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NATIONAL UNIVERSITY OF PHARMACY

faculty for foreign citizens' education

department of clinical pharmacology and clinical pharmacy

QUALIFICATION WORK

on the topic: «**THE IMPACT OF COVID-19 ON ANTIBIOTIC RESISTANCE AT MOHAMED V HOSPITAL IN MEKNES, MOROCCO**»

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Kharkiv – 2024 year

ANNOTATION

Maryem M'hani Ghaouti. The impact of COVID-19 on antibiotic resistance at Mohamed V hospital in Meknes, Morocco. – The manuscript. – National University of Pharmacy of Ministry of Healthcare of Ukraine, Kharkiv, 2024.

The qualification work examines the impact of COVID-19 on antibiotic resistance at Mohamed V Hospital in Meknes, Morocco.

Alternative ways to reduce the risk of antibiotic resistance and ways to improve the effectiveness and safety of patient treatment with antibacterial drugs are proposed.

Qualification work is presented on 40 pages of typewritten text, consists of summary, introduction, 3 chapters, conclusions, references. The work is illustrated with 3 tables, 14 figures. The list of references contains 94 resources.

Key words: antibiotic resistance, COVID-19, Mohamed V hospital, Morocco, healthcare providers, efficacy and safety of therapy.

АНОТАЦІЯ

Маріем М'хані Гауті. Вплив COVID-19 на резистентність до антибіотиків у лікарні Мохамеда V в м. Мекнесі, Марокко. – На правах рукопису. – Національний фармацевтичний університет МОЗ України, Харків, 2023.

У кваліфікаційній роботі вивчено вплив COVID-19 на серезистентність до антибіотиків у лікарні Мохамеда V в м. Мекнесі, Марокко.

Запропоновано альтернативні шляхи зниження ризику антибіотикорезистентності та шляхи підвищення ефективності та безпеки терапії хворих при застосуванні антибактеріальних лікарських засобів.

Кваліфікаційна робота викладена на 40 сторінках машинописного тексту, складається з резюме, вступу, 3 розділів, висновків, списку літератури. Робота проілюстрована 3 таблицями, 14 рисунками. Список літератури містить 94 найменувань.

Ключові слова: антибіотикорезистентність, COVID-19, лікарня Мохамеда V, медичні працівники, ефективність та безпека терапії.

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INTRODUCTION

Relevance of the topic. Antibiotic resistance, an ancient phenomenon, continues to escalate, presenting a dynamic and pressing challenge known as the «resistome». Over recent decades, the surge in antibiotic resistance has emerged as a significant global threat. Essentially, antibiotic resistance occurs when bacteria can survive despite exposure to antimicrobial agents that were previously effective against them [1, 2].

Antibiotic treatment, one of the main approaches of modern medicine for combating infections, experienced a «golden era» from the 1930s to the 1960s, yielding numerous antibiotics. However, this era waned as researchers struggled to keep pace with emerging resistant pathogens. Persistent failures in antibiotic development and the indiscriminate use of antibiotics contribute to the rise of resistance [3, 4].

Antimicrobial resistance (AMR) represents a significant and increasing threat to the health of humans, animals, and the environment worldwide. This threat stems from the emergence, transmission, and persistence of multidrug-resistant (MDR) bacteria, commonly known as «superbugs». MDR bacteria are found throughout the interconnected ecosystems of animals, humans, and the environment, facilitating the exchange of these pathogens within this triad. The rise of AMR, or «the global resistome», can be attributed to various factors including the excessive use of antibiotics in both animals (including those in food production, pets, and aquatic environments) and humans, the unrestricted sale of antibiotics, heightened international travel, inadequate sanitation and hygiene practices, and the release of antibiotics or their remnants into the environment via manure and fecal matter. These factors exert genetic selection pressure, leading to the emergence of MDR bacterial infections within communities. Recent trends in the global consumption of antimicrobials in livestock have highlighted the significant regions of antibiotic use worldwide, indicating potential economic and public health repercussions in the years ahead. Notably, antibiotics are extensively used in food animals such as cattle,

chicken, and pigs, with projections suggesting a substantial increase up to 67% in such usage by 2030, particularly in densely populated countries [5, 6].

Haemophilus influenzae to antibiotics like ampicillin, with the latter also showing resistance to tetracycline and chloramphenicol. The widespread use of antimicrobials, especially in regions where they were readily available without prescription, contributed to the accelerated spread of resistance, particularly in developing countries. Poor hygiene conditions facilitated resistance transmission, while limited healthcare resources restricted access to new and effective antibiotics [7].

MDR bacteria kill approximately 700,000 persons worldwide annually, with MDR tuberculosis alone responsible for 230,000 deaths per year. According to the World Health Organization's (WHO) 2020 Global Report on Tuberculosis, an estimated 10.0 million people contracted TB in 2019, with the majority of cases concentrated in South East Asia, comprising 44% of the global total. In 2019, there were approximately 12,000 reported cases of extensively drug-resistant tuberculosis (XDR-TB), marking a 1.5-time increase compared to 2015 when there were around 8,000 cases. Reports indicate a high mortality rate (ranging from 21% to 70%) among cases of pan drug-resistant (PDR) infections in Gram-negative bacteria, with 81 cases documented, 47 of which were reported within the last five years. Despite these concerning statistics, there is optimism that PDR infections can still be managed effectively at present [8-10].

During the COVID-19 pandemic, antimicrobial resistance (AR) has surged due to the improper use of antibiotics in healthcare facilities and communities. Approximately 72% of COVID-19 patients received antimicrobial treatment, despite only 8% having bacterial or fungal co-infections. Furthermore, various antibiotics, such as azithromycin, have been explored or suggested for treating COVID-19. This misuse of antibiotics, coupled with concerns about the virus, exacerbates the issue, especially in low- and middle-income countries with inadequate antibiotic control measures. Zavala-Flores et al. (2020) revealed that nearly 69% of COVID-19 patients reported using antibiotics (specifically, ceftriaxone and azithromycin) prior

to hospital admission [11-13].

The aim of the study. The aim of the thesis is the study of the impact of COVID-19 on antibiotic resistance at Mohamed V hospital in Meknes, Morocco.

The objectives of the study. Objectives of the work are the following:

1. To investigate the mechanism of antibiotic resistance.
2. To investigate the impact of COVID-19 on antibiotic resistance.
3. To investigate approaches to combat antibiotic resistance in current and future directions.
4. To study the opinion and beliefs of patients with antibiotic resistance concerning antibiotic resistance and COVID-19 influence.
5. To develop practical recommendations for physicians, pharmacists and patients to prevent antibiotic resistance.

Object of research: antibiotic resistance.

Subject of research: impact of COVID-19 on the level of antibiotic resistance.

Research methods. Questionnaire for patients of Mohamed V Meknes hospital with antibiotic resistance developed during the COVID-19 pandemic; statistical.

Structure and scope of qualification work. Qualification work is presented on the 40 pages of typewritten text, consists of summary, introduction, 3 chapters, conclusions, references. The work is illustrated with 3 tables, 14 figures. The list of literature contains 94 references.

CHAPTER 1
MODERN PRESENTATION ABOUT ANTIBIOTIC RESISTANCE (LITERATURE REVIEW)

1.1. The mechanisms of antibiotic resistance

Antibiotic resistance is a natural process driven by microbial ARGs, evolving over billions of years. Bacteria in the environment harbor ARGs, predisposing them to resist newly introduced antibiotics. Even without human intervention, bacteria in permafrost have exhibited resistance. The intrinsic resistance stems from inherent bacterial properties, including structural and functional features that thwart antibiotic action. Antimicrobial resistance mechanisms fall into four main categories: limiting drug uptake, modifying drug targets, deactivating drugs, and expelling drugs actively.

Intrinsic resistance may employ strategies like limiting uptake, drug deactivation, and drug expulsion, while acquired resistance typically involves modifying drug targets, inactivating drugs, and drug efflux. Gram-negative bacteria utilize all mechanisms, whereas gram-positive bacteria may less commonly use drug uptake limitations due to their different structure. Gram-positive bacteria also differ in drug efflux mechanisms, lacking certain types found in gram-negative bacteria. Fig. 1.1. illustrates the general antimicrobial resistance mechanisms [14-18].

As noted, bacteria inherently differ in their ability to limit the antimicrobial uptake. The LPS layer in gram-negative bacteria forms a barrier against certain molecules, granting innate resistance to specific large antimicrobial agents. Mycobacteria, with their lipid-rich outer membrane, facilitate the entry of hydrophobic drugs like rifampicin and fluoroquinolones, while hydrophilic drugs face limited access [19-21].

Bacteria lacking a cell wall, such as Mycoplasma, are intrinsically resistant to drugs targeting the cell wall, including β-lactams and glycopeptides. Gram-positive bacteria, lacking an outer membrane, exhibit less restriction on drug access. For

instance, enterococci's cell wall properties hinder penetration by polar molecules, imparting intrinsic resistance to aminoglycosides. Staphylococcus aureus has developed resistance to vancomycin, partly through thickened cell walls, creating intermediate resistance termed VISA strains [22, 23].

