ELIZABETH MKPOUTO OPAWOYE

EVALUATION OF THE DISTRIBUTION OF MICROORGANISMS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN ISOLATED FROM INTENSIVE CARE UNIT PATIENTS DURING THE PRE-PANDEMIC AND PANDEMIC PERIODS

Nicosia
July, 2023

NEAR EAST UNIVERSITY INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

EVALUATION OF THE DISTRIBUTION OF MICROORGANISMS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN ISOLATED FROM INTENSIVE CARE UNIT PATIENTS DURING THE PRE-PANDEMIC AND PANDEMIC PERIODS

MSC THESIS

ELIZABETH MKPOUTO OPAWOYE

Nicosia

July, 2023

NEAR EAST UNIVERSITY INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

EVALUATION OF THE DISTRIBUTION OF MICROORGANISMS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN ISOLATED FROM INTENSIVE CARE UNIT PATIENTS DURING THE PRE-PANDEMIC AND PANDEMIC PERIODS

MSc. THESIS

ELIZABETH MKPOUTO OPAWOYE

THESIS SUPERVISOR

ASSOC. PROF. DR. AYSE A. SARIOGLU

Nicosia JUNE, 2023

APPROVAL

We certify that we have read the thesis submitted by **Elizabeth Mkpouto Opawoye** Titled **“Evaluation of the Distribution of Microorganisms and their Antibiotic Susceptibility Pattern Isolated from Intensive Care Unit Patients During the Pre-Pandemic and Pandemic Periods”** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Medical Microbiology and Clinical Microbiology.

Examining Committee Name-surname Signature

Head of the Committee: Assoc. Prof. Dr. Meryem Guvener ……………………

Committee Member: Assist. Prof. Dr. Esref Celik ……………………

Committee Member: Assoc. Prof. Dr. Ayse A. Sarioglu ……………………

Approved by the Head of the Department

03/09/2023

Yakın Doğu Üniversitesi Hastanesi
Prof.Dr. Nedim Çakır
Enfekşiyon Hastalıkları ve
Klinik Mikrobiyoloji
Diploma no: 1059

……………………

Title, Name-Surname

Head of Department

Approved by the Institute of Graduate Studies

…../…../20…

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute

NEAR EAST UNIVERSITY INSTITUTE OF GRADUATE STUDIES 1988

DECLARATION

I hereby declare that all information, documents, analysis, and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced informationand data that are not original to this study.

ELIZABETH MKPOUTO OPAWOYE

….. /….. /…..

ACKNOWLEDGEMENT

My utmost gratitude goes to Christ Jesus my savior, who has made it possible for me to go through this program successfully. I am grateful for His grace, strength, and provision during my research work.

My deepest appreciation goes to my thesis supervisor Associate Professor Dr. Ayse Arikan Sarioglu for her help, guidance, and continuous support throughout this research work. Also, for being constantly available when I needed her despite the hour or day,I truly appreciate your efforts Ma.

Additionally, I will like to express my appreciation to Assist. Prof. Victor Markus, Assist. Prof Coston Pwaavodi, Dr. Emmanuel Adewusi, and Dr. Chidi Nwekwo for their unwavering support during the cause of my research work. I am immensely grateful Sirs.

Finally, I would like to thank my lovely and highly supportive Mother, my brothers and sisters, and my Pastor, Frederick Letong for their support and encouragement through this journey.Thank you so much for everything.

ELIZABETH MKPOUTO OPAWOYE

Abstract

Evaluation of the Distribution of Microorganisms and their Antibiotic Susceptibility Pattern Isolated from Intensive Care Unit Patients During the Pre-pandemic and Pandemic Periods

Elizabeth Mkpouto Opawoye

M.Sc., Department of Medical Microbiology and Clinical Microbiology

Supervisor: Assoc. Prof. Ayse Arikan Sarioglu

June 2023

Intensive care units (ICUs) are specialized healthcare environments that offer treatment for seriously sick patients who need constant observation and assistance. These patients are often immunocompromised due to their underlying illnesses, which makes them susceptible to infections. This study aims to evaluate the distribution of microorganisms and trends of antibiotic resistance among critical care unit patients hospitalized before and during the SARS-CoV-2 pandemic. To achieve this, details of patients both male and female from ages 0 to 100 years from various ICU wards of the different departments in the hospital were taken from the electronic microbiology database in the laboratory. A total of 3669 samples from 2018 to 2022 were obtained for the purpose of this study. The most prevalent pathogens isolated during the pandemic were *Pseudomonas aeruginosa* 392(20.3%), *Acinetobacter baumanni/calcoaceticus* complex 359(18.3%), *Klebsiella pneumoniae* 347(18.0%), *Candida spp* 164(8.5%) *Escherichia coli* 145(7.5%) and *Staphylococcus aureus* 106(5.5%). The rates of ESBL, MRSA, and VRE were 43.3%, 55.7%, and 6% respectively. Patients admitted into the ICU (54.7%) showed a significantly high incidence of bacterial pneumonia compared to those admitted to other wards (49.5%) in the hospital. Also, the frequency of growth in inpatients (53.7%) was significantly higher than the growth rate in outpatients (42.4%). Conclusively, the pre-pandemic periods- 2018, and 2019 had growth rates of 52.0%, and 51.6% respectively. The pandemic periods- 2020, 2021, and 2022 had growth rates of 45.5%, 52.2%, and 61.1% respectively. In conclusion, the detection of highly resistant pathogens and the increase in antibiotic resistance, especially after the late stages of the pandemic showed that patients in intensive care should be followed closely and alternative treatment strategies should be developed to limit antibiotic resistance to commonly used antibiotics.

Keywords; ICU, Antibiotic, Drug resistance, Microbial distribution.

Contents

List of Tables

List of Figures

List of Abbreviations

ICU – Intensive care unit

SARS-CoV-2 – Severe acute respiratory syndrome

% - Percentage

HAI – Hospital acquired infection

MDRO – Multi-drug resistant organisms

MRSA – Methicillin resistant *Staphylococcus aureus*

ESBL – Extended spectrum beta-lactamase

NDM – New Delhi metallo-beta-lactamase

NIDDM – Noninsulin-dependent diabetes mellitus

AMR – Antimicrobial resistance

CRE – Carbapenem-resistant Enterobacteriaceae

VRE – Vancomycin-resistant Enterobacteriaceae

AR – Antibiotic resistance

HGT – Horizontal gene transfer

DNA – Deoxyribonucleic acid

MGE- Mobile genetic element

EUCAST – European Committee on Antimicrobial Susceptibility Testing

EMB – Eosin methylene blue

AST – Antibiotic susceptibility test

SPSS – Statistical Package for the Social Sciences

CHAPTER 1

Introduction

1.0 Intensive Care Unit.

Intensive care units (ICUs) are specialized healthcare environments that offer treatment for seriously sick patients who need constant observation and assistance. These patients are often immunocompromised due to their underlying illnesses, which makes them susceptible to infections. Each year, an astounding 5.7 million patients are admitted to ICUs in the United States (Shickel et al., 2021). As a result, the distribution of microorganisms in ICUs is an important factor that can impact patient outcomes.

Healthcare-associated infection (HAI) is typically among the gravest issues in hospital settings and has presented a significant challenge to modern medicine since they increase mortality, lengthens hospital stays, and raises therapeutic costs (Rafa et al., 2021). Healthcare-associated infections harm more than 3 million individuals annually in Europe, 10% of whom needed hospitalization, and up to 1% of whom did not survive (Bouza et al., 2019).

ICUs have a higher incidence of HAIs than other hospital divisions, and many of these HAIs are life-threatening even though they account for only 15–20% of all hospital beds (Duszynska et al., 2020). Research has shown that HAI occurs in 20–50% of patients receiving hospital care in the ICU (Kołpa et al., 2018)(Dadi et al., 2021). This is entirely related to the patient's overall severe medical conditions and comorbidities in the ICU.

Research has shown that the most common sources of microorganisms in ICUs are patients themselves, healthcare workers, and the environment (Tozzo et al., 2022). Patients can harbor a variety of microorganisms, including bacteria, viruses, and fungi, that can be transmitted to other patients or healthcare workers through contact, droplets, or aerosols (Bonadonna et al., 2021). Healthcare workers can also serve as a source of microorganisms, particularly if they are not following proper hand hygiene protocols or if they are carrying pathogens on their clothing or equipment (Department of Health. Victoria, n.d.). The environment in an ICU can also be contaminated with microorganisms, which can be transmitted to patients through contact or inhalation. Additionally, the danger is heightened when intrusive equipment like catheters and intubation tubes are used, as well as when patients must frequently interact directly with medical workers (Blot et al., 2022). Mechanical ventilation, pleural drainage, and the administration of blood and plasma are additional HAI risk factors in intensive care units making the patients vulnerable to infections like bloodstream infections, pneumonia, urinary system illnesses, infection at the surgery site, infections of the soft tissues and skin, and others (Kołpa et al., 2018).

Consequently, patients generally spend time in other hospital wards before their stays in the ICU, where their treatment is typically challenging. During their stay, patients' physiological microbiota is substituted by the hospital microbiota characteristic of a particular ward (Regardless of the hospital ward in which they are being treated) (Liu et al., 2020). Patients who are admitted to intensive care units (ICU) encounter many changes in their gut microbiota as a result of their serious illness, antibiotics, and other ICU-specific medications (Szychowiak et al., 2022). These alterations are linked to a higher risk of infection complications and can be fatal.

Overall, the distribution of microorganisms in the ICUs is highly diverse and varied. Studies found that the most common types of microorganisms found in ICUs were oval-shaped gram-positive bacteria namely Coagulase-negative *Staphylococci, Staphylococcus aureus;* Gram-negative bacilli including *Escherichia coli, Klebsiella pneumoniae,* as well as *Enterobacter spp*; the Non-fermenting gram-negative bacteria namely *Acinetobacter baumanni, Pseudomnas aeruginosa,* and *Stenotrophomonas maltophila*. (Hasanzade et al., 2021) (Chang et al., 2021). Fungi, including *Candida albicans*, *Candida glabrata*, and *Candida krusei* (Prigitano et al., 2022). Finally, viruses, including Adenovirus influenza and Herpes simplex virus, and most recently SARS-CoV-2 (Calderaro et al., 2021).

The issue of antibiotic resistance complicates the treatment of major ICU-related illnesses, including HAIs (Barchitta et al., 2019). The use of antibiotics in the ICU and the alarmingly high frequency of resistant bacteria identified from infections have been the subject of several Polish research (Trejnowska et al., 2018).

1.1 Antimicrobial Resistance

Antimicrobial resistance is a major concern in the ICU, where extremely sick patients are more likely to contract infections due to their weakened immune systems and the usage of invasive medical equipment (Ture et al., 2022). The utilization of antimicrobial therapy in the treatment of these infections can select antimicrobial-resistant microorganisms, leading to the emergence of so-called "superbugs" that are difficult or impossible to treat with standard antimicrobial therapies (Parmanik et al., 2022).

Several factors have been identified as contributing to the emergence of antimicrobial resistance in the ICU setting. One important factor is the overuse and misuse of antimicrobial drugs, this may result in the selection and proliferation of antimicrobial-resistant strains (Chiș et al., 2022). Other factors that have been identified include the presence of invasive medical devices, inadequate infection prevention, and control measures, and inadequate antimicrobial prescribing practices (Harun et al., 2022).

To address the issue of antimicrobial resistance in the ICU setting, it is important to implement evidence-based strategies such as infection prevention and control measures, antimicrobial stewardship programs, and appropriate antimicrobial prescribing practices (Bankar et al., 2022). Other strategies that have been identified as being effective in reducing the risk of antimicrobial resistance in the ICU setting include the implementation of contact isolation protocols, the promotion of hand hygiene, and the proper sterilization and disinfection of medical equipment (Haque et al., 2020a).

Overall, the distribution of microorganisms in ICUs is complex and diverse, with various kinds of microorganisms present in different proportions depending on the specific patient population and the ICU environment. Additionally, antimicrobial resistance is a major concern in the ICU setting due to the high risk of infection in seriously sick patients and the likelihood for the emergence of antimicrobial-resistant "superbugs", particularly during times like the acute covid-19 era when antimicrobial stewardship programs are almost impossible to implement.

The purpose of this study is to analyze the distribution of bacteria and trends of antibiotic resistance among critical care unit patients hospitalized before and during the SARS-CoV-2 pandemic. Consequently, to ascertain the indirect impact of the SARS-

CoV-2 pandemic on the pathogen dispersion at the Near East University Hospital in Northern Cyprus.

CHAPTER II

Literature Review

2.0 Intensive Care Unit Infection.

The distribution of microorganisms in the ICU is complex as well as varied, with different types of microorganisms present in different proportions depending on the specific patient population and the ICU environment. The term "nosocomial infection" or "health-care associated infections" (HAI) refers to illnesses that patients get while undergoing medical care but were neither present at the time of hospital admission nor in the incubation phase at the time of hospitalization (Orhan et al., 2022). Although everyone in the hospital is in danger of infection, patients in the ICU are more susceptible to infection since their immune systems have been impaired and they are exposed to more intrusive surveillance (Sr et al., 2019).

Life-threatening diseases or trauma requiring intensive care are treated in the ICUs. According to reports, the intensive care unit has a 2–5 times greater incidence of nosocomial infections than the general in-patient hospital population (Dasgupta et al., 2015). Hence, all ICU patients are at risk for infection and death from nosocomial infections. According to a surveillance study conducted in 2007, nosocomial infections infected 39% of patients in European Union nations (*Surveillance of Healthcare-Associated Infections in Europe 2007*, 2012), whereas between 24 and 45% of ICU patients in Poland contracted nosocomial infections (Kübler et al., 2012). The most prevalent types of these infections are presented as pneumonia, bloodstream infection, and urinary system illnesses (Rafa et al., 2021).

2.1 Impact of coronavirus in ICUs

The outbreak of severe acute respiratory syndrome (SARS-CoV-2) in the concluding month of 2019 has greatly impacted the population of patients admitted to the ICUs as well as the ICU environment. The pandemic has had a major influence on the world health system since 2019. Following the quick spread of the SARS-CoV-2 infection, about 30% of patients with the infection may develop a severe form of illness that necessitates admission to the ICU (Huang et al., 2020). This has a significant influence on the ICUs, upheaving these intensive wards and significantly altering the microbial flora (Nguyen et al., 2021). This change in the local bacterial and fungal flora was likely brought on by an unanticipated inflow of patients that outnumbered the medical staff and filled more ICU beds than there were before the pandemic (Trentini et al., 2022).

Every medical association created and put into action a variety of infection control and preventive techniques to battle this world health disaster, including the use of masks, using excellent hygiene practices, avoiding crowded places, keeping a social distance, actively identifying and quarantining close contacts, quickly establishing shelter hospitals, and lockdown tactics(Z. Li et al., 2020),(De Georgeo et al., 2021). These forceful countermeasures to the virus have further advantages in the sense of lowering other illnesses. According to research, there was a significant decline in influenza activity in Japan in 2019–2020 as compared to other seasons (Sakamoto et al., 2020), besides that, invasive pneumococcal disease decreased in Taiwan as well as tuberculosis (Juan et al., 2021), (Lai & Yu, 2020).

2.1.1 Varying rate of Co-infection

Since SARS-CoV-2 may impair the immune system, there is a higher chance of fatalities and serious harm brought on by the ever-rising secondary infections, or suspected co-bacterial illnesses, as the case may be. Patients with underlying diseases or those who are immunocompromised experience this with greater severity (Moradi et al., 2021). Research has also shown a rise in organisms that are resistant to multiple drugs on account of the majority of SARS-CoV-2 victims receiving antibiotic and antifungal therapy for confirmed or suspected co-bacterial infections while they were receiving treatmeant in the intensive care unit (Grau et al., 2021). This was almost practically unavoidable given that some patients' COVID-19 condition developed into multiorgan failure(Robba et al., n.d.), there were many constraints on invasive diagnostic techniques as a result of the steps taken to curb the proliferation of SARS-Cov-2 and the skepticism surrounding the novel disease.

Nevertheless, the co-infection rates during the pandemic was substantially lower because of the increased rate of antibiotic usage for victims of COVID-19 illness. In the United Kingdom, an investigation done in the past on 836 patients with COVID-19 infection found that 3.2% of patients had proven bacterial infection during the initial first week after being admitted, rising to 6.1% over the remainder of hospitalization (Sharifipour et al., 2020). Another study found that only 102 of 1495 COVID-19 patients developed bacterial infections during their hospital stay (J. Li et al., 2020).

