An-Najah National University
Faculty of Graduate Studies

QUINOLONE RESISTANCE AMONG *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* BACTERIA ISOLATED AT RAFIDIA AND AN-NAJAH NATIONAL UNIVERSITY HOSPITALS IN NABLUS DISTRICT: PROSPECTIVE CROSS-SECTIONAL STUDY

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This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Infection Diseases Prevention and Control, Faculty of Graduate Studies, An-Najah National University, Nablus - Palestine.

2024

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Dedication

I dedicate this work to my beautiful family, dear father Samer, my loving and giving mother Nadia, my dear brothers, Hakee my companion, Hakeem the beloved, Hameed the compassionate, and little Sadeel, her sister's beloved. Thank you for your constant encouragement and belief in my ability to accomplish all I set out to achieve.

Acknowledgments

My gratitude and praise are given to my God, who has greatly supported me.

I am grateful to all the faculty teachers, instructors, professors, and staff who supported me during my time at the university.

I sincerely appreciate the wonderful, patient, meticulous, successful, and generous Dr. Moatasem Al-Masri, my dear supervisor, who accompanied me throughout the research and long work period and gave me all the necessary instructions and directions with all love, honesty, and sincerity.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

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I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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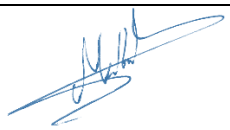
Signature:	
Date:	**24/10/2024**

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Abstract

Background: Quinolone resistance has increased in recent years, particularly among common hospital-acquired pathogens like *Escherichia coli* and *Klebsiella pneumoniae*. Despite being key agents in antimicrobial treatments, quinolones are at risk of reduced effectiveness due to growing resistance.

Objectives: This study aimed to investigate quinolone resistance in *E. coli* and *K. pneumoniae* isolates from two hospitals in Nablus District, Palestine, between June and September 2023. The primary objectives were to determine the prevalence of resistance to ciprofloxacin and levofloxacin, explore associations with demographic and clinical factors, and identify resistance mechanisms.

Methodology: A total of 219 bacterial isolates were analyzed, consisting of 50 *K. pneumoniae* and 169 *E. coli* strains. Resistance to ciprofloxacin and levofloxacin was assessed using disk diffusion, and resistance mechanisms were identified through multiplex PCR. The VITEK 2 system (BioMérieux) was used to identify bacterial species in both hospitals.

Results: *E. coli* exhibited 46.1% resistance to ciprofloxacin and 47.9% to levofloxacin, while *K. pneumoniae* demonstrated higher resistance, with 70% to ciprofloxacin and 68% to levofloxacin. Resistance rates were significantly higher at An-Najah National University Hospital compared to Rafidia Hospital. Older patients (over 65) showed increased resistance to levofloxacin, particularly in *E. coli*. Patients with urinary catheters had a higher frequency of ciprofloxacin-resistant *E. coli* isolates. Chronic conditions, such as hepatic cirrhosis and diabetes, were linked to higher *K. pneumoniae* resistance. In *E. coli*, efflux pumps were the most common resistance mechanism 43.3%, with the OqxA gene most prevalent, followed by enzymatic modification 29.6% and target modification

(26%). For *K. pneumoniae*, efflux pumps (37.8%) and OqxA were also dominant, followed by Aac(6')-Ib-cr and QnrS/QnrB genes responsible for enzymatic and target modifications.

Conclusion: This study highlights the importance of routine antibiotic susceptibility testing and monitoring quinolone resistance. It emphasizes the need for improved surveillance and targeted antimicrobial stewardship to combat quinolone resistance in these hospitals.

Keywords: Escherichia coli, Klebsiella pneumoniae, Quinolone resistance, Resistant genes, Nablus District.

Chapter One

Introduction and literature review

1.1 Overview

The Centers for Disease Control (CDC) predicts that infections with bacteria that are resistant to multiple drugs (MDR) cause 10 million deaths annually worldwide and will result from neglect and if no new antibiotics are discovered and made accessible by 2050, this number exceeds the combined deaths from cancer and heart disease (1). At the moment, infectious diseases rank fourth in the United States and second globally in terms of mortality (1). It was reported that every year 17 million people worldwide pass away from bacterial infections (2).

Palestine faces a significant issue with antimicrobial resistance (AMR); the country has more AMR deaths than tuberculosis and respiratory illnesses, gastrointestinal disorders, violence against others, and non-communicable illness, alongside maternal and conditions affecting newborns (3). In Palestine, there were 1,400 deaths associated with AMR and 346 deaths attributable to AMR in 2019 (3). Palestine has the 104th-highest AMR-related death rate per population of 100,000 (3).

The European Centre for Disease Control and Prevention (ECDC) carried out a study in 2015 that quantified the public health stress of AMR in terms of cases, deaths attributable to the disease, as well as life years adjusted for disability (DALYs), which came to 33,110 deaths in total, and antibiotic-resistant bacterial infections were responsible for 874,541 DALYs, with healthcare-associated infections accounting for 75% of these cases (4). In accordance with information published by the ECDC in the month of November 2022, an additional investigation conducted in the European Union calculated that more than 35 thousand individuals pass away each year from infections resistant to antibiotics. Furthermore, between the years 2012 and 2021, the use of antibiotics with a broad spectrum in healthcare settings increased by 15% (5).

The COVID-19 pandemic may have an unintended impact of increasing antimicrobial resistance. This pandemic has been linked to an increase in MDR bacteria, according to various recent publications (6). Although the cause is complicated, one of the primary causes is that, despite having a relatively small percentage of co- or secondary infections, patients due to coronavirus use antibiotics with greater frequency (6). Antimicrobial

resistance is largely developed and spread by wars and conflicts like the ones in Syria and Iraq (7). Isolates from patients with injuries after the Return on Great March revealed a 300% elevated resistance to specific antimicrobial agents if contrasted with non-injured patients, indicating that this type of resistance is increasing in the Gaza Strip (8). Resistance to antibiotics throughout the conflict is caused by a number of variables, such as limited resources, a high death toll, insufficient preventive measures, environmental contamination from infrastructure breakdown, and the accidental discharge of toxic metals from weapons of mass destruction (8). Insufficient treatment of wastewater in Gaza prior to the start of the conflict on the seventh of October 2023, led to microbial contamination in thirty-four percent of medical facility water systems and surface samples with a significant amount of antibiotic resistance, particularly to cephalosporins and carbapenems (9).

In Palestine, there are five important pathogens when discussing antibiotic resistance (the number of deaths linked to AMR in Palestine in 2019 is indicated in parenthesis): *Pseudomonas aeruginosa* (126), *Streptococcus pneumoniae* (144), *Klebsiella pneumoniae* (203), *Escherichia coli* (217), *and Staphylococcus aureus* (345 deaths) (3). These frequently resulted in bloodstream infections, peritoneal and intraabdominal infections, lower respiratory infections, and all associated thoracic infections (3). This study will concentrate on two common sources of community and hospital infections in Palestine specifically the city of Nablus, *Escherichia coli* and *Klebsiella pneumoniae*, both of which belong to the *Enterobacteriaceae* family and have high levels of antibiotic resistance.

Several community and nosocomial-acquired infections are treated with fluoroquinolones, which are strong antimicrobial agents, on the other hand, there is growing evidence of *Enterobacteriaceae* resistance to fluoroquinolones (10). Fluoroquinolones currently are mainly used as antibacterial drugs, but several in vitro investigations show that they are also effective against a variety of cancer cells, including those found in the bladder and prostate (11). Fluoroquinolones (FQs) work against bacteria by inhibiting DNA synthesis through interactions with topoisomerase IV and DNA gyrase. The two FQs that are prescribed most commonly are levofloxacin and ciprofloxacin (12). About three decades ago, ciprofloxacin was first used in clinical settings, it quickly gained popularity due to its potent ability to combat Gram-negative

bacteria, particularly those belonging to the *Enterobacteriaceae* family (12). It was reported that Levofloxacin is more active against Gram-positive bacteria than ciprofloxacin (12).

In a study conducted in Palestine, 66 isolates of *K. pneumoniae* were gathered from various clinical sources in the country. The isolates revealed high resistance frequencies to both ciprofloxacin and levofloxacin, resistance rates were 44% and 40%, respectively (13). In another study on *E. coli* antibiotic resistance, 41 isolates of the bacteria were collected from three hospitals in the northern West Bank districts. The results indicated a high ciprofloxacin resistance rate of 56% (14).

Bacteria counter the antimicrobial activity of quinolones through a diversity of mechanisms. Plasmid-mediated quinolone resistance (PMQR), mechanisms in the chromosomes caused by changes in the genes that code for quinolone targets (DNA gyrase and topoisomerase IV), enzymatic modification of quinolone and efflux pumps activity are among them (15).

Our study was carried out to fill the gap of knowledge regarding quinolone resistance, particularly its mechanisms, in the Nablus district in Palestine.

1.1.1 *Escherichia coli* characteristics

Escherichia coli is considered to be the most prevalent commensal organism found in warm-blooded animals' digestive tracts, especially humans (16). The bacteria *E. coli* is rod-shaped, oxidase-negative, and Gram-negative. It may grow aerobically or anaerobically, with an optimal temperature of 37°C. It is motile, having peritrichous flagella (1). *Escherichia coli* is among the greatest important pathogens that impact human health and belongs to the *Enterobacteriaceae* family. (16).

Although all *E. coli* strains are a similar kind of bacterium, they vary slightly in some ways. It is possible for various *E. coli* strains to crossbreed and create new strains with a combination of characteristics. The distinct collection of marker compounds that each strain of *E. coli* carries on its surface is used by researchers to categorize it into distinct strains (17).

Meaning of commensal is to live in a situation of advantageous association with hosts, and seldom triggers diseases. However, considering its broad spectrum of disease-causing possibilities, it remains one of the most common bacteria for humans as well as animals

in industrial and medical fields, because of its special characteristics, like the simplicity of dealing with, capacity to thrive in both aerobic and anaerobic circumstances and accessibility of the whole genome sequence (18).

Majority of *E. coli* strains are not dangerous to people, and some are even advantageous. In our guts, a lot of us harbor a population of *E. coli* that helps with digestion and defends against other pathogens. Several significant scientific findings and the essentials of the scientific method have been taught for years to biology learners by scientists using strains of *E. coli* to examine basic biological processes. Industry researchers use different strains of *E. coli* to synthesize essential substances that humans need on a daily basis (17).

But we must consider all the facts and be mindful of the serious hazards before concluding that this bacterium is beneficial.

1.1.2 Infection and diseases caused by *Escherichia coli*

Escherichia coli is a significant component of commensal gut flora and can be found on the floors of hospitals and long-term care facilities. *Escherichia coli* is the most common Gram-negative bacterium in the human gastrointestinal tract because it is not virulent in this setting. Primarily because it can be found in fruits, vegetables, water, soil, and inadequately cooked food. As a consequence, pathogenic strains cause intestinal illnesses when ingested by humans and spread through the fecal-oral route. When found outside of the gastrointestinal tract, *E. coli* can still cause peritonitis, bacteremia, urinary tract infections (UTI), and other illnesses (5,6,7). Additionally, it plays a major role in nosocomial infections like ventilator-associated pneumonia (VAP) and catheter-associated UTIs (16).

Recent studies conducted in Spanish healthcare facilities have shown that *E. coli* is a particularly prevalent pathogen that causes more than 40% of bloodstream infection cases (16). The incidence percentage of adult *E. coli* bacteremia in countries with high incomes was estimated to be 48 per 100,000 person-years globally, with a case-fatality rate (CFR) of 12.4% (22). After passing the age of 60, there was an observed rise in incidence, which

peaked at 319 per 100,000 person-years upon reaching the age of 85 (22). Out of a calculated 48.9 million cases around the world, 11 million people died from sepsis in 2017 (16). Furthermore, the 2010 Global Impact of Foodborne illnesses report from the World Health Organization (WHO) calculated that each year, diarrheagenic *E. coli* causes approximately 111 million diseases and 63,000 deaths worldwide. Additionally, approximately 63,000 cases of hemorrhagic colitis are caused by *E. coli* O157:H7 each year in the United States.

The serologic recognition of the O (lipopolysaccharide, LPS) and H (flagellar) antigens served as the primary basis for classifying *E. coli* (16). A combination of the type of virulence factor present and the clinical symptoms of the host, strains are classified into different pathogenic kinds. Enteric *E. coli* (EPEC) has at least seven major pathotypes, while extraintestinal *E. coli* (ExPEC) has three pathotypes (16).

Children <1 year of age are the main populations affected by EPEC strains that cause diarrhea, especially in situations of hygiene lacking (16). EnteroHaemorrhagic *E. coli* (EHEC) is a food-borne pathogen that usually causes hemolytic uremic syndrome (HUS), or hemorrhagic colitis (16). The most aggressive diarrhoeagenic *E. coli* strains currently present are those that produce Shiga-like toxins, also known as Shiga toxin-producing *E. coli* (STEC), which are comparable to toxins produced by *Shigella dysenteriae* (23). The pathogen most frequently responsible for travelers' diarrhea is EnteroToxigenic *E. coli* (ETEC), which can cause mild to severe watery diarrhea in people of any generation (10,11). Enteroaggregative *E. coli* (EAEC) strains have been linked to chronic diarrhea in humans and have been identified as the source of multiple diarrhea outbreaks across the globe. EAEC is the second most common cause of traveller's diarrhea globally and is commonly linked to diarrhea in HIV-positive patients as well as young kids in developing nations (12,13). Children are more susceptible to diarrhea caused by diffusely adherent *E. coli* (DAEC) (28). In children as well as adults, EnteroInvasive *E. coli* (EIEC) often results in watery diarrhea and sporadically in dysentery (16). Shigella species and EIEC strains are very similar. In recent years, a pathotype called Adherent Invasive *E. coli* (AIEC) has been correlated with Crohn's disease lesions (2,15).

ExPEC are commonly linked to infections that are nosocomial and community-associated. Urinary tract infections (UTIs) tend to be triggered by uropathogenic *E. coli*

(UPEC), around eighty percent of incidents of UTI among people are caused by UPEC (1,16). In developed nations, neonatal meningitis *E. coli* (NMEC) is a primary reason for Gram-negative neonatal bacterial meningitis, and numerous patients encounter neurologic sequelae (31). Multidrug-resistant NMEC strains have recently been noticed to have significantly increased in the past few years (32). According to the latest research, APEC and ExPEC share virulence factors and serogroups, which may point to a potential cause of food-borne illnesses (19, 20).

1.1.3 *Klebsiella pneumoniae* characteristics

The earliest review of *K. pneumoniae* was provided by Carl Friedlander in 1882, once isolated the microbe from the lung tissue of individuals who suffered deaths of pneumonia, he described this bacterium to be an encapsulated bacillus (35). The bacterium did not receive the name *Klebsiella* until 1886 and was initially known as Friedlander's bacillus. Gram-negative, encapsulated, non-motile, facultatively anaerobic, rod-shaped, and lactose-fermenting bacteria called *K. pneumoniae* is present in the atmosphere and is being linked to pneumonia among individuals with diabetes mellitus and alcohol use disorders. *Escherichia coli, Yersinia species, Salmonella species,* and *Shigella* are all members of the *Enterobacteriaceae* family, which also includes *Klebsiella pneumoniae*. In addition to being everywhere, *K. pneumoniae* has been isolated from a range of environments, including soil, sewage, and water.

1.1.4 Infection and diseases caused by *Klebsiella pneumoniae*

Although *Klebsiella* have been identified to be opportunistic pathogens that colonize mucosal surfaces and do not trigger disease, they can spread from mucosae to surrounding tissues and cause potentially fatal infections like sepsis, pneumonia, UTIs, and bloodstream infections (36).

Humans are the primary reservoir for *K. pneumoniae*. In the general public, 1% to 6% of individuals carry the organism in their nasopharynx, and 5% to 38% do so in their stool. The main sources of illness are hospital staff members' hands and the patient's digestive tract. A nosocomial outbreak is one potential consequence. However, it has been discovered that those who are Chinese and suffer from chronic alcoholism are more likely to be colonized. Compared to the rest of the population, hospital patients have a much higher carrier percentage for *K. pneumoniae*. One study found a correlation between the

amount of antibiotics prescribed and the carrier rates in the stools of hospitalized patients, which could reach 77% (27,28). *Klebsiella pneumoniae* causes a couple of different kinds of pneumonia: community-acquired pneumonia and hospital-acquired pneumonia. Community-acquired pneumonia is a fairly common diagnosis, whereas *K. pneumoniae* infection is very rare. Infections with *K. pneumoniae* are estimated to account for 3% to 5% of community-acquired pneumonia cases in the West, but they can account for up to 15% of cases in developing countries such as Africa. *Klebsiella pneumoniae* is responsible for approximately 11.8% of all hospital-acquired pneumonia worldwide. In contrast to 7% of patients not on a ventilator, *K. pneumoniae* causes 8–12% of pneumonia cases in ventilator-dependent patients.

