# NEAR EAST UNIVERSITY

**TURKISH REPUBLIC OF NORTHERN CYPRUS**

**NEAR EAST UNIVERSITY**

**HEALTH SCIENCES INSTITUTE**

# DETERMINATION OF FECAL CARRIAGE RATES OF FLUOROQUINOLONE-RESISTANT *ENTEROBACTERIACEAE* IN HOSPITAL AND COMMUNITY SETTINGS IN NORTHERN CYPRUS

**ABDALLAH SULEIMAN AHMAD ABUZAID**

**MASTER OF SCIENCE THESIS**

**MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM**

**2020 – NICOSIA**


TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

# DETERMINATION OF FECAL CARRIAGE RATES OF FLUOROQUINOLONE-RESISTANT *ENTEROBACTERIACEAE* IN HOSPITAL AND COMMUNITY SETTINGS IN NORTHERN CYPRUS

ABDALLAH SULEIMAN AHMAD ABUZAID

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

2020 – NICOSIA

TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

# DETERMINATION OF FECAL CARRIAGE RATES OF FLUOROQUINOLONE-RESISTANT *ENTEROBACTERIACEAE* IN HOSPITAL AND COMMUNITY SETTINGS IN NORTHERN CYPRUS

ABDALLAH SULEIMAN AHMAD ABUZAID

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

SUPERVISOR

Assoc. Prof. Dr. EMRAH RUH

2020 – NICOSIA

**Thesis Title: Determination of Fecal Carriage Rates of Fluoroquinolone-Resistant *Enterobacteriaceae* in Hospital and Community Settings in Northern Cyprus.**

**Name of the Student:** Abdallah Suleiman Ahmad Abuzaid

**Supervisor:** Assoc. Prof. Dr. Emrah Ruh

**Department:** Medical Microbiology and Clinical Microbiology

## ABSTRACT

**Aim:** The present study was conducted to determine the fecal carriage rates of fluoroquinolone-resistant *Enterobacteriaceae* in hospital and community settings in Northern Cyprus.

**Materials and Methods:** A total of 180 participants (80 hospitalized patients and 100 community-dwellers) were included in this study. Stool sample was collected from each participant. A questionnaire was performed in order to evaluate the potential risk factors that are associated with the colonization of resistant bacteria. Media that contained ciprofloxacin (CIP) at concentration of 1 mg/L were used in order to screen for the colonization of CIP-resistant *Enterobacteriaceae* (CIP-RE). The bacterial isolates were further subjected to disc diffusion test in order to confirm the resistance.

**Results:** In the study, 52 (28.9%) of 180 individuals were found to be colonized with CIP-RE. Resistance rates of CIP-RE isolates to ofloxacin, norfloxacin, levofloxacin and gemifloxacin were 94.2%, 98.1%, 61.5%, and 98.1%, respectively. According to the statistical analysis, age ($p$=0.021) and marital status ($p$=0.035) of the participants were found to be significant factors for the fecal carriage of CIP-RE. Also, a significant association was found between the history of urinary tract infection and intestinal colonization of CIP-RE ($p$=0.011). Moreover, a significant correlation was found between the use of fluoroquinolones in the current hospitalization and fecal carriage of CIP-RE isolates ($p$=0.019).

**Conclusion:** This study indicates that, intestinal colonization with fluoroquinolone-resistant *Enterobacteriaceae* can occur in both hospital and community settings, therefore, control measures should be implemented to prevent further spread of resistant bacteria in Northern Cyprus.

**Key Words:** Fecal Carriage, *Enterobacteriaceae*, Fluoroquinolone, Ciprofloxacin, Antibiotic Resistance.

**Tez Başlığı: Kuzey Kıbrıs'ta Florokinolon Dirençli *Enterobacteriaceae* Türlerinin Hastane ve Toplumdaki Dışkıda Taşıyıcılık Oranlarının Belirlenmesi**

**Öğrencinin Adı:** Abdallah Suleiman Ahmad Abuzaid

**Danışmanı:** Doç. Dr. Emrah Ruh

**Anabilim Dalı:** Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji

## ÖZET

**Amaç:** Bu çalışma florokinolon dirençli *Enterobacteriaceae* türlerinin Kuzey Kıbrıs'ta hastane ve toplumdaki fekal taşıyıcılık oranlarının belirlenmesi amacıyla yapılmıştır.

**Gereç ve Yöntem:** Çalışmaya toplam 180 katılımcı (hastanede yatan 80 hasta ve toplumda yaşayan 100 birey) dahil edilmiştir. Her katılımcıdan dışkı örneği toplanmıştır. Dirençli bakteri kolonizasyonu ile ilişkili olan risk faktörlerinin değerlendirilmesi için bir anket de uygulanmıştır. Siprofloksasine dirençli *Enterobacteriaceae* (CIP-RE) kolonizasyonunun taranması amacıyla 1 mg/L konsantrasyonda siprofloksasin (CIP) içeren besiyerleri kullanılmıştır. Direncin konfirme edilmesi için bakteri izolatlarına daha sonra disk difüzyon testi uygulanmıştır.