2.1.2 Increasing prevalence of antimicrobial resistance.

Numerous current researchers have found an increase in antibiotic resistance patterns of bacteria and other organisms during the COVID-19 period compared to prior years. The prevalence of carbapenem-resistant *Enterobacterales* colonization elevated from 6.7% before the pandemic to 50% during the pandemic, according to several recent papers documenting a growing incidence of antimicrobial resistance; thus, the novelty of this condition masks the exact connection between bacterial infections and the novel coronavirus, resulting in antibiotic over-exposure to COVID-19 patients residing in the ICU (Tiri et al., 2020). Another study conducted in China found *Acinetobacter baumannii* (35.8%; n = 57), *Klebsiella pneumoniae* (30.8%; n = 49), and *Stenotrophomonas maltophilia* (6.3%; n = 10) among the isolated strains from 159 strains obtained from 102 hospitalized SARS-CoV-2 patients who had developed secondary bacterial ailments. Furthermore, carbapenem resistance was found as 91.2% in *Acinetobacter baumannii* and 75.5% in *Klebsiella pneumoniae* (J. Li et al., 2020). During the initial wave of the SARS-CoV-2 pandemic, researchers in a French Intensive care unit found that, of 26 patients, 19 also developed secondary bacterial infections in addition to severe respiratory failure, of which two samples possessed resistance against third-generation cephalosporins and five against amoxicillin/clavulanate (Contou et al., 2020). In a similar vein, there were seventeen multidrug resistant *Acinetobacter baumannii* isolates and one methicillin-resistant *Staphylococcus aureus* (MRSA) which caused secondary infections in a group of 19 COVID-19 victims who needed ICU hospitalization (Sharifipour et al., 2020). The wide spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia, Burkholderia cepacia,* and *Pseudomonas aeruginosa* were suggested to be the causal organisms in 5 conditions of severe SARS-CoV-2 victims with bacterial co-infections as discovered by a group of researchers (Fu et al., 2020). New Delhi metallo-betalactamase (NDM)-producing *Enterobacter cloacae* was also found to be present in 5 SARS-CoV-2 patients in the United States (Nori et al., 2020).

The management of fungal infections and co-infections also got complicated during the acute phase of the SARS-CoV-2 pandemic. An intricate 53-day clinical history of a SARS-CoV-2 patient with a noninsulin-dependent diabetes mellitus (NIDDM) was described by Posteraro et al., the patient experienced multiple incidents of secondary bacteremia by MRSA, *Morganella morganii*, and possibly lethal *Candida glabrata* (Posteraro et al., 2020). After two weeks of caspofungin therapy, the *Candida glabrata* isolates from the patient developed FKS-associated pan-echinocandin resistance (Posteraro et al., 2020). Ten instances and six fatalities out of the 15 critically ill COVID-19 patients who contracted *Candida spp*. bloodstream infections in India were due to multidrug-resistant *Candida auris* (Chowdhary et al., 2020). Furthermore, a patient in Ireland with extreme COVID-19 pneumonia also had a multi-triazole-resistant variant of *Aspergillus fumigatus* in their system (Mohamed et al., 2021). Throughout the COVID-19 pandemic unlike a prior time period, there was a quantitatively marked rise in *Pseudomonas spp*. resistance to carbapenems, piperacillin/tazobactam, and *Enterobacterales spp* to piperacillin/tazobactam, according to a retrospective, research carried out in an ICU in Northern Italy (Zuglian et al., 2022).

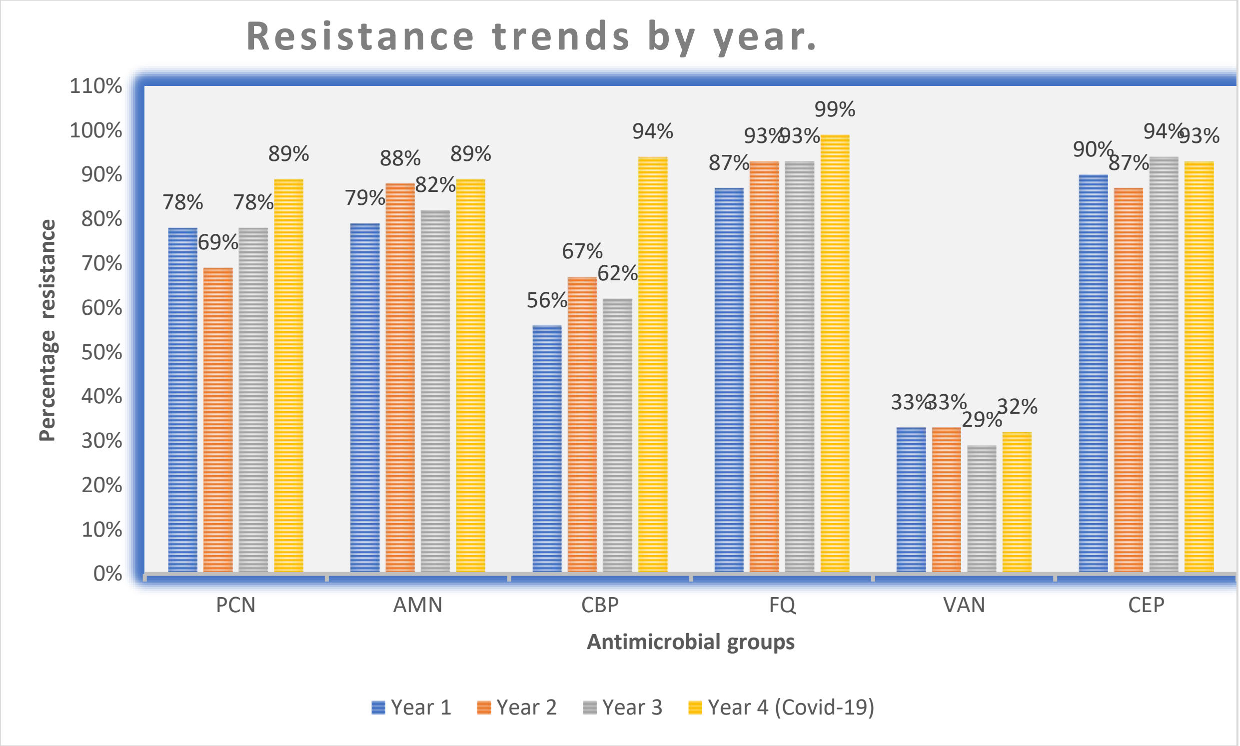


Figure 1.

Trends In Antimicrobial Resistance for the Most Common Antibiotics Tracked Throughout A Four-Year Period. PCN, penicillins; CEP, cephalosporins; AMN, aminoglycosides; FQ, fluoroquinolones; CBP, carbapenems; VAN, vancomycin (Despotovic et al., 2021).

2.1.3 High rate of antibiotic utilization.

According to reports, the usage of antimicrobial drugs is now more important than it was in years past owing to the COVID-19 outbreak. It seems as though secondary bacterial illnesses are frequently to blame for deaths during influenza outbreaks, as observed with the 1918 influenza epidemic and the 2009 H1N1 influenza outbreak (Cox et al., 2020), for example, research conducted in China documenting outcomes and treatment for 191 COVID-19 victims hospitalized in the ICU showed that secondary bacterial infections were responsible for half the amount of fatalities (Zhou et al., 2020) hence, the use of antibiotics throughout the SARS-CoV-2 outbreak was a vital defensive strategy against mortality.

Furthermore, the use of antibiotics in the ICUs as well as the alarmingly high prevalence of resistant bacteria identified from infections have been the subject of several research studies (Rafa et al., 2021). The average percentage of antibiotic usage, according to a review study encompassing 19 publications spanning studies from China, the United States, Brazil, and Denmark, was 74.0%. (2098 of 2834 patients). In addition to antibiotics, 56.9% of patients reportedly received antivirals (1613 of 2834 patients) (Chedid et al., 2021). Moxifloxacin, ceftriaxone, and azithromycin were administered to 89 (64.5%), 34 (24.6%), and 25 (18.1%) patients, respectively, according to results from different research studies encompassing 138 patients admitted to the hospital (D. Wang et al., 2020). Another extensive investigation found that about 637 out of 1099 patients were given intravenous antibiotics (Eastin & Eastin, 2020), while a lower-scale Brazilian group of 72 hospitalized patients revealed that 84.7% had received this same type of treatment (Teich et al., 2020). In general, well over half of patients infected with COVID-19 may require an intravenous antibiotic; in those suffering from serious disease, this fraction may be greater.

This increased rate of antibiotic usage throughout the COVID-19 pandemic particularly in those who contracted the SARS-CoV-2 disease can speculatively be a result of the fact that health professionals routinely administered a wide-spectrum antibiotic amidst the presumption of a viral cause since the typical manifestations of SARS-CoV-2 infection (coughing, flu, chills, elevated temperature, etc.) are also characteristics of pneumonia gotten in a community setting. Moreover, the broad and excessive prescribing of antibiotics may be influenced by the fear and apprehension surrounding the COVID-19 pandemic as well as the lack of potent therapies that can treat SARS-CoV-2 illnesses.

In summary, co-infection with bacteria, fungi, or COVID-19 is conceivable; nevertheless, it might be challenging to distinguish between a solitary COVID-19 infection and co- or secondary infections. Below is a case study of research carried out at the National Taiwan University Hospital within two intervals indicating an increased consumption of antibiotics from January to June 2020 in relation to January to June 2019.

Table 1.

Extended-spectrum Antibiotic Usage at National Taiwan University Hospital Between January and June 2019 and January and June 2020 (Lai et al., 2021).

Antibiotics	**Antibiotic consumption (DDDs/1000 patient-days) by indicated periods**		**Percentage change**
	Jan- June 2019	**Jan-June 2020**	
Beta-lactamase inhibitor combinations / Beta-lactam	373.3	387.6	4.1
Broad-spectrum cephalosporin	763.5	763.7	0.0
Quinolones	182.3	201.8	10.7
Carbapenems	330.4	376	13.8
Aminoglycosides	237	221.3	-6.6
Colistin	63.3	78.4	23.9
Tigecycline	56.3	89.7	59.3
Glycopeptides	340.1	384	12.9
Linezolid	12.1	15.4	27.3
Daptomycin	77.3	95.1	23.0
Fosfomycin	23.7	41.4	74.7

2.2 Multidrug-resistant organisms in ICUs

Antimicrobial resistance (AMR) is a significant threat, particularly in the ICUs, and has become a global concern. AMR is the ability of microorganisms to fight the curative properties of antimicrobial agents, allowing it to become increasingly challenging to eradicate infections caused by them.

The current situation of AMR is alarming, with an estimated 700,000 deaths annually worldwide attributed to AMR infections (WHO, 2020). A rise in the prevalence of both extensively and multi-drug resistant (MDR) pathogens has made the treatment of infectious diseases increasingly challenging. The most common MDR organisms include methicillin-resistant Staphylococcus aureus (MRSA), carbapenem-resistant Enterobacteriaceae (CRE), and vancomycin-resistant Enterococcus (VRE) (Han et al., 2022).

AMR is a complex issue that involves various mechanisms, including the acquisition of resistance genes, mutations in existing genes, and microorganisms capacity to evade the effects of antimicrobial agents (Anthony et al., 2020). Horizontal gene transfer is one of the most important mechanisms by which bacteria acquire resistance genes, leading to the spread of resistance among different bacterial species (Lerminiaux & Cameron, 2019).

Besides the developed immunity, bacteria like *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Enterococcus faecium* have demonstrated innate immunity to conventional antibiotics, leading to the development of extremely aggressive pathogens that make infections difficult to treat (García-Solache & Rice, 2019). In addition, the overuse and misuse of antibiotics in human and animal health, as well as in agriculture, have contributed to the emergence of AMR (Barchitta et al., 2019). To address the issue of AMR, various solutions have been proposed, including the development of new antibiotics, the use of alternative therapies, and the implementation of antimicrobial stewardship programs which aims to promote the rational use of antibiotics, reduce unnecessary prescribing, and improve patient outcomes (Tiri et al., 2020).

However, the development of new antibiotics has been slow, and there is a growing concern that the pipeline of new drugs is insufficient to meet the demand (Klug et al., 2021).

Alternative therapies, such as phage therapy and monoclonal antibodies, have shown promise in preclinical studies, but their clinical efficacy and safety need to be further evaluated (Abedon, 2019). Nonetheless, despite all of the precautions adopted, the apocalypse of resistance continues to pose a threat to healthcare facilities. Routine operations and hospital visits are already becoming more dangerous due to bacterial antibiotic resistance (AR). Given that approximately 25% of HAIs in long-term critical care settings are brought on by bacteria resistant to antibiotics, this plague is a particular concern there (Haque et al., 2020b). As a result of antibiotics' stressors favoring resistance, resistant bacterial populations proliferate.

The elimination of vulnerable microbiota by antibiotics can also lessen competition for nutrients and space hence making these resources readily accessible to resilient opportunistic pathogens (Patangia et al., 2022). Also, a resistant gene may migrate to different cells and bacterial species after replicating in one bacterium, this has contributed to the fast development of AR (Urban-Chmiel et al., 2022).

To effectively combat the growing AR problem, we need to comprehend the manner in which bacteria pick up and spread resistant genes in healthcare situations.

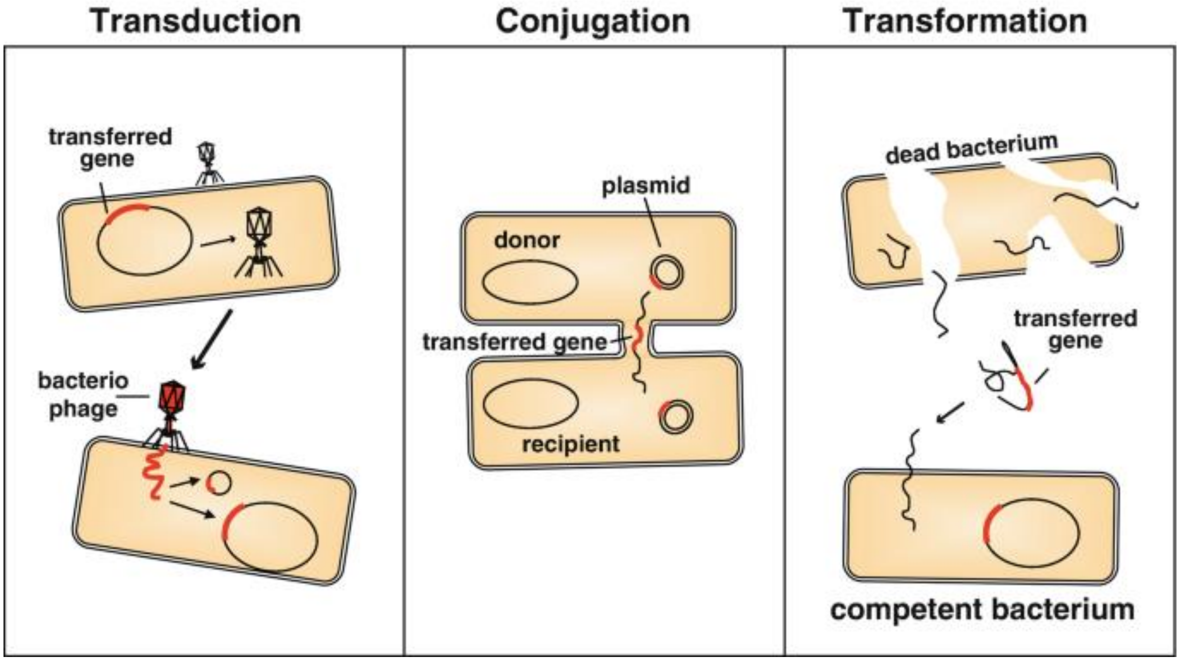
2.3 Horizontal gene transfer.

Microbial organisms can pick up additional genetic traits from origins unrelated to their hereditary lineage thanks to horizontal gene transfer (HGT) (van Dijk et al., n.d.). Microbes may explore and distribute a sizable gene pool through HGT, which may contain features that are advantageous in their immediate surrounding (Woods et al., 2020). For instance, horizontal uptake of antibiotic resistance genes (ARG) permits diversity of genomes and generates a possibility for fast survival gains when bacteria are subjected to intense stressors, including the presence of antimicrobials. Moreover, HGT can produce the genes required for continued existence more quickly than spontaneous mutations (Larsson & Flach, 2022) (Tao et al., 2022). With the transmission of harmful features including virulence genes as well as the tendency to create biofilms, HGT also leads to infectious diseases and epidemics (Michaelis & Grohmann, 2023).