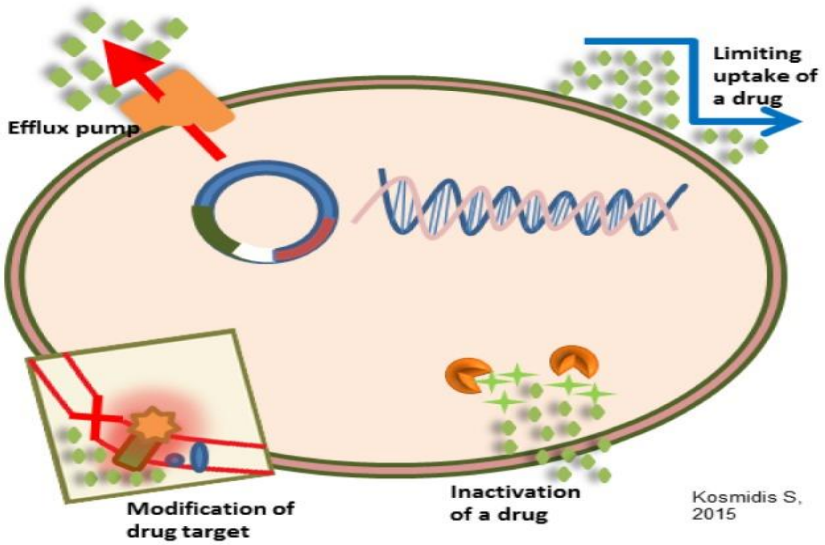

Fig 1.1. General antimicrobial resistance mechanisms.

Bacteria with large outer membranes often rely on porin channels for substance entry into the cell. Gram-negative bacteria typically permit access to hydrophilic molecules through these channels. Porin changes can hinder drug uptake in two primary ways: by reducing the number of porins or by mutations altering porin channel selectivity. Enterobacteriaceae members can develop resistance by diminishing porin numbers or ceasing production of certain porins, particularly as a defense against carbapenems. Mutations affecting the porin channel have been observed in *E. aerogenes*, resulting in resistance to imipenem and certain cephalosporins, as well as in Neisseria gonorrhoeae, leading to resistance against β-lactams and tetracycline [24-27].

The formation of bacterial biofilms is a common occurrence in colonization. These biofilms can be dominated by a single organism, such as *Pseudomonas aeruginosa* in the lungs, or consist of diverse organisms, like those found in the gut's normal flora. For pathogenic bacteria, biofilm formation shields them from the host

immune system and antimicrobial agents. The dense, adhesive biofilm matrix, containing polysaccharides, proteins, and DNA from resident bacteria, impedes antimicrobial access, necessitating higher drug concentrations for efficacy.

Additionally, bacteria within biofilms tend to be sessile, with slow metabolism and cell division rates, rendering antimicrobials targeting actively dividing cells less effective. Importantly, biofilms likely facilitate horizontal gene transfer due to the proximity of bacterial cells, potentially enabling easier sharing of antimicrobial resistance genes within these bacterial communities [28, 29].

Modification of drug targets

In bacterial cells, various components serve as targets for antimicrobial agents, each susceptible to modifications fostering drug resistance. Gram-positive bacteria exhibit one such resistance mechanism against β-lactam drugs, involving alterations in penicillin-binding proteins (PBPs). PBPs, crucial for peptidoglycan synthesis in the cell wall, can undergo changes in number or structure, impacting drug binding. For example, acquisition of the mecA gene in *S. aureus* can lead to structural alterations in PBP2a, diminishing drug affinity or completely preventing drug binding [30, 31].

Glycopeptides like vancomycin and lipopeptides such as daptomycin exert their antimicrobial effects differently. Vancomycin inhibits cell wall synthesis, while daptomycin depolarizes the cell membrane. However, gram-negative bacteria with thick lipopolysaccharide (LPS) layers inherently resist these drugs. In organisms like enterococci (VRE) and *Staphylococcus aureus* (MRSA), vancomycin resistance is a growing concern. This resistance arises from the acquisition of van genes, altering peptidoglycan precursor structures and reducing vancomycin binding. Daptomycin's effectiveness relies on calcium binding. Mutations in genes like mprF alter the cell membrane's surface charge, impeding calcium binding and, consequently, daptomycin's efficacy [32, 33].

Resistance against drugs targeting ribosomal subunits can manifest through various mechanisms. Ribosomal mutation, seen in aminoglycosides and oxazolidinones, alters ribosomal function. Additionally, ribosomal subunit

methylation, typically involving erm genes, affects drugs like aminoglycosides, macrolides (in gram-positive bacteria), oxazolidinones, and streptogramins. These alterations hinder drug binding to the ribosome. The extent of drug interference varies significantly depending on the specific mechanism employed [34, 35].

Resistance to drugs that target nucleic acid synthesis, such as fluoroquinolones, occurs through alterations in DNA gyrase (in gram-negative bacteria, e.g., gyrA) or topoisomerase IV (in gram-positive bacteria, e.g., grlA). These mutations induce structural changes in gyrase and topoisomerase, reducing or abolishing the drug's ability to bind to these components [36, 37].

Resistance to drugs that inhibit metabolic pathways involves mutations in enzymes such as DHPS (dihydropteroate synthase) and DHFR (dihydrofolate reductase) that are part of the folate biosynthesis pathway. Additionally, resistance can arise from the overproduction of resistant DHPS and DHFR enzymes (in sulfonamides-DHPS and trimethoprim-DHFR). Sulfonamides and trimethoprim bind to their respective enzymes because they structurally resemble the natural substrates (sulfonamides mimic p-amino-benzoic acid, while trimethoprim mimics dihydrofolate). These drugs competitively inhibit enzyme activity by binding to the active site. Mutations, often near the active site, cause structural changes in the enzyme, interfering with drug binding while still allowing the natural substrate to bind [38, 39].

Bacteria have two primary methods to render drugs ineffective: degradation of the drug itself or transferring a chemical group to the drug. The β-lactamases constitute a significant group of enzymes that hydrolyze drugs. Another example of drug inactivation through hydrolysis is tetracycline, catalyzed by the tetX gene [34, 40].

The inactivation of drugs through the transfer of chemical groups most frequently involves the transfer of acetyl, phosphoryl, and adenyl groups. Numerous transferases have been discovered, with acetylation being the most widely employed mechanism. It is utilized against various drugs such as aminoglycosides, chloramphenicol, streptogramins, and fluoroquinolones. Phosphorylation and

adenylation, on the other hand, are primarily employed against aminoglycosides [40, 41].

The β-lactam drugs constitute the most extensively utilized group of antimicrobial agents. All members of this group share a distinctive core structure featuring a four-sided β-lactam ring. Resistance to these drugs typically arises through three primary mechanisms: hindering the interaction between the target PBP and the drug, often by altering the drug's binding capacity to the PBP (achieved through modifications to existing PBPs or acquisition of new PBPs); the presence of efflux pumps capable of expelling β-lactam drugs; and the hydrolysis of the drug by β-lactamase enzymes [42, 43].

β-lactamases, originally termed penicillinases and cephalosporinases, deactivate β-lactam drugs by catalyzing hydrolysis at a specific site within the β-lactam ring, resulting in ring opening. This alteration prevents the drugs from binding to their target penicillin-binding protein (PBP) receptors. Widely distributed, β-lactamases encompass enzymes capable of neutralizing various β-lactam drugs. Among gram-negative bacteria, β-lactamase production stands as the primary resistance mechanism against these drugs, particularly affecting penicillin and cephalosporin efficacy [33,44].

Recently, there has been a rise in β-lactamases that target carbapenems (carbapenemases), predominantly found in Enterobacteriaceae. Two main types of carbapenemases exist: *Klebsiella pneumoniae* carbapenemases (KPCs) and Carbapenem-Resistant Enterobacteriaceae (CRE) enzymes. KPCs, categorized as serine Class A (functional group 2f) β-lactamases, resist all β-lactam drugs but may still be impacted by β-lactamase inhibitors. Conversely, CRE strains harbor metallo-β-lactamases (MBLs) in Class B (functional group 3a), capable of hydrolyzing all β-lactam drugs without susceptibility to β-lactamase inhibitors. The IMP-1 and VIM-1 types are among the most widespread CREs, with a newly identified MBL, NDM-1, emerging mainly in E. coli strains. Infections linked to CRE strains have been associated with in-hospital mortality rates as high as 71% [42-46].

There is a significant focus on developing more potent β-lactamase inhibitor

combinations, particularly to tackle CRE strains. One such combination, ceftolozane/tazobactam, primarily targets *P. aeruginosa* and demonstrates efficacy against gram-negative ESBL producing strains. Additionally, newer β-lactamase inhibitors with distinct structures from traditional β-lactam drugs are emerging. Avibactam, the first of these inhibitors, has been approved for use with ceftazidime against gram-negative bacteria and is being explored for its effectiveness with aztreonam against CREs. Another non-β-lactam structured inhibitor, vaborbactam, was approved in 2017 for use with meropenem against gram-negative bacteria causing complicated urinary tract infections (cUTIs). Unfortunately, none of the newer combination drugs are directly aimed at combating CREs. Overcoming metallo-β-lactamases proves challenging due to their structural and mechanistic diversity across three distinct groups [47, 48].

Bacteria possess chromosomally encoded genes for efflux pumps, some of which are consistently active, while others are triggered or heightened in expression under specific environmental conditions or upon exposure to particular substances. Typically, high-level resistance arises from mutations altering the transport channels. Efflux pumps primarily serve to expel toxic substances from bacterial cells, and many can transport a diverse range of compounds, making them multi-drug (MDR) efflux pumps. The effectiveness of these pumps in conferring resistance is often influenced by the available carbon source [19, 49].