**Bulgular:** Çalışmada 180 kişiden 52 (%28,9)'sinin CIP-RE ile kolonize olduğu saptanmıştır. CIP-RE izolatlarının ofloksasin, norfloksasin, levofloksasin ve gemifloksasine direnç oranları sırasıyla %94,2, %98,1, %61,5, ve %98,1 olarak bulunmuştur. İstatistiksel değerlendirme sonucunda, katılımcıların yaş ($p$=0.021) ve medeni durumunun ($p$=0.035) CIP-RE fekal taşıyıcılığı açısından anlamlı faktörler oldukları tespit edilmiştir. Ayrıca, idrar yolu enfeksiyonu öyküsü ile CIP-RE intestinal kolonizasyonu arasında anlamlı bir ilişki bulunmuştur ($p$=0.011). Hastanede yatış sırasında florokinolon kullanımı ile CIP-RE izolatlarının fekal taşıyıcılığı arasında da anlamlı bir ilişki bulunmuştır ($p$=0.019).

**Sonuç:** Bu çalışma florokinolon dirençli *Enterobacteriaceae* türlerinin intestinal kolonizasyonunun hem hastane hem de toplumda görülebileceğini, bu nedenle, Kuzey Kıbrıs'ta dirençli bakterilerin yayılımının engellenmesi için kontrol önlemlerinin uygulanması gerektiğini göstermektedir.

**Anahtar Kelimeler:** Fekal Taşıyıcılık, *Enterobacteriaceae,* Florokinolon, Siprofloksasin, Antibiyotik Direnci.

# 1. INTRODUCTION AND AIM

Antibiotic resistance has become a global issue which occurs quickly and become a threat to the use of antibiotics to treat bacterial infections (Ventola, 2015). Despite the initial use of antibiotics effectively in the treatment of many bacterial diseases decades ago, resistance emerged not long after the discovery of the agents (Da silva et al., 2007; Ventola, 2015). Studies indicated that antibiotic-resistant infections can cause over 10 million deaths yearly by 2050 if the problem is not solved (Jasovský et al., 2016). Antibiotic resistance is a term that refers to when bacteria get used to an antibiotic which leads to that it would no longer response to it (Davies and Davies, 2010). Use, misuse and abuse of antibiotic agents have been blamed for the development and spread of drug-resistant microscopic organisms. Emergence of resistance was not a surprise as it was predicted soon after the discovery of the antibiotics which affected the treatment of many diseases and conditions such as surgery and other bacterial infectious agents of humans and animals (Livermore, 2003).

Hospital settings have been recognized as the breeding grounds for the wide-spread of antibiotic resistance. This is because of the continuous use of antibiotics in the hospitals, and also presence of antibiotics in the wastewater which subsequently may get into environment and humans (Adegoke et al., 2017).

It has been observed that the recent increase of antimicrobial resistance among the members of *Enterobacteriaceae* as fecal carriage is becoming a global health concern (Huang et al., 2018). Focusing has been on the monitoring of antibiotic resistance among pathogenic bacteria, however, there is an increasing interest in profiling commensal bacteria, which are considered significant reservoir of antibiotic resistance genes which could easily disseminate to pathogenic bacterial strains (Huang et al., 2018).

There have been studies that indicated such bacteria are presented as multidrug-resistant organisms some of which require extensive treatment of antibiotics, hence

infections caused by such bacteria require expensive antibiotics and subsequently would contribute to high mortality and increased hospital budgets (Bradford, 2001; Abdallah et al., 2017). The fecal carriage of resistant bacteria occurs in both hospital and community settings (Lukak et al., 2015).

In a cross-sectional study by Maslow et al. (2005), in a particular long-term care health setting to assess the prevalence and colonization rate of fluoroquinolone-resistant *Escherichia coli*, the result revealed that a significant number of the participants harbored fluoroquinolone-resistant *E. coli* 51% (25/49). In a similar study on patients receiving quinolone treatment in Ankara, Turkey, a total of 150 patients were enrolled to assess the rate and risk factors of the fecal carriage of quinolone-resistant *E. coli*. The study indicated an increase in the quinolone-resistant *E. coli* strains in fecal flora with an increase of quinolone treatment. This justifies the fact that excessive exposure to treatment of antibiotic is a risk factor of increase of resistance rate (Yagci et al., 2009). In another comparative work to assess the fluoroquinolone-resistant *E. coli* and *Klebsiella pneumoniae* among adults who have previously used the antibacterial agent and children without the history of the antibiotic administration, surprisingly, even the children with no clear history of quinolones administration was reported to carry fluoroquinolone-resistant *Enterobacteriaceae*. This could be attributed to the wide spread of resistant isolates in the environment (Huang et al., 2015). *Enterobacteriaceae* are known to exhibit resistance to a high number of antibiotics including fluoroquinolones via well-known mechanisms such as a mutation in the genes encoding for DNA-gyrase or topoisomerase IV which lead to decreased binding to quinolones (Rodriguez-Martinez et al., 2011; Ruiz, et al., 2012). The authors further added that another important mechanism is achieved via plasmid-mediated fluoroquinolone resistance (PMQR) which includes Qnr (QnrA, QnrB, QnrC, QnrD) and AAC(6')-Ib-cr enzymes (Rodriguez-Martinez et al., 2011; Ruiz, et al., 2012).

