetes for approximately 13 years without any treatment to control the disease. During the initial consultation, the patient related no use of any antibiotic therapy for his non-healing wound to the right foot. The patient was admitted to the hospital and further clinical and medical imaging revealed the presence of a neuro-ischemic ulcer complicated with infection and osteomyelitis. The presence of vascular compromise and osteomyelitis were diagnosed by the vascular surgeon on call through the macroscopic visualization of a large area of tissue necrosis, accompanied by abscess and radiological analysis respectively. Initial laboratory analysis included a complete blood count, accompanied by a fasting glucose level which was requested shortly thereafter. The patient's hemoglobin was 8.8 mg/dL (reference value: 11.0-16.5 mg/dL), hematocrit was 25.8% (reference value: 42-54%) and white blood cell count was $23.5 \times 10^3/mm^3$ (reference value: $3.5\text{-}10.0 \times 10^3/mm^3$) with granulocyte percentage equivalent to 85.6% (reference value: 43.0-76.0%). The differential count showed a segmented neutrophil percentage of 80% (reference value: 54-62%/2,700-6,200/mm^3), and 1% of eosinophils (reference value:2-5%/100-500/mm^3). The random plasma glucose level corresponded to 305 mg/dL (reference value: 70-99mg/dL). Automated equipment (Abbott Cell-dyn1700 Diagnosis and Bioplus BIO2000) were used to obtain the results of the complete blood count and glucose levels, respectively.

7. CONCLUSION

Drug-resistance is a common clinical problem that desperately needs to be solved. A better understanding of its mechanisms will be helpful in developing efficient methods to overcome drug resistance. Even, periodic antibiotic resistance surveys could also help all health

care providers and the local population on the best treatment strategies. The continuous advances in the development of new and potent high-throughput technologies will definitively allow the discovery of new compounds with antibiotic adjuvant activity.

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