The majority of bacteria possess a variety of efflux pumps, categorized into five main families based on their 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. Most of these efflux pump families consist of single-component pumps responsible for transporting substrates across the cytoplasmic membrane. However, the RND family stands out as multi-component pumps, predominantly found in gram-negative bacteria. These pumps function in conjunction with a periplasmic membrane fusion protein (MFP) and an outer membrane protein (OMP-porin) to expel substrates

across the entire cell envelope [19, 20, 50, 51].

In certain cases, different efflux family members collaborate with various cellular components to function as multicomponent pumps in gram-negative bacteria. For instance, MacB, a member of the ABC family, operates alongside MacAB-TolC as a tripartite pump to expel macrolide drugs. Similarly, EmrB, a member of the MFS, functions with EmrAB-TolC as a tripartite pump to remove nalidixic acid in *E. coli* [52, 53].

Table 1.1 shows a summary of the antimicrobial resistance mechanisms that are used against the various drugs.

Antibiotic resistance can arise through horizontal acquisition of resistance genes, facilitated by plasmids or transposons, recombination of foreign DNA into the chromosome, or mutations in various chromosomal loci. In molecular evolutionary biology, mutation rate refers to the rate of mutation per nucleotide, locus, or genome per generation, categorizing mutations as favorable, unfavorable, or neutral. In contrast, mutation frequency encompasses all mutants in a population, regardless of when the mutation occurred, providing a snapshot of the population's history before selection [54].

In the context of antibiotic resistance, the mutation rate is often defined as the frequency at which detectable mutants emerge in a bacterial population under a specific antibiotic concentration in vitro. This measurement focuses on the number of mutant cells rather than the number of mutation events, capturing selectively advantageous mutations that confer visible antibiotic resistance. Assessing mutation rates is crucial for predicting the emergence of antibiotic-resistant bacteria. However, in discussions surrounding antibiotics, the term «mutation rate» is sometimes oversimplified, portraying it as an inherent property of a new antimicrobial drug in its interaction with target bacteria, where a «low mutation rate» is seen as advantageous. This notion is misleading; instead, it's essential to recognize the multifaceted nature of mutation rates and advocate for more sophisticated methods to predict the emergence of mutational resistance to antibiotics.

Table 1.1

Antimicrobial resistance mechanisms

Drug	Drug Uptake Limitation	Drug Target Modification	Drug Inactivation
β-Lactams	Decreased numbers of porins, no outer cell wall	Gram pos- alterations in PBPs	Gram pos, gram neg- β-lactamases
Carbapenems	Changed selectivity of porin		
Cephalosporins	Changed selectivity of porin		
Glycopeptides	Thickened cell wall, no outer cell wall	Modified peptidoglycan	
Lipopeptides	Modified net cell surface charge		
Aminoglycosides	Cell wall polarity	Ribosomal mutation, methylation	Aminoglycoside modifying enzymes, acetylation,
Tetracyclines	Decreased numbers of porins	Ribosomal protection	Antibiotic modification, oxidation
Chloramphenicol		Ribosomal methylation	Acetylation of drug
Lincosamides		Gram posribosomal methylation	
Macrolides		Ribosomal mutation, methylation	

Since the groundbreaking studies by Luria and Delbrück, it has been evident that assessing mutation rates is challenging. Distinguishing the observed frequency of mutants from the true mutation rate is difficult, leading to the development of fluctuation tests to analyze the presence of jackpots of preexisting mutants in tested populations. In the realm of antibiotic resistance, the complexity is heightened by the fact that the phenotype may not consistently reflect the same genotypes among selected mutants, as mutations in various genes can yield similar antibiotic resistance

phenotypes. For instance, when determining the mutation rate for quinolone resistance, it actually represents a combination of mutation rates for genes encoding GyrA, GyrB, ParA, ParC, and several multidrug resistance (MDR) systems. Consequently, the calculated «phenotypic» mutation rate results from multiple "genotypic" mutation events. Moreover, mutations at different loci lead to varying changes in minimum inhibitory concentrations (MICs), contributing to the stable maintenance of heterogeneous antibiotic resistance expression classes in bacterial populations [55-58].

In recent years, a surge in published research has highlighted that the mutation process within bacterial populations is dynamic rather than static. Various factors form a complex network influencing the rate and types of mutants selected under antibiotic pressure. Mutation rates can significantly fluctuate depending on the concentration of a specific antibiotic during selection. Physiological conditions, such as the availability of certain carbon sources or bacterial stress, may also regulate mutation rates in bacteria. Moreover, the presence of mutations generating mutator phenotypes and certain antibiotics' ability to enhance mutability further complicate studies on the effects of population dynamics on the emergence of antibiotic-resistant mutants. These sources of variability pose significant challenges to predicting the «true» mutation rate solely through simple experimental procedures commonly employed in laboratory settings [59-63].

Horizontal gene transfer (HGT) plays a crucial role in rapidly disseminating antibiotic resistance genes (ARGs) across bacterial populations. Various mechanisms facilitate the movement of these genes between strains and species, including conjugation via plasmids, transduction via bacteriophages, and natural transformation through extracellular DNA uptake. Consequently, ARGs can spread to unrelated pathogens via HGT, leading to outbreaks fueled by the transfer of resistance genes.

HGT is vital for bacterial survival and serves as a primary mechanism for ARG transfer. The three primary mechanisms of bacterial HGT encompass intracellular conjugation (facilitated by plasmids and integrative conjugative

elements), natural transformation (mediated by bacteriophages), and transduction (involving the uptake of extracellular DNA (Fig. 1.2) [64].

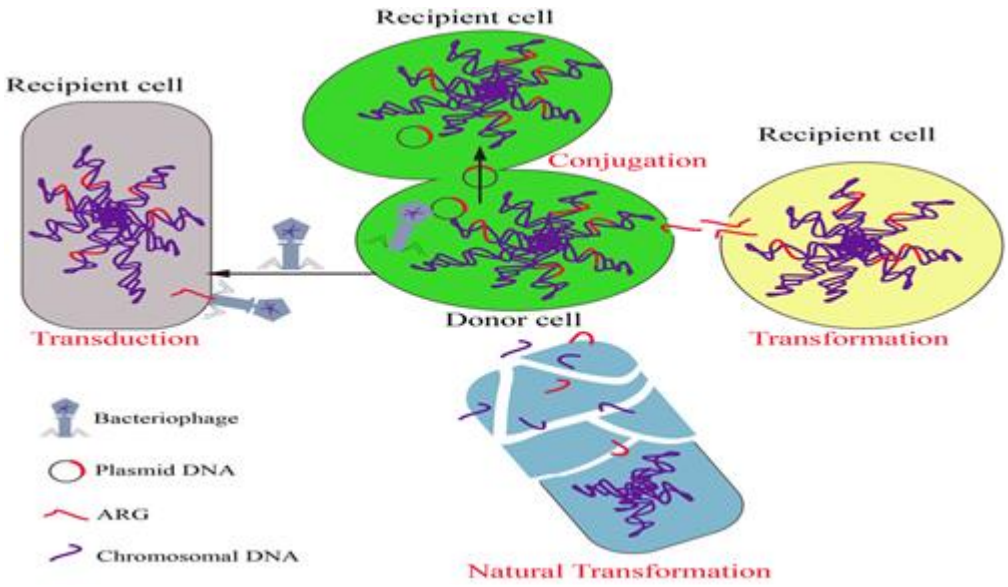

Fig.1. 2. The main mechanisms of bacterial HGT.

Horizontal transfer of antibiotic resistance genes involves the movement of these genes from donor to recipient cells through three main mechanisms: conjugation, transformation, and transduction. Conjugation occurs when two bacteria come into direct contact, facilitating the exchange of DNA, typically via plasmids. This process allows recipient cells to acquire resistance genes.Transformation entails the incorporation of resistance genes into the chromosomes or plasmids of recipient cells. This occurs through lysis, where DNA is released by one bacterium and absorbed by another.Transduction occurs when resistance genes are transferred from one bacterium to another by bacteriophages. These genes can then be integrated into the chromosomes of recipient cells.Natural transformation involves the direct uptake and integration of extracellular DNA by bacteria.These processes contribute to the dissemination of antibiotic resistance genes among bacterial populations.

The World Health Organization (WHO) and scientific literature have

documented numerous drug-resistant microbes, including vancomycin-resistant Enterococcus (VRE), imipenem-resistant *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA), cephalosporin-resistant *Escherichia coli*, clarithromycin-resistant *Helicobacter pylori*, fluoroquinolone-resistant *Campylobacter spp*., fluoroquinolone-resistant *Salmonellae,* cephalosporin-resistant and fluoroquinolone-resistant *Neisseria gonorrhoeae,* penicillin-non-susceptible *Streptococcus pneumoniae*, ampicillin-resistant *Haemophilus influenzae*, fluoroquinolone-resistant *Shigella spp*., as well as resistant strains of *Klebsiella*, *Serratia*, *Proteus*, and *Broccoli* [65].

These pathogens have become increasingly resistant to treatments and therapeutic regimens, posing a relentless public health concern. Not only have current microbial strains developed resistance to individual medicines, but they also exhibit resistance to multiple drugs, leading to widespread dissemination within communities. Additionally, reports indicate that bacterial isolates have evolved resistance against colistin and carbapenems, considered last-line stronghold antibiotics against Gram-negative bacteria (GNB), thereby posing a serious threat [66, 67].