Resistance of *Enterobacteriaceae* to fluoroquinolones is not limited to the isolates from hospital or clinical origin but also isolates from community, hence community-acquired *Enterobacteriaceae* can also exhibit resistance to fluoroquinolones.

Antibiotics and antibiotic-resistant bacteria disseminate into community through surface water, ground water, drinking water, human waste, animal waste as well as other agricultural practices (Costanzo et al., 2005). Furthermore, use of antibiotics facilitates the occurrence and spread of antibiotic-resistant bacteria in the community. Previous studies revealed that fecal carriages of the isolates that are resistant to fluoroquinolones are common in the community (Maslow et al., 2004; Lautenbach et al., 2005).

*Enterobacteriaceae* isolates that are resistant to fluoroquinolones have been reported worldwide, hence this poses a threat to treatment of infections related to such pathogens (Lautenbach et al., 2005; Yagci et al., 2009).

Studies are conducted for understanding the occurrence, colonization rate and antibiotic resistance profiles of fluoroquinolone-resistant *Enterobacteriaceae* across the world. Thus, the primary aim of this study was to detect the colonization rates of fluoroquinolone-resistant *Enterobacteriaceae* in hospital and community settings in Nicosia, Northern Cyprus. The secondary purpose of the study was to evaluate the possible risk factors that are associated with the intestinal colonization of fluoroquinolone-resistant *Enterobacteriaceae* isolates.

# 2. GENERAL INFORMATION

## 2.1. *Enterobacteriaceae*

*Enterobacteriaceae* is a family of large genera that share similarities biochemically, genetically and even to some extend morphologically. These qualities allowed them to be grouped in the same family. The most known members are *E. coli, Klebsiella, Enterobacter, Salmonella, Shigella, Proteus, Citrobacter, Providencia, Serratia, Hafnia,* and *Yersinia* (Walker et al., 2018). The microbiological features of these organisms include being gram-negative, short rods, non-sporulating, and facultative anaerobes. These sets of organisms cause diseases ranging from primary infections to opportunistic infections (Walker et al., 2018). As the name depicts, these organisms are common colonizers of the gastrointestinal tract (GIT) which play a vital role in managing human wellness, and in animals they also form part of their normal flora. Important to note *Enterobacteriaceae* are also commonly spread in the environment. Studies also showed that some of these members can clearly be pathogenic by causing infections in GIT and urinary tract; they are also implicated in infections of central nervous system and bloodstream infections (Brolund et al., 2010). *Enterobacteriaceae* species cause number of diseases with much impact on general public health. For instance, nosocomial infections are mainly caused by , *K.pneumoniae,* and *Proteus* spp. (Xia et al., 2016).

### 2.1.1. *Escherichia coli*

These organisms are found in human gut and are generally harmless but some cause diarrhea due to the ingestion of contaminated water or food. The causing agents of diarrhea such as enteropathogenic *E. coli* (EPEC) leads to infant diarrhea, others include enterotoxigenic *E. coli* (ETEC) which cause diarrhea that resembles cholera, and furthermore they result in diarrhea associated with travellers. Another type is enteroinvasive *E. coli* (EIEC) that remarkably resembles *Shigella* species in terms of

disease, dysentery. Enterohemorrhagic *E. coli* (EHEC) especially serotype O157:H7 is at the control for causing a bloody diarrhea and hemorrhagic colitis. These organisms can spread systemically via bloodstream leading to hemolytic-uremic syndrome, a condition that leads to kidney failure and fatal outcome (Sharif et al., 2017).

*E. coli* is known to have O antigen (lipopolysaccharide) and H antigen (flagellar) that determines its serotypes. On this basis, *E. coli* is grouped into four broad phylogenetic groups, which are A, B1, D and B2. The commensal strains of *E. coli* are usually found in the A and B1 phylogenetic groups. The specific pathogenic lineages responsible for extra intestinal infections are mainly derived from B2 group (Kaper et al., 2004).

### 2.1.2. *Klebsiella pneumoniae*

*K. pneumoniae* is predominantly found in soil and water environment but it also colonizes human gut. In fact it is one of the first invaders of gut of the newborn but less commonly associated with the flora of adults (Kumarasamy et al., 2012). Arnold et al. (2004) describe this important organism as gram-negative, nonmotile, rod-shaped, facultative anaerobic, lactose-fermenting, and encapsulated bacterium. Despite being part of the normal flora, it can also perturb both human and animal lungs if aspirated, reach to the alveoli causing bloody sputum especially among immunosuppressed people (Arnold et al., 2004).

*K. pneumoniae* gains attention as the most significant member of the genus *Klebsiella* in *Enterobacteriaceae* for its ability to cause nosocomial infections. There are also other species that play important role in clinical settings, such as *K. oxytoca* and *K. rhinoscleromatis* (Tang et al., 2017).