In addition to places like wastewater treatment facilities, the study of HGT has advanced thanks to a number of studies that have been able to follow and quantify rates and causes of HGT in clinical settings where infections are most prevalent. Conjugation, transduction, and natural transformation have been determined to be the main mechanisms of HGT in bacteria. It has also been determined that these three are mostly responsible for the propagation of AR in healthcare settings (W. Li & Zhang, 2022). Furthermore, the three most important precursors of conjugation, transduction, and natural transformation, respectively, are plasmids, bacteriophages, and extracellular DNA (Golz & Stingl, 2021).

Figure 2**.**

General Ways of Horizontal Gene Transfer. Conjugation, Transformation, Transduction. (red represents DNA fragments).



(Blokesch, 2016).

Bacteriophages and plasmids are two common genetically encoded parts of bacteria. Although natural transformation is more irregular in distribution, the ability to undergo spontaneous transformation existed before the development of the bacterial Gram-positive and Gram-negative taxa (*3.1*, 2016). Phylogenetically divergent species can also transmit genes from one another via any of the three pathways, but closely linked creatures are preferred (Lerminiaux & Cameron, 2019).

Plasmids and bacteriophages are ubiquitous, and natural transformation occurs across a wide range of habitats and settings due to the widespread dispersion of phylogenetic groups that make up the natural world. In view of the foregoing, the same processes will be used to transfer ARGs between patients, in therapeutic settings, throughout human societies, and in practically every possible natural setting (Michaelis & Grohmann, 2023). Although there is a dearth of information on the origins, processes, or frequency of ARG transmission among medical reservoirs (Tao et al., 2022), There are other instances where patients and clinical surfaces have both shown the precise same ARG to be present at the same time (Aleem et al., n.d.) (Sukhum et al., 2022), (Avershina et al., 2021). A while ago hospital statistical information, as well as computational simulations, were used to determine the incidence of blaOXA-48 plasmid distribution involving *Klebsiella pneumoniae* and *Escherichia coli* inside the host patient (Haverkate et al., 2015). Forecasts regarding DNA frequency of transfer are provided by these investigations, which may then be experimentally verified and improved.

2.3.1 Conjugation by Plasmids.

Mobile genetic elements such as plasmids exist outside of chromosomes and multiply on their own. Plasmids or transposons are responsible for the process of conjugation. It consists of a receiving cell that lacks a conjugative plasmid and the donating bacteria that does (*3.1*, 2016). Cell-to-cell interface between live bacteria results in the transfer of genetic information from a donor to a receiver. It often includes a conjugation bridge or sex pilus in Gram-negative bacteria (*3.1*, 2016). When these self-serving genetic components transport traits that are beneficial to recipient cell, for instance, ARGs during the use of antimicrobials, their resilience is enhanced (Zwanzig, 2021). As a result, several ARGs are present on plasmids (Khezri et al., 2020).

Particular instances demonstrating the way HGT aggravates AR issues in healthcare facilities include plasmid-mediated defense against beta-lactam antibiotics. Penicillin, carbapenems, and cephalosporins are only a few examples of beta-lactam antibiotics that extended-spectrum-lactamases (ESBLs) and carbapenemase breaks down to cause resistance (Aryal et al., 2020). In the family of Enterobacteriaceae, *Pseudomonas*, and *Acinetobacter*, beta-lactam gene sequences that confer resistance are frequently found on plasmids and spread by conjugation in phylogenetically divergent or closely related species (Kyriakidis et al., 2021), (J. Wang et al., 2019), (Paul et al., 2020), (Rensing et al., 2019).

Subsequently, the identification of homologous ARG-carrying plasmids in healthcare recipients and on hospital surfaces, disease outbreaks have been seen to be long-lasting due to plasmid replacement across reservoirs (Silago & Mshana, 2022). Carbapenem-resistant strains were shown to have come into existence via hospital-acquired transmission or external dissemination, according to genomic profiling (Wendel et al., 2022). Monitoring epidemics and enhancing infection prevention protocols in hospitals are both greatly impacted by the granularity of results provided by genome sequencing in combination with medical records. Nevertheless, the rare ambient tracking for ARGs makes it difficult to substantiate epidemiological linkages.

The ability of plasmids obtained from hospital AR outbreaks to spread through conjugation amongst different varieties of bacteria has been demonstrated in laboratory studies. By way of illustration, conjugation allowed hospital isolates of *K. pneumoniae* to transfer plasmids containing the medically significant extended-spectrum-beta-lactamase (ESBL)-encoding genes blaOXA-48, blaNDM-1, blaKPC-2, and blaSHV-1 to laboratory *E. coli* (Lerminiaux & Cameron, 2019). The evolution of resistance in microorganisms such as *Staphylococcus aureus* is also impacted by plasmid transfer. *S. aureus* with methicillin resistance is a tenacious ICU colonizer in addition to being a common source of infection obtained from hospitals, and its infections are challenging to combat with typical antibiotics (Ali Alghamdi et al., 2023). MRSA along with other bacterial infections that are resistant to other antibiotics are subsequently treated with vancomycin as a last option. Though MRSA was the parent strain, vancomycin-resistant *Staphylococcus aureus* (VRSA) developed from it by horizontal transfer of an *Enterococcus faecalis* plasmid (Cong et al., 2019). This incident offers a crucial illustration of the extent to which HGT aggregates resistance traits in DNA sequences, resulting in the formation of the infamous "superbugs" that cannot be effectively treated with antibiotics.

Antibiotics have the potential to induce conjugation, which is especially important in healthcare environments where antibiotics are routinely administered. It has been discovered that exposing numerous Gram-negative bacteria to minimum inhibitory doses of a combination of two antibiotics from the aminoglycoside family or a combination of antibiotics that offers wide antimicrobial coverage, promotes conjugation (Sun et al., 2022) (Shahid et al., 2023). Ultimately, antibiotics have the potential to regulate DNA transcription that participates in virulence, repair of genomes, and genome transfer by functioning as chemical impulses (Briffotaux et al., 2019). Additionally, they can indirectly alter the basic structure of cell walls or promote the amplification of SOS response proteins to trigger conjugative morphological characteristics (Crane et al., n.d.) (Cylke et al., 2022).

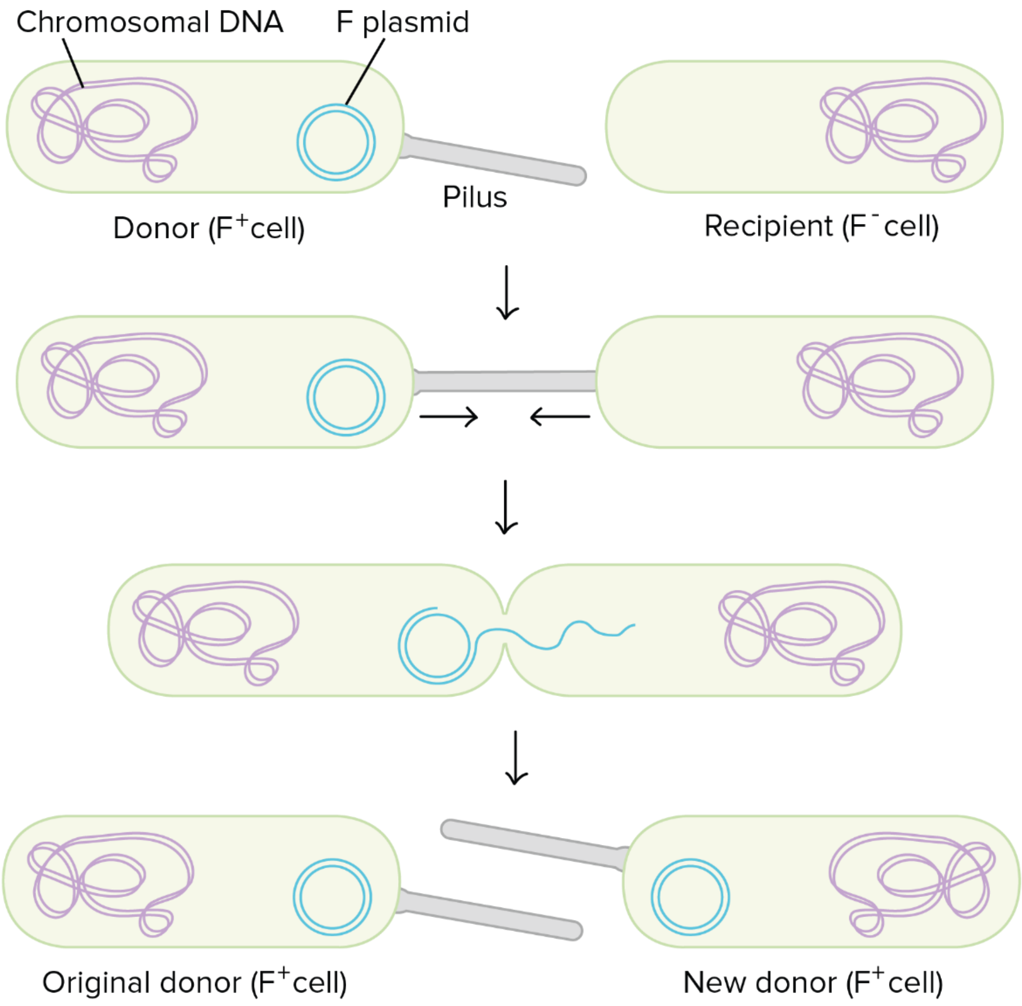


Figure 3: Conjugation by plasmid (*Conjugation, Transformation & Transduction | Bacteria (Article)*, n.d.)**.**

2.3.2 Transduction by bacteriophages.

Transduction is recognized as a possible factor in the transmission of ARGs, particularly among organisms of common species (Tao et al., 2022). Transduction is another HGT technique that involves the utilization of bacteriophages, autonomously reproducing several copies of virus-infecting bacteria that have the ability to wrap of portions host genetic information in their capsid and infuse it into an alternate host when an external stimulation induces cell death. As a result, the virus-infected cell's freshly injected genome may recombine with the DNA of the recipient cell to produce one of two types, a lytic or lysogenic cycle (Bello-López et al., 2019).

Also, due to their molecular architecture and the activity of specific enzymes, bacteriophages can allow for the entry of other MGEs into their genomes, including virulence or genomic islands, and "jumping genes" from other kinds of bacteria. These kinds of occurrences normalize recombination, which can help explain the complex genetic layout of phages and the adaptability of their DNA sequence (Qi et al., 2023).

Staphylococcus aureus more frequently exhibits resistance transduction (Turner et al., 2019). Other species of bacteria that have the mecA gene transfer it to *Staphylococcus aureus*, establishing a superbug known as Methicillin-resistant *Staphylococcus aureus* (MRSA) (Tao et al., 2022). Phageφ80α In addition to mediating the transfer of penicillin and tetracycline resistance genomes to the multidrug-resistant *Staphylococcus aureus* strain USA300, Phageφ80α may further facilitate the spread of resistance traits to the *Staphylococcus aureus* species that are not susceptible to phages (Tao et al., 2022). In gram-negative bacteria, several ARGs, as well as ESBL genes, have been seen to be transmitted by transduction from *Pseudomonas* isolates obtained from a medical facility to other *Pseudomonas spp* in the lab (Qin et al., 2022).

According to studies, mice who received antibiotics exhibited greater numbers of bacteriophages having ARGs in their digestive systems than mice who did not get antibiotics, suggesting that antibiotics are possibly responsible for the transduction of ARGs (Y. Zhang et al., 2022). In addition, certain sophisticated phages may encode ARGs by forming what is known as phage-plasmids, which combine the capabilities of a plasmid and a phage. It was discovered that 60 phage-plasmids harboring 184 ARGs were resistant to about 5 broad-spectrum antibiotics (Pfeifer et al., n.d.). The transduction of plasmids-carry ARGs to *Staphylococcus aureus* has a prospect of being more active compared to methicillin resistance acquired by transduction by the transfer of chromosomes (J. Zhang et al., 2021). Thus, ARGs can be spread by both transduction of chromosomal DNA and transduction of plasmids-bearing ARGs and in both cases, antibiotics are likely to speed up the process.

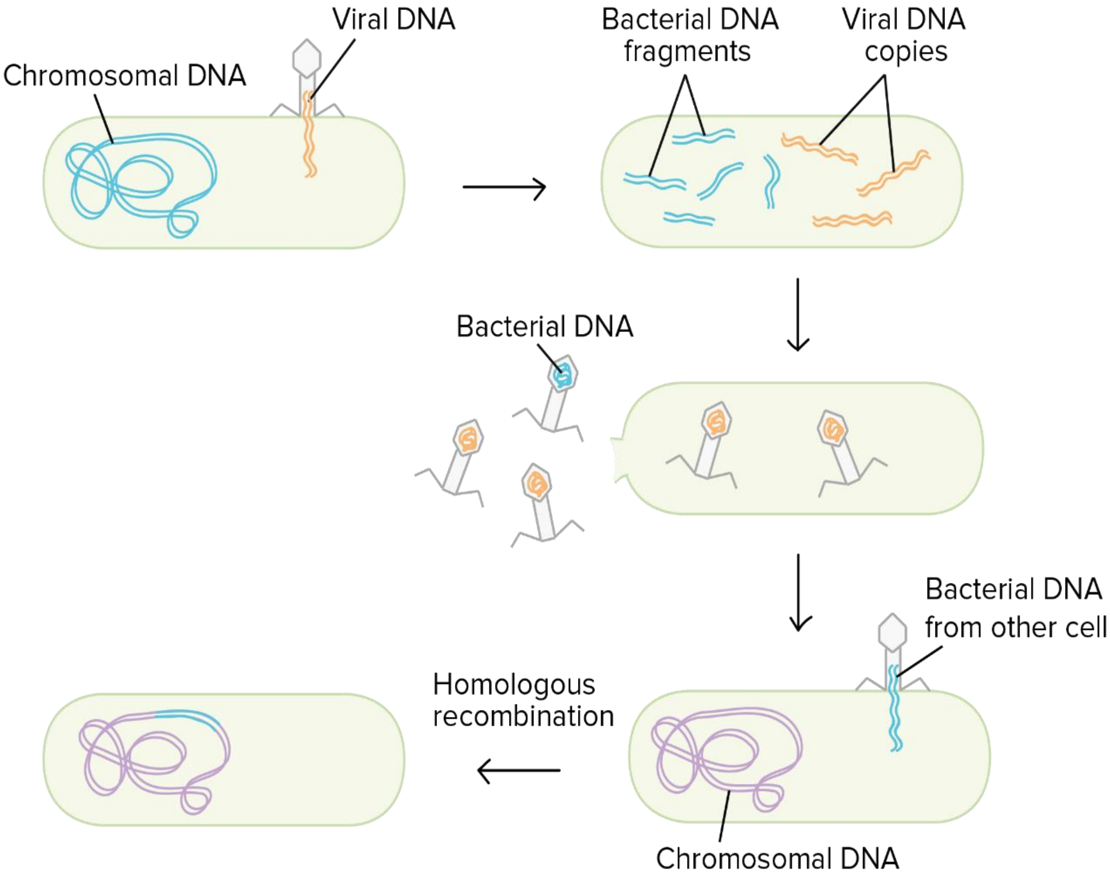


Figure 4: Transduction by bacteriophages (*Conjugation, Transformation & Transduction | Bacteria (Article)*, n.d.)

2.3.3 Natural transformation by extracellular DNA.

The process of Transformation in bacteria is a mutation in genes that occurs in a cell following the immediate assimilation, integration, and proliferation of foreign DNA by two or more genetically similar bacterial species, and the process is carried out chromosomally by encoded proteins (Michaelis & Grohmann, 2023). This alien genome is "without any covering," and it may exist in an ecosystem where the bacterium flourishes. It can enter the bacterial selectively permeable membrane when the bacterium is in a "competent" condition, which can be brought on by a shortage of vital minerals or a high cell mass. For transformation to occur, the DNA must move from the outer layer to the cytoplasmic wall and subsequent passage through the cytoplasmic membrane via an intricately intact membrane pathway (Bello-López et al., 2019).