1.2. The impact of COVID-19 on antibiotic resistance

During the COVID-19 pandemic, there has been a significant rise in antimicrobial usage, primarily because of the absence of effective treatment approaches. While COVID-19 itself is caused by a virus and is not treatable with antibiotics, secondary bacterial infections like pneumonia or superinfections may occur, requiring the use of antibiotics [68].

Excessive antibiotic use in treating COVID-19 patients stemmed from several factors: heightened anxiety surrounding a novel disease, similarity in symptoms to pneumonia, and increased mortality rates in communities with lower immunity. Furthermore, bacterial co-infection occurred in nearly 16% of cases, leading to a surge in antibiotic prescriptions, notably broad-spectrum ones, which rose to over 72% during the pandemic [69-71].

The surge in antibiotic consumption during COVID-19 has significantly fueled the proliferation of antimicrobial resistance (AMR). Increased hospitalization rates are quietly exacerbating the growing prevalence of AMR, resulting in approximately 700,000 global deaths annually. Moreover, the extensive deployment of antibiotics to manage pandemics could further escalate the development of resistant pathogens. Current projections indicate that by 2050, the current trajectory of antibiotic use in COVID-19 could lead to around 10 million deaths. This evidence underscores the urgent need to bolster antimicrobial stewardship (AMS) efforts and establish stringent antibiotic usage policies [72-76].

Research on antimicrobial resistance (AMR) has highlighted concerns regarding the inflammatory effects of prescribed medications. Consequently, various nations have developed guidelines for antimicrobial usage amid the pandemic. Nevertheless, the World Health Organization (WHO) advises against antibiotic use for patients with mild to moderate bacterial or COVID-19 infections. For severe cases, WHO recommends only low-potency antibiotics, and for elderly patients, antibiotics listed by WHO should be prescribed. However, these guidelines are deemed inadequate in curbing AMR emergence. There is a need for evidence-based antimicrobial stewardship (AMS) guidelines during and post-COVID-19. Analyzing pandemic spread trends and global antibiotic usage since the COVID-19 onset is imperative [77-79].

Based on our PRISMA keyword search, a total of 130 articles were chosen for the final systematic synthesis. Among these studies, the majority were conducted in the USA, followed by the UK, India, Italy, and China.

In terms of antimicrobial prescription trends, there was a slight increase in the number of antimicrobial types prescribed in 2020 and 2021 compared to 2019. However, the number of patients using antimicrobials saw a significant surge during this period. In 2019, there were 645 different antimicrobials being used, but by 2021, this number had nearly quadrupled to 2503. Concurrently, the total number of admitted patients and available beds in the surveyed wards also increased from 2019 to 2021, aligning with the rise in COVID-19 cases (Fig. 1.3) [94].

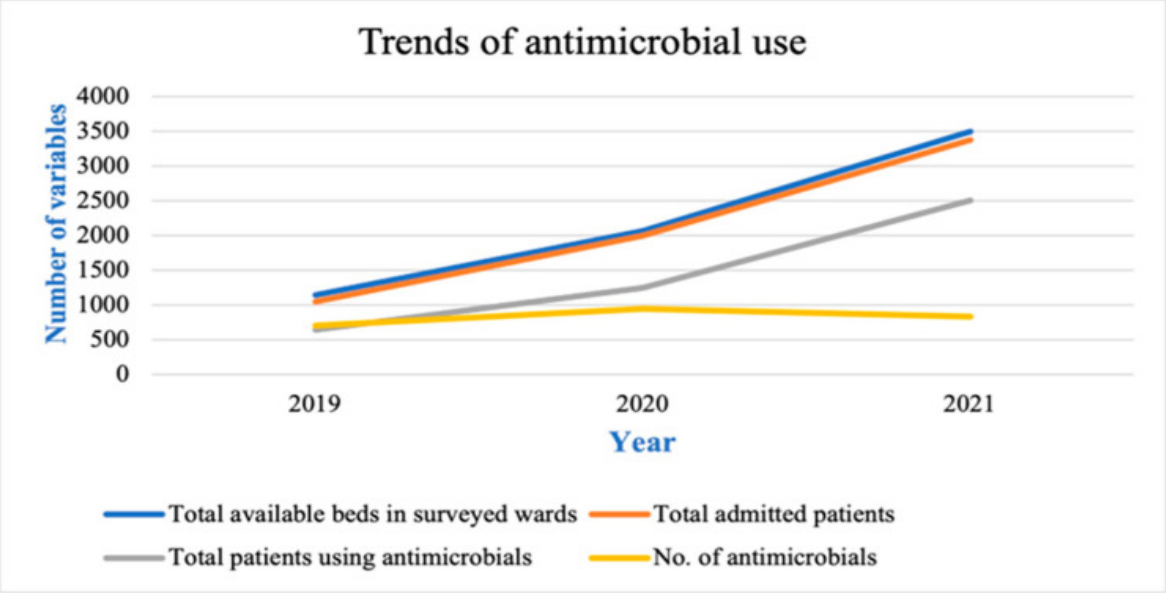

Fig. 1.3. Trends of antimicrobial use in hospitalized COVID-19 patients along with total available beds in surveyed hospital wards.

In total, 47.6% of patients experienced severe or critical illness, with the remainder having mild or moderate cases. Antibiotics were prescribed for nearly 78% of patients. There was a slight variation in antibiotic prescription rates between patients with severe or critical conditions and those with mild or moderate cases (77.4% and 76.8% respectively; see Figure 1.4) [94].

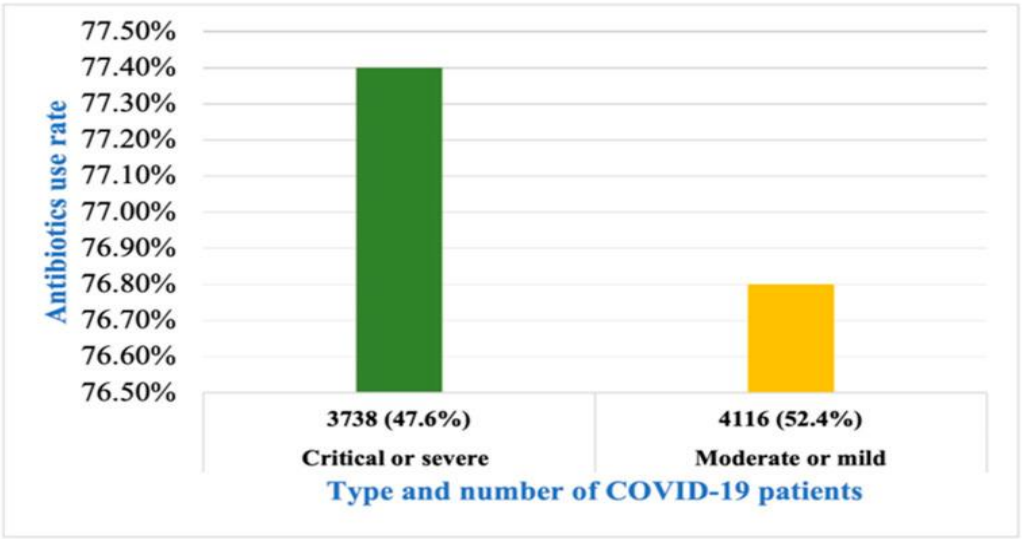

Fig. 1.4. Rate of antibiotic use in COVID-19 patients according to the severity of illness.

Cases where all patients were prescribed antibiotics exhibited a higher

mortality rate compared to cases where most patients did not receive antibiotics. The length of hospital stay (LOS) was longer in the group where the majority, but not all, patients were administered antibiotics. Conversely, the discharge rate was highest among patients who did not receive antibiotics compared to the group where most patients were prescribed antibiotics (see Fig. 1.5) [94].

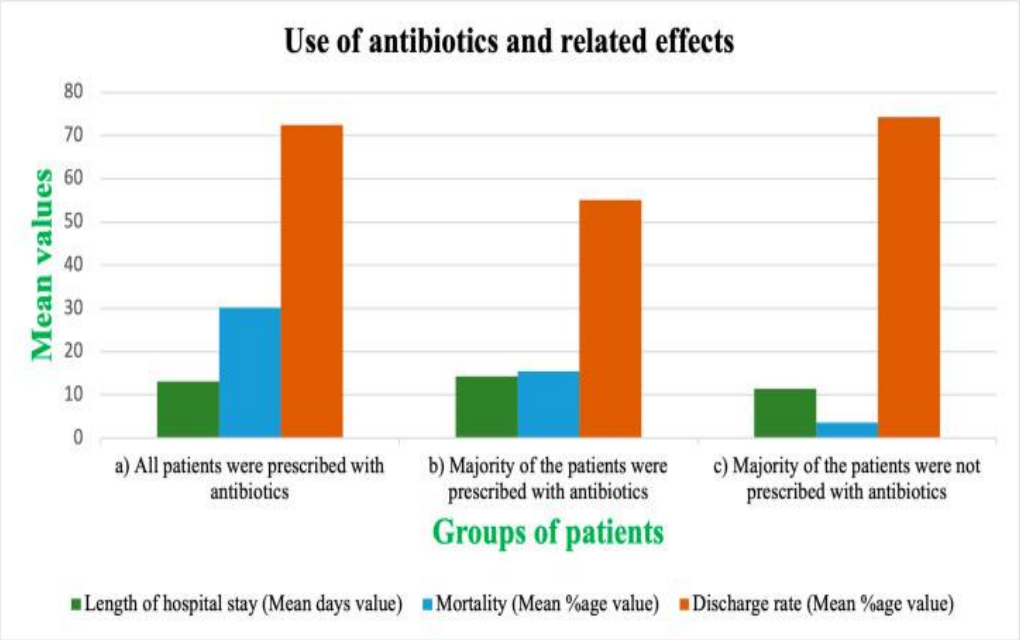

Fig. 1.5. Use of antibiotics and related effects.