There is an increased interest on *K. pneumoniae* recently due to its ability to become more resistant to broad-spectrum antibiotics. This bacterium has the ability to produce enzymes that confers resistance to broad-spectrum antibiotics such as fluoroquinolones and beta-lactams. Particularly, it can also produce extended-spectrum

beta-lactamase enzymes (ESBLs) and become highly resistant against different beta-lactam antibiotics. Thus, production of such enzymes will break down beta-lactam class of antibiotics leading to difficulty in treatment (Dsouza et al., 2017).

The infections develop in humans regardless of age, but infants are known to be at high risk, together with elderly and immunocompromised people. The initial pathogenesis is achieved by the presence of cell wall receptors, the capsular polysaccharide and endotoxin. Several studies reported that capsule is the significant virulence factor that facilitates the pathogenesis in pathogenic *Klebsiella* species (Chang et al., 2000; Turton et al., 2008; Lin et al., 2014).

In a previous study, Turton et al., (2010) emphasized that this polysaccharide capsule acts as a barrier and helps in evasion of phagocytosis. Furthermore, another study revealed that acute and fatal infection caused by *K. pneumoniae* occurred due to the capsular serotype K2 (Turton et al., 2010).

### 2.1.3. *Serratia*

This genus is another member of *Enterobacteriaceae* that shares similar characteristics with each other both phenotypically and on DNA sequence. Microbiological properties of the organism include being gram-negative with length of 0.9-2.0 µm surrounded with peritrichous flagella and it tends to be facultative anaerobe with two system of metabolisms; respiratory and fermentative pathways. Most species are indole-negative with the exception of *S. odorifera* strains, citrate-positive and most strains have ability to produce DNase and hydrolyze gelatin (Berlanga and Viñas 2000). Biochemically, this bacterium does not hydrolyze urase or does not have the ability to produce $H_2S$, however, they have ability to reduce nitrate and ferment carbohydrates such as sucrose and, maltose. *Serratia* species are found in various habitats and they can be isolated from water, plants, mammals, and most importantly on hospitalized patients (Hu and Zhao, 2009).

*S. marcescens* is a classical opportunistic pathogen that results in infections among immunosuppressed individuals. Such pathogenesis is achieved via a number of pathogenicity factors (Hu and Zhao, 2009).

Kurz et al. (2003) describes that the fimbriae of *Serratia* species are proteinous sets of appendages that are characteristically thinner and shorter than a flagellum. The authors stated that there are five types of fimbriae which are associated with the genus and each has ability to produce at least one to three types of hemaglutinin. There exist type l fimbriae that can produce mannose-sensitive hemaglutinin (MS-HA) which can be found in most of the strains of *S. marcescens* regardless of the source; environments or clinical isolates. The presence of such type of fimbriae gives advantage to the organisms and capacity to attach to host cells during pathogenesis, however such type is commonly found in clinical isolates than in environmental isolates (Kurz et al, 2003).

Another important virulence determinant produced by *Serratia* is siderophore which can be produced by both clinical and environmental isolates of *S. marcescens.* Specifically, they produce enterobactin (Abbas and Hegazy, 2017).

Other virulence factors include O antigens which are situated in lipopolysaccharide surface, and particularly O16 and O14 are associated with *S. marcescens* infections. Extracellular enzymes also play significant role in the pathogenesis of these species. For instance, it was demonstrated by previous studies that extracellular enzymes are implicated in the development of pneumonia and keratitis (Fraga et al., 2007).

### 2.1.4. *Proteus*

Species of *Proteus* are members of *Enterobacteriaceae* family which are gram-negative bacilli; they are mostly seen in human intestinal tract as being part of normal flora (Armbruster et al., 2018). It inhabits many environments which include soil, long-term care facilities and emergency clinics, water and sewage, but it is found mainly as a commensal of GIT (Adeolu et al., 2016). *Proteus* has ability to cause different infections

owing to its ability to form biofilms which also increases its resistance profile. The infections include wound, eye and GIT infections, and most commonly urinary tract infections (UTI) that are observed in catheterized patients (Jacobsen et al., 2008). The genus *Proteus* consists of five species: *P. mirabilis, P. vulgaris, P. hauseri, P. penneri* and *P. myxofaciens.* Among these, *P. myxofaciens* has less importance as a human pathogen (Kalra et al., 2011).

*Proteus* species are opportunistic bacterial pathogens which under perfect conditions result in UTI, mainly affecting the upper urinary tract, causing infections like stone formation in kidney or bladder (urolithiasis), acute pyelonephritis and cystitis. Unusual cases of bacteremia, related to UTI, caused by *Proteus* spp. have been described by Kalra et al (2011). The authors further expand on infections such as sepsis and wound infections, meningitis in neonates or infants, and rheumatoid arthritis. In the same trend, other studies reported endocarditis and brain abscesses caused by *Proteus* species (Fujihara et al., 2011; Rozalski et al., 2012; Mohammed et al., 2016).