Pseudomonas, Acinetobacter, Staphylococcus, Streptococcus, Neisseria, and *Haemophilus* are a few medically significant antibiotic-resistant organisms that have the capacity to absorb DNA and undergo natural transformation. Escherichia and Klebsiella are significant contributors to both community-acquired and nosocomial infections gotten as a result of antibiotic resistance (Caneiras et al., 2019) (Nimer, 2022). Despite neither species having shown spontaneous transformation in the lab, their natural competence in the environment has been speculated (Komiyama & Maeda, 2020). Natural transformation may help ARGs spread throughout numerous important disease-causing microorganisms since this speculation holds for the other gram-negative bacteria as well (Averhoff et al., 2021).

It has been noted that horizontal gene transfer occurs often in aeromonads and its incidence has lately increased. Despite the lack of research and knowledge regarding natural transformation in the Aeromonas genus, a cohort study was conducted on Aeromonas species obtained from bodies of water to ascertain whether the microbial isolates proved to be competent for natural transformation and to determine the ideal environment for transformation in an experimental system to outline the progressive sequences of transformability within the genera. According to their research, various species of Aeromonas are naturally capable of transforming under the studied settings, and the ideal parameters for transformation are similar to the ones present in their original ecosystem (Bello-López et al., 2019). In lab settings, antibiotics can boost the overall amount of transformation in some bacteria (Kunhikannan et al., 2021), indicating that by just being present, antibiotics can promote HGT and the spread of resistant DNA. For instance, the expression of competence genes and the frequency of transformation were both increased when quinolones were added to Streptococcus cells (Lerminiaux & Cameron, 2019). While dwelling in biofilms, several strains of bacteria exhibit natural competence, and quorum sensing in biofilms may further encourage competence in nearby organisms. As a result, the continued existence of biofilms and the possibility of being exposed to extreme doses of antibiotic drugs may work in concert to promote natural competence and transformation (Michaelis & Grohmann, 2023).

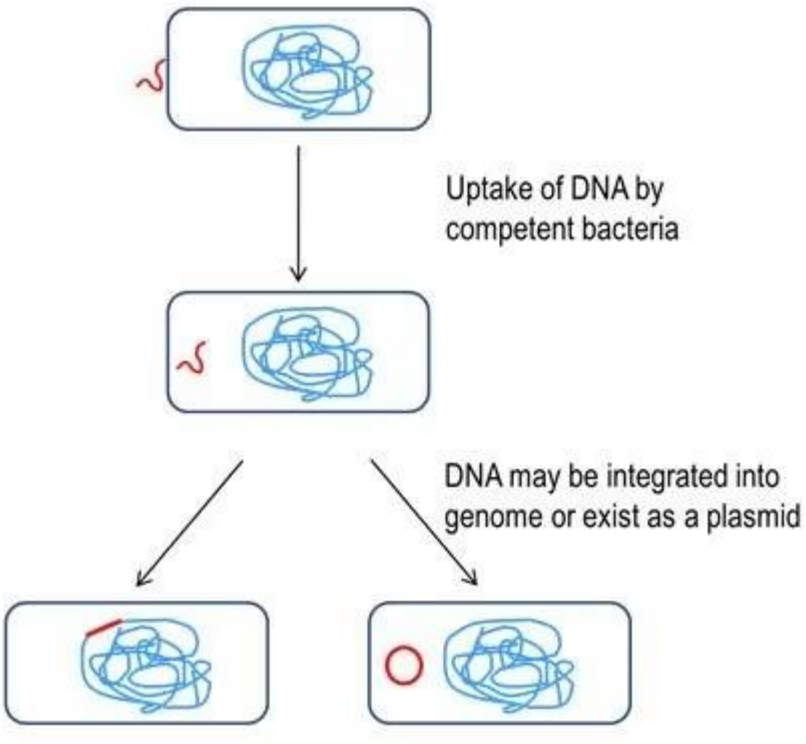


Figure 5: Transformation in bacteria (Avery et al., 1944).

CHAPTER 3

Materials and Methods

3.1 Study group

This retrospective investigation was done at Near East University Hospital. All patients admitted to the ICU wards who had bronchoalveolar lavage and/or tracheal aspiration procedures performed on them were included in the research. The research timeframes covered the preceding pandemic years of 2018–2019 and the pandemic years of 2020–2022. Age, sex, patient number, and antimicrobial treatment were all extracted from the patient's medical records with ethical consent. The most abundant bacterial species that may be harmful to the respiratory tract were grouped based on their profile of antibiotic resistance, in accordance with The European Committee on Antimicrobial Susceptibility Testings (EUCAST) criteria, and antibiotics that were found to be intermediate in strength were deemed resistant.

3.2 Tools and equipment

- Petri-dish
- Inoculating loop
- Test-tubes
- Test-tube racks
- VITEK 2 compact system (Biomerieux)
- Automatic pipette (Gilson Pipetman.Dk60063, Biyomedikal 2179. Made in France)
- Autoclave (model OT40L. Miive Steam Art. Biyomedikal 2189)
- Incubator (Heraeus Thermo Scientific. Biyomedikal 2184)
- Microscope slides
- Electronic microscope
- Sterile swab

3.2.1 Chemicals

- Blood agar- Becton, Dickinson and Company, France.
- Eosin methylene blue agar (EMB)- Becton, Dickinson and Company, France.

3.3 Inclusion criteria

There was no limitation on the age boundaries, according to the data of patients in this research, which ranged in age from 0 to 95. Sputum and aspirate fluids were the only samples used in this experiment.

3.4 Exclusion criteria

For the sole purpose of this investigation, aspirate samples obtained from patients hospitalized in hospital wards aside from the ICU wards of the various departments were disregarded.

3.5 Data collection

Each of the patients was assessed based on age, gender, hospital department, sample type, resistance as well as sensitivity to antimicrobial drugs.

3.5.1 Culture and antibiotic tests

Aspirate samples that were sent to the Microbiology Laboratory with labels detailing the patient’s name, barcode number, date of sample collection, and type of sample, were entered into a record book as well as the electronic microbiology database.

All aspirate samples were inoculated on blood agar and eosin methylene blue agar and were incubated for about 24-48 hours at 37°C. VITEK 2 (Biomerieux) compact automated system was used for identification and antibiotic susceptibility tests (AST) of microorganisms in cultures with growth. VITEK 2 GN and VITEK 2 GP cards were used for identification.

Also, VITEK 2 AST-N325, VITEK 2 AST-N327, VITEK 2 AST-N326, VITEK 2 AST-P641, and VITEK 2 AST-P640 cards were used to measure antibiotic susceptibility. The antibiotic card that was used in the study; Amikacin, Tobramycin, Ampicillin, Aztreonam, Cefepime, Cefixime, Cefoxitin, Ceftazidime, Ceftriaxone, Cefuroxime, Ciprofloxacin, Clindamycin, Colistin, Daptomycin, Ertapenem, Erythromycin, Gentamycin, Imipenem, Levofloxacin, Linezolid, Meropenem, Netilmicin, Nitrofurantoin, Tazobactam, Teicoplanin, Tetracycline, Tigecycline, Trimethoprim-Sulphamethoxazole, Vancomycin.

Antibiotic susceptibility tests were evaluated according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria and antibiotics detected as intermediate were considered resistant.

3.6 Statistical data analysis

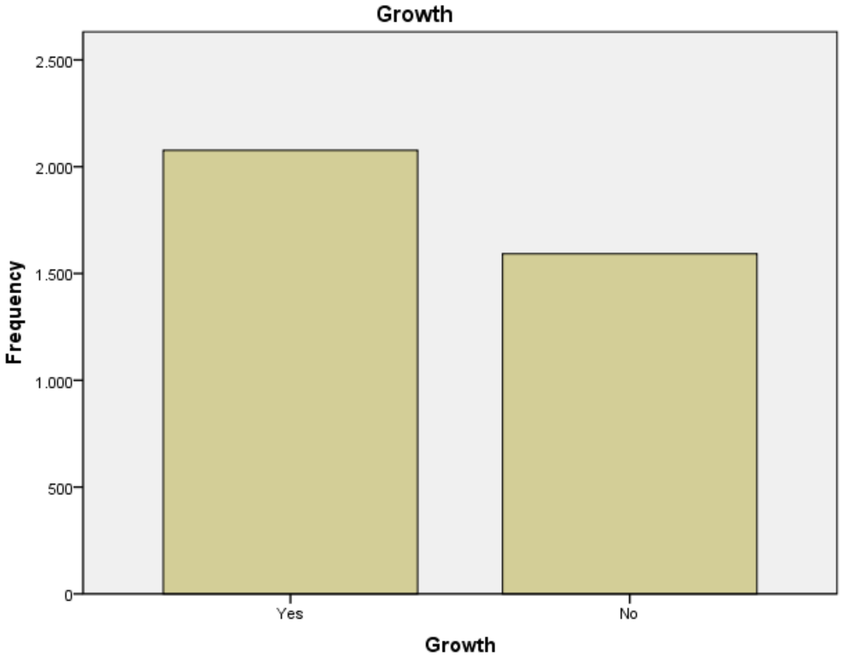
The Pearson chi-square test was the testing method for the results in this study, with an SPSS version 27 for all statistical analyses. A P value of 0.005 was considered significant.

CHAPTER IV

Results

Growth was observed in 52.7% (n: 1932) of a total of 3669 aspirate and sputum samples evaluated in the Microbiology Laboratory of NEU Hospital between 2018-2022. In addition, there was no growth in 47.3% (n: 1737) of the samples. 63% (n: 2312) of the patients were male, 37% (n: 1357) were female, and the mean age was 66.75±18.77 (range 0-100 years).

Figure 6: *The total distribution of microorganisms across the two time periods (2018-2022)*



64.9% (n: 1254) of the patients with growth were male, 35.1% (n: 678) were female, and the mean age was 69.63±17.17 (range 0-100 years). When gender and growth status were compared, it was determined that the rate of growth in aspirate/sputum cultures of the males was more than females (p=0.012). Accordingly, we can say that male gender is statistically more prone to bacterial pneumonia.

Table 2: *Comparison of gender and growth status across the two time periods*

			Gender		
			Male	Female	Total
Growth	Yes	Count	1254	678	1932
		Expected Count	1217,4	714,6	1932,0
		% within Growth	64,9%	35,1%	100,0%
		% within Gender	**54,2%**	**50,0%**	52,7%
	No	Count	1058	679	1737
		Expected Count	1094,6	642,4	1737,0
		% within Growth	60,9%	39,1%	100,0%
		% within Gender	45,8%	50,0%	47,3%
Total		Count	2312	1357	3669
		Expected Count	2312,0	1357,0	3669,0
		% within Growth	63,0%	37,0%	100,0%
		% within Gender	100,0%	100,0%	100,0%

p-Value = 0.012

When the mean age of the patients with and without growth was compared, it was observed that the mean age of the patients with growth was significantly higher than the patients without growth (p=<0.0001). Accordingly, the mean age of those with growth was 69.63±17.17, and the mean age of those who did not have a growth was 63.54±19.93. These results show us that the risk of bacterial pneumonia increases in parallel with age

Table 3: *Mean age distribution of growth across the two time periods*

Growth	Mean	N	Std. Deviation	
dimension1	**Yes**	**69,63**		**17,165**
	No	**63,54**		**19,925**

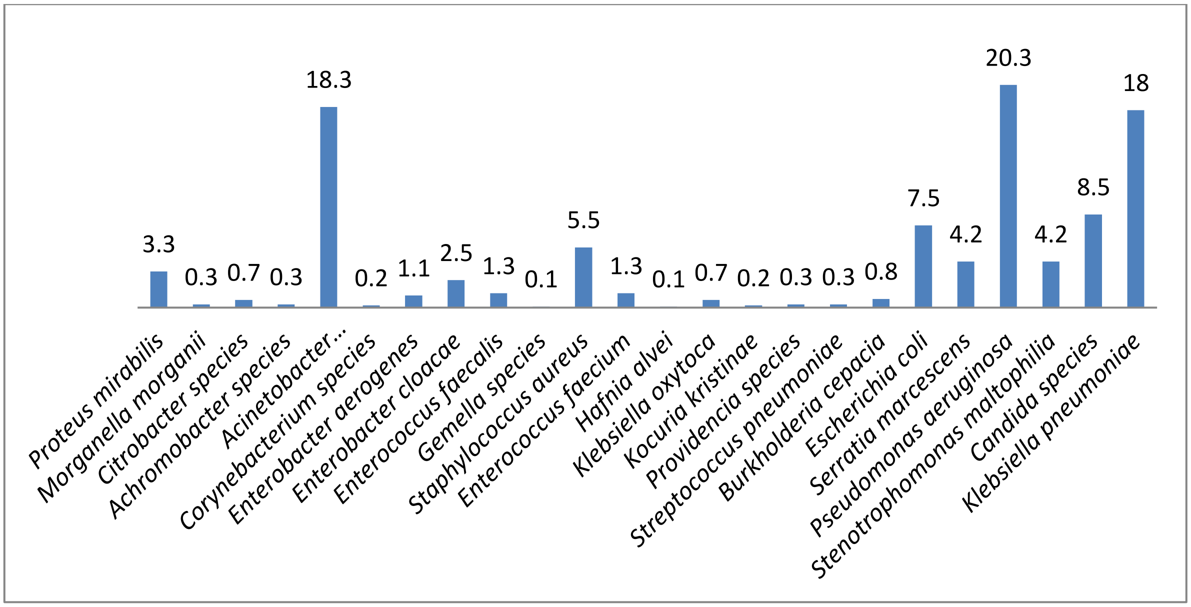
p-Value = 0.000

Of the cultures that grew, 82.7% (n: 1597) were gram negative, 8.9% (n: 171) were gram positive, and 8.5% (n: 164) were Candida species. The distribution of microorganisms is as follows. Accordingly, the most common microorganisms were *Pseudomonas aeruginosa* (20.3%, 392), *Acinetobacter baumannii/calcoaceticus complex* (18.3%, 359) and *Klebsiella pneumoniae* (18.0, 347), respectively.

Table 4: *Total distribution of microorganisms across the two time periods*

Microorganisms	Frequency	Percent
Proteus mirabilis	63	3,3
Morganella morganii	5	0,3
Citrobacter species	13	0,7
Achromobacter species	6	0,3
Acinetobacter baumannii/calcoaceticus complex	359	18,6
Corynebacterium species	4	0,2
Enterobacter aerogenes	21	1,1
Enterobacter cloacae	49	2,5
Enterococcus faecalis	25	1,3
Gemella species	2	0,1
Staphylococcus aureus	106	5,5
Enterococcus faecium	25	1,3
Hafnia alvei	1	0,1
Klebsiella oxytoca	14	0,7
Kocuria kristinae	4	0,2
Providencia species	5	0,3
Streptococcus pneumoniae	5	0,3
Burkholderia cepacia	15	0,8
Escherichia coli	145	7,5
Serratia marcescens	81	4,2
Pseudomonas aeruginosa	392	20,3
Stenotrophomonas maltophilia	81	4,2
Candida albicans	164	8,5
Klebsiella pneumoniae ssp pneumoniae	347	18,0
Total	1932	100,0

Figure 7: *Total distribution of microorganisms across the two time periods.*



The frequency of growth in the cultures of inpatients was found to be significantly higher than in outpatients (p=<0.0001).