Infections with *K. pneumoniae* are especially troublesome in healthcare facilities for newborns, older adults, and people with compromised immune systems (36). Moreover, this pathogen is to blame for a sizable portion of community-acquired infections globally (36). The capacity for dissemination and the high morbidity and mortality rate of these illnesses are characteristics that set them distinct (36). Infections like these are thought to be caused by hypervirulent *Klebsiella* strains, as well as current epidemiological research suggests that these kinds of strains have particular genetic features in common (36). Managing patients with the infection requires an awareness of the mechanisms underlying *K. pneumoniae* pathogenicity and transmission, as these variables are changing along with the virulence of the organisms and characteristics of the infected patients' demographics (36).

After *Staphylococcus aureus* and *Clostridium difficile*, *Klebsiella* is the third most frequent reason for healthcare-associated infections (HAIs) in the United States, accounting for 9.9% of cases (39). Life-threatening infections such as bloodstream infections, pneumonia, and UTIs are caused by *K. pneumoniae* (39). As HA pneumonia has been described as pneumonia that develops more than 48 hours after being hospitalized, *Klebsiella* have been found to be the third most common cause of this illness in the United States (39). In patients who were admitted to intensive care units (ICUs), *Klebsiella* can also be an important contributory factor of ventilator-associated pneumonia (VAP) (40). VAP accounts for eighty-three percent of hospital-acquired (HA) pneumonia cases (36). It has been reported in some cases that *K. pneumoniae* pneumonia can have a mortality rate around of fifty percent (41).

Klebsiella pneumoniae is one of the most common causes of bloodstream infections (BSI) triggered by Gram-negative bacteria (31,28). When it comes to community-acquired (CA) *Klebsiella* BSI, diabetes mellitus and liver disease had the strongest correlation, but cancer is the main underlying medical condition linked to hospital-acquired BSI (41). BSI may occur as a primary infection without a known cause. But BSI is frequently a secondary infection brought on by an established cause spreading through the bloodstream. Urinary catheters, urinary tract, gastrointestinal tract, and respiratory sites are frequent locations of secondary BSI (41).

The urinary tract is where *K. pneumoniae* infections occur most frequently (42). Diabetes mellitus is linked to UTI caused by *K. pneumoniae*, just like it is to other types of infections (36). *Klebsiella pneumoniae* is also the cause of catheter-associated urinary tract infections (CAUTIs). The capacity of *K. pneumoniae* to establish biofilms and stick to catheters is believed to make it easier to cause CAUTIs (36). Infections at surgical sites as well as wounds are also caused by *Klebsiella pneumoniae*.

Generally affecting older kids and babies, acute meningitis caused by bacteria is a serious central nervous system infection. Gram-negative pathogens typically cause lethal bacterial meningitis. *Klebsiella pneumoniae* can cause meningitis with a dismal prognosis. Patients conducting neurosurgical techniques with or without cerebrospinal fluid (CSF) leakage, people who have suffered a head injury, and patients suffering from diabetes mellitus as well as *K. pneumoniae* bacteremia are among the numerous individuals who are vulnerable to *K. pneumoniae* meningitis (41).

Ultimately, it can be concluded that infections with *K. pneumoniae* are an endemic opportunistic infectious agent that significantly stress medical facilities.

1.1.5 Antimicrobial resistance (AMR)

Antibiotic use laid the groundwork for modern medical advancements. This period of the 20th century was dubbed the "antibiotic era", and infectious illnesses was thought to have been eradicated by the conclusion of the previous century. In a similar way, antibiotics have been critical for the efficacy of intrusive procedures such as transplantation of organs, oncology, immunomodulatory agents' therapies in rheumatology, and numerous other medical fields (43).

The widespread availability of therapy with antibiotics has substantially decreased mortality in youngsters, which has led to a rise in the average lifespan (44). Still, a growing percentage of bacteria are developing resistance to several antibiotics that are still in consumption, leading to multidrug-resistant (MDR) bacteria (44). The Nobel Prize-winning physician and microbiologist Alexander Fleming cautioned against the prospective hazards of overusing and abusing antibiotics, which could cause antibiotic resistance to develop (45). Antibiotic-resistant bacteria constitute an imminent danger to contemporary healthcare because of the lack of novel antibiotics and the rise in MDR bacteria that are leading to unsuccessful treatments (44).

AMR is found among bacteria, viruses, fungi, and parasites changing over time to a stage where they are immune to antimicrobial drugs. This increases the risk of disease dissemination, severe illness, and death while making infections harder to treat.

1.1.6 The AMR crisis's effects on human society

Growing numbers of infectious diseases that are incurable will have major consequences for the economy as more productive people are absent from the job market for a longer duration of time (46). Furthermore, the burden of caring for the sick will increase and put pressure on the healthcare systems, the community, and the families. The majority of countries' national outcomes will be lower than they are now due to the downstream consequences resulting from this labor loss and a greater burden on health services, which will also have implications on society and culture. The possible consequences of incurable, deadly, and virulent pandemics would definitely at least match the repercussions of the 1918 worldwide Spanish flu epidemic, which claimed the lives of at least 50 million humans, alongside the overall impacts brought on by rising morbidity (47).

1.1.7 Antimicrobial resistance in *Escherichia coli*

Multidrug resistance in *E. coli* is a worrying issue that is spreading throughout the world in human as well as veterinary medicine. *Escherichia coli* can acquire resistance genes, mainly through horizontal gene transfer, even though it is susceptible to almost all clinically used antibiotics. Horizontal gene transfer (HGT) is the process by which genetic information is transferred from one organism to another. It is a mechanism that propels the evolution of pathogens by enabling the spread of antibiotic resistance genes within

bacteria (aside from genes passed from parents to children, which is called vertical gene transfer).

Multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL)-producing *E. coli* are becoming more prevalent, which is concerning due to they are resistant to a variety of antimicrobial medications, involving a broad spectrum of β-lactams (48). This increasing resistance may limit existing therapies and affect the future outlook of *E. coli* infections (49).

The acquisition of genes encoding carbapenemases (which confer resistance to carbapenems), plasmid-mediated quinolone resistance (PMQR) genes (which confer resistance to fluoroquinolones), extended-spectrum β-lactamases (which confer resistance to broad-spectrum cephalosporins), mcr genes (which confer resistance to polymyxins), and 16S rRNA methylases (which confer pan-resistance to aminoglycosides) are also linked to among the most problematic processes in *E. coli* (50).

1.1.8 Antimicrobial resistance in *Klebsiella pneumoniae*

Klebsiella pneumoniae is distinguished by its unique ability to elude or avoid the effects of antimicrobial drugs. Furthermore, due to the growing issue of antibiotic resistance in the world the World Health Organization (WHO) has designated *K. pneumoniae* to be one of the organisms with the greatest priority as well as encouraging studies and the creation of new antibiotics (51). Furthermore, the US Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), and Public Health England (PHE) have designated *K. pneumoniae* to be one of these MDR organisms that pose a critical risk to the well-being of humans (52).

For a while now, the expression "superbug" has been commonly used to characterize certain strains of bacteria, particularly those that are extensively and multidrug-resistant and immune to the majority of commonly used antibiotics (53). *Klebsiella pneumoniae* has been one of the largest and most popular superbugs that have appeared in the past twenty years, it can carry different colistin resistance gene mcr-1, carbapenemases, and extended-spectrum beta-lactamases (ESBL), it has also become extensively drug-resistant (XDR) and multidrug-resistant (MDR) (53).

1.1.9 Quinolone antibiotics

The class of drugs known as quinolone antibiotics is diverse and has a wide range of therapeutic uses, the synthetic antibiotic nalidixic acid, which has been in use since the 1960s, is the source of all of them, the fact that these substances are all synthetic allows for the synthesis of drug with numerous modifications to the fundamental quinolone molecule (54). In this class, a large number of novel antibiotics are being developed, frequently with broader and distinct clinical uses as well as greater bacterial coverage, frequently prescribed drugs like theophylline and antacids can have significant interactions, and children, pregnant women, and nursing mothers should not take such antibiotics (54).

Common fluoroquinolones consist of delafloxacin, moxifloxacin, gemifloxacin, norfloxacin (discontinued), ciprofloxacin, and levofloxacin. The quinolones' spectrum of action differs in a few significant ways, ciprofloxacin doesn't work on *Streptococcus pneumoniae* bacteria, moxifloxacin is useful in treating anaerobes together with delafloxacin, but is ineffective against *Pseudomonas aeruginosa*, the only quinolone that works against methicillin-resistant *Staphylococcus aureus* (MRSA) is delafloxacin (55).

Adults with gastrointestinal and intraabdominal diseases, sexually transmitted diseases, skin and soft tissue diseases, urinary tract infections, prostatitis, bone and joint diseases, pyelonephritis, and community-acquired and nosocomial pneumonia can all benefit from using quinolones, according to permission by the Food and Drug Administration (FDA) indications (54). Quinolones like levofloxacin, gatifloxacin, and moxifloxacin are also being used more frequently off-label to treat drug-resistant tuberculosis or in patients who are intolerant to other anti-tuberculosis medications (54).

1.1.10 Quinolone resistance in *Escherichia coli* and *Klebsiella pneumoniae*

Quinolones are now widely used in medical treatment for humans and animals due to their strong action against Gram-negative bacteria, such as *E. coli* (56). It was first anticipated that the resistance of quinolones would remain uncommon because of their exceptional in-vitro activity, nevertheless, since they have been introduced into veterinary and human medicine, the percentage of resistant strains in Gram-negative bacteria, such as *E. coli*, has increased (57). Also, it has been reported frequently that clinical strains of *K. pneumoniae* are now resistant to several antibiotics, which include fluoroquinolones (58).

Escherichia coli uses chromosomal target site mutations as one of its resistant mechanisms against quinolones. The gyrase, which is made up of two GyrA along with two GyrB subunits, is the main target of (fluoro)quinolones in *E. coli.* A secondary target is topoisomerase IV. There are two ParC along with two ParE subunits in this enzyme (57). Also, in *K. pneumoniae* fluoroquinolones resistance occurs by multiple mechanisms, the main mechanism involves a chromosomal mutation at the quinolone resistance determining regions (QRDR), which are encoded by the topoisomerase IV (genes ParC and ParE) and DNA gyrases (GyrA and GyrB) (57).

Plasmid-borne resistance mechanisms are an additional means of resistance that *E. coli* employs. The discovery of QnrA1, the initial plasmid-mediated quinolone resistance (PMQR) determinant, in the year 1997 raised grave concerns regarding the potential worldwide spread of PMQR genes (57). Several resistance mechanisms encoded in plasmids have been discovered. These include (1) Qnr-like proteins (QnrA, QnrB, QnrC, QnrD, and QnrS) that protect DNA from quinolone compounds binding (59). Also, the Qnr plasmid confers low-level quinolone resistance in *K. pneumoniae* while protecting ciprofloxacin targets from inhibition. Numerous Qnr genes, such as QnrA, QnrS, QnrB, QnrC, and QnrD, have been identified in bacterial strains from around the world (60). (2) The aminoglycoside acetyltransferase AAC(6')-Ib-cr is encoded by a gene found in integrons, transposons, and plasmids in *Enterobacteriaceae* like *E. coli* and *K. pneumoniae*. Certain fluoroquinolones, such as enrofloxacin and ciprofloxacin are altered by aminoglycoside acetyltransferase AAC(6')-Ib-cr (78,79). (3) Certain microorganisms such as *E. coli* and *K. pneumoniae* contain particular efflux pump encoding genes, QepA and OqxAB, which discharge fluoroquinolone from cells of bacteria, thereby enhancing resistance (78,79).

In previous research around the world, studies of *E.coli* clinical isolates quinolone-resistant in Shanghai and Paris have detected QnrA in 8% and 0.3% of examined isolates, respectively. A study that included ceftazidime-resistant and quinolone-resistant *K. pneumoniae* isolates from the United States. In this study, the QnrA gene was detected in 11% of strains (61). Nine distinct PMQR genes (QnrA, AAC(6′)-Ib-cr, QnrB, OqxA, QepA, QnrE, QnrS, OqxB, and QnrD) were identified in 61 articles that comprised a systematic review of plasmid-mediated quinolone resistance (PMQR) among *Enterobacteriales* in Latin America. The most commonly reported genes were AAC(6′)-

Ib-cr (37/61, 60.6%) and QnrB (26/61, 42.6%). Furthermore, it was discovered that *E. coli* contained the PMQR gene the most frequently (40/61, 65.6%), followed by *K. pneumoniae* (24/61, 39.3%) (57).

A systematic review on the topic of Plasmid-mediated quinolone resistance in *Enterobacteriaceae* in Mediterranean countries, including Palestine, reveals the following findings (Table 1) (62):

Table 1

Prevalence of Quinolone-Resistant Genes in Mediterranean Countries

Country	Organism	Origin	Year of isolation	PMQR	Rate (%)	Reference
Algeria	*E. Coli*	Clinical	2011	AAC(6′)Ib-cr	22.2	(63)
Croatia	*E. Coli*	Clinical	2002-2005	AAC(6′)Ib-cr	3.3	(64)
				AAC(6′)Ib-cr	5	
				AAC(6′)Ib-cr	3	
Egypt	*E. Coli*	Clinical, Community	2007	QnrB	23.3	(65)
				AAC(6′)Ib-cr	23.3	
France	*Enterobacteriaceae*	Clinical	2002-2005	QnrA	2.2	(66)
				QnrS	1.6	
Greece	*E. Coli*	Clinical	2011	AAC(6′)Ib-cr	65.7	(67)
Israel	*K. pneumoniae*	Clinical	2004-2006	AAC(6′)Ib-cr	13	(68)
Italy	*E. Coli*	Clinical, Community	2004-2006	AAC(6′)Ib-cr	11	(69)
Morocco	*K. pneumoniae*	Clinical	2011	QnrB	1.2	(70)
				QnrS	1.8	
				AAC(6′)Ib-cr	3.6	
Palestine	*K. pneumoniae*	Clinical	2013	QnrB	12.5	(71)
				QnrS	31.2	
				AAC(6′)Ib-cr	43.7	
Spain	*E. coli*	Clinical	2006	QnrA	0.7	(72)
	K. pneumoniae			QnrB	1.3	
				QnrS	1.5	
Tunisia	*Enterobacteriaceae*	Clinical	2005-2007	QnrA6, QnrB1, QnrB2, QnrS1	16	(73)
Turkey	*K. pneumoniae*	Clinical	2006-2010	AAC(6′)Ib-cr	13.6	(74)

1.2 Problem statement

The antimicrobial resistance (AMR) trouble refers to the increasing global prevalence of infectious diseases that affect humans and can't be handled with any antimicrobial agent presently on the market. The current state of affairs will have a disastrous impact on humanity as a whole due to the increase in the frequency and severity of both deadly and damaging diseases. Antibiotic resistance has been deemed a global public health

emergency by agencies such as the World Health Organization (WHO) and the United Nations (UN) (75).

An estimated 1.27 million deaths worldwide in 2019 were attributed to antibiotic resistance (76). It is predicted that as AMR levels rise, it will rank among the primary causes of mortality by 2050, overtaking cancer (76). Additionally, according to the Centers for Disease Control and Prevention (CDC), approximately 23,000 people die from antibiotic-resistant diseases in the US every year, affecting more than 2 million individuals (77).

Research is needed to close the knowledge gaps about the frequency of infections brought on by pathogens resistant to antibiotics according to the WHO's Global Action Plan on AMR (75). There is a lack of information on the quantity and frequency of infections brought via these microbes.

Inadequate prevention and control of infections in healthcare institutions, inappropriate and excessive use of antibiotics in humans and animals, and inadequate access to pure water, sanitary facilities, and proper hygiene are the main causes of antimicrobial resistance (75). Additionally, the causes and effects of AMR are made worse by inadequate access to prompt and accurate diagnosis (78).

This crisis is caused by three primary variables: (1) the global human population, which is huge and connected, (2) the rising incidence of AMR phenotypes within microbes, which is a progressive reaction to the widespread consumption of antibiotics, and (3) the widespread and often unwarranted utilization of antibiotics by humans, resulting in an intense selective pressure which triggers the evolutionary response in the world of microbes (76).

AMR in bacteria belonging to the family *Enterobacteriaceae* (for example, *Citrobacter species*, *Enterobacter species*, *Escherichia coli*, *Serratia marcescens*, *Klebsiella species*, *Proteus species*, and others) has increased dramatically over the last few decades. Intestinal flora known as *Enterobacteriaceae* are significant pathogens in both community and nosocomial settings. Human-to-human spread is common for *Enterobacteriaceae*, which can also pick up AMR through plasmids or different mobile resistance components (80).

Two significant *Enterobacteriaceae* members that can become resistant to different antibiotic classes are *E. coli* and *K. pneumoniae*. The analysis of epidemiological data related to MDR *E. coli* and *K. pneumoniae* across the globe demonstrates that these two pathogens are significantly more prevalent with distinct variations throughout numerous countries (79). These bacteria have been shown to be resistant to a wide variety of antibiotics, such as broad-spectrum cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins. After fluoroquinolones were first made available to the public, chromosomal modifications in topoisomerase IV or DNA gyrase have been identified as the primary cause of fluoroquinolone resistance in *Enterobacteriaceae*, particularly in *E. coli* and *K. pneumoniae* (79).