The microbiological characteristic that is known for these organisms is the swarming nature that appears as concentric rings of growth coming from a single colony or inoculum. Understanding this at cellular level, it can be proven to the bacterial modification from "swimmer cells" in broth to "swarmer cells" on a surface like agar, in a process that involve cellular elongation and increased flagellin synthesis (Gibbs et al., 2008).

A virulence factor, the uroepithelial cell adhesin (UCA), was initially discovered in an uropathogenic isolate of *P. mirabilis* which helps the organism with increased binding to uroepithelial cells (Ronald, 2003). Also, Fraser et al. (2004) showed that flagella-negative mutant strains of *P. mirabilis* was not as virulent as the flagellated strains, and this indicated the role of flagella in the pathogenesis of the organism. In a recent detailed explanation of these virulence factors, Armbruster et al. (2018) stated that the bacterial adherence to epithelial cells and catheter was accomplished by 17 various fimbriae, while MR/P fimbriae was common among these.

### 2.1.5. *Enterobacter*

This genus consists of gram-negative, facultative anaerobic bacteria which are motile due to their flagella. Another important feature of these organisms is their ability to synthesize an enzyme called ornithine decarboxylase. The presence of this enzyme serves as a differential characteristic with the closely related *Enterobacteriaceae* member *Klebsiella* (Akingbade et al., 2013). Its name originates from the fact that it is mostly found in intestine of animals. The presence of the organisms in intestines made it more likely to be found in soil and water, and moreover, they are also found in plants. On human context, they are opportunistic pathogens and the species include *Enterobacter cloacae, E. aerogenes, E. gergoviae* and *E. agglomerans* (Akingbade et al., 2013).

*Enterobacter* may produce severe diseases such as disease in abdomen, urinary tract, and meningeal, eye, bone, lower respiratory tract and surgical site infections. Another importance of these organisms is the appearance of ESBL and AmpC enzymes, which result in emergence of multidrug resistance (Hong et al., 2012).

*Enterobacter* spp. have either type 1 or type 3 mannose sensitive hemagglutinins, and unusually make mannose resistant hemagglutinin. Another factor they use is their ability to produce many types of siderophores including the hydroxamate siderophore aerobactin that is repeatedly associated with microbial types which generate invasive infection. In addition, the organisms have the ability to produce several toxins, however the toxins may be found in single strains or few numbers of the isolates (Kim et al., 2008; Akingbade et al., 2013).

### 2.1.6. *Citrobacter*

*Citrobacter* spp. are present in both human and animals feces as an element of the normal flora. Microscopically, *Citrobacter* spp. exist in rods that are organized either in single or pairs or both. They are motile because of the presence of pertrichous flagella. Colonies in general appear grey, flat and slightly wet, but they can also exist as

mucoid or rough strains (Ranjan and Ranjan, 2013). Biochemically, some strains of this genus may give agglutination in the presence of *Salmonella* polyvalent antisera, and this could possibly hinder the identification and give a wrong diagnosis. *Citrobacter* is positive for catalase, indole, and nitrate reduction tests (Kumari et al., 2018).

### 2.1.7. *Salmonella*

One of the characteristics of this species is its ability to ferment glucose and mannose but not lactose or sucrose. They can readily grow on simple media as well as selective media which contain suppressors of other coliforms (Cheesbrough, 2006).

This organism shares characteristic with other *Enterobacteriaceae* in terms of possessing O and H antigens, however, some members exhibit capsular antigens called Vi which is associated with high virulence capacity. The presence of H antigens and flagella turn the organism to motile and losing of O antigen results in change of a colony from smooth to rough. Additionally, the bacterial colony that has Vi antigen may be lost either partly or totally (Adeleke et al., 2006).

*Salmonella* can be infective to man and animals but the most common infective species in humans include *S. typhi* and *S. paratyphi.* The main source of transmission to humans is via consumption of contaminated food or water. The disease presentations in humans are in three forms; enteric fever, septicemia and gastroenteritis, however, mixed presentation is also possible (Hardjo, 2017). The infectious dose that is required to establish clinical outcomes ranges from 1000 to 1 million organisms. With an average incubation period of one to two weeks after ingestion of the organism, it can bypass the acidic condition of stomach and invade intestinal epithelium via the Peyer's patches. This will result in penetration and translocation into the intestinal lymphoid follicles and subsequently to other organism. The organism can be situated in the reticuloendothelial sites of liver and spleen and then spread resulting in secondary bacteremia (Hardjo, 2017).

### 2.1.8. *Shigella*

*Shigella* is the etiological agent of dysentery, and it is a gram-negative facultatively intracellular pathogen. These members of *Enterobacteriaceae* are grouped into four species which consist of *S. dysenteriae*, *S. flexneri*, *S. boydii,* and *S. sonnei*, and are sometimes grouped as A, B, C, and D respectively based on their O-polysaccharide antigen sequencing (Prabhurajeshwar and Kelmani, 2018). They are characteristically non-motile and non-spore forming and also non-encapsulated. These bacteria differ from closely related member of the *Enterobacteriaceae, E. coli*, due to the pathogenicity, physiology, specifically by their inability to use lactose, and using serological techniques. For the cultures, specific or differential media, like MacConkey, Xylose Lysine Deoxycholate (XLD), and Salmonella-Shigella media can be used (Teneja 2007).