The use of antibiotics in COVID-19 patients divided into three groups (a) all patients using antibiotics, (b) the majority of the patients using antibiotics, and (c) the majority of the patients not using antibiotics) and related effects (length of hospital stay, mortality, and discharge rate).

1.3. Approaches to combat antibiotic resistance in current and future directions

Infections caused by antibiotic-resistant pathogens present a growing threat to humanity. Exploring innovative approaches to address the antimicrobial resistance

crisis is crucial for any global response to this issue, preventing a potential return to the pre-penicillin era of medicine. To combat AMR, various approaches and strategies are currently being developed and implemented:

- New Antibiotic Discovery;
- Antibiotic Adjuvants for the Inhibition of Resistance;
- Antivirulent Therapy;
- Vaccination;
- Phage Therapy.

A significant challenge in the discovery of new antibiotics lies in the lengthy and costly drug production process. Currently, it takes around 15 years from the initial discovery of a promising compound in the laboratory to its selection and utilization as a therapeutic agent. Consequently, researchers often focus on modifying or repurposing existing drugs rather than discovering entirely new antibiotics [80, 81].

Fig. 1.6. demonstrates the approaches for new drug discovery against multidrug resistance.

In addition to the quest for new antibiotics, it's crucial to safeguard our current drug arsenal. A strategy to preserve existing drugs involves the use of antibiotic adjuvants. These adjuvants serve the dual purpose of blocking resistance and enhancing the effectiveness of current medications.

Antibiotic adjuvants are primarily employed in combination therapy. These therapeutics demonstrate their effectiveness by modulating active transport, enhancing drug absorption, influencing drug metabolism in the intestine or liver, boosting immune activity, and reducing elimination rates. Generally, antibiotic adjuvants fall into two categories: class I and class II. Class I adjuvants are further subdivided into class I-A and class I-B [82-84].

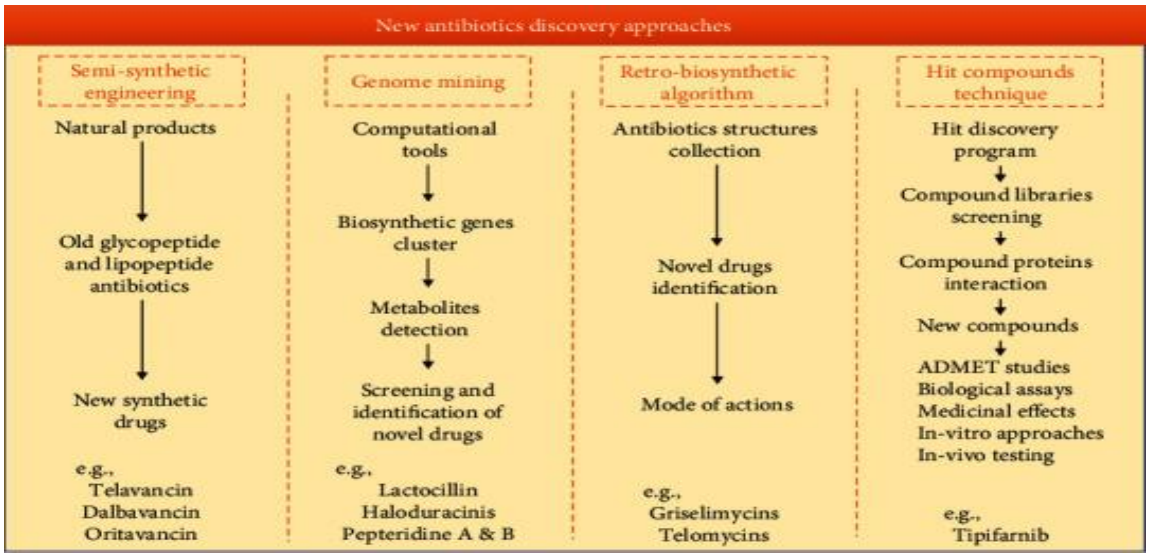

Fig. 1.6. Schematic diagram showing the approaches for new drug discovery against multidrug resistance.

Antivirulent therapy aims to reduce bacterial virulence without impeding pathogen development by utilizing quorum-sensing (QS) inhibitors. Prokaryotic organisms utilize QS for cell-to-cell communication at high concentrations, triggering prokaryotic adaptive immunity. By employing QS inhibitors, adaptive immunity can be suppressed, leading to nonpathogenicity. The mechanisms by which QS elicits a response in Gram-positive bacteria and Gram-negative bacteria (GNB) differ; Gram-positive bacteria utilize oligopeptides, while GNB employ N-acyl-L-homoserine lactones. Quorum quenching can be achieved through sequestration, competition, and signal destruction. This approach highlights the inhibition mechanism of QS inhibitors in controlling bacterial biofilm formation [85, 86].

Vaccines play a crucial role in combating antimicrobial resistance (AMR) by reducing the need for antibiotics. Diseases like pertussis and diphtheria, against which we've vaccinated for years, have seen decreased clinical obstacles due to low infection rates. However, the real impact lies in tackling bacteria with high mortality rates and resistance potential. By lowering infection rates, vaccines decrease antibiotic prescriptions, thus reducing selective pressure, a major driver of resistant

gene emergence. Studies show that reducing antibiotic use correlates with lower resistance rates. Unlike drugs, vaccines stimulate immune responses and can be tailored to target specific microorganisms, minimizing their impact on the body's bacterial flora. For instance, the Hib conjugate vaccine has successfully decreased cases of bacteremia, pneumonia, and meningitis caused by Hib, leading to reduced antibiotic usage and impeding the development of resistance [87, 88].

Bacteriophages act as a biocontrol measure against antimicrobial resistance (AMR). Despite being overlooked during the antibiotic era, the resurgence of AMR has reignited interest in phage therapy. This approach utilizes specific bacteriophages as an alternative to antibiotics. Phages with lytic cycles are preferred for biocontrol as they directly lyse pathogens, unlike lysogenic phages, which may contribute to the spread of antimicrobial-resistant genes rather than eliminating them [89-91].

Researchers, led by experts from Imperial College London, have discovered a method to weaken antibiotic-resistant bacteria, including *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, by targeting a protein crucial for driving resistance formation [92]. Antibiotic-resistant bacteria possess various proteins that render antibiotics ineffective. These resistance proteins must adopt specific shapes to function properly. Dr. Mavridou's team identified DsbA, a bacterial protein that assists in folding resistance proteins into their functional shapes to counteract antibiotics.

In a proof-of-concept study, the researchers inhibited DsbA using chemicals unsuitable for direct use in humans, effectively halting the formation of resistance proteins. The team aims to develop safe inhibitors that can replicate this protective effect in human patients [93].

Conclusions for chapter 1

1. Antibiotic resistance is increasing at an alarming rate and is now widely recognized as a global issue that requires urgent attention.
2. There is a significant gap between the beliefs and understanding of differentiating between COVID-19 infection and bacterial coinfection, led to widespread use of antibiotics, contributing to antibiotic resistance.
3. There is a need for new effective approaches because, despite several strategies being deployed, resistance levels are still of huge concern.

CHAPTER 2
MATERIALS AND METHODS

The experimental part of the master thesis was conducted in collaboration with Mohamed V hospital in Meknes, Morocco.

For the purposes of the master thesis a questionnaire was developed for surveying of patients with antibiotic resistance developed during the COVID-19 pandemic (table. 2.1.)

This chapter included questions concerning efficiency of antibiotic therapy as well as the safety, that from the point of view of Mohamed V hospital patients they are the most important for the effectiveness and safety of the treatment during the COVID-19 pandemic and after.

The study was conducted in the period from October 14, 2023 to March 02, 2024. Our study included hospital patients who were diagnosed with COVID-19 infection.

The questionnaire included questions of a general nature regarding the age, gender, professional activity of the respondent, as well as special questions related directly to the use of the antibiotic therapy in normal life and to treat COVID-19 infection being carried out. Particular attention was also paid to the assessment of antibiotic resistance between patients with coronavirus disease.

Also, based on the results of the survey, practical recommendations were developed to improve the efficiency and safety of antibiotic therapy using that the prevention of antibiotics resistance is the key for controlling its spread among Moroccan population. We have formulated the principles of Rational use when using antibiotics treatments, which are of practical importance not only for patients using antibiotics , but also for medical and pharmaceutical specialists.

The methodological basis of the study is the principles of objectivity and consistency. The work uses a complex of general scientific and special methods: theoretical, generalization, data systematization, comparison, methods of studying literary sources, analysis, questionnaire method, statistical methods, etc.