Economic results, wildlife, the environment, and human health are all impacted by antibiotic resistance. AMR infection can lead to serious illnesses, long-term hospitalizations, increased medical expenses, increased prices for second-line medications, and therapeutic breakdowns. For instance, it has been estimated that AMR costs Europe more than nine billion euros a year (80). In addition, excluding the roughly $35 billion in productivity losses each year, the CDC estimates that AMR contributes a 20-billion-dollar excess to direct medical expenses in the United States. In addition, excluding the roughly thirty-five billion dollars in productivity losses each year, the CDC estimates that AMR contributes a twenty billion dollar excess to direct medical expenses in the United States (77).

Although beneficial, the current management strategies to regulate antibiotic usage and teach the medical community about the concerns have not fully addressed the challenge of achieving a decrease in the overall amount of antibiotics used by humans. The primary issue is that, particularly with regard to Palestine, there is a deficiency or absence of reliable evidence that clarifies and resolves the problem of antibiotic resistance. As a result, it was crucial to carry out this study, and we focused on quinolone antibiotics to learn more about possible risk factors of resistance development and mechanisms of resistance by two kinds of bacteria that are among the most important microorganisms of our day and that pose a threat to human health, which are *E. coli* and *K. pneumoniae*.

1.3 Study significance

The foundation of treatment is a suitable antimicrobial regimen. But promptly during the course of an infection, the organism that causes it usually remains unknown, and treatment decisions are made based mostly on experience (empirical treatment). For this reason, doctors frequently select broad-spectrum antibiotics to combat a variety of potential infectious agents. It is crucial to figure out whether there is a real correlation between this approach and patient outcomes because it may raise costs, cause side effects, and antibiotic resistance (81).

Broad spectrum antimicrobial agents meant antibiotics that worked against Gram-positive as well as Gram-negative bacteria. This was different from penicillin, which works mainly toward Gram-positive microorganisms, while streptomycin, which works basically toward Gram-negative microorganisms.

Every patient receiving broad-spectrum prescriptions may experience a rise in antibiotic-related toxic effects as well as expenses (82). Additionally, they might cause rash or diarrhea as adverse reactions. Proof from meta-analysis and systematic review based on studies, the 30-day and in-hospital mortality rates are greater among individuals with serious infections. when empiric antibiotics are administered inappropriately (83).

Improved antibiotic prescription procedures could be developed by taking into account the burden and predictive effects of using unsuitable empiric treatment for serious illnesses. Thus, it is crucial to emphasize the use of antimicrobial susceptibility testing (AST), a lab technique carried out by clinical laboratory technicians to determine which antibiotic regimen is particularly beneficial for a given patient. More broadly, it facilitates the assessment of therapeutic services offered by clinics, hospitals, as well as government programs aimed at controlling and preventing infectious diseases.

Quinolones find widespread application in medical treatment as antimicrobial agents. Nevertheless, resistance has extended to several pathogens that affect humans. Hence, earlier exposure to quinolones is among the most important risk variables linked to quinolone resistance, which physicians should be conscious of it (85).

Two of the most significant bacteria that are resistant to quinolones are *Escherichia coli* and *Klebsiella pneumoniae*. According to the microorganism and agent being

investigated, broad surveillance studies of clinically relevant isolates in North America as well as Europe have found fluoroquinolone resistance in an extensive variety of isolates (1 to 20%) of *E. coli* and *K. pneumoniae* (84).

Lastly, in order to ensure the accurate and reliable use of quinolone antibiotics, we need to continuously monitor trends in both local and national resistance (85). Also, Reducing unneeded prescriptions and maintaining successful therapy of patients with infections are two goals of proper antibiotic usage in hospitals according to systematic review research on measures to enhance hospital inpatients access to antibiotic prescriptions (86). It was important to carry out this study in an attempt to decrease the inappropriate use of antibiotics called quinolones because antibiotic miss use could eventually cause a rise in the prevalence of antibiotic-resistant bacteria, particularly *E. coli* and *K. pneumoniae,* which would have disastrous effects on patients, society, and the world as a whole.

1.4 Study general aim

This research attempts to ascertain the prevalence of quinolone antibiotic resistance, risk factors, and mechanisms of resistance (through gene detection) among *E. coli* and *K. pneumoniae* bacteria in Nablus hospitals.

1.5 Study objectives

The investigation was carried out to:

1. Find the prevalence of resistance of *E. coli* and *K. pneumoniae* to quinolone antibiotics (ciprofloxacin and levofloxacin) at NNUH and Rafidia Hospital in Nablus District.
2. Evaluate the association of quinolone resistance to various patient variables such as age, gender, clinical symptoms, and specimen type.
3. Find out the presence of any possible risk factors such as hospitalization and comorbidities for the development of quinolone resistance in the patients at NNUH and Rafidia Hospital in Nablus District.
4. Study mechanisms of quinolone antibiotics (ciprofloxacin and levofloxacin) resistance of *E. coli* and *K. pneumoniae* isolated from patients at NNUH and Rafidia Hospital in Nablus District.

5. Determine the frequency of genes of quinolone-resistant (QnrA, QnrB, QnrS, Aac(6')-Ib-cr, QepA, OqxA, and OqxB) among *E. coli* and *K. pneumoniae* isolated from patients at NNUH and Rafidia Hospital in Nablus District.

1.6 Research questions

1. What is the prevalence of resistance of *E. coli* and *K. pneumoniae* to quinolone antibiotics (ciprofloxacin and levofloxacin) at NNUH and Rafidia Hospital in Nablus District?
2. Is quinolone resistance associated with different patient variables such as age, gender, clinical symptoms, and specimen type?
3. Are there any possible risk factors such as hospitalization and comorbidities for the development of quinolone resistance in the patients at NNUH and Rafidia Hospital in Nablus District?
4. What are the mechanisms of quinolone antibiotics (ciprofloxacin and levofloxacin) resistance of *E. coli* and *K. pneumoniae* isolated from patients at NNUH and Rafidia Hospital in Nablus District?
5. What is the frequency of genes of quinolone-resistant (QnrA, QnrB, QnrS, Aac(6')-Ib-cr, QepA, OqxA, and OqxB) among *E. coli* and *K. pneumoniae* isolated from patients at NNUH and Rafidia Hospital in Nablus District?

1.7 Research hypothesis

This study will examine several research hypotheses, some of which are shown below.

P-value < 0.05 will be considered significant.

There is a significant difference related to:

1. Resistance rate of *E. coli* and *K. pneumoniae* to quinolone antibiotics (ciprofloxacin and levofloxacin).
2. Quinolone resistance among isolates of Rafidia Hospital (Secondary care hospital) compared to that of An-Najah National University Hospital (Tertiary care hospital).
3. Development of quinolone-resistant and various patient variables such as age, gender, clinical symptoms, and specimen type.

4. The development of quinolone-resistant and medical history, chronic diseases, signs and symptoms, and hospitalization.
5. Different mechanisms of quinolone antibiotics (ciprofloxacin and levofloxacin) resistance of *E. coli* and *K. pneumoniae* isolated from patients at NNUH and Rafidia Hospital in Nablus District.

Chapter Two
Methodology

2.1 Study design and setting

A cross-sectional prospective study design was conducted about the prevalence of quinolone antibiotic resistance and mechanisms of resistance among *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients admitted to Rafidia Hospital and An-Najah National University Hospital in Nablus District. Both hospitals are located in Nablus city. Rafidia Hospital is a secondary care hospital, while An-Najah National University Hospital is a tertiary care hospital.

The sample collection period was from the first day of June 2023 until the last day of September 2023. The study was conducted at An-Najah National University laboratories for molecular analysis and susceptibility testing.

2.2 Study population and sample size

The study population was defined as Palestinians in North West Bank specifically in the city of Nablus. The study target population includes all samples from patients infected with *E. coli* bacteria and *K. pneumoniae* bacteria, among inpatients and outpatients who were admitted to Rafidia Hospital and An-Najah National University Hospital during the study period (during four months, from June 2023 to September 2023). All bacterial culture isolates were collected regardless of the patient's age, gender, diagnosis, previous medical history, and specimen type from the microbiology section in the laboratories of the mentioned hospitals. A convenience sampling method was employed to choose all available samples that met the specified criteria from the previously mentioned hospitals. A questionnaire of data collection (Appendix D) was prepared to collect data of the patients from whom bacterial samples were obtained. Clinical data and epidemiological data of the patient from whom the bacteria were isolated, were collected with medical staff cooperation from the computer department of the mentioned hospitals.

2.2.1 Inclusion criteria

All patients infected with *E. coli* and *K. pneumoniae* bacteria, including inpatients and outpatients who were admitted to Rafidia Hospital and An-Najah National University

Hospital irrespective of the patient's age, gender, diagnosis, previous medical history and type of specimen.

2.2.2 Exclusion criteria

There were no exclusion criteria.

2.2.3 Sample size

All samples were obtained during the collection period.

2.2.4 Storage of samples

Following the successful permission for sample collection from hospitals, the aforementioned criteria were informed to technicians working in the two hospital laboratories for the purpose of collecting samples for our study. In the above-mentioned hospitals, culture isolates of *E. coli* and *K. pneumoniae* were preserved in a regular refrigerator at a temperature of 2-4°C until they were received within three days in bulk, and then preserved in 20% glycerol nutrient broth at -80 °C in the Microbiology Laboratory at the Faculty of Medicine and Health Sciences at An-Najah National University.

2.3 Study Variables

2.3.1 Background Variables

- Age
- Gender: Male or Female
- Hospital wards: Outpatients, Inpatients, General Surgery, Emergency, Pediatrics, Burns, Neonates, Urology, Intensive care unit (ICU), Internal medicine, Orthopaedic, Gynaecology.

2.3.2 Dependent variables (outcome variables)

- Antibiotic susceptibility result, which is determined by the disk diffusion method. Ciprofloxacin and levofloxacin were used, and then it was determined whether the bacteria *E. coli* and *K. pneumoniae* were resistant to these antibiotics.

- A positive result of the polymerase chain reaction (PCR) for resistance genes among strains resistant to quinolone antibiotics (ciprofloxacin and levofloxacin) to determine the resistance mechanisms used by examined bacteria:
    - Target modification mechanism: Qnr genes (qnrA, qnrB, qnrS genes).
    - Enzymatic modification mechanism: aac(6')-Ib-cr.
    - Efflux pumps mechanism: QepA, OqxA, and OqxB genes.

2.3.3 Independent variables (risk factor variables)

1. Specimen type from which the bacteria were isolated: All types of samples were accepted and included: urine, wound swab, nasal swab, fluid, ear swab, cerebrospinal fluid (CSF), skin graft, blood, and sputum.
2. Clinical symptoms (At the time of sample collection): diarrhea, fever (body temperature > 38 degrees Celsius) or a history of fever and chills within 24 hours before presentation, abdominal pain, cough, bloody stool, chest pain, shortness of breath, vomiting, and fatigue.
3. Chemotherapy.
4. Previous hospitalization.
5. Comorbidities: Hepatic cirrhosis, diabetes mellitus, cardiovascular disease, neurogenic bladder, transplantation, renal dysfunction, congestive heart failure, neurologic disease, urinary catheter, cancer, allergy, and hypertension.

All the previously mentioned variables were taken from the patient's records (patient medical file). Confidentiality of patient's data was maintained and secured based on IRB approval.

2.4 Measurement tool and data collection

2.4.1 Preservation and culture of bacterial isolates

As we mentioned previously, the cultures of *E. coli* and *K. pneumoniae* were collected from the Nablus area, specifically from Rafidia Hospital and An-Najah National University Hospital. The complete identification of the bacterial isolates was provided from the laboratories of these two hospitals by the VITEK 2 system (BioMérieux).

In order to examine bacterial isolates by susceptibility and/or PCR, the samples that were preserved in 20% glycerol nutrient broth were cultured first on nutrient agar using sterile cotton swap and incubated at 37 °C, for 24 hours. Second, the bacterial isolates were subcultured from nutrient agar to the MacConkey agar (MAC), using the four-quadrant method, and then incubated at 37 °C for 24 hours. Both *E. coli* and *K. pneumoniae* can grow on this selective media because they are Gram-negative non-fastidious rods.

Escherichia coli colonies on MacConkey (Figure 1, A) agar appear pink (rabid lactose fermenter), flat, circular, and with an entire margin. *Klebsiella pneumoniae* colonies on MacConkey agar appear large, mucoid, and pink (Figure 1, B).

Figure 1

E. coli and *K. pneumoniae Cultured on MacConkey Agar*

A. *E. coli* *B. K. pneumoniae*

2.4.2 Antibiotic susceptibility testing

Mueller-Hinton agar was used for susceptibility testing, using the Kirby-Bauer disc diffusion method. Cultured isolates were suspended at a density of 0.5 McFarland in sterile normal saline. In order to verify the concentration of the bacterial suspension, we additionally utilize a spectrophotometer device. According to published research, the standard spectrophotometer method's absorbance measuring range is roughly 0.08 to 0.12 absorbance units (Au), the absorbance was measured at a wavelength of 600 nm (87). To standardize microbial testing, McFarland assists in maintaining and ensuring that the

number of bacteria remains within a specified range during susceptibility testing. To transfer the inoculum, a sterile cotton swab was dropped into the suspension and evenly distributed across the plate's surface. We use ATCC (The American Type Culture Collection) as a control for *E. coli* and *K. pneumoniae.* Plates were incubated at 37 °C for twenty-four hours. The concentrations of antibiotics used were 5 µg/disc of ciprofloxacin and 5 µg/disc of levofloxacin. We measure the zone of inhibition following incubation to ascertain whether the bacteria is susceptible, intermediate, or resistant.

The inhibition zones for the selected antibiotics (ciprofloxacin and levofloxacin) were taken following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI)(88), and it was as follows (Table 2):

Table 2

Inhibition Zone Diameters for Quinolone Antibiotics (Ciprofloxacin and Levofloxacin)

Antimicrobial Agent	Disk Content	Zone Diameter (mm)		Resistant
		Susceptible	Intermediate	
Ciprofloxacin	5 µg	≥ 26	22-25	≤ 21
Levofloxacin	5 µg	≥ 21	17-20	≤ 16

2.4.3 Bacterial DNA Extraction

The extraction of deoxyribonucleic acid (DNA) from the *E. coli* and *K. pneumoniae* samples that were resistant to quinolones (ciprofloxacin and levofloxacin) was performed using the boiling method of DNA extraction (89). In this procedure, 500 µl of sterile distilled water was added into a sterile Eppendorf tube. Then, one big colony from the bacterial plate we picked up and suspended it in the Eppendorf tube containing the distilled water. This was followed by well mixing until the suspension was homogeneous. The bacterial suspension was boiled at 100°C in a water bath for 10 minutes and cooled on ice. To the bacterial suspension, an equal volume of chloroform was added followed by well mixing and centrifuged at 15,000 g for 30 seconds. The upper layer (supernatant) was pipetted into a sterile new Eppendorf tube, which was stored at -20°C.

2.4.4 Polymerase chain reaction (PCR) test

Multiplex PCR followed by agarose gel electrophoresis method was selected for molecular characterization of quinolones (ciprofloxacin and levofloxacin) resistant genes in *E. coli* and *K. pneumoniae* isolates. The selection of primers was based on previously published studies, as indicated in Table 3. The primers were prepared at 100 pmol/µl in sterile distilled water and then the solution was stored at a temperature of -20°C. From this solution, a 10 pmol/µl solution with sterile distilled water was prepared and placed at -20°C until it was used in the master mix.

Table 3

Primers Sequences for Quinolones Resistant Genes

Primer	Sequence (5' → 3')	Amplification size (base pair)	References
qnrA	Forward: ATTTCTCACGCCAGGATTTG Reverse: GATCGGCAAAGGTTAGGTCA	516	(65)
qnrB	Forward: GATCGTGAAAGCCAGAAAGG Reverse: ACGATGCCTGGTAGTTGTCC	469	(65)
qnrS	Forward: ACGACATTCGTCAACTGCAA Reverse: TAAATTGGCACCCTGTAGGC	417	(65)
aac(6')-Ib-cr	Forward: TTGCGATGCTCTATGAGTGGCTA Reverse: CTCGAATGCCTGGCGTGTTT	482	(72)
QepA	Forward: GCAGGTCCAGCAGCGGGTAG Reverse: CTTCCTGCCCGAGTATCGTG	281	(69)
OqxA	Forward: CTCGGCGCGATGATGCT Reverse: CCACTCTTCACGGGAGACGA	392	(62)
OqxB	Forward: TTCTCCCCCGGCGGGAAGTAC Reverse: CTCGGCCATTTTGGCGCGTA	512	(62)

2.4.4.1 Multiplex PCR reaction mixture

PCR test for one sample included 20 µl of Master Mix, which was mixed with 5 µl of extracted DNA to get a final volume of 25 µl for each sample. Each PCR reaction for a single sample consisted of 0.4 pmol/µl of forward and 0.4 pmol/µl of reverse primers of quinolone resistant genes, 5 µl of extracted DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 2.5-unit Taq DNA polymerase. The components of the PCR reaction were obtained from Sigma (USA). After the detection of each resistant gene, all runs were repeated with it as

a positive control. Negative control using sterile distilled water was also carried out during each run.