The disease caused by the organism is shigellosis which is known as an issue globally, with mortality especially reported in countries with less cleanliness and high population density (Taneja et al., 2012). Like the other members of *Enterobacteriaceae, Shigella* strains are also implicated with multidrug resistance globally. Over the years, there is an increasing rise in the emergence of resistance by *Shigella* species which endangers the treatment possibilities of shigellosis, however, the distribution of the resistance varies according to the geographic location, thus it is important to check serotype differences (Bhattacharya et al., 2012).

## 2.2. Antibiotics: Mechanisms of Action and Resistance

In a historical perspective, the discovery of antibiotics in the late 1920's dramatically changed the way human diseases are treated as well as revolutionized the the modern medicine. The discovery of penicillin, which has saved millions of lives, was made by scientist Alexander Fleming in 1928. One of the earliest researchers in the field was Selman Waksman, who defined the term "antibiotic" as a substance that is capable of killing bacterial infectious agents (salabi and Allaaeddin, 2011; Larsson, 2014). In a wider definition, Kummerer (2009) simply defines antibiotics as organic molecules that

can kill microbes or stop the growth by acting on a specific target on bacteria. Hence, such agents can be used in targeting the bacterial structure or its metabolic system. This is possible because there are differences between bacteria and humans, the former being prokaryotes while the latter eukaryotes (Kummerer, 2009).

Antibiotics in general are important in treating bacterial infections in humans and animals; therefore it is important to preserve their efficacy (Allen et al., 2010). Generally, antibiotics that share structural similarities are known to exhibit similar mode of action, level of effectiveness, and coverage of organisms. Furthermore, antibiotics are grouped as broad-spectrum that can act on wide range organisms; or as narrow-spectrum which target a limited group of bacteria. Antibiotics can either cause cell death (bactericidal drugs) or only stop cell growth (bacteriostatic drugs) (Allen et al., 2010; Kohanski et al., 2010).

The targets of antibiotics are the bacterial cell wall (cell wall inhibitors), cell membrane (cell membrane synthesis inhibitors), protein synthesis (protein synthesis inhibitors), nucleic acid (nucleic acid synthesis inhibitors) and metabolic pathway (anti-metabolites) (salabi and Allaaeddin, 2011).

### 2.2.1. The inhibitors of cell wall

Bacterial cell wall contains peptidoglycan layer that gives the mechanical strength, and helps bacteria multiply and survive in environmental conditions (Kohanski et al., 2010). Therefore, different antibiotic agents can be directed towards the bacterial cell wall synthesis as target. Generally, both gram-negative and gram-positive bacteria share basic similarities such as N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). Muramic acid holds a pentapeptide, and in the third amino acid there is L-lysine in gram-positive bacteria and meso-diaminopimelicin acid in gram-negative bacteria. In an extensive explanation, the major cell wall agents include β-lactams, fosfomycin, glycopeptides, and bacitracin which selectively inhibit different stage of cell wall

synthesis. Use of these agents can affect the shape and size of bacteria and subsequently induce stress responses and result in cell lysis (Kohanski et al., 2010).

Glycopeptides are effective only against gram-positive bacteria, because large molecular size of the agents cannot pass through gram-negative bacteria (Finch et al., 2010; Kohanski et al., 2010). During the phase of bacterial cell wall synthesis, the building block molecules need to be transported to the membrane, and this dephosphorylization is required to allow addition of phosphate group, but an agent, bacitracin can block this step, hence prevent further cell wall synthesis. However, the major side effect of this drug is its toxicity in humans (Finch et al. 2010). The final step in cell wall synthesis, transpeptidation is prevented by important class of antibiotics, β-lactams. It is well established that cross-link of peptidoglycan is responsible for the cell wall rigidity (Josephine et al., 2004).

### 2.2.2. The inhibitors of bacterial protein synthesis

Ribosomes are involved in the translation in the following manner; initiation, elongation and termination which lead to protein synthesis. Both bacteria (prokaryotes) and humans (eukaryotes) follow universal way in processing genetic information, but the slight difference between bacterial and human ribosome make it possible to attack bacteria not the host cell. The bacterial ribosome consists of two ribonucleoprotein subunits, the 30S and 50S; while eukaryotic ribosomes are composed of 40S and 60S subunits (Mukhtar and Wright, 2005). Agents in these groups are generally grouped into two subclasses; those that inhibit 50S and those that inhibit 30S subunits. Examples of 50S inhibitors include macrolides (erythromycin), streptogramins (dalfopristin), lincosamide (clindamycin) and oxazolidinone (linezolid); while 30S inhibitors include tetracyclines and aminoglycosides (Katz and Ashley, 2005). The mode of action of 50S inhibitors can be either preventing initial step of protein translation, for example oxazolidinones; or preventing peptidyltransferase enzyme such as chloramphenicol, thus stop chain elongation step and this account for bacteriostatic nature of chloramphenicol (Kohanski et al., 2010). In terms of 30S inhibitors, for example tetracycline's target is

known to be preventing aminocyl tRNA to the A site, subsequently stop elongation, and similar to chloramphenicol, it is bacteriostatic in nature (Kohanski et al., 2010).