Table 5: Frequency distribution of growth in inpatients and outpatient

			In/Out		Total
			Outpatient	**Inpatient**	
Growth	Yes	Count	140	1792	1932
		Expected Count	173,8	1758,2	1932,0
		% within Growth	7,2%	92,8%	100,0%
		% within In/Out	**42,4%**	**53,7%**	52,7%
	No	Count	190	1547	1737
		Expected Count	156,2	1580,8	1737,0
		% within Growth	10,9%	89,1%	100,0%
		% within In/Out	57,6%	46,3%	47,3%

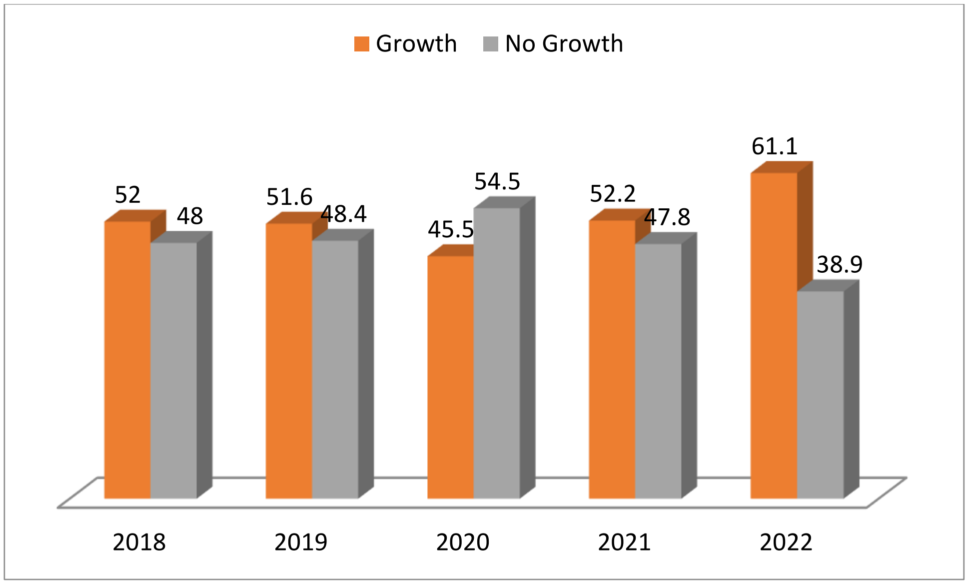
p-Value = 0.000

Considering the distribution by years, the highest growth rate was determined in 2022. Accordingly, the highest rate was found in 2022 with the rate of 61.1% and this difference was found to be significant (p=<0.0001).

Table 6: *Growth rate during the different time periods.*

			Year					Total
			2018	2019	2020	2021	2022	
Growth	Yes	Count	270	313	363	467	519	1932
		Expected Count	273,3	319,6	420,2	471,3	447,6	1932,0
		%within Growth	14,0%	16,2%	18,8%	24,2%	26,9%	100,0%
		% within Year	**52,0%**	**51,6%**	**45,5%**	**52,2%**	**61,1%**	52,7%
	No	Count	249	294	435	428	331	1737
		Expected Count	245,7	287,4	377,8	423,7	402,4	1737,0
		% within Growth	14,3%	16,9%	25,0%	24,6%	19,1%	100,0%
		% within Year	48,0%	48,4%	54,5%	47,8%	38,9%	47,3%
Total		Count	519	607	798	895	850	3669
		Expected Count	519,0	607,0	798,0	895,0	850,0	3669,0
		% within Growth	14,1%	16,5%	21,7%	24,4%	23,2%	100,0%
		% within Year	100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

Figure 8: *Growth rate during the different time periods.*



A statistically significant difference was found in the hospitalization of the patients to the intensive care unit and the growth in their cultures. Accordingly, the rate of growth in the patients hospitalized in the intensive care unit was 54.7% (n: 1234), while the rate in the patients who were not hospitalized in the intensive care unit was 49.5% (n: 698) (p=0.002). It is clear that hospitalization in intensive care increases the risk of bacterial pneumonia.

Table 7: *Comparison of microbial growth in patients admitted to the ICU ward and other wards in NEU hospital.*

			Patients admitted		Total
			ICU wards	Non-ICU	
Growth	Yes	Count	1234	698	1932
		Expected Count	1189,0	743,0	1932,0
		% within Growth	63,9%	36,1%	100,0%
		% within ICU	**54,7%**	**49,5%**	52,7%
	No	Count	1024	713	1737
		Expected Count	1069,0	668,0	1737,0
		% within Growth	59,0%	41,0%	100,0%
		% within ICU	45,3%	50,5%	47,3%

p-Value = 0.002

46.6% (n: 744) of Gram-negative bacteria were enteric and 53.4% (n: 853) were non-enteric bacteria. Among enteric bacteria, *Klebsiella pneumoniae* (46.6%, 347/744) and *Escherichia coli* (19.5%, 145/744) were most frequently isolated. Among non-enteric bacteria, *Pseudomonas aeruginosa* (46.0%, 392/853) and *Acinetobacter baumannii* (42.1%, 359/853) were the leading ones, respectively.

The rate of ESBL in enteric bacteria was 43.3%. The antibiotic resistance patterns in these bacteria are given in the table below;

Table 8: *Antibiotic resistance patterns in enteric bacteria*

Antibiotic	**Sensitive (%)**	**Resistant (%)**
Amikacin	87.5	12.5
Ampicillin	8.9	91.1
Aztreonam	60.6	39.4
Cefepime	55.8	44.2
Ceftazidime	56.3	43.7
Ceftriaxone	50.5	49.5
Cefuroxime	36.9	63.1
Ciprofloxacin	56.2	43.8
Ertapenem	72.2	27.8
Gentamicin	85.1	14.9
Imipenem	82.5	17.5
Meropenem	83.7	16.3
TZP	66.4	33.6
Tigecycline	72.2	27.8
SXT	64.4	35.6

The resistance rates in non-enteric bacteria are as follows;

Table 9: *Antibiotic resistance patterns in non-enteric bacteria*

Antibiotic	**Sensitive (%)**	**Resistant (%)**
Amikacin	87.5	12.5
Ampicillin	8.9	91.1
Aztreonam	60.6	39.4
Cefepime	55.8	44.2
Ceftazidime	56.3	43.7
Ceftriaxone	50.5	49.5
Cefuroxime	36.9	63.1
Ciprofloxacin	56.2	43.8
Ertapenem	72.2	27.8
Gentamicin	85.1	14.9
Imipenem	82.5	17.5
Meropenem	83.7	16.3
TZP	66.4	33.6
Tigecycline	72.2	27.8
SXT	64.4	35.6

The most frequently isolated Gram-positive bacteria were *Staphylococcus aureus* (62%, 106/171) and *Enterococcus spp*. (29.2%, 50/171). The rate of MRSA within the *Staphylococcus aureus* was 55.7% (59/106). The VRE rate was 6% (3/50).

Table 10: *Antibiotic resistance patterns in gram positive bacteria.*

Antibiotic	**Sensitive (%)**	**Resistant (%)**
Ciprofloxacin	73.6	26.4
Clindamycin	64.6	35.4
Daptomycin	92.1	7.9
Erythromycin	54.5	45.5
Gentamicin	84.2	15.8
Levofloxacin	76.0	24.0
Linezolid	98.1	1.9
Teicoplanin	91.5	8.5
Tetracycline	59.3	40.7
Tigecycline	99.2	0.8
SXT	65.4	34.6
Vancomycin	95.8	4.2

Table 11: *Comparison of growth rate in the pre-pandemic and pandemic periods*

			Pandemic		Total
			Pre-pandemic	During pandemic	
Growth	Yes	Count	583	1349	1932
		Expected Count	592,9	1339,1	1932,0
		% within Growth	30,2%	69,8%	100,0%
		% within Pandemic	**51,8%**	**53,0%**	52,7%
	No	Count	543	1194	1737
		Expected Count	533,1	1203,9	1737,0
		%within Growth	31,3%	68,7%	100,0%
		% within Pandemic	48,2%	47,0%	47,3%
Total		Count	1126	2543	3669
		Expected Count	1126,0	2543,0	3669,0
		% within Growth	30,7%	69,3%	100,0%
		% within Pandemic	100,0%	100,0%	100,0%

Accordingly, while the growth rate in cultures was 51.8% before the pandemic, this rate was 53% during the pandemic. The difference between the two ratios was not statistically significant (p=0.477).

CHAPTER V

Discussion

ICU patients are seriously unwell and have weak immune systems. Throughout medical evaluation and therapy, they experience a variety of stressful surgical treatments. Unlike patients in different wards, ICU patients have a greater prevalence of hospital-acquired infections because they consume high-dose hormonal substances and broad-ranging antibiotics more often, more frequently, and for more extended periods of time (Han et al., 2022), leaving ICU patients highly vulnerable to infections caused by multidrug resistant bacteria.

The SARS-CoV-2 outbreak that occurred more recently presented a bigger problem for the healthcare sector. Aside from the disease itself, there has been a sharp rise in the prevalence of antibiotic resistance, which is especially connected to an elevated level of antimicrobial agent usage during the period (Lai et al., 2021).

In order to determine the effects of COVID-19 pandemic in the ICU of Near East Hospital, this retrospective study investigated the distribution of pathogens involved in nosocomial infections in the ICU and the level of drug resistance to various antibiotics during the pre-pandemic and pandemic era. Out of the 3669 patients analyzed, only 1932(52.7%) of the patient samples were colonized by microorganisms as seen in Figure 6. The growth of microorganisms was more prevalent in the male patients having 1254(64.9%) number of growth than in female patients with 678(35.1%) and it was statistically, significant (p-value = 0.012) as shown in Table 2. Based on this data we can say that male gender is statistically more prone to bacterial pneumonia than female gender. These findings are similar to the data obtained in the investigation of Sharifipour et al., (2020), in which case out of the 19 patients evaluated, 11 (58%) of them were male and 8 (42%) were female. Also, (Chen et al., 2020b) had similar findings however the reason was likely due to occupational exposure of which majority of the infected workers were males.

In a broad sense, a higher percentage of ICU hospitalizations, deaths, and HAIs are linked to older ages. The findings in this study as seen in Table 3 were indicative of higher risk for bacterial pneumonia in older patients and negligible risk for patients aging less than 20. With a mean age of 69.63±17.17 and a significant value of p=0.000, the findings were significant. This information is consistent with a prior research (Moradi et al., 2021) that found that bacterial co-infection of the respiratory tract was most common in those between the ages of 60 and 70. This is largely because elderly people have weaker immune systems and are therefore more susceptible to infections.

Among the different species isolated from the ICU patients, the most prevalent pathogen isolated during the pandemic were the gram negative bacteria. Among which were *Pseudomonas aeruginosa*, *Acinetobacter baumanni/calcoaceticus* complex, and *Klebsiella pneumoniae*. The most prevalent fungal species was *Candida spp*. *Escherichia coli* among the gram negative enteric bacteria showed more prevalence (second to *Klebsiella pneumoniae*) and the most prevalent gram positive bacteria were *Staphylococcus aureus*, and *Enterococcus spp.* as shown in Table 4 and Figure 7. This findings align with various other research carried out in the ICU (J. Li et al., 2020), (Ibrahim et al., 2021), (Dasgupta et al., 2015). Although (Chang et al., 2021) reported that out of 267 pathogens isolated, the most prevalent were *A. baumanni* (n=89), *S. aureus* (n=52), *K. pneumoniae* (n=25), *P. aeruginosa* (n=22).

HAIs have since been a serious problem in healthcare settings even before the SARS-CoV-2 pandemic. From the results obtained, there was a significant increase in the frequency of microbial growth of patients who were admitted into the hospital than patients who were not admitted (Table 5). Also, ICU hospitalization showed a significant increase in growth compared to patients admitted to other wards (49.5%) in the hospital as seen in Table7. This is possibly due to the fact that patients who are admitted to the hospital are already at risk of nosocomial infection especially those who have prolonged hospital stay and those who end up being admitted into the ICU. This findings are in accordance with (Kołpa et al., 2018), whose report showed that patients who stayed longer than 20days in the ICU had HAIs compared to those who had lesser number of days.

The distribution of microorganisms was seen to be more prevalent in the year 2022 having a frequency of 61.1% when compared to previous years. However, the year 2020 which was the peak of the pandemic showed a drastically reduced frequency of growth as seen in Figure 8 and Table 6. This is mostly due to the infection control and preventive measures put in place at the time, as well as the fact that patients were overexposed to broad-spectrum antibiotics during the pandemic due to the novelty of the disease, which led to a decline in other bacterial respiratory tract-associated diseases. This result is similar to those obtained by (Zuglian et al., 2022), who according to their research revealed that a number of bacterial species linked to respiratory tract infections, particularly Enterobacterales (22.9%, p=0.002), significantly decreased in patients admitted to the ICU during the pandemic. This was congruent with the decreased frequency of co-bacterial infection among COVID-19 patients. Other recent investigators (Lai et al., 2021), reported that several respiratory infections such as tuberculosis, seasonal influenza e. t. c. reduced in frequency during the COVID-19 pandemic due to the same reasons.

In enteric bacteria, the ESBL rate was 43.3%, the rate of MRSA within the *Staphylococcus aureus* was 55.7% and the rate of VRE was 6%. These microorganisms are crucial to the serious complications of infections in intensive care units. Patients who spend a minimum of seven days and more in the ward are at an increased risk of developing MRSA (> 2.5–4 times) (Sharifipour et al., 2020). Multiple research investigations have also demonstrated a substantial death rate among patients referred to intensive care units due to bacterial pneumonia brought about by MRSA.

The enteric and the gram-positive bacteria in this investigation demonstrated high levels of antibiotic sensitivity as seen in Tables 8 and 10 when compared to their resistivity, however this does not negate the fact that there was resistance to the antibiotics used for example, ampicillin had the highest rate of resistance (91.1%). The high rate of sensitivity could be as a result of the introduction of different treatment protocols imbibed by the ICU medical workers which involved the use of combination therapies to combat infections caused by resistant strains during the pandemic as evaluated by (Sharifipour et al., 2020).

Nevertheless, the resistance rate in non-enteric bacteria showed a considerable high level of resistivity compared to their sensitivity as shown in Table 9. The predominant organism is this group is the *Acinetobacter baumanni/calcoaceticus* complex which from other recent studies has become an ICU menace due to its increasing resistance against antibiotics (Ibrahim et al., 2021). A recent study carried out by (Han et al., 2022) showed that the resistance rate of *A. baumanni* to Ceftaziidime in 2016 which was 89.44% increased to 94.32% in 2017. Its resistance rate to meropenem also increased from 67.05% to 77.38% from 2017 to 2018.

In comparing the pre-pandemic and pandemic periods, it was found that the incidence of growth during the pandemic period was not statistically different from the growth rate during the pre-pandemic period (as shown in Table 11). This was basically due to the high rate of utilization of antimicrobials during the pandemic period that resulted in reduced microbial growth. This result aligns with the findings of (Chen et al., 2020a) who reported that In China, Well over 70% of patients admitted to the ICU, especially COVID-19 patients in an initial study of 99 cases were administered antibiotic medication, while around 15% of them received antifungal therapy.

CHAPTER VI

Conclusion

The emergence of the COVID-19 pandemic has undoubtedly caused a change in every sector of the world system, healthcare facilities, more specifically the ICUs have been greatly affected. There has been a variation in the distribution of microbiological respiratory isolates before and during COVID-19 pandemic.

An unanticipated and inescapable effect of the COVID-19 pandemic is the development of antibiotic resistance, particularly in patients hospitalized to the intensive care unit. The root reason is complex and includes, among other things, the increased incidence of antimicrobial agent usage during the COVID-19 pandemic as compared to pre-pandemic times. This as a result has led to a much bigger concern which is the inability of standard antimicrobial therapies to be effective against microbial infection leading to the emergence of what is called the “super bugs”.

In conclusion, the detection of highly resistant pathogens and the increase in antibiotic resistance, especially after the late stages of the pandemic showed that patients in intensive care should be followed closely and alternative treatment strategies should be developed to limit antibiotic resistance to commonly used antibiotics.

Recommendation

To address the issue of antimicrobial resistance in the ICU setting, it is important to implement evidence-based strategies such as infection prevention and control measures, antimicrobial stewardship programs, and appropriate antimicrobial prescribing practices.

Other strategies that have been identified as being effective in reducing the risk of antimicrobial resistance in the ICU setting include the implementation of contact isolation protocols, the promotion of hand hygiene, and the proper sterilization and disinfection of medical equipment.

The emergence of MDROs during the pandemic may be avoided with the proper prescription and optimized use of antibiotics in accordance with the guidelines of antimicrobial stewardship, in conjunction with accurate diagnosis and vigorous measures to combat infection.

This retrospective study examines the patterns of drug resistance for pathogenic microorganisms associated with nosocomial infections in the ICU over the course of two time periods. These findings offer a conceptual framework for hospitals to manage drug-resistant illnesses, enhancing the reliability of patient assessment and treatment, the potency of antibiotics, and the prudent use of medicines while easing the burden on patients, their families, and the general economy.