Multiplex PCR was carried out for three separate groups of primers each group having a specific annealing temperature. The first primer group (annealing temperature: 53°C) was specific to the target modification mechanism, which included Qnr genes (qnrA, qnrB, and qnrS). The second group (annealing temperature: 55 °C) targeted enzymatic modification mechanism (aac(6')-Ib-cr gene). The third group (annealing temperature: 62 °C) targeted the efflux pump mechanism (QepA, OqxA, and OqxB genes).

The program of the PCR (TECHNE, UK) starts with a step of denaturation for 5-minute of duration at 95°C, then 35 cycles that include denaturation at 95°C for 1 minute, annealing for 1 minute with a suitable temperature shown above, and extension for 1 minute at 72°C. After completion of the 35 cycles a final extension step of 5 minutes at 72°C was carried out.

The electrophoresis of PCR product was done on a 1.4% agarose gel, where 1.4 g of agarose was added to 100 ml of TBE 1X buffer, the mixture was then heated in the microwave until it became completely clear and poured in an electrophoresis equipment with no bubbles. The products were electrophoresed alongside a (5 µL)100bp DNA ladder (BIO-HELIX) and stained with Novel Juice, a DNA staining reagent (BIO-HELIX). In more details, a sample of 10 µl was taken from each product, mixed with 2 µl Novel Juice, and then put in for electrophoresis. Each group of samples was electrophoresed for approximately 1 hour, and then to observe DNA bands, a UV transilluminator device was used.

2.5 Statistical data analysis

Statistical Package for Social Science (SPSS) software version 25 was used for data analysis. Descriptive and frequency statistics were used to summarize different variables and presented in the form of texts, charts, and tables. Categorical variables were described as frequencies and proportions, and the associations between the categorical variables were assessed by Chi-square and Fisher exact tests. A *P*-value < 0.05 was considered significant.

2.6 Ethical approval

This research proposal was submitted to the IRB committee at An-Najah National University-College of Graduate Studies and obtained their approval (Appendix A). In addition, those responsible for scientific research in the Palestinian Ministry of Health were communicated in order to collect patient culture samples from the microbiology department in the laboratory and patient data from the records department and the computer department at Rafidia Governmental Hospital (Appendix B). Furthermore, acceptance was obtained from clinical scientific research members at An-Najah National University Hospital in order to collect patient culture samples from the hospital laboratory and collect patient data from records (Appendix C). Patient information was kept confidential and secure. Each sample was signed with a specific symbol instead of a name.

Chapter Three

Results and Discussion

3.1 Bacterial Isolates

From the first of June 2023 to the last day of September 2023, 219 bacterial isolates were collected. One positive culture was included from each patient from a variety of clinical specimen types. Bacterial isolates included 169 isolates of *E. coli* and 50 isolates of *K. pneumoniae*. As shown in Table 4, 156 and 63 isolates were collected from Rafidia Hospital and An-Najah National University Hospital, respectively.

Escherichia coli isolates were found in a variety of clinical materials including 104 hospitalized patients across 10 departments and 65 outpatients. Table 4 shows that the majority of the *E. coli* isolates were found in urine specimens 68.6%, followed by wound swabs 26.7%. Females had a higher prevalence of *E. coli* infection 66.9% than males 33.1%.

Most isolates of *K. pneumoniae* 62% have been collected from inpatients. The frequency of *K. pneumoniae* isolates among hospitalized patients varied throughout the wards, with the general surgery unit having a notable high frequency (5 isolates) and the remaining units having different frequencies ranging from 1 to 4 (Table 4). As indicated in Table 4, most of the *K. pneumoniae* isolates were found in urine (58%) and wound swabs (28%). Furthermore, *K. pneumoniae* infections occur more frequently in females (56%) in comparison to males 44%.

Table 4

Source of Isolates and Patients Demographic Characteristics

Variable	Total No.	*E. coli* No[1] (%[2])	*K. pneumoniae* No (%[3])
Source			
Rafidia Hospital	156	134 (79.3%)	22 (44%)
An-Najah National University Hospital	63	35 (20.7%)	28 (56%)
Units (Wards)			
Outpatients	84	65 (38.5%)	19 (38%)
Inpatients	135	104 (61.5%)	31 (62%)
General surgery	19	14 (8.3%)	5 (10%)
Emergency	15	11 (6.5%)	4 (8%)
Pediatrics	22	19 (11.2%)	3 (6%)
Burns	10	7 (4.1%)	3 (6%)
Neonates	8	5 (3%)	3 (6%)
Urology	11	9 (5.3%)	2 (4%)
Intensive care unit (ICU)	11	10 (5.9%)	1 (2%)
Internal medicine	5	3 (1.8%)	2 (4%)
Orthopedic	16	12 (7.1%)	4 (8%)
Gynecology	18	14 (8.3%)	4 (8%)
Specimen			
Urine	145	116 (68.6%)	29 (58%)
Wound swab	59	45 (26.7%)	14 (28%)
Nasal swab	1	1 (0.6%)	0 (0%)
Fluid	2	2 (1.2%)	0 (0%)
Ear swab	1	0 (0%)	1 (2%)
Cerebrospinal fluid (CSF)	1	1 (0.6%)	0 (0%)
Skin graft	1	1 (0.6%)	0 (0%)
Blood	7	3 (1.8%)	4 (8%)
Sputum	2	0 (0%)	2 (4%)
Gender			
Male	78	56 (33.1%)	22 (44%)
Female	141	113 (66.9%)	28 (56%)
Age groups			
0-2 years	26	20 (11.8%)	6 (12%)
3-14 years	35	32 (18.9%)	3 (6%)
15-39 years	66	51 (30.2%)	15 (30%)
40-65 years	60	42 (24.9%)	18 (36%)
> 65 years	32	24 (14.2%)	8 (16%)
Total	219	169	50

[1] No: Number, [2] % of the total number of *E. coli* isolates, [3]% of total number of *K. pneumoniae* isolates

In the present study, patients were grouped into 5 age groups (0-2 years, 3-14 years, 15-39 years, 40-65 years, and > 65 years) as shown in Table 4. The age group 15-39 years possessed the highest frequency of *E. coli* infection 30.2%, whereas the group aged 40-65 years had a higher frequency of *K. pneumoniae* 36%.

3.2 Susceptibility of *E. coli* and *K. pneumoniae* isolates to quinolone antibiotics

The resistance to ciprofloxacin and levofloxacin was determined by disc diffusion method as shown in Figure 2 below. Table 5 shows the percentage of resistance to ciprofloxacin and levofloxacin among the bacterial isolates. We considered isolates with intermediate resistance as resistance ones. A total of 78 46.2% *E. coli* isolates were resistant to ciprofloxacin. A slightly higher frequency 47.9% of resistance to levofloxacin was found among *E. coli* isolates. The percentage of *K. pneumoniae* isolates resistant to ciprofloxacin 70% was significantly higher (P=0.008) than that of *E. coli*. In addition, the percentage of isolates resistant to levofloxacin among *K. pneumoniae* 68% was significantly higher (P=0.022) than that of *E. coli*.

Table 5

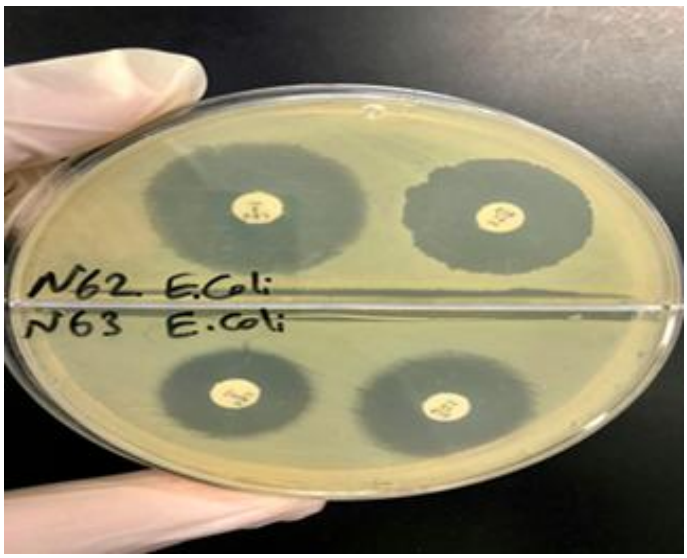
Susceptibility Testing Results of Ciprofloxacin and Levofloxacin Antibiotics

Bacterial species	No.*	CIP*		LEV*	
		Resistant No.* (%)	Susceptible No.* (%)	Resistant No.* (%)	Susceptible No.* (%)
E. coli	169	78 (46.2%)	91 (53.9%)	81 (47.9%)	88 (52.1%)
K. pneumoniae	50	35 (70%)	15 (30%)	34 (68%)	16 (32%)
Total	219	113	106	115	104

* No.: Number; CIP: Ciprofloxacin; LEV: Levofloxacin

Figure 2

Inhibition Zones of Bacterial Isolate in Susceptibility Testing of Ciprofloxacin and Levofloxacin



In the present study, *E. coli* and *K. pneumoniae* isolated in hospitals showed relatively high percentages of resistance to both ciprofloxacin and levofloxacin (Table 6). *E. coli* isolates from the An-Najah National University Hospital (tertiary care hospital) had the highest rates of ciprofloxacin and levofloxacin resistance 60% and 54.3%, respectively. This rate was significantly (*P*= 0.000 and *P*= 0.008, respectively) greater than that of the isolates obtained from Rafidia Hospital. Additionally, the isolates of *K. pneumoniae* from An-Najah National University Hospital had the highest rate of ciprofloxacin resistance 92.9%, which was also significantly higher than the isolates from Rafidia Hospital (*P*= 0.001).

Frequency of *E. coli* ciprofloxacin resistance strains isolated from the outpatients in An-Najah National University Hospital (NNUH, tertiary care hospital) was 46.2%, which was significantly higher than that of outpatients in Rafidia Hospital (Secondary care hospital) (43.6%; *P*= 0.029). Similarly, *E. coli* levofloxacin resistance frequency isolated from the outpatients in NNUH 61.5% was significantly higher than that of outpatients in Rafidia hospital (56.4%; *P*=0.033). Furthermore, frequency of *E. coli* ciprofloxacin resistance strains isolated from the inpatients in NNUH (68.2%) was significantly higher than that of inpatients in Rafidia Hospital (34.7%; *P*= 0.031). On the other hand, *E. coli* levofloxacin resistance frequency in the inpatients in NNUH (50%) was not significantly higher than that of inpatients in Rafidia Hospital (32.6%; *P*=0.078).

Table 6 shows that the frequency of *K. pneumoniae* ciprofloxacin resistance strains isolated from outpatients in NNUH 91% was significantly higher than that of outpatients in Rafidia Hospital (22.2%; *P*=0.016). Furthermore, frequency of *K. pneumoniae* ciprofloxacin resistance strains isolated from the inpatients in NNUH 94.1% were significantly higher than that of inpatients in Rafidia Hospital (53.8%; *P*= 0.025). On the other hand, *K. pneumoniae* levofloxacin resistance frequency isolated from the outpatients in NNUH 81.8% was not significantly higher than that of outpatients in Rafidia hospital (33.3%; *P*=0.075). Also, *K. pneumoniae* levofloxacin resistance frequency among the inpatients in NNUH (70.6%) was not significantly higher than that among inpatients in Rafidia Hospital (61.5%; *P*=0.09).

Escherichia coli strains isolated from the general surgery unit had the highest frequency (64.3%) of ciprofloxacin resistance when compared to isolates from other departments and outpatients. Ciprofloxacin resistance among *E. coli* isolated from the general surgery unit was significantly higher than that of the emergency department (54.5%; *P*=0.002) and the outpatients (36.9%; *P*= 0.016), and not significantly higher than that of pediatrics (21.1%; *P*= 0.087), ICU (60%; *P*= 0.079), and gynecology (50%; *P*= 0.093). On the other hand, the intensive care unit had the highest frequency of levofloxacin resistance (50%). In more detail, levofloxacin resistance frequency in the ICU was significantly higher than that of the outpatients (49.2%; *P*=0.018), emergency (45.5%; *P*=0.022), and general surgery (42.9%; *P*=0.037).

Table 6 in appendix E shows that the *K. pneumoniae* bacteria isolated from outpatients had high resistance against both ciprofloxacin (78.9%) and levofloxacin (94.7%). Ciprofloxacin resistance among *K. pneumoniae* isolated from the outpatients was significantly higher than that of the general surgery department (60%; *P*=0.039), and not significantly higher than that of the emergency department (50%; *P*=0.067), gynecology department (57%; P= 0.081), and orthopedic department (57%; *P*= 0.088). On the other hand, levofloxacin resistance frequency in the outpatients was not significantly higher than that of the general surgery unit (40%; *P*=0.097), and emergency department (50%; *P*=0.11).

Escherichia coli isolated from urine had a higher level of resistance to ciprofloxacin and levofloxacin (44% and 47.4%, respectively) than wound swabs (31.1% and 22.2%,

respectively) as shown in Table 6. This difference was with no statistical significance (*P*=0.097 and *P*= 0.21, respectively). However, among *K. pneumoniae* isolates, wound swabs had a higher resistance rate to ciprofloxacin and levofloxacin (71.4% and 92.9%, respectively) than urine (65.5% and 51.7%, respectively) as shown in Table 6. This difference was with no statistical significance (*P*=0.1 and *P*= 0.079, respectively). The number of other types of specimens was limited and ignored in comparison.

When antibiotic resistance rates for *E.coli* isolates from males and females were analyzed, it was found that ciprofloxacin resistance was significantly higher (*P*=0.008) in male isolates 53.6% than in female isolates 36.3%. In addition, frequency of levofloxacin resistance among *E. coli* isolates was close in both genders (Table 6). Furthermore, as Table 6 shows, female isolates of *K. pneumoniae* had greater rates of ciprofloxacin and levofloxacin resistance (75% and 78.6%, respectively) than males without significant association (*P*=0.062).

The mean age of patients infected by ciprofloxacin-resistant *E. coli* (41.3 ± 18.7 years) and *K. pneumoniae* (44.3 ± 21.3 years) isolates were insignificantly (*P*= 0.175 and *P*= 0.122, respectively) higher from that of patients with ciprofloxacin susceptible *E. coli* (30.0 ± 13.6 years) and *K. pneumoniae* (34.4 ± 16.2 years) isolates. In addition, the mean age (41.7 ± 19.1 years) of patients from whom levofloxacin-resistant *E. coli* was isolated, was insignificantly (*P*= 0.172) higher than that (29.11 ± 11.7 years) of patients with levofloxacin susceptible *E. coli* isolates. Levofloxacin-resistant *K. pneumoniae* strains were isolated from patients with a mean age (44.6 ± 20.2 years), which was insignificantly (*P*=0.095) higher than that of levofloxacin susceptible *K. pneumoniae* isolates (34.2 ± 16.1 years).

The antibiotic resistance frequencies of *K. pneumoniae* and *E. coli* isolates across various age groups are shown in Table 6 above. *E. coli* ciprofloxacin resistance highest percentage 50% was found in the age groups 0–2 and >65 years. The 0-2 and >65 years age groups were significantly (*P*= 0.017 and *P*= 0.023, respectively) higher than that of the 40–65 years age group. Similarly, the *E. coli* isolated from patients over 65 years of age had the highest levofloxacin resistance 62.5%, which was also significantly (*P*= 0.007) higher than that of the 40–65 year age group. With regard to *K. pneumoniae*, the 40–65 years old age group had the highest percentage of ciprofloxacin resistance (83.3%), while the

15–39 years old age group had the highest percentage of levofloxacin resistance 80%. The differences were without statistical significance.

The study included patients with various diagnoses on which symptoms and signs mainly depend. This hindered the detection of correlations between clinical symptoms of patients and *E. coli* and *K. pneumoniae* quinolone resistance. In more detail, we cannot judge the relationship between bacterial resistance to quinolone antibiotics (ciprofloxacin and levofloxacin) and the signs and symptoms that the patient had during the sample collection period.