Table 2.1

Questionnaire for Mohamed V hospital patients

with antibiotic resistance and COVID-19 impact

1.	Sex and age	
2.	Do use or take antibiotics?	Yes No
3.	Had you COVID-19?	Yes No
4.	Did you use antibiotics to treat COVID-19?	Yes No
5.	Did you take the antibiotic based on?	1 – With a doctor's prescription 2 – Without doctor's prescription
6.	If without doctor's prescription was it based on:	1 – Pharmacist's advice 2 – Your own experience
7.	What antibiotic did you use ?	1 – Azithromycin 2 – Amoxicillin 3 – Cefixime 4 – Gentamicin 5 – Other
8.	What was the duration of your antibiotic treatment?	For three days For four days For six days Other
9.	Did you stop taking antibiotics before finishing the full course of treatment, or did you continue until the end?	Yes, I stop before the end No, I always finish the full course
10.	Did you take multiple antibiotics or just a single one to treat COVID-19?	Just single antibiotics multiple antibiotics
11.	Did you receive guidance or recommendations from healthcare professionals regarding antibiotic use during the COVID-19 pandemic?	Yes No
12.	Had you ever experienced antibiotic resistance? (you may have an antibiotic-resistant infection if you don’t get better after treatment with standard antibiotics)?	Yes, I had personally experienced antibiotic resistance. No, I had not personally experienced antibiotic resistance.

Conclusions for chapter 2

1. The questions concerning the criteria of efficiency and safety of antibiotic use during COVID-19 and about the causes of antibiotic resistance, that from the point of view of the Mohamed V hospital patients, are the most important for the effectiveness of antibiotic use. In the questionnaire for Mohamed V hospital patients the questions and answers were adopted for better understanding by Mohamed V Hospital patients.

2. For the purposes of the survey were pooled 60 patients who presented in Mohamed V hospital in Meknes, Morocco.

CHAPTER 3
ANALYSING OF ANTIBIOTIC RESISTANCE DURING COVID-19 PANDEMIC AT MOHAMED V HOSPITAL IN MEKNES

3.1. Survey of cases concerning efficiency and safety of antibiotic therapy

The total number of surveyed hospital visitors was 60. The inclusion criteria were: diagnosis with COVID-19, use of antibiotics, experiencing antibiotic ineffectiveness- (antibiotic resistance); volunteering to take part in the survey.

The main characteristics of the surveyed hospital patients are presented in the table 3.1.

Table 3.1

Characteristics of surveyed hospital patients

#	Patients characteristics	Indicator	% from total amount
1.	Sex		
	Female	25	41.7
	Male	35	58.3
2.	Minimal age, years	20	
3.	Maximal age, years	65	
4.	Hospital patients, who used antibiotics to treat COVID-19	47	78.3
5.	Experience antibiotics resistance Yes No	 43 17	 71.7 28.3
6.	Total amount of hospital patients surveyed	60	100

From all surveyed hospital patients, 25 are females (41.7%) and 35 are male (58,3%). The average age was 42.5, the youngest respondent was 20 years old and the oldest – 65 years old. 78.3% of patients used antibiotic to treat COVID-19. From the surveyed hospital patients, 71.7% experienced antibiotic resistance, which is a high percentage compared to the 28.3% who did not experience antibiotic resistance.

Factors, that lead to antibiotic resistance

Irrational consumption

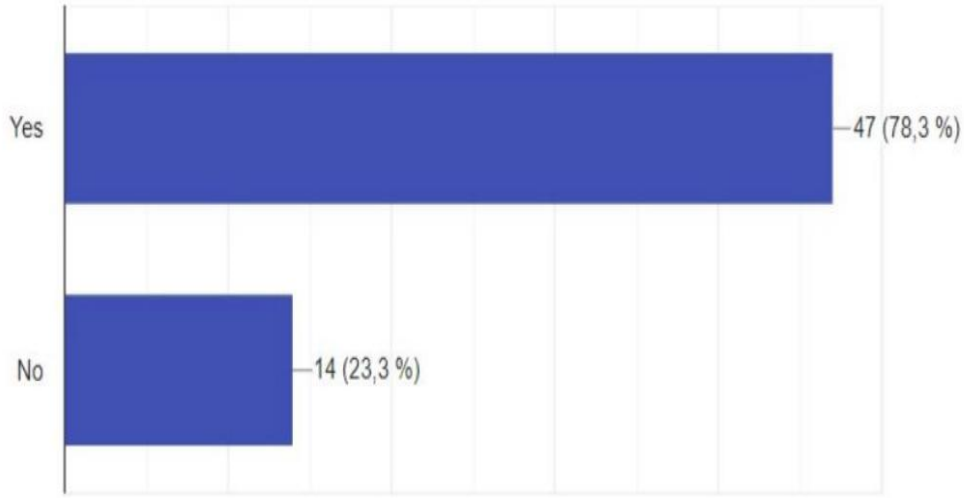

Fig.3.1. Patients who used antibiotics to treat COVID-19

According to the survey results (Fig. 3.1), 47 (78.3%) used the antibiotics to treat COVID-19 infection, which can be explained by the difficulty of differentiating between COVID-19 viral infection and bacterial coinfection leading widespread use of antibiotics. According to different analysis the number of antibiotics types increased only slightly in 2020 and 2021 compared to 2019, but the number of patients using antibiotics increased tremendously (a four-fold increase). The total number of admitted patients also increased from 2019 to 2021. This indicates a positive relationship between the number of COVID-19 patients and the antibiotics consumption, contributing to AMR.

The survey results concerning the frequency of antibiotic use: single vs. multiple times are presented in the Fig. 3.2.

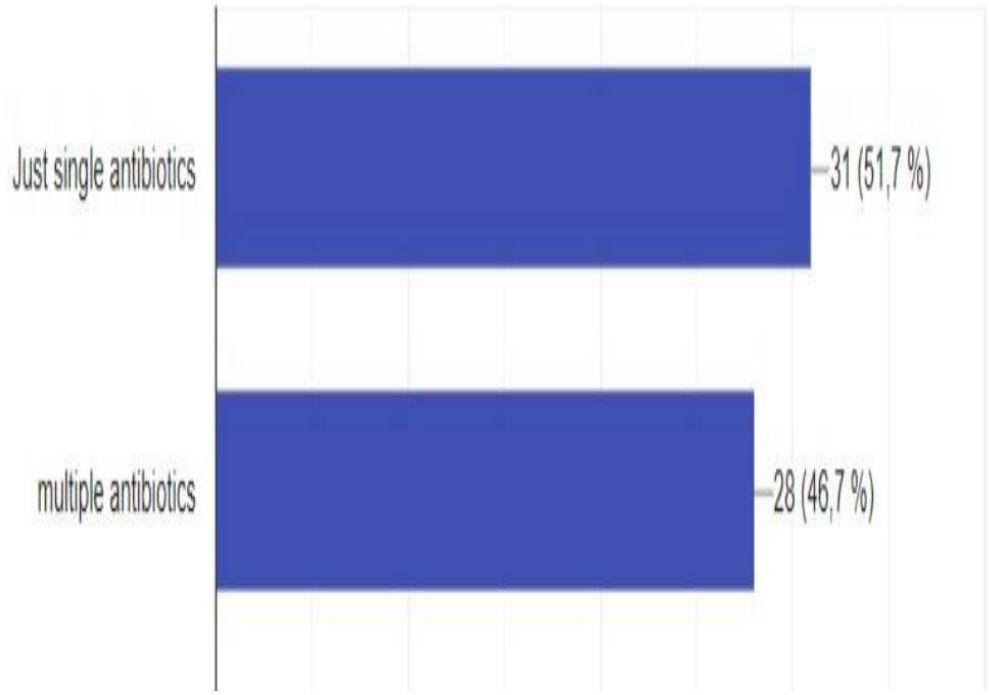

Fig. 3.2. Comparison of antibiotic use: single vs. multiple times.

According to the survey results (Fig. 3.2) 31 (51.7%) used just single antibiotic, while 28 (46.7%) used multiple antibiotics . In analyzing the results , it becomes evident that the use of antibiotics for multiple times in the treatment of COVID-19 indicates a certain level of ineffectiveness of the treatment. This finding can be attributed to the development of antibiotic resistance, particularly in cases of co-infection with bacterial pathogens. Or in cases of pure COVID-19 infection, the use of antibiotics does not yield effective results.

The survey results concerning the access to antibiotics use are presented in Fig. 3.3.

According to the survey results (Fig. 3.3) 28 patient (46.7%) took the antibiotic with doctor's prescription; 21 (35%) based on h advice and 12 (20%) by their own view. A higher rate of AMR could be predicted in Morocco which is considered as a low- and middle-income countries because of a lack of awareness and stewardship programs, poor lab facilities, and a lack of proper rules for accessing antibiotics without prescription. COVID-19 can be more easily spread to areas that are more populated and lack proper hygiene facilities.

The survey results concerning the types of antibiotic used to treat COVID-19 infection are presented in Fig. 3.4.