REFERENCES

3.1: Horizontal Gene Transfer in Bacteria. (2016, March 5). Biology LibreTexts. https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Kaiser)/Unit_2%3A_Bacterial_Genetics_and_the_Chemical_Control_of_Bacteria/3%3A_Bacterial_Genetics/3.1%3A_Horizontal_Gene_Transfer_in_Bacteria

Abedon, S. T. (2019). Phage-Antibiotic Combination Treatments: Antagonistic Impacts of Antibiotics on the Pharmacodynamics of Phage Therapy? *Antibiotics*, *8*(4), Article 4. https://doi.org/10.3390/antibiotics8040182

Aleem, M., Azeem, A. R., Rahmatullah, S., Vohra, S., Nasir, S., & Andleeb, S. (n.d.). Prevalence of Bacteria and Antimicrobial Resistance Genes in Hospital Water and Surfaces. *Cureus*, *13*(10), e18738. https://doi.org/10.7759/cureus.18738

Ali Alghamdi, B., Al-Johani, I., Al-Shamrani, J. M., Musamed Alshamrani, H., Al-Otaibi, B. G., Almazmomi, K., & Yusnoraini Yusof, N. (2023). Antimicrobial resistance in methicillin-resistant staphylococcus aureus. *Saudi Journal of Biological Sciences*, *30*(4), 103604. https://doi.org/10.1016/j.sjbs.2023.103604

Anthony, W. E., Burnham, C.-A. D., Dantas, G., & Kwon, J. H. (2020). The Gut Microbiome as a Reservoir for Antimicrobial Resistance. *The Journal of Infectious Diseases*, *223*(Suppl 3), S209–S213. https://doi.org/10.1093/infdis/jiaa497

Aryal, S. C., Upreti, M. K., Sah, A. K., Ansari, M., Nepal, K., Dhungel, B., Adhikari, N., Lekhak, B., & Rijal, K. R. (2020). Plasmid-Mediated AmpC β-Lactamase CITM and DHAM Genes Among Gram-Negative Clinical Isolates. *Infection and Drug Resistance*, *13*, 4249–4261. https://doi.org/10.2147/IDR.S284751

Averhoff, B., Kirchner, L., Pfefferle, K., & Yaman, D. (2021). Natural transformation in Gram-negative bacteria thriving in extreme environments: From genes and genomes to proteins, structures and regulation. *Extremophiles*, *25*(5), 425–436. https://doi.org/10.1007/s00792-021-01242-z

Avershina, E., Shapovalova, V., & Shipulin, G. (2021). Fighting Antibiotic Resistance in Hospital-Acquired Infections: Current State and Emerging Technologies in Disease Prevention, Diagnostics and Therapy. *Frontiers in Microbiology*, *12*, 707330. https://doi.org/10.3389/fmicb.2021.707330

Avery, O. T., MacLeod, C. M., & McCarty, M. (1944). STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES. *Journal of Experimental Medicine*, *79*(2), 137–158. https://doi.org/10.1084/jem.79.2.137

Bankar, N. J., Ugemuge, S., Ambad, R. S., Hawale, D. V., & Timilsina, D. R. (n.d.). Implementation of Antimicrobial Stewardship in the Healthcare Setting. *Cureus*, *14*(7), e26664. https://doi.org/10.7759/cureus.26664

Barchitta, M., Quattrocchi, A., Maugeri, A., La Rosa, M. C., La Mastra, C., Sessa, L., Cananzi, P., Murolo, G., Oteri, A., Basile, G., & Agodi, A. (2019). Antibiotic Consumption and Resistance during a 3-Year Period in Sicily, Southern Italy. *International Journal of Environmental Research and Public Health*, *16*(13), 2253. https://doi.org/10.3390/ijerph16132253

Bello-López, J. M., Cabrero-Martínez, O. A., Ibáñez-Cervantes, G., Hernández-Cortez, C., Pelcastre-Rodríguez, L. I., Gonzalez-Avila, L. U., & Castro-Escarpulli, G. (2019). Horizontal Gene Transfer and Its Association with Antibiotic Resistance in the Genus Aeromonas spp. *Microorganisms*, *7*(9), 363. https://doi.org/10.3390/microorganisms7090363

Blokesch, M. (2016). Protocols for Visualizing Horizontal Gene Transfer in Gram-Negative Bacteria Through Natural Competence. In T. J. McGenity, K. N. Timmis, & B. Nogales (Eds.), *Hydrocarbon and Lipid Microbiology Protocols: Single-Cell and Single-Molecule Methods* (pp. 189–204). Springer. https://doi.org/10.1007/8623_2015_46

Blot, S., Ruppé, E., Harbarth, S., Asehnoune, K., Poulakou, G., Luyt, C.-E., Rello, J., Klompas, M., Depuydt, P., Eckmann, C., Martin-Loeches, I., Povoa, P., Bouadma, L., Timsit, J.-F., & Zahar, J.-R. (2022). Healthcare-associated infections in adult intensive care unit patients: Changes in epidemiology, diagnosis, prevention and contributions of new technologies. *Intensive & Critical Care Nursing*, *70*, 103227. https://doi.org/10.1016/j.iccn.2022.103227

Bonadonna, L., Briancesco, R., Coccia, A. M., Meloni, P., Rosa, G. L., & Moscato, U. (2021). Microbial Air Quality in Healthcare Facilities. *International Journal of Environmental Research and Public Health*, *18*(12), Article 12. https://doi.org/10.3390/ijerph18126226

Bouza, E., Alonso, S., Asensio, A., De Juan, G., García Lucio, C., Larrosa, C., López-Iglesias, J., Muñoz, P., Sierra, R., Perianes, J., De la Serna, J. L., Palomo, E., & Gracia, D. (2019). Information on nosocomial infections in the mainstream media: An opinion document. *Revista Espanola De Quimioterapia: Publicacion Oficial De La Sociedad Espanola De Quimioterapia*, *32*(2), 165–177.

Briffotaux, J., Liu, S., & Gicquel, B. (2019). Genome-Wide Transcriptional Responses of Mycobacterium to Antibiotics. *Frontiers in Microbiology, 10*. https://www.frontiersin.org/articles/10.3389/fmicb.2019.00249

Calderaro, A., Buttrini, M., Montecchini, S., Piccolo, G., Martinelli, M., Dell'Anna, M. L., Di Maio, A., Arcangeletti, M. C., Maccari, C., De Conto, F., & Chezzi, C. (2021). Detection of SARS-CoV-2 and Other Infectious Agents in Lower Respiratory Tract Samples Belonging to Patients Admitted to Intensive Care Units of a Tertiary-Care Hospital, Located in an Epidemic Area, during the Italian Lockdown. *Microorganisms*, *9*(1), 185. https://doi.org/10.3390/microorganisms9010185

Caneiras, C., Lito, L., Melo-Cristino, J., & Duarte, A. (2019). Community- and Hospital-Acquired Klebsiella pneumoniae Urinary Tract Infections in Portugal: Virulence and Antibiotic Resistance. *Microorganisms*, *7*(5), 138. https://doi.org/10.3390/microorganisms7050138

Chang, Y., Jeon, K., Lee, S.-M., Cho, Y.-J., Kim, Y. S., Chong, Y. P., & Hong, S.-B. (2021). The Distribution of Multidrug-resistant Microorganisms and Treatment Status of Hospital-acquired Pneumonia/Ventilator-associated Pneumonia in Adult Intensive Care Units: A Prospective Cohort Observational Study. *Journal of Korean Medical Science*, *36*(41), e251. https://doi.org/10.3346/jkms.2021.36.e251

Chedid, M., Waked, R., Haddad, E., Chetata, N., Saliba, G., & Choucair, J. (2021). Antibiotics in treatment of COVID-19 complications: A review of frequency, indications, and efficacy. *Journal of Infection and Public Health*, *14*(5), 570–576. https://doi.org/10.1016/j.jiph.2021.02.001

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., & Zhang, L. (2020a). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China:

A descriptive study. *Lancet (London, England)*, *395*(10223), 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., & Zhang, L. (2020b). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *The Lancet*, *395*(10223), 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7

Chiș, A. A., Rus, L. L., Morgovan, C., Arseniu, A. M., Frum, A., Vonica-Țincu, A. L., Gligor, F. G., Mureșan, M. L., & Dobrea, C. M. (2022). Microbial Resistance to Antibiotics and Effective Antibiotherapy. *Biomedicines*, *10*(5), 1121. https://doi.org/10.3390/biomedicines10051121

Chowdhary, A., Tarai, B., Singh, A., & Sharma, A. (2020). Multidrug-Resistant Candida auris Infections in Critically Ill Coronavirus Disease Patients, India, April-July 2020. *Emerging Infectious Diseases*, *26*(11), 2694–2696. https://doi.org/10.3201/eid2611.203504

Cong, Y., Yang, S., & Rao, X. (2019). Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features. *Journal of Advanced Research*, *21*, 169–176. https://doi.org/10.1016/j.jare.2019.10.005

Conjugation, transformation & transduction | Bacteria (article). (n.d.). Khan Academy. Retrieved 25 April 2023, from https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/mutations-ap/a/genetic-variation-in-prokaryotes

Contou, D., Claudinon, A., Pajot, O., Micaëlo, M., Longuet Flandre, P., Dubert, M., Cally, R., Logre, E., Fraissé, M., Mentec, H., & Plantefève, G. (2020). Bacterial and viral co-infections in patients with severe SARS-CoV-2 pneumonia admitted to a French ICU. *Annals of Intensive Care*, *10*(1), 119. https://doi.org/10.1186/s13613-020-00736-x

Cox, M. J., Loman, N., Bogaert, D., & O'Grady, J. (2020). Co-infections: Potentially lethal and unexplored in COVID-19. *The Lancet. Microbe*, *1*(1), e11. https://doi.org/10.1016/S2666-5247(20)30009-4

Crane, J. K., Alvarado, C. L., & Sutton, M. D. (n.d.). Role of the SOS Response in the Generation of Antibiotic Resistance In Vivo. *Antimicrobial Agents and Chemotherapy*, *65*(7), e00013-21. https://doi.org/10.1128/AAC.00013-21

Cylke, C., Si, F., & Banerjee, S. (2022). Effects of antibiotics on bacterial cell morphology and their physiological origins. *Biochemical Society Transactions*, *50*(5), 1269–1279. https://doi.org/10.1042/BST20210894

Dadi, N. C. T., Radochová, B., Vargová, J., & Bujdáková, H. (2021). Impact of Healthcare-Associated Infections Connected to Medical Devices—An Update. *Microorganisms*, *9*(11), 2332. https://doi.org/10.3390/microorganisms9112332

Dasgupta, S., Das, S., Chawan, N. S., & Hazra, A. (2015). Nosocomial infections in the intensive care unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India. *Indian Journal of Critical Care Medicine : Peer-Reviewed, Official Publication of Indian Society of Critical Care Medicine*, *19*(1), 14. https://doi.org/10.4103/0972-5229.148633

De Georgeo, M. R., De Georgeo, J. M., Egan, T. M., Klee, K. P., Schwemm, M. S., Bye-Kollbaum, H., & Kinser, A. J. (2021). Containing SARS-CoV-2 in hospitals facing finite PPE, limited testing, and physical space variability: Navigating resource constrained enhanced traffic control bundling. *Journal of Microbiology,* *Immunology and Infection*, *54*(1), 4–11. https://doi.org/10.1016/j.jmii.2020.07.009

Department of Health. Victoria, A. (n.d.). *Infection control—Standard and transmission-based precautions*. State Government of Victoria, Australia. Retrieved 8 March 2023, from https://www.health.vic.gov.au/infectious-diseases/infection-control-standard-and-transmission-based-precautions

Duszynska, W., Rosenthal, V. D., Szczesny, A., Zajaczkowska, K., Fulek, M., & Tomaszewski, J. (2020). Device associated –health care associated infections monitoring, prevention and cost assessment at intensive care unit of University Hospital in Poland (2015–2017). *BMC Infectious Diseases*, *20*(1), 761. https://doi.org/10.1186/s12879-020-05482-w

Eastin, C., & Eastin, T. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *The Journal of Emergency Medicine*, *58*(4), 711–712. https://doi.org/10.1016/j.jemermed.2020.04.004

Fu, Y., Yang, Q., Xu, M., Kong, H., Chen, H., Fu, Y., Yao, Y., Zhou, H., & Zhou, J. (2020). Secondary Bacterial Infections in Critical Ill Patients With Coronavirus Disease 2019. *Open Forum Infectious Diseases*, *7*(6), ofaa220. https://doi.org/10.1093/ofid/ofaa220

García-Solache, M., & Rice, L. B. (2019). The Enterococcus: A Model of Adaptability to Its Environment. *Clinical Microbiology Reviews*, *32*(2), e00058-18. https://doi.org/10.1128/CMR.00058-18

Golz, J. C., & Stingl, K. (2021). Natural Competence and Horizontal Gene Transfer in Campylobacter. In S. Backert (Ed.), *Fighting Campylobacter Infections: Towards* *a One Health Approach* (pp. 265–292). Springer International Publishing. https://doi.org/10.1007/978-3-030-65481-8_10

Grau, S., Echeverria-Esnal, D., Gómez-Zorrilla, S., Navarrete-Rouco, M. E., Masclans, J. R., Espona, M., Gracia-Arnillas, M. P., Duran, X., Comas, M., Horcajada, J. P., & Ferrández, O. (2021). Evolution of Antimicrobial Consumption During the First Wave of COVID-19 Pandemic. *Antibiotics*, *10*(2). https://doi.org/10.3390/antibiotics10020132

Han, Y., Zhang, J., Zhang, H.-Z., Zhang, X.-Y., & Wang, Y.-M. (2022). Multidrug-resistant organisms in intensive care units and logistic analysis of risk factors. *World Journal of Clinical Cases*, *10*(6), 1795–1805. https://doi.org/10.12998/wjcc.v10.i6.1795

Haque, M., McKimm, J., Sartelli, M., Dhingra, S., Labricciosa, F. M., Islam, S., Jahan, D., Nusrat, T., Chowdhury, T. S., Coccolini, F., Iskandar, K., Catena, F., & Charan, J. (2020a). Strategies to Prevent Healthcare-Associated Infections: A Narrative Overview. *Risk Management and Healthcare Policy*, *13*, 1765–1780. https://doi.org/10.2147/RMHP.S269315

Haque, M., McKimm, J., Sartelli, M., Dhingra, S., Labricciosa, F. M., Islam, S., Jahan, D., Nusrat, T., Chowdhury, T. S., Coccolini, F., Iskandar, K., Catena, F., & Charan, J. (2020b). Strategies to Prevent Healthcare-Associated Infections: A Narrative Overview. *Risk Management and Healthcare Policy*, *13*, 1765–1780. https://doi.org/10.2147/RMHP.S269315

Harun, Md. G. D., Anwar, M. M. U., Sumon, S. A., Hassan, Md. Z., Mohona, T. M., Rahman, A., Abdullah, S. A. H. M., Islam, M. S., Kaydos-Daniels, S. C., & Styczynski, A. R. (2022). Rationale and guidance for strengthening infection prevention and control measures and antimicrobial stewardship programs in Bangladesh: A study protocol. *BMC Health Services Research*, *22*, 1239. https://doi.org/10.1186/s12913-022-08603-0

Hasanzade, H., Gürün Kaya, A., Çiledağ, A., Erol, S., Çiftçi, F., Güriz, H., & Kaya, A. (2021). [The distribution of microorganisms and antibiotic resistance profile in pulmonary critical care unit patients: A single-centre study]. *Tuberkuloz Ve Toraks*, *69*(1), 9–20. https://doi.org/10.5578/tt.20219902

Haverkate, M. R., Dautzenberg, M. J. D., Ossewaarde, T. J. M., van der Zee, A., den Hollander, J. G., Troelstra, A., Bonten, M. J. M., & Bootsma, M. C. J. (2015). Within-Host and Population Transmission of blaOXA-48 in K. pneumoniae and E. coli. *PloS One*, *10*(10), e0140960. https://doi.org/10.1371/journal.pone.0140960

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, *395*(10223), 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

Ibrahim, S., Al-Saryi, N., Al-Kadmy, I. M. S., & Aziz, S. N. (2021). Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals. *Molecular Biology Reports*, *48*(10), 6987–6998. https://doi.org/10.1007/s11033-021-06690-6

Juan, H.-C., Chao, C.-M., Lai, C.-C., & Tang, H.-J. (2021). Decline in invasive pneumococcal disease during COVID-19 pandemic in Taiwan. *Journal of Infection*, *82*(2), 282–327. https://doi.org/10.1016/j.jinf.2020.09.018