The percentage of patients in this study with each comorbidity is shown in Table 6 above. For *E. coli* ciprofloxacin resistant isolates, patients with urinary catheters 91.7% were the most common. *Escherichia coli* ciprofloxacin resistant bacterium isolated from patients with urinary catheters 91.7% had a significantly higher frequency than that of hypertension patients (85.5%; *P*=0.013), diabetes mellitus patients (84.8%; *P*=0.017), cancer patients (81.9%; *P*=0.022), and hepatic cirrhosis patients (64.1%; *P*=0.028). However, patients with urinary catheters had an insignificantly higher frequency than that of congestive heart failure patients (42.9%; *P*=0.52), neurologic disease patients (45.5%; *P*=0.41), transplantation patients (66.7%; *P*=0.38), neurogenic bladder patients (75%; *P*=0.29), cardiovascular disease patients (81.8%; *P*=0.24), allergy patients (56.5%; *P*=0.17), and renal dysfunction patients (58.6%; *P*=0.093). On the other hand, the highest percentage of *K. pneumoniae* ciprofloxacin resistant isolates 77.8% was found in patients with hepatic cirrhosis. *Klebsiella pneumoniae* ciprofloxacin-resistant bacteria that were isolated from hepatic cirrhosis patients had a significantly higher frequency than that of hypertension patients (65.2%; *P*=0.003), cancer patients (61.9%; *P*=0.019), and diabetes mellitus patients (58.9%; *P*=0.032). However, hepatic cirrhosis patients had an insignificantly higher frequency than that of congestive heart failure patients (40%; *P*=0.53), transplantation patients (57.1%; *P*=0.36), neurogenic bladder patients (25%; *P*=0.2), cardiovascular disease patients (53.8%; *P*=0.27), neurologic disease patients (42.9%; *P*=0.13), allergy patients (47.4%; *P*=0.11), and renal dysfunction patients (40%; *P*=0.091).

The highest percentage of levofloxacin resistant isolates of both *E. coli* and *K. pneumoniae* (89.1% and 71.8%, respectively) were found in patients with diabetes

mellitus as shown in Table 6. *Escherichia coli* levofloxacin-resistant bacteria, which were isolated from diabetes mellitus patients 89.1% had a significantly higher frequency than that of hypertension patients (76.3%; *P*=0.013), cancer patients (73.6%; *P*=0.016), patient with the urinary catheter (77.8%; *P*=0.021), and hepatic cirrhosis patients (51.3%; *P*=0.028). However, diabetes mellitus patients had an insignificantly higher frequency of levofloxacin-resistant *E.coli* than that of congestive heart failure patients (28.6%; *P*=0.33), transplantation patients (41.7%; *P*=0.3), neurologic disease patients (81.8%; *P*=0.26), neurogenic bladder patients (56.3%; *P*=0.21), cardiovascular disease patients (68.2%; *P*=0.19), allergy patients (69.6%; *P*=0.11), and renal dysfunction patients (75.9%; *P*=0.078). *Klebsiella pneumoniae* levofloxacin-resistant bacteria, which were isolated from diabetes mellitus patients (71.8%) had a significantly higher frequency than that of hypertension patients (56.5%; *P*=0.009), cancer patients (52.4%; *P*=0.016), hepatic cirrhosis patients (59.3%; *P*=0.023), and patient with the urinary catheter (48%; *P*=0.031). However, diabetes mellitus patients had an insignificantly higher frequency than that of congestive heart failure patients (20%; *P*=0.28), transplantation patients (57.1%; *P*=0.25), neurologic disease patients (42.9%; *P*=0.17), neurogenic bladder patients (37.5%; *P*=0.14), cardiovascular disease patients (38.5%; *P*=0.12), allergy patients (57.9%; *P*=0.094), and renal dysfunction patients (60%; *P*=0.088).

In the present study, analysis of antibiotic resistance rates for *E. coli* isolates from nosocomial infection patients had insignificant higher rates of ciprofloxacin resistance 59.4% and levofloxacin resistance 65.6% than those without nosocomial infections (37.9% and 37.2%, respectively). However, *K. pneumoniae* isolated from patients without nosocomial infections had higher frequencies of ciprofloxacin resistance 71.8% and levofloxacin resistance 66.7% than patients with nosocomial infections (63.6% and 54.5%, respectively). The differences were without statistical significance.

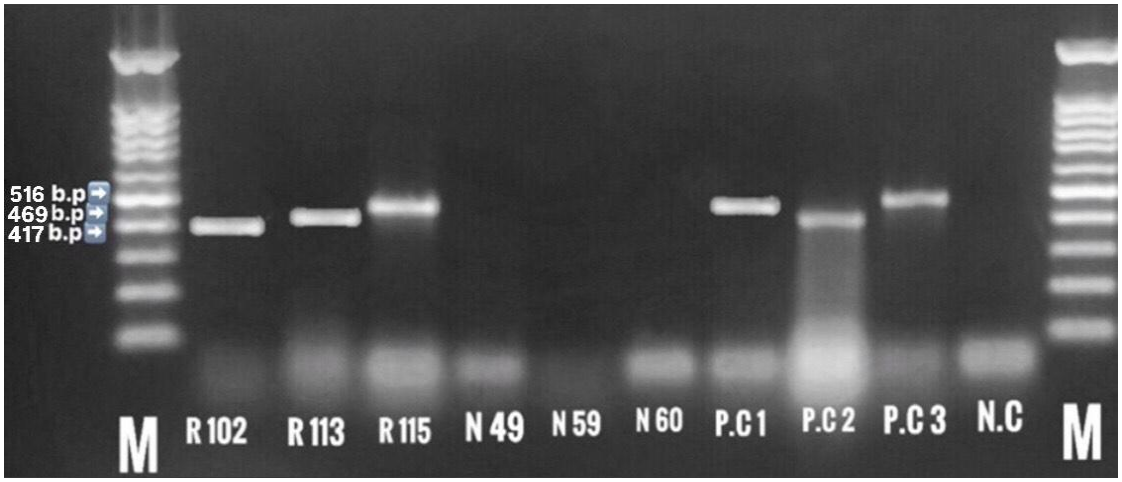
3.3 Mechanisms of quinolone (ciprofloxacin and levofloxacin) resistance

The ciprofloxacin- levofloxacin susceptibility test (disk diffusion method) and detection of resistance genes by PCR were combined to predict the most probable resistance mechanism. We examined the samples that gave a resistant or intermediate resistant result to ciprofloxacin or levofloxacin and included 81 isolates of *E. coli* and 37 isolates of *K. pneumoniae* bacteria. Multiplex-PCR was carried out with a reaction mixture containing primers in three groups, as follows: 1. Target modification mechanism (qnrA, qnrB, and

qnrS genes) 2. Enzymatic modification mechanism (aac(6')-Ib-cr gene) 3. Efflux pump mechanism (QepA, OqxA, and OqxB genes). Representative results are shown in Figures 3, 4, and 5. The genes of the target modification mechanism, which were qnrS, qnrB, and qnrA were detected in 15, 12, and 5, respectively, among *E. coli* and *K. pneumoniae* isolates together, as shown in Table 7. The enzymatic modification mechanism (aac(6')-Ib-cr) gene was detected in a total of 36 *E. coli* and *K. pneumoniae* isolates. Efflux pump mechanism genes were detected in a total of 49 examined *E. coli* and *K. pneumoniae* isolates exhibiting resistance to ciprofloxacin and levofloxacin. Most of these isolates carried the oqxA gene (29 isolates), as demonstrated in Table 7.

Figure 3

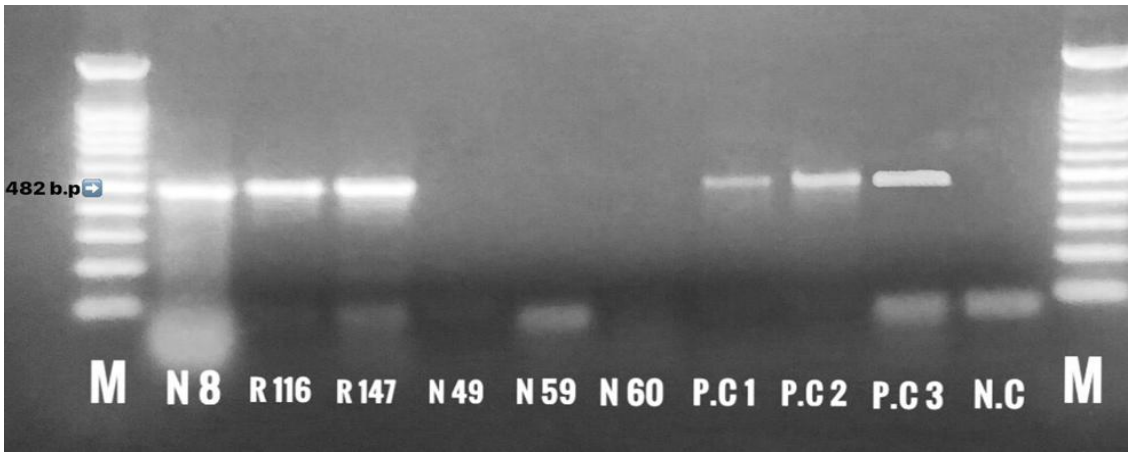
Gel Electrophoresis for Representative Samples, with Positive Results for the Target Modification Mechanism (qnrA, qnrB, and qnrS genes)



M: DNA ladder; R 102: Sample qnrS positive; R 113: Sample qnrB positive; R 115: Sample qnrA positive; N 49, N 59 and N 60: Negative samples for examined genes; P.C 1: Positive control qnrB (469 b.p); P.C 2: Positive control qnrS (417 b.p); P.C 3: Positive control qnrA (516 b.p); N.C: Negative control.

Figure 4

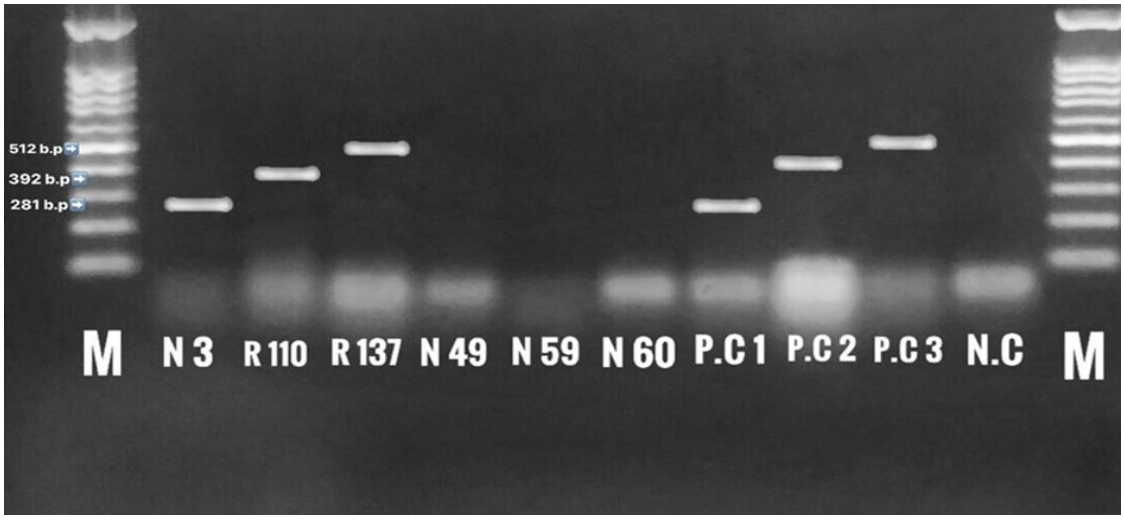
Gel Electrophoresis for Representative Sample with Positive Results for the Enzymatic Modification Mechanism (aac(6')-Ib-cr gene)



Note: M: DNA ladder; N 8, R 116 and R 147: Positive samples of aac(6')-Ib-cr; N49, N 59, N 60: Negative samples for all primers in this study; P.C 1, P.C 2, P.C 3: Positive controls (482 b.p); N.C: Negative control.

Figure 5

Gel Electrophoresis for Representative Sample with Positive Results for the Efflux Pump Mechanism (QepA, OqxA, and OqxB genes)



Note: M: DNA ladder; N 3: Sample qepA positive; R 110: Sample oqxA positive; R 137: Sample oqxB positive; N 49, N 59, and N 60: Negative samples for all examined genes; P.C 1: Positive control qepA (281 b.p); P.C 2: Positive control oqxA (392 b.p); P.C 3: Positive control oqxB (512 b.p); N.C: Negative control.

Table 7 shows the frequency of quinolone resistance genes and possible mechanism of resistance among quinolone resistant *E. coli* and *K. pneumoniae* isolates. Among *E. coli* isolates the most common mode of resistance was the efflux pump mechanism 43.3% with OqxA 23.5% being the most common gene. This was followed by enzymatic modification by Aac(6')-Ib-cr enzyme 29.6% and target modification mechanism 26% in which the QnrS gene was the most common 13.6%. The frequency of the efflux pump

mechanism among *E. coli* isolates 43.3% was significantly higher than that of the enzymatic modification mechanism (29.6%; *P*=0.017) and target modification mechanism (26%; *P*=0.021). The frequency of the enzymatic modification mechanism among *E. coli* isolates 29.6% was significantly higher than that of the target modification mechanism (26%; *P*= 0.027).

Seven isolates had two genes of resistance together; four of them were *E. coli* and three were *K. pneumoniae*. Among the four *E.coli* isolates, two isolates had target modification QnrS gene with enzymatic modification Aac(6)'-Ib-cr gene, one isolate had QnrS with QnrB genes, which were genes of target modification mechanism, and finally one isolate had enzymatic modification Aac(6)'-Ib-cr gene with efflux pump OqxA gene.

Table 7

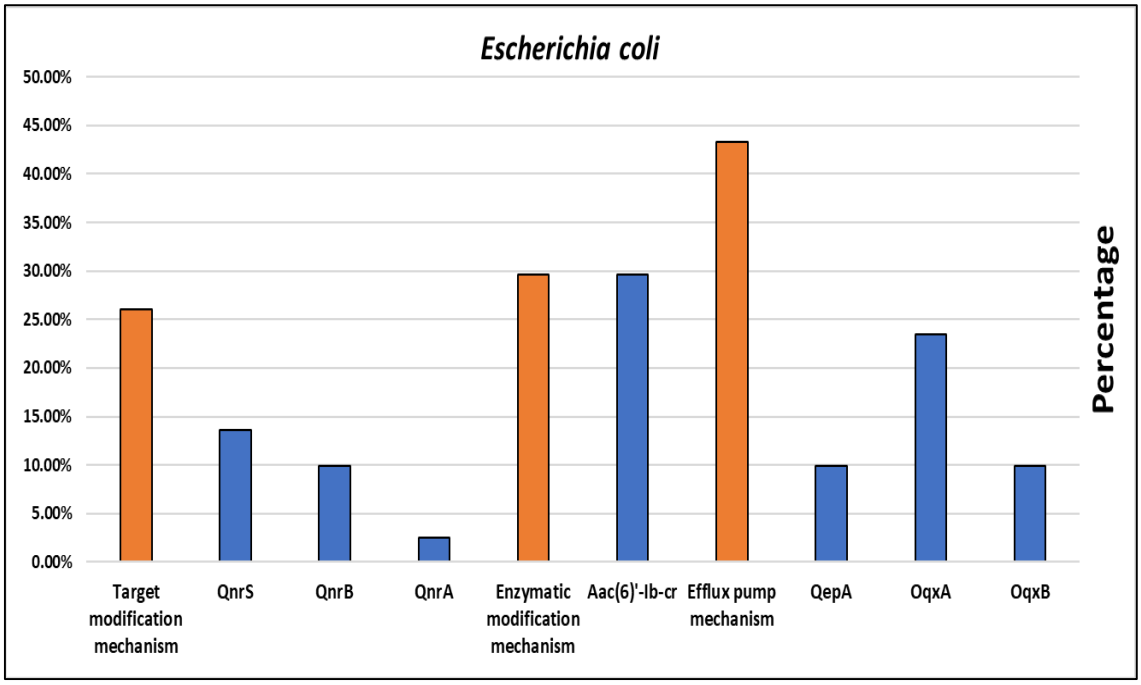
Genes of Quinolone (Ciprofloxacin and Levofloxacin) Resistance among Escherichia coli and Klebsiella pneumoniae Isolates

Type of bacteria	E.I*	Detected Gene (%)									
		*T.M**	QnrS	QnrB	QnrA	*E.M**	Aac(6)'-Ib-cr	*E.P**	QepA	OqxA	OqxB
E. coli	81	21 (26%)	11 (13.6%)	8 (9.9%)	2 (2.5%)	24 (29.6%)	24 (29.6%)	35 (43.3%)	8 (9.9%)	19 (23.5%)	8 (9.9%)
K. pneumoniae	37	11 (29.7%)	4 (10.8%)	4 (10.8%)	3 (8.1%)	12 (32.4%)	12 (32.4%)	14 (37.8%)	1 (2.7%)	10 (27.0%)	3 (8.1%)

* E.I: Examined isolates; T.M: Target modification mechanism; E.M: Enzymatic modification mechanism; E.P: Efflux pump mechanism.