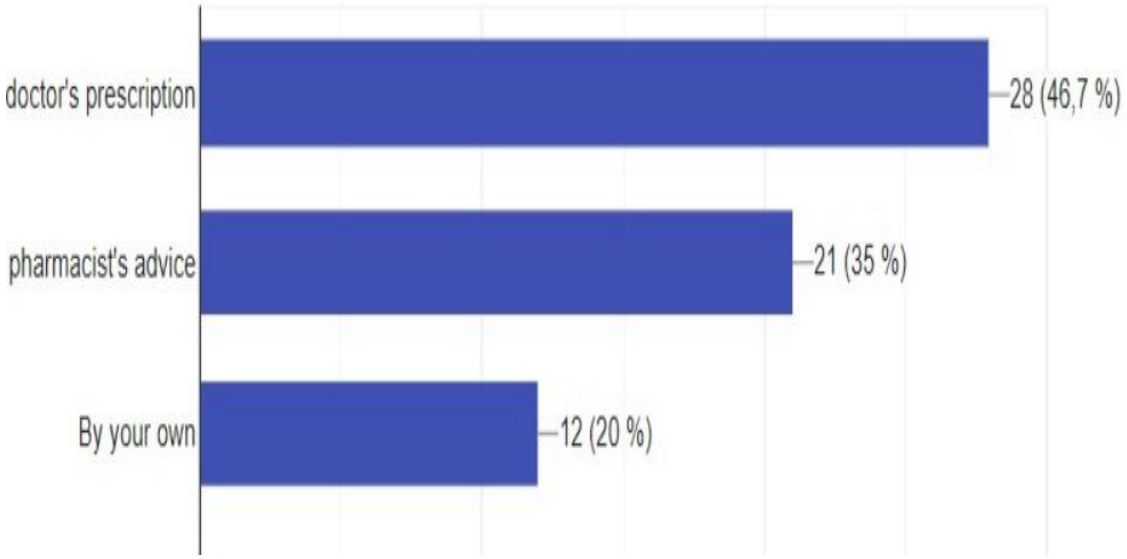

Fig. 3.3. Use of antibiotics according to doctor's prescription, pharmacist's advice, by the patient s own view.

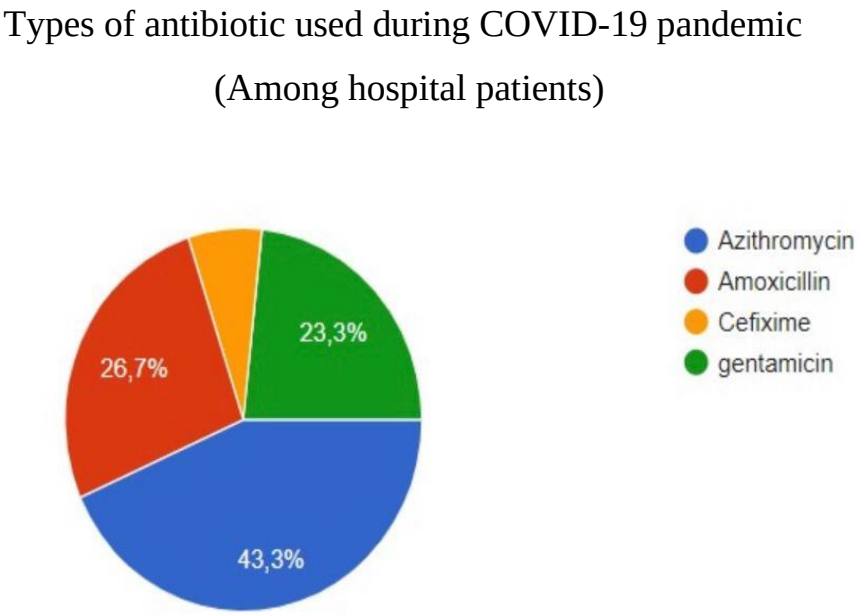

Fig. 3.4. Analysis of antibiotics used to treat COVID-19.

Based on the survey results, it seems that azithromycin is the most commonly used antibiotic at 43.3%, followed by amoxicillin at 26.7% and gentamicin at 23.3%, and 6.7 % cefixime. The improper access to antibiotics contributes to their increased consumption, especially azithromycin. Some patients believe that it can treat COVID-19, which leads to its high usage. Additionally, data analysis shows that doctors in Morocco prescribed azithromycin in 80% of COVID-19 cases. This may be due to misdiagnosis and the fear of bacterial coinfection.

The survey results concerning the completion of full course treatment of antibiotic are presented in Fig. 3.5.

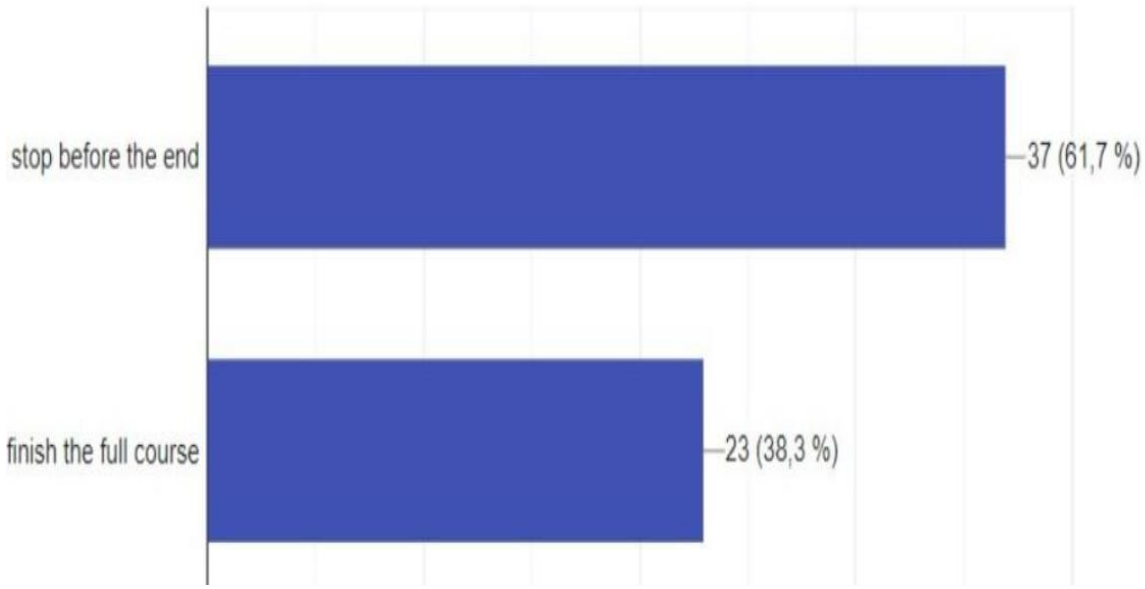

Fig. 3.5. The number of patients who stopped taking the antibiotic before completing the full course, as well as the number of patients who finished the entire course.

According to the survey results, 37 patients, which is about 61.7%, stopped taking the antibiotic before completing the full course. On the other hand, 23 patients, which is about 38.3%, successfully finished the entire course of antibiotic

treatment. The majority of patients 61.7% didn't finish the full course of antibiotics, this finding helps explain that stopping the treatment course before its end can allow remaining bacteria to multiply and potentially develop resistance. While the minority of patients 38.3%, which completed the full course of treatment helps ensure that all of the bacterial pathogens are killed or prevented from multiplying.

The survey results concerning the duration of course treatment of antibiotic is presented in Fig. 3.6.

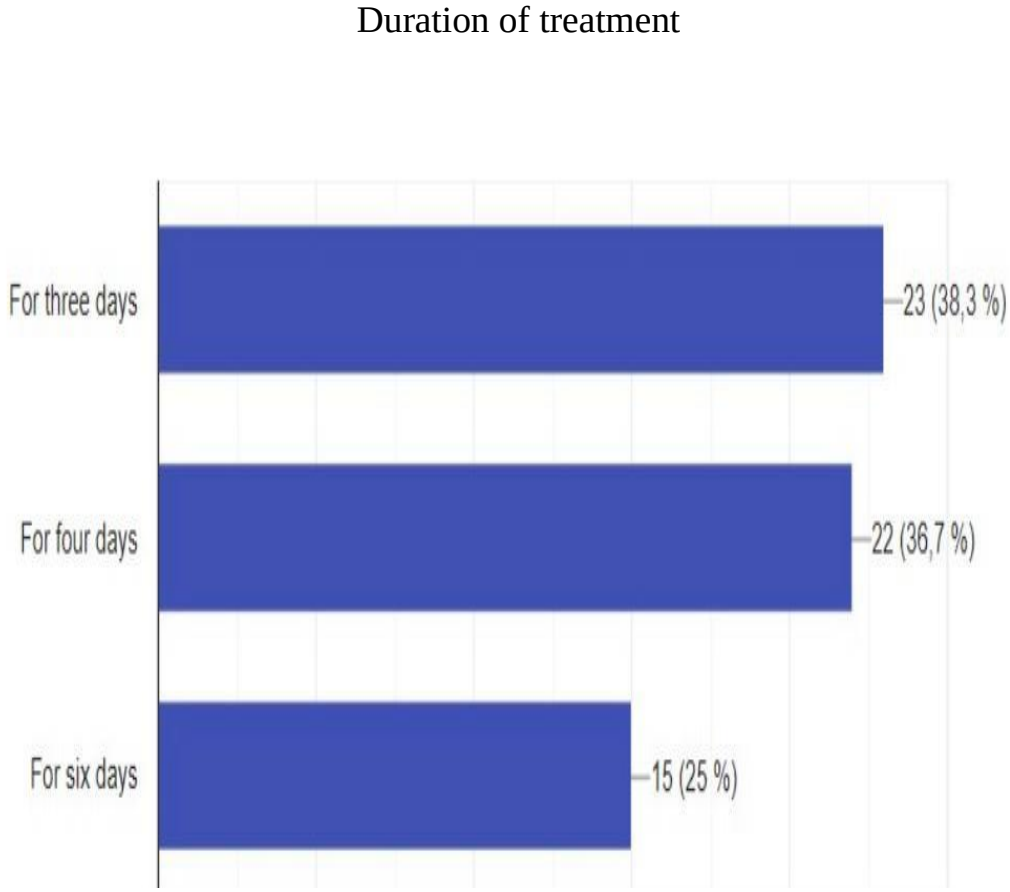

Fig.3.6. The duration of the treatment course.