Khezri, A., Avershina, E., & Ahmad, R. (2020). Plasmid Identification and Plasmid-Mediated Antimicrobial Gene Detection in Norwegian Isolates. *Microorganisms*, *9*(1), 52. https://doi.org/10.3390/microorganisms9010052

Klug, D. M., Idiris, F. I. M., Blaskovich, M. A. T., von Delft, F., Dowson, C. G., Kirchhelle, C., Roberts, A. P., Singer, A. C., & Todd, M. H. (2021). There is no market for new antibiotics: This allows an open approach to research and development. *Wellcome Open Research*, *6*, 146. https://doi.org/10.12688/wellcomeopenres.16847.1

Kołpa, M., Wałaszek, M., Gniadek, A., Wolak, Z., & Dobroś, W. (2018). Incidence, Microbiological Profile and Risk Factors of Healthcare-Associated Infections in Intensive Care Units: A 10 Year Observation in a Provincial Hospital in Southern Poland. *International Journal of Environmental Research and Public Health*, *15*(1), 112. https://doi.org/10.3390/ijerph15010112

Komiyama, Y., & Maeda, S. (2020). Natural Transformation in Escherichia coli. *Methods in Molecular Biology (Clifton, N.J.), 2075,* 179–187. https://doi.org/10.1007/978-1-4939-9877-7_13

Kübler, A., Duszynska, W., Rosenthal, V. D., Fleischer, M., Kaiser, T., Szewczyk, E., & Barteczko-Grajek, B. (2012). Device-associated infection rates and extra length of stay in an intensive care unit of a university hospital in Wroclaw, Poland: International Nosocomial Infection Control Consortium's (INICC) findings. *Journal of Critical Care*, *27*(1), 105.e5-105.e10. https://doi.org/10.1016/j.jcrc.2011.05.018

Kunhikannan, S., Thomas, C. J., Franks, A. E., Mahadevaiah, S., Kumar, S., & Petrovski, S. (2021). Environmental hotspots for antibiotic resistance genes. *MicrobiologyOpen*, *10*(3), e1197. https://doi.org/10.1002/mbo3.1197

Kyriakidis, I., Vasileiou, E., Pana, Z. D., & Tragiannidis, A. (2021). Acinetobacter baumannii Antibiotic Resistance Mechanisms. *Pathogens*, *10*(3), 373. https://doi.org/10.3390/pathogens10030373

Lai, C.-C., Chen, S.-Y., Ko, W.-C., & Hsueh, P.-R. (2021). Increased antimicrobial resistance during the COVID-19 pandemic. *International Journal of Antimicrobial Agents*, *57*(4), 106324. https://doi.org/10.1016/j.ijantimicag.2021.106324

Lai, C.-C., & Yu, W.-L. (2020). The COVID-19 pandemic and tuberculosis in Taiwan. *Journal of Infection*, *81*(2), e159–e161. https://doi.org/10.1016/j.jinf.2020.06.014

Larsson, D. G. J., & Flach, C.-F. (2022). Antibiotic resistance in the environment. *Nature Reviews. Microbiology*, *20*(5), 257–269. https://doi.org/10.1038/s41579-021-00649-x

Lerminiaux, N. A., & Cameron, A. D. S. (2019). Horizontal transfer of antibiotic resistance genes in clinical environments. *Canadian Journal of Microbiology*, *65*(1), 34–44. https://doi.org/10.1139/cjm-2018-0275

Li, J., Wang, J., Yang, Y., Cai, P., Cao, J., Cai, X., & Zhang, Y. (2020). Etiology and antimicrobial resistance of secondary bacterial infections in patients hospitalized with COVID-19 in Wuhan, China: A retrospective analysis. *Antimicrobial Resistance & Infection Control*, *9*(1), 153. https://doi.org/10.1186/s13756-020-00819-1

Li, W., & Zhang, G. (2022). Detection and various environmental factors of antibiotic resistance gene horizontal transfer. *Environmental Research*, *212*, 113267. https://doi.org/10.1016/j.envres.2022.113267

Li, Z., Chen, Q., Feng, L., Rodewald, L., Xia, Y., Yu, H., Zhang, R., An, Z., Yin, W., Chen, W., Qin, Y., Peng, Z., Zhang, T., Ni, D., Cui, J., Wang, Q., Yang, X., Zhang, M., Ren, X., … Li, S. (2020). Active case finding with case management: The key to tackling the COVID-19 pandemic. *The Lancet*, *396*(10243), 63–70. https://doi.org/10.1016/S0140-6736(20)31278-2

Liu, W., Cheng, M., Li, J., Zhang, P., Fan, H., Hu, Q., Han, M., Su, L., He, H., Tong, Y., Ning, K., & Long, Y. (2020). Classification of the Gut Microbiota of Patients in Intensive Care Units During Development of Sepsis and Septic Shock. *Genomics, Proteomics & Bioinformatics*, *18*(6), 696–707. https://doi.org/10.1016/j.gpb.2020.06.011

Michaelis, C., & Grohmann, E. (2023). Horizontal Gene Transfer of Antibiotic Resistance Genes in Biofilms. *Antibiotics*, *12*(2), Article 2. https://doi.org/10.3390/antibiotics12020328

Mohamed, A., Hassan, T., Trzos-Grzybowska, M., Thomas, J., Quinn, A., O'Sullivan, M., Griffin, A., Rogers, T. R., & Talento, A. F. (2021). Multi-triazole-resistant Aspergillus fumigatus and SARS-CoV-2 co-infection: A lethal combination. *Medical Mycology Case Reports*, *31*, 11–14. https://doi.org/10.1016/j.mmcr.2020.06.005

Moradi, N., Kazemi, N., Ghaemi, M., & Mirzaei, B. (2021). Frequency and antimicrobial resistance pattern of bacterial isolates from patients with COVID-19 in two hospitals of Zanjan. *Iranian Journal of Microbiology*, *13*(6), 769–778. https://doi.org/10.18502/ijm.v13i6.8078

Nguyen, N. T., Chinn, J., Nahmias, J., Yuen, S., Kirby, K. A., Hohmann, S., & Amin, A. (2021). Outcomes and Mortality Among Adults Hospitalized With COVID-19 at US Medical Centers. *JAMA Network Open*, *4*(3), e210417. https://doi.org/10.1001/jamanetworkopen.2021.0417

Nimer, N. A. (2022). <p>Nosocomial Infection and Antibiotic-Resistant Threat in the Middle East</p>. *Infection and Drug Resistance*, *15*, 631–639. https://doi.org/10.2147/IDR.S351755

Nori, P., Szymczak, W., Puius, Y., Sharma, A., Cowman, K., Gialanella, P., Fleischner, Z., Corpuz, M., Torres-Isasiga, J., Bartash, R., Felsen, U., Chen, V., & Guo, Y. (2020). Emerging Co-Pathogens: New Delhi Metallo-beta-lactamase producing Enterobacterales Infections in New York City COVID-19 Patients. *International Journal of Antimicrobial Agents*, *56*(6), 106179. https://doi.org/10.1016/j.ijantimicag.2020.106179

Orhan, Ö., Yılmaz, K., Gözü Pirinççioğlu, A., Solmaz, M., & Karakoç, F. (2022). Antibiotic Susceptibility of Microorganisms Grown in Tracheal Aspirate Cultures of Pediatric Intensive Care Patients. *Cureus*, *14*(7), e26934. https://doi.org/10.7759/cureus.26934

Parmanik, A., Das, S., Kar, B., Bose, A., Dwivedi, G. R., & Pandey, M. M. (2022). Current Treatment Strategies Against Multidrug-Resistant Bacteria: A Review. *Current Microbiology*, *79*(12), 388. https://doi.org/10.1007/s00284-022-03061-7

Patangia, D. V., Anthony Ryan, C., Dempsey, E., Paul Ross, R., & Stanton, C. (2022). Impact of antibiotics on the human microbiome and consequences for host health. *MicrobiologyOpen*, *11*(1), e1260. https://doi.org/10.1002/mbo3.1260

Paul, D., Chanda, D. D., Chakravarty, A., & Bhattacharjee, A. (2020). An insight into analysis and elimination of plasmids encoding metallo-β-lactamases in Pseudomonas aeruginosa. *Journal of Global Antimicrobial Resistance*, *21*, 3–7. https://doi.org/10.1016/j.jgar.2019.09.002

Pfeifer, E., Bonnin, R. A., & Rocha, E. P. C. (n.d.). Phage-Plasmids Spread Antibiotic Resistance Genes through Infection and Lysogenic Conversion. *MBio*, *13*(5), e01851-22. https://doi.org/10.1128/mbio.01851-22

Posteraro, B., Torelli, R., Vella, A., Leone, P. M., De Angelis, G., De Carolis, E., Ventura, G., Sanguinetti, M., & Fantoni, M. (2020). Pan-Echinocandin-Resistant Candida glabrata Bloodstream Infection Complicating COVID-19: A Fatal Case Report. *Journal of Fungi (Basel, Switzerland)*, *6*(3), 163. https://doi.org/10.3390/jof6030163

Prigitano, A., Perrone, P. M., Esposto, M. C., Carnevali, D., De Nard, F., Grimoldi, L., Principi, N., Cogliati, M., Castaldi, S., & Romanò, L. (2022). ICU environmental surfaces are a reservoir of fungi: Species distribution in northern Italy. *The Journal of Hospital Infection*, *123*, 74–79. https://doi.org/10.1016/j.jhin.2022.02.006

Qi, Q., Rajabal, V., Ghaly, T. M., Tetu, S. G., & Gillings, M. R. (2023). Identification of integrons and gene cassette-associated recombination sites in bacteriophage genomes. *Frontiers in Microbiology*, *14*. https://www.frontiersin.org/articles/10.3389/fmicb.2023.1091391

Qin, S., Xiao, W., Zhou, C., Pu, Q., Deng, X., Lan, L., Liang, H., Song, X., & Wu, M. (2022). Pseudomonas aeruginosa: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduction and Targeted Therapy*, *7*(1), Article 1. https://doi.org/10.1038/s41392-022-01056-1

Rafa, E., Wałaszek, M. Z., Wałaszek, M. J., Domański, A., & Różańska, A. (2021). The Incidence of Healthcare-Associated Infections, Their Clinical Forms, and Microbiological Agents in Intensive Care Units in Southern Poland in a Multicentre Study from 2016 to 2019. *International Journal of Environmental Research and Public Health*, *18*(5), 2238. https://doi.org/10.3390/ijerph18052238

Rensing, K. L., Abdallah, H. M., Koek, A., Elmowalid, G. A., Vandenbroucke-Grauls, C. M. J. E., al Naiemi, N., & van Dijk, K. (2019). Prevalence of plasmid-mediated AmpC in Enterobacteriaceae isolated from humans and from retail meat in Zagazig, Egypt. *Antimicrobial Resistance & Infection Control*, *8*(1), 45. https://doi.org/10.1186/s13756-019-0494-6

Robba, C., Battaglini, D., Pelosi, P., & Rocco, P. R. M. (n.d.). Multiple organ dysfunction in SARS-CoV-2: MODS-CoV-2. *Expert Review of Respiratory Medicine*, 1–4. https://doi.org/10.1080/17476348.2020.1778470

Sakamoto, H., Ishikane, M., & Ueda, P. (2020). Seasonal Influenza Activity During the SARS-CoV-2 Outbreak in Japan. *JAMA*, *323*(19), 1969–1971. https://doi.org/10.1001/jama.2020.6173

Shahid, A., Muzammil, S., Aslam, B., Ashfaq, U. A., Hayat, S., Bilal, M., Rajoka, M. S. R., Nisar, M. A., & Khurshid, M. (2023). Chapter 2 - Antibiotics and antibiotic-resistant bacteria in the environment: Sources and impacts. In P. Singh & M.

Sillanpää (Eds.), *Degradation of Antibiotics and Antibiotic-Resistant Bacteria from Various Sources* (pp. 39–65). Academic Press. https://doi.org/10.1016/B978-0-323-99866-6.00002-7

Sharifipour, E., Shams, S., Esmkhani, M., Khodadadi, J., Fotouhi-Ardakani, R., Koohpaei, A., Doosti, Z., & Ej Golzari, S. (2020). Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. *BMC Infectious Diseases*, *20*(1), 646. https://doi.org/10.1186/s12879-020-05374-z

Shickel, B., Davoudi, A., Ozrazgat-Baslanti, T., Ruppert, M., Bihorac, A., & Rashidi, P. (2021). Deep Multi-Modal Transfer Learning for Augmented Patient Acuity Assessment in the Intelligent ICU. *Frontiers in Digital Health*, *3*, 640685. https://doi.org/10.3389/fdgth.2021.640685

Silago, V., & Mshana, S. E. (2022). Whole Genome Sequencing Reveals Presence of High-Risk Global Clones of Klebsiella pneumoniae Harboring Multiple Antibiotic Resistance Genes in Multiple Plasmids in Mwanza, Tanzania. *Microorganisms*, *10*(12), Article 12. https://doi.org/10.3390/microorganisms10122396

Sr, L., Oj, S.-R., S, R., & Af, S. (2019). Chlorhexidine bathing of the critically ill for the prevention of hospital-acquired infection. *The Cochrane Database of Systematic Reviews*, *8*(8). https://doi.org/10.1002/14651858.CD012248.pub2

Sukhum, K. V., Newcomer, E. P., Cass, C., Wallace, M. A., Johnson, C., Fine, J., Sax, S., Barlet, M. H., Burnham, C.-A. D., Dantas, G., & Kwon, J. H. (2022). Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates. *Communications Medicine*, *2*(1), Article 1. https://doi.org/10.1038/s43856-022-00124-5

Sun, R., Yu, P., Zuo, P., & Alvarez, P. J. J. (2022). Bacterial Concentrations and Water Turbulence Influence the Importance of Conjugation Versus Phage-Mediated Antibiotic Resistance Gene Transfer in Suspended Growth Systems. *ACS Environmental Au*, *2*(2), 156–165. https://doi.org/10.1021/acsenvironau.1c00027

Surveillance of healthcare-associated infections in Europe 2007. (2012, February 15). https://www.ecdc.europa.eu/en/publications-data/surveillance-healthcare-associated-infections-europe-2007

Szychowiak, P., Villageois-Tran, K., Patrier, J., Timsit, J.-F., & Ruppé, É. (2022). The role of the microbiota in the management of intensive care patients. *Annals of Intensive Care*, *12*(1), 3. https://doi.org/10.1186/s13613-021-00976-5

Tao, S., Chen, H., Li, N., Wang, T., & Liang, W. (2022). The Spread of Antibiotic Resistance Genes In Vivo Model. *The Canadian Journal of Infectious Diseases & Medical Microbiology = Journal Canadien Des Maladies Infectieuses et de La Microbiologie Médicale*, *2022*, 3348695. https://doi.org/10.1155/2022/3348695

Teich, V. D., Klajner, S., Almeida, F. A. S. de, Dantas, A. C. B., Laselva, C. R., Torritesi, M. G., Canero, T. R., Berwanger, O., Rizzo, L. V., Reis, E. P., & Cendoroglo Neto, M. (2020). Epidemiologic and clinical features of patients with COVID-19 in Brazil. *Einstein (Sao Paulo, Brazil)*, *18*, eAO6022. https://doi.org/10.31744/einstein_journal/2020ao6022

Tiri, B., Sensi, E., Marsiliani, V., Cantarini, M., Priante, G., Vernelli, C., Martella, L. A., Costantini, M., Mariottini, A., Andreani, P., Bruzzone, P., Suadoni, F., Francucci, M., Cirocchi, R., & Cappanera, S. (2020). Antimicrobial Stewardship Program, COVID-19, and Infection Control: Spread of Carbapenem-Resistant Klebsiella

Pneumoniae Colonization in ICU COVID-19 Patients. What Did Not Work? *Journal of Clinical Medicine*, *9*(9), E2744. https://doi.org/10.3390/jcm9092744

Tozzo, P., Delicati, A., & Caenazzo, L. (2022). Human microbiome and microbiota identification for preventing and controlling healthcare-associated infections: A systematic review. *Frontiers in Public Health*, *10*, 989496. https://doi.org/10.3389/fpubh.2022.989496