Figure 6

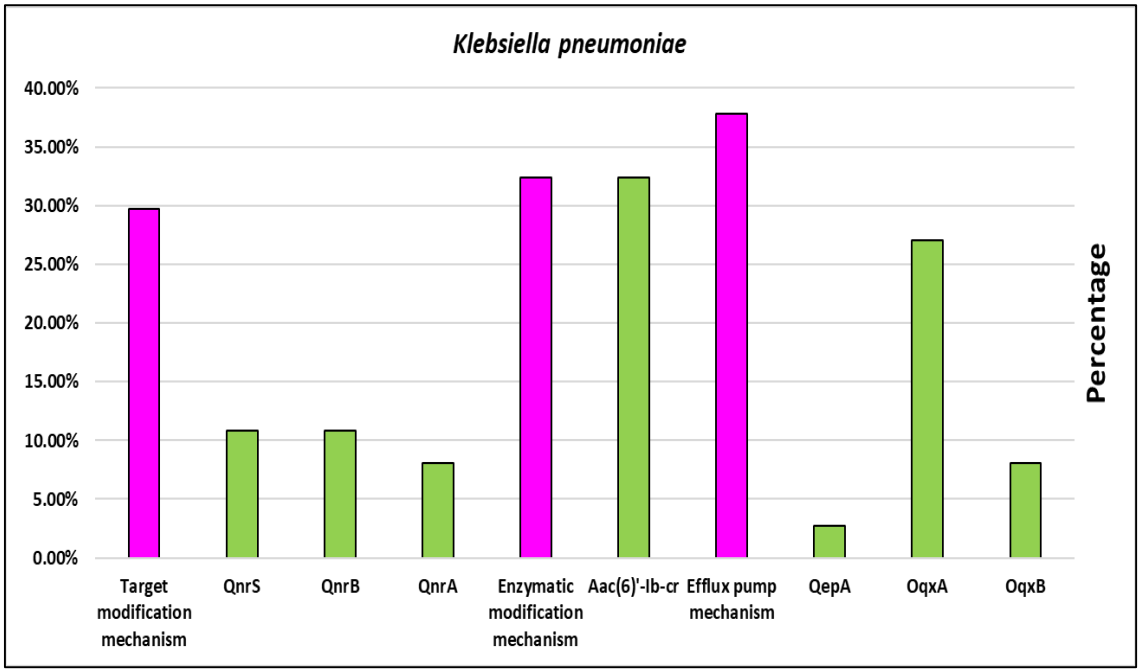
The Percentage of Genes of Quinolone (ciprofloxacin-levofloxacin)Resistance among Quinolone Resistant Escherichia coli Isolates



Similar to *E.coli*, the efflux pump mechanism of resistance was the most common mode of resistance among quinolone resistant *K. pneumoniae* 37.8% with the OqxA gene exhibiting the highest frequency 27%. Furthermore, the frequency of the Aac(6')-Ib-cr gene 32.4% was in the second position after the efflux pump mechanism of resistance and target modification in the third position 29.7% with QnrS and QnrB being the most commonly detected genes 10.8%. The frequency of the efflux pump mechanism among *K. pneumoniae* isolates 37.8% was significantly higher than that of the enzymatic modification mechanism (32.4%; *P*= 0.009) and target modification mechanism (29.7%; *P*=0.016). The frequency of the enzymatic modification mechanism among *K. pneumoniae* isolates 32.4% was significantly higher than that of the target modification mechanism (29.7%; *P*= 0.022). Three *K. pneumoniae* isolates had two genes of resistance together (two isolates had enzymatic modification Aac(6)'-Ib-cr gene with efflux pump OqxA gene and one isolate had OqxA gene with QepA, which were efflux pump mechanism genes).

Figure 7

The Percentage of Genes of Quinolone (Ciprofloxacin-Levofloxacin) among Quinolone Resistant Klebsiella pneumoniae Isolates



3.2 Discussions

Antimicrobial resistance is a global issue acknowledged as a risk to patient safety and public health. Since empirical antimicrobial therapy may fail, it results in elevated mortality and morbidity, fewer possibilities for treatment, and higher costs. Antimicrobial resistance has more pressure due to the improper use of antibiotics. It has been demonstrated that national programs that track the utilization and development of resistance to antibiotics are an effective way to maintain the efficacy of antibiotics in many different nations (76).

Quinolone antibiotics have strong effects against Gram-negative bacteria like *Escherichia coli* and *Klebsiella pneumoniae*, and they are now frequently used in the medical treatment of humans and animals (15). It has been reported around the world that the proportion of quinolones resistant strains of *E. coli* has greatly increased recently (90). Furthermore, clinical strains of *K. pneumoniae* have been indicated to be resistant to fluoroquinolones on several occasions (58).

This present study reported that 46.2% of the *E. coli* isolates had ciprofloxacin resistance, this is a relatively high frequency. Among 166 *E. coli* isolates, a study carried out in

Pakistan in 2015 found that the rate of ciprofloxacin resistance was 40% (91). A global systematic review and meta-analysis of observational studies revealed that the prevalence of *E. coli* isolates resistant to the antibiotic ciprofloxacin varied over time, as shown by the following: In a cross-sectional study conducted in India in 2007, 69% of sixty-one *E. coli* isolates were found to be resistant to ciprofloxacin; in 2013, the proportion was 53% among 397 isolates. According to a 2010 cohort study conducted in Turkey, 30% of the 464 isolates had ciprofloxacin resistance. During the year 2012, the publication of several cross-sectional studies displayed comparatively high rates of *E. coli* ciprofloxacin resistance worldwide. Where rates in Cameroon, Iran, and Colombia were 26%, 40%, and 38%, respectively. Additionally, many studies found that *E. coli* isolates from the USA (2013), Mexico (2011), Nigeria (2013), and China (2014) had higher rates of ciprofloxacin resistance; the percentages were 41%, 55%, 80%, and 71%, respectively (92).

In Palestine, especially the Gaza Strip, a study conducted from January to June 2004 reported that resistance to ciprofloxacin among *E. coli* isolates was 12.0% (93). Another study indicated that the rate of ciprofloxacin resistance was 12.5% among 80 *E. coli* strains isolated from Rafidia Hospital in Nablus, Northern Palestine, throughout the 1996–2000 period (94). Furthermore, a cross-sectional study conducted in Nablus, Palestine, between November 2009 and April 2010 revealed that out of 243 *E. coli* isolates, the rate of ciprofloxacin resistance was 17.2%, and this study emphasizes the necessity of creating regional guidelines where prescription decisions should take elevated antibiotic resistance into account (95).

In the present study, the levofloxacin resistance rate among *E. coli* isolates was 47.9%. Through the years, *E. coli* resistance to levofloxacin has changed at different rates all over the world. According to multiple studies conducted across European countries, the percentage of *E. coli* resistance to levofloxacin in 2020 was 7.9% in Sweden and in 2020 was 52% in Turkey (96). In Asian countries, during 2019, India had a 69% resistance rate and Korea had a 25% (97). Levofloxacin-resistant *E. coli* was found in the United States in 1% of cases in 1994, but in inpatients and outpatients in 2014, the frequency varied from 2% to 27% (98). Levofloxacin resistance was found in 23.4% of *E. coli* isolates in the Japanese population in 2012 (99). Also, a previous retrospective study conducted in South Korea between 2005 and 2009 examined the rate of levofloxacin

resistance in 509 patients who had been infected with *E. coli*, according to the study, the annual rates of *E. coli* levofloxacin resistance were 29.5% in 2005, 26.5% in 2006, 40.2% in 2007, 43.2% in 2008, and 31.7% in 2009 (100). Also, the study emphasized that levofloxacin resistance rates of *E. coli* were high at over 25% from 2005 to 2009 years (100).

Klebsiella pneumoniae bacteria in this study additionally displayed a high level (70%) of ciprofloxacin resistance. The rates of ciprofloxacin resistance in *K. pneumoniae* isolates have also shown an interesting evolution over time, as observed worldwide. According to a recent retrospective study conducted in Ethiopia and published on 27 March 2024, the ciprofloxacin resistance rate among 266 *K. pneumoniae* isolates elevated significantly between 2017 and 2021, rising from 41% to 90% (101). Another investigation conducted in Iran between 2016 and 2017 found that 40% of the 75 *K. pneumoniae* isolates were resistant to ciprofloxacin (102). According to a study conducted in the Gaza Strip in 2005, 17.6% of *K. pneumoniae* isolates in Palestine were resistant to ciprofloxacin (93). In 2019, 66 isolates from various hospitals in the Northern West Bank of Palestine had a 40% resistance rate to ciprofloxacin (13).

In our study, 68% of the *K. pneumoniae* isolates were resistant to levofloxacin, which is regarded as a high percentage. Levofloxacin resistance in *K. pneumoniae* has been found at high rates in previous studies conducted all over the world. For example, a study conducted in Basrah, Iraq in 2022 found that 78.9% of 120 *K. pneumoniae* isolates had resistance (103). Additionally, a study in China in 2013 included 144 isolates of *K. pneumoniae* demonstrating that the resistance rate was 65.3% (12), which is extremely similar to the percentage found in the current study. According to a study in Palestine, specifically in 2019, 66 *K. pneumoniae* isolates have been identified to have a 44% levofloxacin resistance rate (13).

Therefore, even though the current study found that *E. coli* isolates had a high rate of ciprofloxacin resistance 46.2% and levofloxacin resistance 47.9% and that *K. pneumoniae* isolates had 70% ciprofloxacin resistance and 68% levofloxacin resistance, compared to previous years when the rates were relatively lower, Palestine is currently experiencing a increasing rate of quinolone resistance due to the lack of standardized criteria for the use of antibiotics, in contrast to other countries that have stewardship

programs, protocols, and monitoring systems for the consumption and application of antibiotics.

Variation of ciprofloxacin and levofloxacin resistance levels may be explained by time factors (global trends of antimicrobial resistance), variety of resistance mechanisms, geographical location, patient characteristics, local antibiotic policies, how the health authority monitors and tracks the consumption or application of antibiotics, epidemiological factors, and the strains' origin (104). These variations in the rates of resistance highlight the significance of ongoing observation of the emergence of drug resistance in bacterial strains.

According to the current study, hospital ward type appears to be related to differences in antibiotic resistance frequencies across the various hospitals under investigation. In more details, bacterial isolates at An-Najah National University Hospital (tertiary care hospital) had a greater percentage of ciprofloxacin resistance (60% in *E. coli* isolates and 92.9% in *K. pneumoniae* isolates) and levofloxacin resistance (54.3% in *E. coli* isolates and 75% in *K. pneumoniae* isolates. This could be explained by the fact that a considerable proportion of patients at this medical facility, were treated for infections, as a result, they receive various antibiotics, which raises the possibility of antibiotic resistance. Complex cases that are transferred from other hospitals are also treated at this hospital, due to the duration lengthy of treatment, the patient will have received a wide range of medications. Additionally, this hospital treats cancer patients and patients undergoing chemotherapy, which accounts for a significant portion of the quinolone resistance observed in our study. Rafidia Hospital, on the other hand, mostly treats surgical patients; patients who are admitted there are probably for sugary reasons rather than infection treatment. As a result, it was found that resistance rates were reduced and peaked at 50%.

In the current study, we observed high rates of resistance to ciprofloxacin 64.3% and levofloxacin 42.9% in the general surgery unit among *E. coli* isolates, as well as in *K. pneumoniae* isolates, where the rate of resistance to ciprofloxacin was 60% and to levofloxacin was 40%. This can be explained by the fact that many surgeons using quinolones as prophylactic therapy.

In the intensive care units (ICU) of the hospitals where the isolates were obtained for this study, we found a high rate of ciprofloxacin resistance 60% and levofloxacin resistance

(50%) among *E. coli* isolates. This is because antibiotic resistance has become a major concern for patients in critical care due to the overuse and misuse of antimicrobials. Because antimicrobial therapy has a high failure rate, treating these patients is challenging. This problem is linked to high mortality rates, disadvantageous prognoses, extended hospital stays, and higher medical costs. Patients who stay in the ICU for longer than 24 hours have a 19% infection rate (105). Over the past few years, patients in intensive care units are still showing high levels of fluoroquinolone resistance, and nosocomial isolates of *K. pneumoniae* have demonstrated an increase in this resistance. What's more concerning are recent reports that bacteria that cause community-acquired infections, like *E. coli*, are becoming more resistant to fluoroquinolones overall (104).

In the present investigation, we found that *E. coli* ciprofloxacin resistance isolates were significantly higher (*P*=0.008) in male isolates 53.6% than in female isolates 36.3%. A study conducted in 2021 in New Jersey, United States, found that 53% of the *E. coli* isolates were resistant to ciprofloxacin in males (106). Additionally, a cross-sectional study in Ghana using 135 *E. coli* isolates from July 2018 to June 2019 found that isolates from male participants (80%) had a higher prevalence of ciprofloxacin and levofloxacin resistant genes compared to isolates from female participants 75.7%, a significant difference (*P*=0.0127) was observed in the prevalence of ciprofloxacin and levofloxacin resistant *E. coli* infections in men compared to females (107). Thus, it is possible that male sex presents an independent risk factor for quinolone resistance. Quinolone resistant uropathogens may be more common in the male urological system; for example, research has demonstrated a high frequency of quinolone resistance in the microorganisms causing acute prostatitis following transrectal prostate biopsy. Quinolones are frequently used as preventative measures in prostate biopsies, so male sex and quinolone resistance may be related, partially because men are more likely to have been exposed to antibiotics over their lifetime (106). However, in the current study female isolates of *K. pneumoniae* had greater rates of ciprofloxacin and levofloxacin resistance (75% and 78.6%, respectively) without significant association (*P*=0.062). According to an Iranian study, the percentage of female *K. pneumoniae* isolates 65.9% that were resistant to quinolones was higher than the percentage of male isolates 44.2% (108).

Escherichia coli isolated from urine had a higher level of resistance to ciprofloxacin and levofloxacin (44% and 47.4%, respectively) than wound swabs (31.1% and 22.2%, respectively) as shown in Table 6. This difference was with no statistical significance (*P*=0.097 and *P*= 0.21, respectively). A study conducted in 2021 in Mansoura city, Egypt, demonstrated a significant correlation between *E. coli* isolated from urine and quinolone resistance (109). Quinolones were found to be one of the most significant therapeutic regimens used for the treatment of uropathogens (109). Therefore, this might be responsible for the high frequency of quinolone resistance among urinary tract infection isolates, especially in developing countries.

Concerning age groups, the *E. coli* ciprofloxacin-resistant bacterium highest percentage (50%) was found in the age groups 0–2 and over 65 years. The 0-2 and over 65 years age groups were significantly (*P*= 0.017 and *P*= 0.023, respectively) higher than that of the 40–65-years age group. Similarly, the *E. coli* isolated from patients over 65 years of age had the highest levofloxacin resistance 62.5%, which was also significantly (*P*= 0.007) higher than that of the 40–65 years age group. The immune systems' ability in these age groups may be the cause of this (immunocompromised group). In a cross-sectional study conducted in Ghana in 2022, *E. coli* isolates showed extremely high ciprofloxacin resistance rates 83.3% among the 0-2 years age group, and the difference was statistically significant (*P*=0.0127) compared to the 40–65 years age group (107). Additionally, the results of a study conducted in North America regarding *E. coli* resistance to quinolones among >65 years patients, showed a high quinolones resistance rate 73.1% (110).

Concerning quinolone risk factors, a meta-analysis study, and a systematic review in 2020 regarding risk factors for quinolone resistance in *E. coli* isolates was carried out. This review includes twenty-seven studies, mainly published in PubMed up to April 2019, with 67,019 participants. The odds ratio (OR) with a 95% confidence interval (CI) was used to analyze risk factors (90). An odds ratio larger than 1 (one) is considered a positive association because it indicates a greater probability of the event occurring in the exposed group compared to the non-exposed group. According to this study, the following risk factors were correlated with infections by quinolones-resistant *E. coli*: male gender (OR= 1.41), use of antibiotics (OR=2.74), previous hospitalization (OR=2.06), a urinary catheter (OR=4.39), chemotherapy (OR=7.67), neurogenic bladder (OR=8.66), diabetes mellitus (O =1.62), hepatic cirrhosis (OR=2.05), congestive heart failure (OR=5.63),

cardiovascular disease (OR=1.76), hypertension (OR=5.83), renal dysfunction (OR= 2.47), and neurologic disease (OR = 2.80) (90). In our study, significant correlations (*P*-value < 0.05) were found between quinolone resistance in *E. coli* isolates and the following risk factors: age, male gender, diabetes mellitus, and previous antibiotic use.