### 2.2.3. Nucleic acid synthesis inhibitors

Antibiotics inhibit the nucleic acid synthesis by using direct action on DNA gyrase (fluoroquinolones) or RNA polymerase (rifamycins). Broadly, the drugs directly affect the double helix which also contributes to its toxicity to mammalian cells because they interfere with enzymes that are associated with DNA replication, hence a very high selective toxicity is needed to avoid affecting host cell (Drlica et al., 2008). In a related mechanism of action, rifampicin inhibit sRNA synthesis through inhibition of DNA-dependent RNA polymerase.One advantage of this drug is the high selective toxicity to only bacterial enzymes without affecting eukaryotic RNA polymerase, it is a broad-spectrum antibiotic and an important agent for the treatment (Campbell et al., 2001).

The targets of fluoroquinolone are two enzymes: DNA gyrase and topoisomerase IV. These antibiotics interfere with the supercoiling activity of the enzyme, and as a result, this leads to damaged DNA reproduction (at decreased concentrations) and apoptosis (at lethal dose) (Dorman and Corcoran, 2009).

Fluoroquinolones are important agents in the treatment of children with cystic fibrosis complicated with bronchopulmonary disease caused by *Pseudomonas aeruginosa*. These antibiotics are also used in serious (UTIs), otitis media caused by *P.aeruginosa*, shigellosis, disseminated form of salmonellosis, and *Campylobacter jejuni* infections (Hooper, 2001). In addition, the agents are used for prophylaxis during neutropenia, general treatment of febril, neutropenic children with cancer, and also for treating bacterial septicemia and multidrug-resistant mycobacterial disease (Hooper, 2001).

Fluoroquinolones have been used as therapeutic agents since the 1980s as they show higher effectiveness up to 100-fold than their original compound, nalidixic acid. Fluoroquinolones have wide therapeutic indications and are also used as prophylaxis.

Although fluoroquinolones originated from quinolones, they differ structurally from quinolones by the replacing of the eighth carbon atom at the backbone with a nitrogen atom and fluorine atom added at the sixth position. This makes it an additional powerful antibiotic and enhances its activity. (Arnold et al., 2013).

Mechanism of action of fluoroquinolones is known to attack DNA synthesis by forming a complex of drug-enzymes, hence affect cleaving and resealing of DNA strands, subsequently block replication at the replication fork. The lethal effect of fluoroquinolones is a double-step process that involves irreversible complex of, topoisomerase-drug-DNA and formation of a double break by denaturation of topoisomerase. Therefore, it is obvious that the cellular point of attack is type II topoisomerase and DNA gyrase (Hopper, 2001; Aldred et al., 2014). Nalidixic acid is known to be very toxic and its use was discouraged. Among the first modified generations such as norfloxacin, which had a broader activity, a poor tissue penetration was reported (Aldred et al., 2014). Further modifications led to the development of newer members such as ciprofloxacin, levofloxacin, moxifloxacin, and sparfloxacin which had broader activity and good pharmakinetics (Mitscher, 2005).

### 2.3. Fluoroquinolones

Quinolones are structural molecules derived from the heterocyclic aromatic compound quinoline, originated from bark of Cinchona tree in 1811 (Heeb et al., 2011). These groups of broad-spectrum antibiotics are widely isolated in natural sources with different medicinal properties which led to the development of synthetic drugs such as fluoroquinolone antibiotics (Drlica and Zhao, 1997).

The quinolones use important targets for their actions. These are DNA gyrase and DNA topoisomerase lV (Gomez et al., 2003). These enzymes control important processes of cell growth and division; gyrase controls supercoiling of DNA and lessens topological stress increasing from the translocation of transcription and replication complexes along DNA by introducing negative supercoils, while topoisomerase IV

resolves conjugate chromosomes following DNA replication (Drlica and Zhao, 1997). Recent studies indicated that quinolone activity is beyond antibacterial, which is not surprising because they have wide activity against different pathogens (Gomez et al., 2003; Paul et al., 2007). Pommier et al., (2010) reported that DNA topoisomerases were the major target of important anticancer drugs and antibacterials. Both drugs cause defect in their target which result in the break of DNA double-strand.

There are four generations of fluoroquinolone antibiotics. These antibiotics and their generations are given in Table 1 (Idowu and Schweizer, 2017).