The survey results concerning the patients experiencing the antibiotic resistance are presented in Fig. 3.7.

Prevalence of Antibiotic resistance (Among hospital patients)

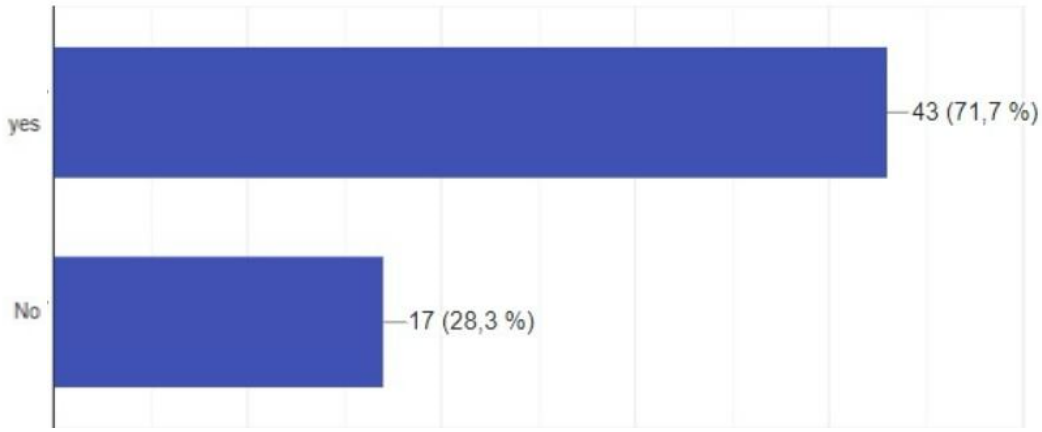

Fig.3.7. Percentage of patients experiencing antibiotic resistance.

According to the survey results, out of the 43 patients surveyed, a staggering 71.7% experienced antibiotic resistance. This high percentage can be attributed to various factors such as misdiagnosis, easy accessibility without a prescription, not completing the full course of antibiotics, and a lack of awareness about the serious problem of antibiotic resistance, which poses a threat to people's lives.

The survey results concerning the receiving of guidance and recommendations on antibiotic use among the hospital patients are presented in Fig. 3.8.

Guidance and recommendations on antibiotic (Among hospital patients)

Fig. 3.8. Patients who received guidance and recommendations on antibiotic use and did not receive.

According to the survey results we see that out of the total patients surveyed, 23 patients, which accounts for 38.3%, received guidance and recommendations on antibiotic use. On the other hand, a larger group of 37 patients, making up 61.7%, did not receive such guidance. This data highlights the need for improved efforts in providing guidance and recommendations to patients regarding antibiotic use, it can help the efficacy and safety of antibiotics by reducing the risk of antibiotic resistance and promoting better overall health outcomes. It emphasizes the need for healthcare professionals to actively engage with patients, empowering them with the rational use of antibiotics during the treatment for obtaining of effective results.

3.2. Discussion of the obtained results

The consensus among all patients was that COVID-19 have a significant impact on the efficacy of antibiotics effect, mainly in terms of increase of antibiotics resistance. Many factors were examined in the survey for better understanding of the factors leading to the spread of antibiotic resistance in Morocco and the consequences of COVID-19 pandemic on the antibiotic resistance.

Then comes the Morocco and factors contributing to antibiotics resistance. The accessibility and affordability of antibiotics, without doctor s prescription contribute to their increased consumption, especially azithromycin. The recommendation of antibiotics uses by physicians without any medical or biological indication of secondary bacterial infection, thus the antibiotic attacks the pathogenic and non-pathogenic bacteria in the body promoting the development of bacterial resistance. As a result, bacteria next time when taking antibiotic become hard to kill.

Lastly, the irrational use of antibiotics was highly remarkable between patients, according to the survey results most of patients receive any recommendations and guidance concerning the proper use of antibiotics which explain why 61.7% didn’t complete the full course of treatment. This incomplete treatment course can lead to bacterial survival, and the surviving germs start developing resistance through mutation and horizontal gene transfer, ultimately

leading to antibiotic resistance.

In summary, the COVID-19 has contributed to an increase of antibiotic resistance, due to various factors. The misdiagnosis of secondary infections while COVID-19 infection by physicians, along with unrestricted access to antibiotics in Morocco, leading to an increase of antibiotic use mostly without practicing the rational use during the treatment course.

3.3. Practical recommendations for physicians and patients to prevent antibiotic resistance

The prevention is the main key of antibiotics resistance controlling, by promoting Rational use of antibiotics, [physicians] in this case to communicate in an efficient way about the antibiotic resistance, the prevention, and do follow ups to provide the patient with effective results.

The physician should be able to prescribe the antibiotics that are needed: using antibiotics appropriately. For reducing the unnecessary and over prescribing antibiotics, for example, when antibiotics are prescribed for viral infection, it is irrational because antibiotics do not work against viruses.

Educate the patient about the causes and factors of antibiotic resistance: Antibiotic resistance occurs when bacteria develop the ability to survive the effects of antibiotics. This can happen due to several factors, such as the overuse and misuse of antibiotics, not completing the full course of treatment, and the use of antibiotics in agriculture and livestock

Practice good hygiene: For physician, performing of hand hygiene before and after all patients contact, by using of alcohol-based hand rubs or washing hands has proven efficacy in prevention of infection. This factor can restrict the spread of infection and thereby the antibiotic resistance. For patients, by simple means like covering mouth while coughing or sneezing, infection spread can be reduced.

Promoting the rational use of antibiotics by discussing the rational use of antibiotics we refer to the correct, proper and appropriate use of antibiotic. It implies

the appropriate choice of antibiotics, in the proper dose frequency, duration of the full course treatment, right information to the patient including advices, right follow-up.

Compliance to the antibiotic regime: improved compliance definitely can improve the rate of infection control. Patient education on compliance with antibiotics is must [30]. Using established regimes for prophylactic use of antibiotics in high-risk cases and for the shortest duration possible can minimize risk of AMR.

Follow up with patients: Follow up with patients to ensure that their antibiotic therapy is working effectively. Adjust the treatment plan as needed to avoid antibiotic resistance and achieve the best possible outcome.

Conclusions for chapter 3

1. The misdiagnosis of secondary infections while COVID-19 infection by physicians, contributed to an increase in the inappropriate consumption of antibiotics.

2. According to the survey 78.3% of patients used the antibiotics to treat COVID-19. According to different analysis the number of patients using antibiotics increased tremendously during COVID-19 pandemic. The total number of admitted patients also increased from 2019 to 2021. This indicates a positive relationship between the number of COVID-19 patients and the antibiotics consumption, leading to bacterial resistance to antibiotics.

3. Unrestricted access to antibiotics in Morocco. Based on the survey results (55%) of patients took the antibiotic without doctor`s prescription, leading to an increase of antibiotic use mostly without respecting the appropriate duration of the full course treatment, about 61.7%, of patients stopped taking the antibiotic before completing the full course.

4. The lack of guidance and recommendations concerning the rational use of antibiotics is one of the main factors of antibiotic resistance. According to the survey up to 61.7%, patient did not receive any guidance concerning the correct use of antibiotics, it highlights the need for improved efforts in providing guidance and recommendations to patients regarding antibiotic use treatment, during the treatment course.

5. The efficacy of treatment changed from a patient to another. According to the survey results, out of the 43 patients surveyed, a staggering 71.7% experienced antibiotic resistance, which leads to antibiotic therapy ineffectiveness. And the need for the use of multiple antibiotics. According to the survey 46.7% of patients used multiple antibiotics. In analyzing the results, it becomes evident that the use of antibiotics for multiple times in the treatment of COVID-19 indicates a certain level of ineffectiveness of the treatment.

6. The rational use of antibiotics is the key for prevention of antibiotic resistance. Physicians play a crucial role in promoting this practice. It is important for patients to practice rational. By doing so physicians and patients can contribute to the efficacy and safety of antibiotics.

CONCLUSIONS

1. Antibiotic resistance is increasing at an alarming rate and is now widely recognized as a global issue that requires urgent attention. There is a significant gap between the beliefs and understanding of differentiating between COVID-19 infection and bacterial coinfection, led to widespread use of antibiotics, contributing to antibiotic resistance

2. The misdiagnosis of secondary infections while COVID-19 infection by physicians, contributed to an increase in the inappropriate consumption of antibiotics. According to the survey 78.3% of patients used the antibiotics to treat COVID-19. According to different analysis the number of patients using antibiotics increased tremendously during COVID-19 pandemic. The total number of admitted patients also increased from 2019 to 2021. This indicates a positive relationship between the number of COVID-19 patients and the antibiotics consumption, leading to bacterial resistance to antibiotics.

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