In the current study, out of 81 examined *E. coli* isolates, the percentage of quinolone resistant genes is as follows: 1. Target modification mechanism 26% including QnrA 2.5%, QnrB 9.9%, and QnrS 13.6% genes 2. Enzymatic modification mechanism 29.6% including Aac(6')-Ib-cr 29.6% gene 3. Efflux pump mechanism 43.3% including QepA 9.9%, OqxA 23.5%, and OqxB 9.9% genes. According to a 2020 study conducted in China, the enzymatic modification mechanism (Aac (6′)-Ib-cr gene) had the highest rate 57.1% of quinolone resistance among the different genes among *E. coli* isolates, followed by the efflux pump mechanism 48.7% with OqxA gene 39.2% and OqxB genes 9.5% and finally target modification mechanism was the last QnrS gene 12.6%. In addition, QnrA, QnrB, QnrC, QnrD, and QepA genes were not found (111). Furthermore, among 135 *E. coli* isolates, a study conducted in Ghana between July 2018 and June 2019 found that the most common resistance mechanism was the enzymatic modification mechanism (Aac(6')-Ib-cr gene 48.9%, followed by target modification mechanism QnrS gene 25.6% (107). However, among 134 *E. coli* isolates in a study conducted in Mansoura City, Egypt in 2021, the active efflux pump genes OqxA and QepA had the highest prevalence 72.2%, followed by the enzymatic modification mechanism gene Aac(6 r′)-Ib-cr 66.7%, and the target modification mechanism genes Qnr 61.11% (109). A study in 2020 conducted in the Gaza Strip in Palestine revealed that four isolates out of 69 *E. coli* were found to carry the enzymatic modification mechanism (Aac(6′)-Ib-cr gene) (112). A subsequent investigation conducted in Gaza in December 2022 revealed that out of 42 isolates, 12 of the *E. coli* isolates had the enzymatic modification mechanism (Aac(6′)-Ib-cr gene (113).

The current study revealed that the efflux pump mechanism had the highest percentage 37.8% among *K. pneumoniae* with the OqxA gene 27% being most common, followed by the enzymatic modification mechanism with Aac(6)'-Ib-cr gene 32.4%, Gene frequencies for target modification mechanism gene QnrS and QnrB were lower 10.8%. Additionally, QnrA and OqxB are present in *K. pneumoniae* isolates at small rates 8.1%. QepA gene possessed the lowest rate 2.7%. Indian study conducted in 2020 among 110

K. pneumoniae isolates resistant to levofloxacin and ciprofloxacin, the highest prevalent mechanism was the enzymatic modification mechanism with Aac(6')-Ib-cr gene 89%, followed by efflux pump OqxA gene 58% and target modification mechanism with QnrS gene 12%. The OqxB gene 6.3%, QnrB gene 1.8%, and QnrA gene 0.9% had the lowest frequencies (114). According to an additional investigation, 43 *K. pneumoniae* isolates that were collected in Baghdad between June and December 2019 had the following prevalence of ciprofloxacin and levofloxacin resistant mechanism: the highest prevalence was enzymatic modification mechanism with Aac(6')-Ib-cr gene 51.8%, followed by efflux pump gene QepA 40.7%, finally, the target modification mechanism with QnrS gene 37% and QnrB gene 2.5% (115). Additionally, a study conducted in southwest Iran in 2022 found that out of 92 clinical isolates of *K. pneumoniae*, the highest ciprofloxacin resistance mechanism was the enzymatic modification mechanism due to Aac(6')-Ib-cr enzyme 88% (116). A study conducted in 2020 in the Gaza Strip in Palestine revealed that out of 27 *K. pneumoniae* isolates, two isolates had the enzymatic modification mechanism with Aac(6′)-Ib-cr gene and two isolates had the target modification mechanism with QnrS gene identified (71).

In July 2024, a new study conducted in the Gaza Strip, Palestine, found that Aac(6′)-Ib-cr 26.6%, QnrS 20.8%, QnrA 18.8%, and QnrB 6.4% were the most common quinolone-resistant genes among 146 gram-negative isolates from patients in three Palestinian hospitals (117). Based on research conducted in Palestine, we can now summarize the quinolone-resistant genes found in isolates of *E. coli* and *K. pneumoniae* were enzymatic modification mechanism (Aac(6’)-Ib-cr gene) and target modification mechanism (QnrA, QnrB, and QnrS genes). The efflux pump mechanism (QepA, OqxA, and OqxB genes) was not recognized, and no research has been conducted regarding it up until now. Therefore, we are the first to report the predominance of efflux pump among quinolone resistant *E. coli* and *K. pneumoniae* in the studied Nablus hospitals.

The variations in sample collection, study design, sample population, patient inclusion criteria, environmental factors, and personal hygiene might be responsible for these variations in proportions between studies.

Chapter Four

Conclusion, recommendations, and limitations

4.1 Conclusion

Patterns of antibiotic susceptibility to levofloxacin and ciprofloxacin among *Escherichia coli* and *Klebsiella pneumoniae* isolates show that these drugs are still effective, but resistance rates are concerning, particularly for the next few years. The most common quinolone mechanism of resistance among both *E. coli* and *K. pneumoniae* was the efflux pump mechanism, and the OqxA gene was most frequently detected. In addition, the enzymatic modification mechanism of resistance was second in frequency with the Aac(6′)-Ib-cr gene, followed by the target modification mechanism of resistance with the QnrS gene was most commonly found. *Escherichia coli* and *K. pneumoniae* ciprofloxacin resistant bacteria isolated at An-Najah National University Hospital were significantly higher than the isolates obtained from Rafidia Hospital. Ciprofloxacin resistance among *E. coli* isolated from the general surgery unit was significantly higher than that of the emergency ward and the outpatients. However, *E. coli* levofloxacin resistance frequency in the intensive care unit was significantly higher than that of the outpatients, emergency, and general surgery. *Escherichia coli* and *K. pneumoniae* isolation rate from urine was significantly higher than that of wound swabs. *Escherichia coli* ciprofloxacin resistance was significantly higher in male isolates than in female isolates. *Escherichia coli* isolated from patients over 65 years of age had the highest levofloxacin resistance frequency, which was also significantly higher than that of the 40–65-year age group. *Escherichia coli* ciprofloxacin-resistant bacterium isolated from patients with urinary catheters had a significantly higher frequency than that of hypertension patients, cancer patients, diabetes mellitus patients, and hepatic cirrhosis patients. However, *K. pneumoniae* ciprofloxacin resistant bacterium isolated from hepatic cirrhosis patients had a significantly higher frequency than that of hypertension patients, cancer patients, and diabetes mellitus patients.

4.2 Recommendations

Limiting exposure to *Escherichia coli* and *Klebsiella pneumoniae* as well as the spread of infection is crucial for both prevention and management. This can be achieved by implementing efficient infection prevention control programs that focus on controlling the transmission of infection through contact and isolation precautions. The other

component of prevention, which is successful in lowering infection rates, is antibiotic stewardship, which serves as a justification for the use of antibiotics. Additionally, increasing awareness of the methods of infection transmission and preventative measures through health education reaches everyone in the population, both individually and in institutions.

We emphasize that when choosing the right antibiotic, medical professionals need to pay closer attention to the current circumstances. In our situation, before deciding on a prescription, it appears necessary to conduct an antibiotic susceptibility test. Additionally, it is important to consider the variations in the resistance rates across various hospitals, departments, genders, age groups, sample types, and patient histories (comorbidities, chemotherapy, hospitalization, etc.).

In conclusion, it appears that to determine the most effective drug of treatment, the relevant government departments should focus more on tracking the rates of resistance in the nation's clinical facilities and improving current antibiotic stewardship programs because we are missing standardization guidelines on antibiotic use. The well-known proverb "An ounce of prevention is worth a pound of cure," sums up everything.

4.3 Limitations

We encountered several challenges while conducting this research, including the challenge of collecting samples, the challenge of getting hospital approval, the challenge of supplying the required materials for this study, and the restricted financial resources.

Generalizing the study's findings may be challenging due to the small sample size, the study's restriction to the city of Nablus, and the short sample collection period of four months. In future studies, more cases from various Palestinian cities in the Gaza Strip and the West Bank, including Ramallah, Bethlehem, Jenin, Tulkarm, Hebron, and Qalqilya, should be collected over a longer period, additionally, the relationship between patient outcomes in terms of treatment, complications, and symptoms should be examined, which make generalization easier.

List of Abbreviations

Abbreviation	Meaning
CDC	Centers for Disease Control
MDR	Multi-Drug Resistant
AMR	Antimicrobial Resistance
ECDC	European Centre for Disease Control and Prevention
DALYs	Disability-Adjusted Life Years
FQs	Fluoroquinolones
PMQR	Plasmid-Mediated Quinolone Resistance
UTI	Urinary Tract Infections
VAP	Ventilator-Associated Pneumonia
CFR	Case-Fatality Rate
WHO	World Health Organization
LPS	Lipopolysaccharide
EPEC	Enteric *Escherichia coli*
EHEC	EnteroHaemorrhagic *E. coli*
HUS	Hemolytic Uremic Syndrome
STEC	Shiga Toxin-Producing *E. coli*
ETEC	EnteroToxigenic *E. coli*
EAEC	Enteroaggregative *E. coli*
DAEC	Diffusely Adherent *E. coli*
EIEC	EnteroInvasive *E. coli*
AIEC	Adherent Invasive *E. coli*
UPEC	UroPathogenic *E. coli*
NMEC	Neonatal Meningitis *E. coli*
HAIs	Healthcare-Associated Infections
ICUs	Intensive Care Units
BSI	Bloodstream Infections
CA	Community-Acquired
CAUTIs	Catheter-Associated Urinary Tract Infections
CSF	Cerebrospinal Fluid
HGT	Horizontal Gene Transfer
ESBL	Extended-Spectrum β-Lactamase
PHE	Public Health England

Abbreviation	Meaning
ESBL	Extended-Spectrum Beta-Lactamases
XDR	Extensively Drug-Resistant
MRSA	Methicillin-Resistant Staphylococcus Aureus
FDA	Food and Drug Administration
QRDR	Quinolone Resistance Determining Regions
UN	United Nations, USA: United States of America
AST	Antimicrobial Susceptibility Testing
PCR	Polymerase Chain Reaction
MAC	MacConkey Agar
Au	Absorbance Units
ATCC	The American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
SPSS	Statistical Package for Social Science
STDs	sexually transmitted diseases
OR	Odds Ratio
CI	Confidence Interval

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Appendices

Appendix A

IRB-Approval

An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas . March 2023/16

IRB Approval Letter

Title of Research:

Quinolone Resistance among *Escherichia coli* and *Klebsiella pneumoniae* Bacteria Isolated at Rafidia and An-Najah National University Hospitals in Nablus District : Prospective Cross-Sectional study

Submitted by:
Marah Samer Khatatbeh

Supervisor:
Moatasem Al-Masri

Approved:
23rd. Mar. 2023

Your Study Title "**Quinolone Resistance among *Escherichia coli* and *Klebsiella pneumoniae* Bacteria Isolated at Rafidia and An-Najah National University Hospitals in Nablus District : Prospective Cross-Sectional study**" .reviewed by An-Najah National University IRB committee and was approved on 23rd. Mar. 2023

Hasan Fitian, MD

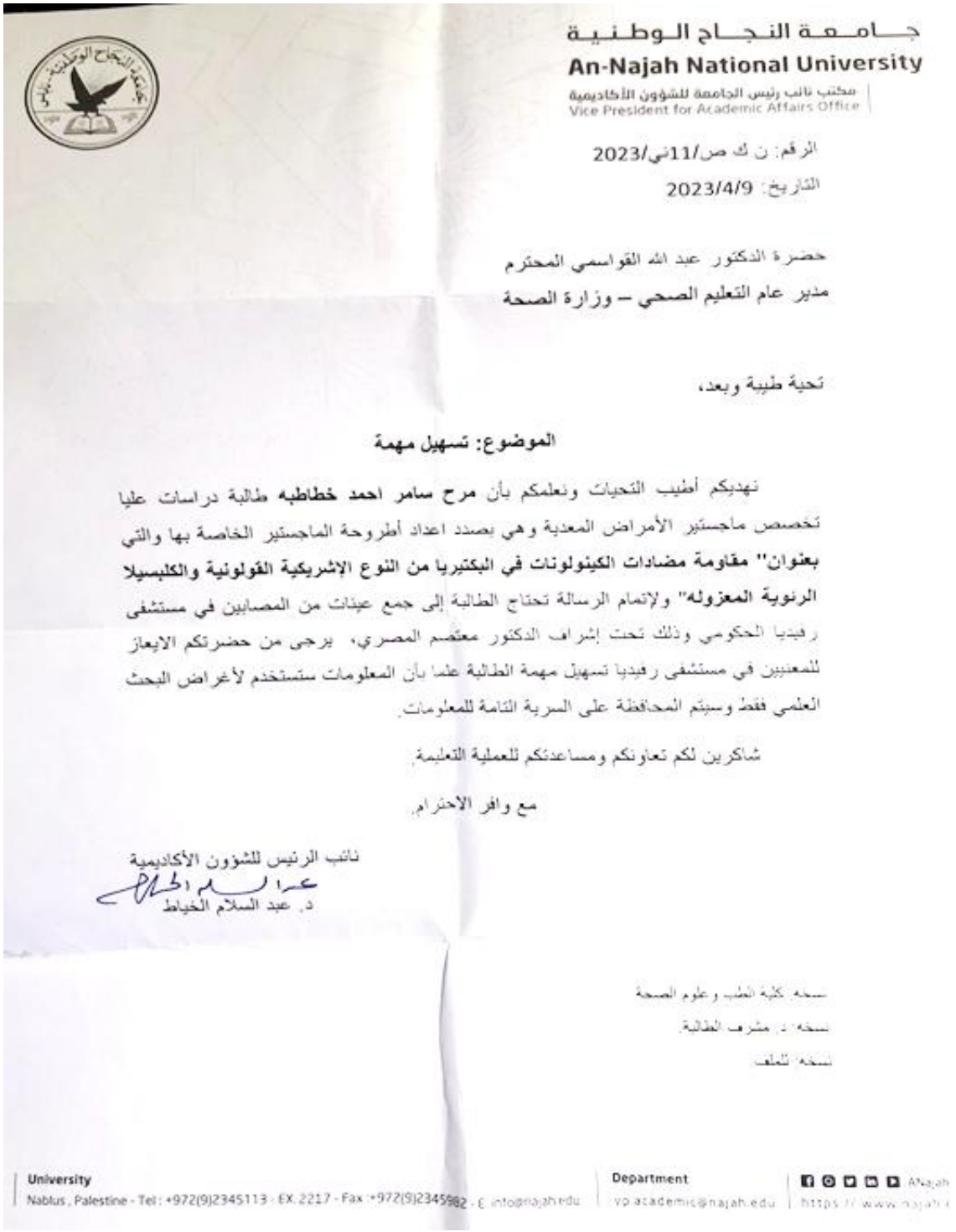
IRB Committee Chairman

IRB
Institutional Review Board

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

Appendix B

Ministry of Health Correspondence



جـــامـــعـــة النـجـــاح الـوطـنـيـة
An-Najah National University
مكتب نائب رئيس الجامعة للشؤون الأكاديمية
Vice President for Academic Affairs Office

الرقم: ن ك ص/11ني/2023
التاريخ: 2023/4/9

حضرة الدكتور عبد الله القواسمي المحترم
مدير عام التعليم الصحي – وزارة الصحة

تحية طيبة وبعد،

الموضوع: تسهيل مهمة

نهديكم أطيب التحيات ونعلمكم بأن **مرح سامر احمد خطاطبه** طالبة دراسات عليا تخصص ماجستير الأمراض المعدية وهي بصدد اعداد أطروحة الماجستير الخاصة بها والتي **بعنوان" مقاومة مضادات الكينولونات في البكتيريا من النوع الإشريكية القولونية والكلبسيلا الرئوية المعزوله"** ولإتمام الرسالة تحتاج الطالبة إلى جمع عينات من المصابين في مستشفى رفيديا الحكومي وذلك تحت إشراف الدكتور معتصم المصري، يرجى من حضرتكم الايعاز للمعنيين في مستشفى رفيديا تسهيل مهمة الطالبة علما بأن المعلومات ستستخدم لأغراض البحث العلمي فقط وسيتم المحافظة على السرية التامة للمعلومات.

شاكرين لكم تعاونكم ومساعدتكم للعملية التعليمية.

مع وافر الاحترام.