Trejnowska, E., Deptuła, A., Tarczyńska-Słomian, M., Knapik, P., Jankowski, M., Misiewska-Kaczur, A., Tamowicz, B., Śmiechowicz, J., Antończyk, R., Armatowicz, P., Sułkowski, W., & Durek, G. (2018). Surveillance of Antibiotic Prescribing in Intensive Care Units in Poland. *The Canadian Journal of Infectious Diseases & Medical Microbiology = Journal Canadien Des Maladies Infectieuses Et De La Microbiologie Medicale*, *2018*, 5670238. https://doi.org/10.1155/2018/5670238

Trentini, F., Marziano, V., Guzzetta, G., Tirani, M., Cereda, D., Poletti, P., Piccarreta, R., Barone, A., Preziosi, G., Arduini, F., Della Valle, P. G., Zanella, A., Grosso, F., Del Castillo, G., Castrofino, A., Grasselli, G., Melegaro, A., Piatti, A., Andreassi, A., … Merler, S. (2022). Pressure on the Health-Care System and Intensive Care Utilization During the COVID-19 Outbreak in the Lombardy Region of Italy: A Retrospective Observational Study in 43,538 Hospitalized Patients. *American Journal of Epidemiology*, *191*(1), 137–146. https://doi.org/10.1093/aje/kwab252

Ture, Z., Güner, R., & Alp, E. (2022). Antimicrobial stewardship in the intensive care unit. *Journal of Intensive Medicine*. https://doi.org/10.1016/j.jointm.2022.10.001

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., Holland, T. L., & Fowler, V. G. (2019). Methicillin-resistant

Staphylococcus aureus: An overview of basic and clinical research. *Nature Reviews Microbiology*, *17*(4), Article 4. https://doi.org/10.1038/s41579-018-0147-4

Urban-Chmiel, R., Marek, A., Stępień-Pyśniak, D., Wieczorek, K., Dec, M., Nowaczek, A., & Osek, J. (2022). Antibiotic Resistance in Bacteria—A Review. *Antibiotics*, *11*(8), 1079. https://doi.org/10.3390/antibiotics11081079

van Dijk, B., Hogeweg, P., Doekes, H. M., & Takeuchi, N. (n.d.). Slightly beneficial genes are retained by bacteria evolving DNA uptake despite selfish elements. *ELife*, *9*, e56801. https://doi.org/10.7554/eLife.56801

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., & Peng, Z. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. *JAMA*, *323*(11), 1061–1069. https://doi.org/10.1001/jama.2020.1585

Wang, J., Xu, T., Ying, J., Zhou, W., Chen, Q., Qian, C., Zhu, X., Shen, K., Li, P., Li, K., Bao, Q., & Lu, J. (2019). PAU-1, a Novel Plasmid-Encoded Ambler Class A β-Lactamase Identified in a Clinical Pseudomonas aeruginosa Isolate. *Infection and Drug Resistance*, *12*, 3827–3834. https://doi.org/10.2147/IDR.S225288

Wendel, A. F., Malecki, M., Mattner, F., Xanthopoulou, K., Wille, J., Seifert, H., & Higgins, P. G. (2022). Genomic-based transmission analysis of carbapenem-resistant Pseudomonas aeruginosa at a tertiary care centre in Cologne (Germany) from 2015 to 2020. *JAC-Antimicrobial Resistance*, *4*(3), dlac057. https://doi.org/10.1093/jacamr/dlac057

Woods, L. C., Gorrell, R. J., Taylor, F., Connallon, T., Kwok, T., & McDonald, M. J. (2020). Horizontal gene transfer potentiates adaptation by reducing selective constraints on the spread of genetic variation. *Proceedings of the National Academy of Sciences*, *117*(43), 26868–26875. https://doi.org/10.1073/pnas.2005331117

Zhang, J., He, X., Shen, S., Shi, M., Zhou, Q., Liu, J., Wang, M., & Sun, Y. (2021). Effects of the Newly Isolated T4-like Phage on Transmission of Plasmid-Borne Antibiotic Resistance Genes via Generalized Transduction. *Viruses*, *13*(10), Article 10. https://doi.org/10.3390/v13102070

Zhang, Y., Guo, Y., Qiu, T., Gao, M., & Wang, X. (2022). Bacteriophages: Underestimated vehicles of antibiotic resistance genes in the soil. *Frontiers in Microbiology*, *13*, 936267. https://doi.org/10.3389/fmicb.2022.936267

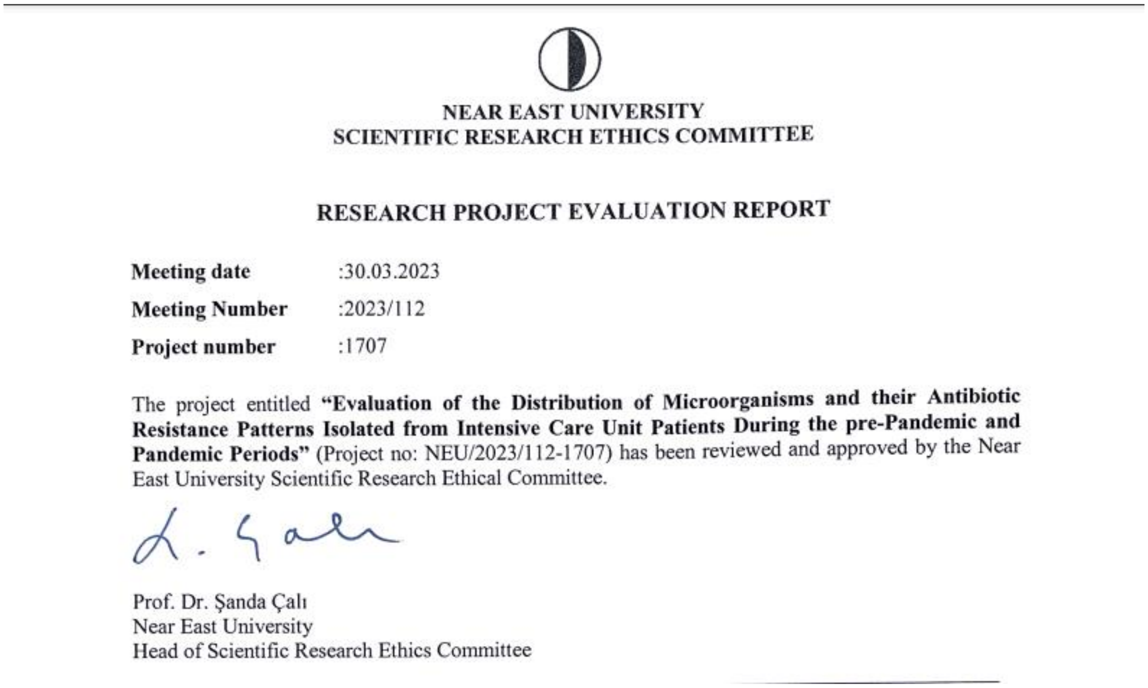
Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., & Cao, B. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *The Lancet*, *395*(10229), 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3

Zuglian, G., Ripamonti, D., Tebaldi, A., Cuntrò, M., Riva, I., Farina, C., & Rizzi, M. (2022). The changing pattern of bacterial and fungal respiratory isolates in patients with and without COVID-19 admitted to intensive care unit. *BMC Infectious Diseases*, *22*(1), 185. https://doi.org/10.1186/s12879-022-07176-x

Zwanzig, M. (2021). The ecology of plasmid-coded antibiotic resistance: A basic framework for experimental research and modeling. *Computational and Structural Biotechnology Journal*, *19*, 586–599. https://doi.org/10.1016/j.csbj.2020.12.027

APPENDICES

Appendix A



NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date	:30.03.2023
Meeting Number	:2023/112
Project number	:1707

The project entitled **"Evaluation of the Distribution of Microorganisms and their Antibiotic Resistance Patterns Isolated from Intensive Care Unit Patients During the pre-Pandemic and Pandemic Periods"** (Project no: NEU/2023/112-1707) has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Appendix B

Curriculum Vitae

Name Surname: Elizabeth Mkpouto Opawoye

Nationality: NIGERIA

Title: Masters in Medical Microbiology and Clinical Microbiology

Address: Dogu sokak 1/3 yeni kent ogrenci yurdu. Yeni kent. Daire 55

Mobile Phone: +905338322984,

E-Mail: lizzyopawoye@gmail.com

Education:

Degree	**Field**	**Class of Degree**	**University**	**Year**
Bachelor	Microbiology	First class honors (4.50/5.0)	Ahmadu Bello University	2014-2019
Master	Medical Microbiology and Clinical Microbiology.	High honors (4.0/4.0)	Near East University	2021-2023

TEACHING EXPERIENCE

YUSUF MAITAMA SULE UNIVERSITY (Teaching Assistant) 2019-2020

- Tasked with the responsibility of coordinating students' laboratory research work.
- Assisted in the preparation of laboratory equipment and reagents for each laboratory session.
- Assessed practical workbooks of the students

- Assisted in lecturing and teaching undergraduate students.

RESEARCH EXPERIENCE

Masters Research 2021-2023

Department of Medical Microbiology and Clinical Microbiology, Near East University, TRNC, Lefkosa, Turkey

- Carrying out research on the impact of the COVID-19 pandemic on the microbial profile and antibiotic susceptibility pattern of pathogens isolated from the ICU of Near East University Hospital.
- Analyzing the data using statistical software.

Undergraduate Research 2018-2019

- Researched on the prevalence and antibiograms of *Citrobacter spp* and *Pseudomonas aeruginosa* isolated from the urine samples of students in a tertiary institution in Nigeria
- To check the prevalence of asymptomatic UTI among apparently healthy students in a tertiary institution.
- Carried out biochemical Identification and characterization of the isolated microorganisms
- Carried out antibiotic susceptibility tests using the disc diffusion method.
- Analyzed the data using statistical software.

RESEARCH AND TEACHING INTERESTS

- Host-pathogen interaction,
- Immunology and bacteriology,
- Microbial pathogenesis and antibiotic resistance.
- Healthcare-associated infections etc.

CURRENT RESEARCH WORKS AND MANUSCRIPT PREPARATION

- Recent Advances in Clinical Assessment, Management, and Prevention Strategies of Pediatric Urinary Tract Infections.
- Role of Artificial sweeteners in antibiotic resistance of gut bacteria.

Publications

Opawoye, E., Markus, V., & Henry, G. B. (2023). Antibiogram of asymptomatic bacteriuria among university students in Nigeria. *The Bioscientist Journal* (under review).

CONFERENCES ATTENDED

- 1st Annual symposium and AGM of Nigerian Society for Microbiology 26th-27th July 2018, Ahmadu Bello University, Nigeria. EMERGING AND RE-EMERGING MICROBIAL DISEASES: IMPLICATION ON HEALTHCARE DELIEVERY.
- DESAM Research Institute workshop 29th July, 2022, Near East University, TRNC. One Kit One Product.

SCHOLARSHIPS/AWARDS

- Near East University Masters Degree Scholarship **2021-2023**

COMMUNITY INVOLVEMENT/MEMBERSHIP

- Near East Christian Fellowship
  2021-Present
- Secretary, Good Manufacturing Practices, Seven-Up Bottling Company, Kano State, Nigeria **2020-2021**
- State Assistant Coordinator, Winners Corpers Fellowship, (NYSC), Kano State, Nigeria **2019-2020**
- Member of Sustainable Development Goals (SDGs) in Kano State, Nigeria
  2019-2020

COMPUTER SKILLS

- Excellent in Computer fundamentals such as Microsoft Office Word, Excel, PowerPoint,
- Proficient in SPSS statistical software.

PERSONAL QUALITIES

- Excellent Interpersonal Relationships and communication skills with people.
- Positive Team playing
- Strong leadership skills.
- High strength of character and Integrity.
- Dedicated to tasks and duties, Result oriented, flexible, and punctual to duties and responsibilities.
- Love Researching new information, and enthusiastic about inventions that will help people.
- Reading, listening to music/singing/meeting new people.
- Seeing movies, playing volleyball and football.

LANGUAGES SPOKEN

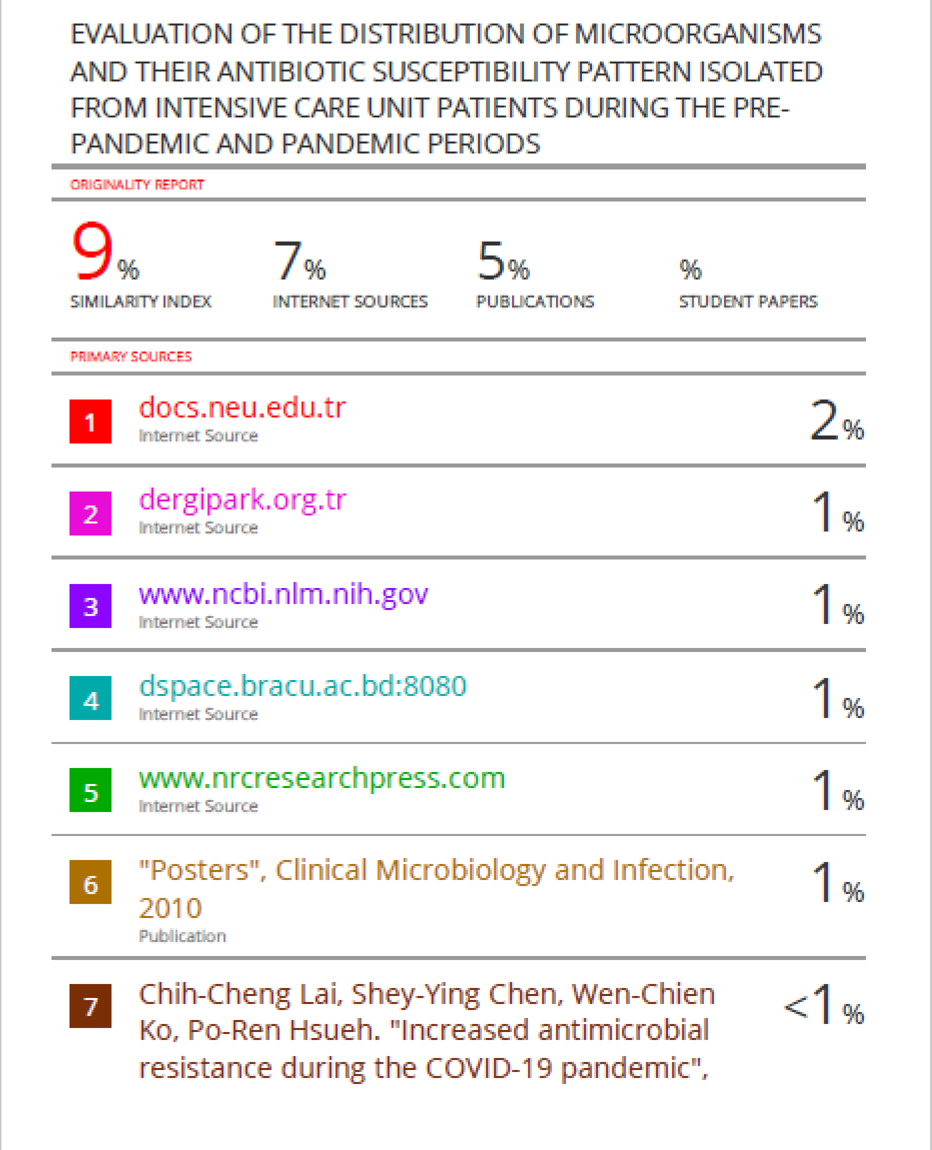
- Fluent in the English Language.

REFEREES.

(Available on request).

Appendix C

Turnitin similarity report



EVALUATION OF THE DISTRIBUTION OF MICROORGANISMS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN ISOLATED FROM INTENSIVE CARE UNIT PATIENTS DURING THE PRE-PANDEMIC AND PANDEMIC PERIODS

ORIGINALITY REPORT

9%	7%	5%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	docs.neu.edu.tr Internet Source	2%
2	dergipark.org.tr Internet Source	1%
3	www.ncbi.nlm.nih.gov Internet Source	1%
4	dspace.bracu.ac.bd:8080 Internet Source	1%
5	www.nrcresearchpress.com Internet Source	1%
6	"Posters", Clinical Microbiology and Infection, 2010 Publication	1%
7	Chih-Cheng Lai, Shey-Ying Chen, Wen-Chien Ko, Po-Ren Hsueh. "Increased antimicrobial resistance during the COVID-19 pandemic",	<1%