نائب الرئيس للشؤون الأكاديمية
د. عبد السلام الخياط

نسخه: كلية الطب وعلوم الصحة
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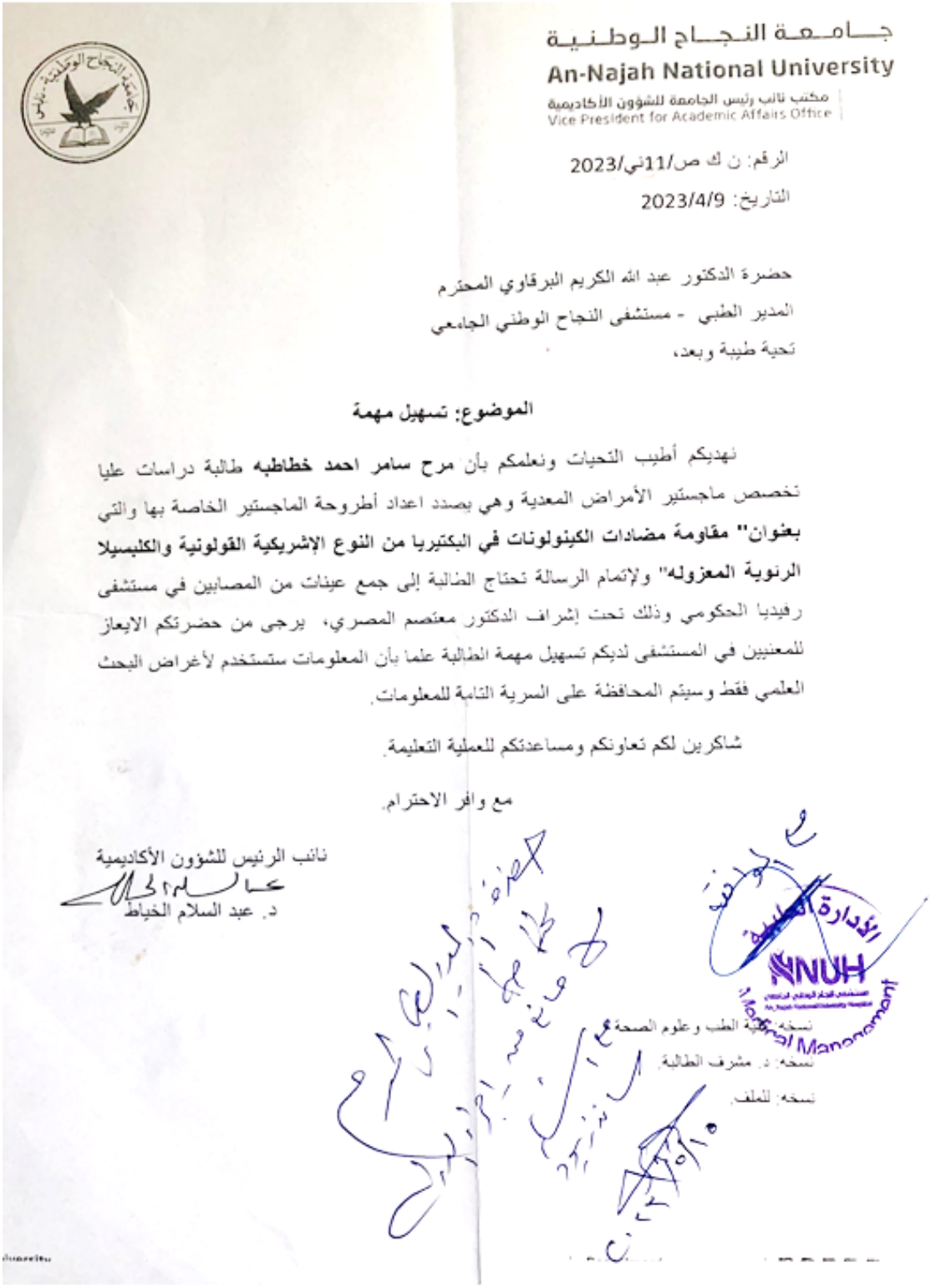
Appendix C

An-Najah National University Hospital Correspondence



جـــامـعـة النـجـــاح الـوطـنـيـة
An-Najah National University
مكتب نائب رئيس الجامعة للشؤون الأكاديمية
Vice President for Academic Affairs Office

الرقم: ن ك ص/11ني/2023
التاريخ: 2023/4/9

حضرة الدكتور عبد الله الكريم البرقاوي المحترم
المدير الطبي - مستشفى النجاح الوطني الجامعي
تحية طيبة وبعد،

الموضوع: تسهيل مهمة

نهديكم أطيب التحيات ونعلمكم بأن **مرح سامر احمد خطاطبه** طالبة دراسات عليا تخصص ماجستير الأمراض المعدية وهي بصدد اعداد أطروحة الماجستير الخاصة بها والتي **بعنوان" مقاومة مضادات الكينولونات في البكتيريا من النوع الإشريكية القولونية والكليسيلا الرئوية المعزوله"** ولإتمام الرسالة تحتاج الطالبة إلى جمع عينات من المصابين في مستشفى رفيديا الحكومي وذلك تحت إشراف الدكتور معتصم المصري، يرجى من حضرتكم الايعاز للمعنيين في المستشفى لديكم تسهيل مهمة الطالبة علما بأن المعلومات ستستخدم لأغراض البحث العلمي فقط وسيتم المحافظة على السرية التامة للمعلومات.

شاكرين لكم تعاونكم ومساعدتكم للعملية التعليمية.

مع وافر الاحترام.

نائب الرئيس للشؤون الأكاديمية
د. عبد السلام الخياط

الإدارة الطبية
NUH
Medical Management

نسخه: كلية الطب وعلوم الصحة
نسخه: د. مشرف الطالبة
نسخه: للملف

Appendix D

Questionnaire for data collection of Escherichia coli and Klebsiella pneumoniae samples

General characteristic				
Gender (Male \ Female)…….. **Name……** **Age……...**		**Hospital……..** **Ward……..** **Specimen type……...** **Type of bacteria (*E.coli* \ *K.pneumoniae*)……...** **Month of sample collection……..**		
Clinical symptoms (At the time of sample collection)	**1. Diarrhea**	**2. Fever > 38° or a history of fever and chills within 24 hours before presentation**	**3. Abdominal Pain-Stomach cramps**	
	4. Cough	**5. Bloody stool**	**6. Chest pain**	
	7. Shortness of breath	**8. Vomiting**	**9. Fatigue**	
Previous hospitalization in the last 6 months **Used any type of quinolone antibiotics in the last 6 months**	**Yes-----No** **Yes-----No**			
	Hepatic cirrhosis	**Diabetes mellitus**	**Cardiovascular disease**	**Neurogenic bladder**
Comorbidities were significantly associated with the acquisition of quinolones-resistant (QR)	**Urinary tract abnormality**	**Renal dysfunction**	**Congestive heart failure**	**Neurologic disease**
	Transplantation	**Cancer**	**Hypertension**	**Allergy**
Use one or more of these antibiotics (It has a greater impact on the emergence of resistance of Gram-negative bacteria)	Cephalosporins	Carbapenems		
Vitek result for quinolone antibiotic (ciprofloxacin)	**Susceptible**	**Intermediate**	**Resistant**	

Appendix E

Tables

Table 6

Clinical Data of Ciprofloxacin and Levofloxacin Resistant E. coli and K. pneumoniae Isolates

Variable	*Escherichia coli*			*Klebsiella pneumoniae*		
	No. isolates*	CIP-R* (%)	LEV-R* (%)	No. isolates*	CIP-R* (%)	LEV-R* (%)
• Source:						
Rafidia hospital	134	50 (37.3%)	53(39.6%)	22	9 (40.9%)	11 (50%)
Outpatients (Rafidia)	39	17 (43.6%)	22 (56.4%)	9	2 (22.2%)	3 (33.3%)
Inpatients (Rafidia)	95	33 (34.7%)	31 (32.6%)	13	7 (53.8%)	8 (61.5%)
An-Najah National University Hospital (NNUH)	35	21 (60%)	19 (54.3%)	28	26 (92.9%)	21 (75%)
Outpatients (NNUH)	13	6 (46.2%)	8 (61.5%)	11	10 (91%)	9 (81.8%)
Inpatients (NNUH)	22	15 (68.2%)	11 (50%)	17	16 (94.1%)	12 (70.6%)
• Units:						
Outpatients (All)	65	24 (36.9%)	32 (49.2%)	19	15 (78.9%)	18 (94.7%)
Inpatients (All)	104	47 (45.2%)	40 (38.5%)	31	20 (64.5%)	14 (45.2%)
General surgery	14	9 (64.3%)	6 (42.9%)	5	3 (60%)	2 (40%)
Emergency	11	6 (54.5%)	5 (45.5%)	4	2 (50%)	2 (50%)
Pediatrics	19	4 (21.1%)	6 (31.6%)	3	2 (66.7%)	1 (33.3%)
Burns	7	3 (42.9%)	2 (28.6%)	3	2 (66.7%)	2 (66.7%)
Neonates	5	2 (40%)	2 (40%)	3	1 (33.3%)	1 (33.3%)
Urology	9	3 (33.3%)	3 (33.3%)	2	2 (100%)	1 (50%)
Intensive care unit (ICU)	10	6 (60%)	5 (50%)	1	1 (100%)	1 (100%)

Internal medicine	3	1 (33.3%)	0 (0%)	2	1 (50%)	1 (50%)
Orthopedic	12	6 (50%)	5 (41.7%)	4	3 (75%)	2 (50%)
Gynecology	14	7 (50%)	6 (42.9%)	4	3 (75%)	1 (25%)
• Specimen:						
Urine	116	51 (44%)	55 (47.4%)	29	19 (65.5%)	15 (51.7%)
Wound swab	45	14 (31.1%)	10 (22.2%)	14	10 (71.4%)	13 (92.9%)
Nasal swab	1	1 (100%)	1 (100%)	0	0 (0%)	0 (0%)
Fluid	2	1 (50%)	1 (50%)	0	0 (0%)	0 (0%)
Ear swab	0	0 (0%)	0 (0%)	1	1 (100%)	1 (100%)
Cerebrospinal fluid (CSF)	1	1 (100%)	1 (100%)	0	0 (0%)	0 (0%)
Skin graft	1	1 (100%)	1 (100%)	0	0 (0%)	0 (0%)
Blood	3	2 (66.7%)	3 (100%)	4	3 (75%)	2 (50%)
Sputum	0	0 (0%)	0 (0%)	2	2 (100%)	1 (50%)
Total	169	71 (42%)	72 (42.6%)	50	35 (70%)	32 (64%)
• Gender:						
Male	56	30 (53.6%)	25 (44.6%)	22	14 (63.6)	10 (45.5%)
Female	113	41 (36.3%)	47 (41.6%)	28	21 (75%)	22 (78.6%)
• Age groups:						
0-2 years	20	10 (50%)	12 (60%)	6	3 (50%)	4 (66.7%)
3-14 years	32	14 (43.8%)	13 (40.6%)	3	1 (33.3%)	1 (33.3%)
15-39 years	51	19 (37.3%)	20 (39.2%)	15	11 (73.3%)	12 (80%)
40-65 years	42	16 (38.1%)	12 (28.6%)	18	15 (83.3%)	11 (61.1%)
> 65 years	24	12 (50%)	15 (62.5%)	8	5 (62.5%)	3 (37.5%)

• Clinical symptoms:						
Diarrhea	47	32 (68.1%)	35 (74.5%)	9	8 (88.9%)	8 (88.9%)
Fever >38	28	25 (89.3%)	21 (75%)	13	11 (84.6%)	9 (69.2%)
Abdominal Pain	32	29 (90.6%)	27 (84.4%)	5	4 (80%)	4 (80%)
Cough	9	7 (77.8%)	5 (55.6%)	31	25 (80.6%)	29 (93.5%)
Bloody stool	13	8 (61.5%)	11 (84.6%)	3	2 (66.7%)	2 (66.7%)
Chest pain	5	4 (80%)	2 (40%)	27	22 (81.5%)	25 (92.6%)
Shortness of breath	7	5 (71.4%)	5 (71.4%)	18	16 (88.9%)	13 (72.2%)
Vomiting	17	13 (76.5%)	16 (94.1%)	7	4 (57.1%)	6 (85.7%)
Fatigue	28	19 (67.9%)	22 (78.6%)	20	13 (65%)	17 (85%)
• Comorbidities:						
Hepatic Cirrhosis	39	25 (64.1%)	20 (51.3%)	27	21 (77.8%)	16 (59.3%)
Diabetes Mellitus	46	39 (84.8%)	41 (89.1%)	39	23 (58.9%)	28 (71.8%)
Cardiovascular Disease	22	18 (81.8%)	15 (68.2%)	13	7 (53.8%)	5 (38.5%)
Neurogenic Bladder	16	12 (75%)	9 (56.3%)	8	2 (25%)	3 (37.5%)
Transplantation	12	8 (66.7%)	5 (41.7%)	7	4 (57.1%)	4 (57.1%)
Renal Dysfunction	29	17 (58.6%)	22 (75.9%)	20	8 (40%)	12 (60%)
Congestive Heart Failure	7	3 (42.9%)	2 (28.6%)	5	2 (40%)	1 (20%)
Neurologic Disease	11	5 (45.5%)	9 (81.8%)	14	6 (42.9%)	6 (42.9%)
Urinary Catheter	36	33 (91.7%)	28 (77.8%)	25	16 (64%)	12 (48%)
Cancer	72	59 (81.9%)	53 (73.6%)	42	26 (61.9%)	22 (52.4%)
Allergy	23	13 (56.5%)	16 (69.6%)	19	9 (47.4%)	11 (57.9%)
Hypertension	76	65 (85.5%)	58 (76.3%)	46	30 (65.2%)	26 (56.5%)
Total	169	71 (42%)	72 (42.6%)	50	35 (70%)	32 (64%)

* No. isolates: Number of isolates; CIP-R: Ciprofloxacin resistant; LEV-R: Levofloxacin resistant

جامعة النجاح الوطنية
كلية الدراسات العليا

مقاومة مضادات الكينولونات في البكتيريا من النوع الإشريكية القولونية والكلبسيلا الرئوية المعزولة في مستشفى رفيديا ومستشفى النجاح الوطني الجامعي في مقاطعة نابلس: دراسة مستقبلية مقطعية

إعداد
مرح سامر أحمد خطاطبة

إشراف
د. معتصم المصري

قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجه الماجستير في مكافحة وضبط العدوى، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس – فلسطين

2024

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الملخص

الخلفية: زادت مقاومة الكينولونات في السنوات الأخيرة، وخاصة بين مسببات الأمراض الشائعة المكتسبة من المستشفيات مثل الإشريكية القولونية والكلبسيلا الرئوية. وعلى الرغم من كونها عوامل رئيسية في العلاجات المضادة للميكروبات، فإن الكينولونات معرضة لخطر انخفاض الفعالية بسبب المقاومة المتزايدة.

الأهداف: هدفت هذه الدراسة إلى التحقيق في مقاومة الكينولونات في عزلات الإشريكية القولونية والكلبسيلا الرئوية من مستشفيين في محافظة نابلس في فلسطين، بين يونيو وسبتمبر 2023. وكانت الأهداف الأساسية هي تحديد انتشار مقاومة السيبروفلوكساسين والليفوفلوكساسين، واستكشاف الارتباطات بالعوامل الديموغرافية والسريرية، وتحديد آليات المقاومة.

المنهجية: تم تحليل ما مجموعه 219 عزلة بكتيرية، تتكون من 50 سلالة من الكلبسيلا الرئوية و169 سلالة من الإشريكية القولونية. تم تقييم مقاومة السيبروفلوكساسين والليفوفلوكساسين باستخدام الانتشار القرصي، وتم تحديد آليات المقاومة من خلال تفاعل البوليميراز المتسلسل المتعدد. تم استخدام نظام VITEK 2 (BioMérieux) لتحديد الأنواع البكتيرية في كلا المستشفيين. تم اختيار تصميم دراسة مقطعية.

النتائج: أظهرت الإشريكية القولونية مقاومة بنسبة 46.1% للسيبروفلوكساسين و47.9% لليفوفلوكساسين، في حين أظهرت الكلبسيلا الرئوية مقاومة أعلى، بنسبة 70% للسيبروفلوكساسين و68% لليفوفلوكساسين. كانت معدلات المقاومة أعلى بشكل ملحوظ في مستشفى النجاح الوطني الجامعي مقارنة بمستشفى رفيديا. أظهر المرضى الأكبر سنًا (فوق 65 عامًا) زيادة في مقاومة الليفوفلوكساسين، وخاصة في الإشريكية القولونية. كان لدى المرضى الذين لديهم قسطرة بولية تواتر أعلى من عزلات الإشريكية القولونية المقاومة للسيبروفلوكساسين. ارتبطت الحالات المزمنة، مثل تليف الكبد والسكري، بمقاومة أعلى للكلبسيلا الرئوية. في الإشريكية القولونية، كانت مضخات التدفق هي آلية المقاومة الأكثر شيوعًا 0.43%، مع انتشار جين OqxA، يليه التعديل الأنزيمي 0.29% وتعديل الهدف 26%. بالنسبة للكلبسيلا الرئوية، كانت مضخات التدفق (37.8% وOqxA هي الآلية السائدة أيضًا، تليها جينات Aac(6')-Ib-cr وQnrB , QnrS المسؤولة عن التعديلات الأنزيمية والهدف.

الخلاصة: تسلط هذه الدراسة الضوء على أهمية اختبار حساسية المضادات الحيوية الروتينية ومراقبة مقاومة الكينولونات. وتؤكد على الحاجة إلى تحسين المراقبة والإدارة المستهدفة للمضادات الحيوية لمكافحة مقاومة الكينولونات في هذه المستشفيات.

الكلمات المفتاحية: الإشريكية القولونية، الكلبسيلا الرئوية، مقاومة الكينولونات، الجينات المقاومة، منطقة نابلس.