Table 2.1. Fluoroquinolones and their generations (Adapted from Idowu and Schweizer, 2017).

| **Generations** | **Fluoroquinolone** |
|---|---|
| First generation | Nalidixic acid,<br>Cinoxacin (discontinued),<br>Flumequine |
| Second generation | Lomefloxacin (discontinued),<br>Norfloxacin,<br>Enoxacin,<br>Ofloxacin,<br>Ciprofloxacin |
| Third generation | Levofloxacin,<br>Sparfloxacin (discontinued),<br>Gatifloxacin (discontinued) |
| Fourth generation | Trovafloxacin (discontinued),<br>Moxifloxacin,<br>Gemifloxacin |

Introduction of the first quinolone, nalidixic acid into the market started the era of an excellent class of antibiotic, however, the hope was dashed for its activity against only gram-negative bacteria and complicated with poor oral absorption. However, its high urinary concentration makes it good for the treatment of UTI. Not long after the milestone, number of pathogens developed resistance (Dalhoff, 2012) The major breakthrough in fluoroquinolones came with discovery of second generation quinolone called norfloxacin that was achieved by modification of the 6-fluorinated compound with a piperazine ring situated at position 7 were added to original quinolones compound(Robert and Paul, 2000). Furthermore, Robert and Paul, 2000 emphasized that the discovery of norfloxacin has improved its activity to both gram-negative and has gram-positive bacteria, nonetheless, pharmacokinetic profile and therapeutic potentialof norfloxacin are not suitable for clinical use. The most successful and widely used compound of the class is ciprofloxacin and the use of the fluoroquinolones for the therapy of invasive diseases has become widely recognized (Robert and Paul, 2000).

The advantages of fluoroquinolones are their wide-spectrum activity including gram-positive and gram-negative pathogens and their good absorption in the gastrointestinal track, reaching adequate blood levels to allow their use in systemic infections (Fuzi et al., 2020). Introduction of the third and the fourth generation quinolones, for example levofloxacin and gemifloxacin respectively has improved features such as broad-spectrum activity against gram-positive bacteria, anaerobes as well as gram-negative bacteria. Although fluoroquinolones have become promising agents for the treatment of diseases there are also issues such as side effects and rapid emergence of resistance (Renau et al., 2003).

### 2.4. Fluoroquinolone Resistance in *Enterobacteriaceae*

Resistance to fluoroquinolones poses threat to the treatment of infections caused by *Enterobacteriaceae*. Fluoroquinolones such as ciprofloxacin and levofloxacin are used in UTI; however, this effective way of treatment is being challenged by the emergence of resistance. The most important strategy employed by these organisms is

through chromosomal alteration in the quinolone resistance- determining region (QRDR) of specific genes encoding DNA gyrase known as *gyrA* and *gyrB*; and in the topoisomerase IV, the genes are *parC* and *parE*. In addition to these, another mechanism of resistance exhibited by organisms to quinolones is through plasmid-mediated resistance known as PMQR (Kao et al., 2016).

Resistance to fluoroquinolones also occurs due to the efflux pumps. Increased expression of mechanical pumps by the organisms suggested that it is the initial mechanism of resistance to fluoroquinolones which will allow the mutation of the genes, resulting to higher resistance to the drugs (Osei and Amoaka, 2017).

The plasmid-mediated quinolone resistance includes *qnr* genes such as *qnrA,B,C,D* and *qnrS*. Another gene that is reported to have conferred resistance to quinolones is *aac(6')-Ib-cr*, a variant of aminoglycoside acetyltransferase which acetylates members of fluoroquinolones such as ciprofloxacin and norfloxacin (Strahilevitz et al., 2009). In addition, *qepA* is known to facilitate resistance via plasmid, although it was reported to be low-level quinolone resistance but it is known to help chromosome-mediated quinolone resistance in *Enterobacteriaceae (*Yang et al., 2014).

A study from South Africa addressed the genomic characteristics of fluoroquinolone resistance in *Enterobacteriaceae*. The results showed the following mechanisms employed by the *Enterobacteriaceae* to resist the drugs. Efflux pumps, plasmid-mediated quinolone resistance genes and the mutations detected in *gyrA*, *gyrB*, *parC* and *parE*. The authors concluded that vertical and horizontal transmissions of high-level fluoroquinolone resistance exist in *Enterobacteriaceae* (Osei and Amoako, 2017).

In another study, Leski et al. (2016) suggested that the main cause of high-level fluoroquinolone resistance in *Enterobacteriaceae* is the mutations in QRDRs of, *gyrA* and *parC* genes. In addition to this, as reported previously, reduced uptake and increased efflux decrease the drug concentration and result in resistance.

In an extensive review by Strahilevitz et al. (2006), the distribution of plasmid mediated quinolone resistance worldwide showed that it can be in two-way directions; vertically and horizontally. Moreover, the study agreed with the previous reports that indicated that Qnr proteins were able to protect DNA gyrase from quinolones and the presence of AAC(6')-Ib-cr, a variant aminoglycoside acetyltransferase modified ciprofloxacin efficiently and reduced its action. This appears to have started more lately, but might be even more common than the Qnr proteins. This brought the concept of multiple mechanisms to be used by *Enterobacteriaceae* to be resistant to fluoroquinolones, ranging from low level to high level fluoroquinolone resistance (Rodriguez-Martinez et al., 2011). Levels of fluoroquinolone resistance were reported from different parts of the world. For example, level of fluoroquinolone resistance in clinical *E. coli* isolates in Hong Kong was found to be 40%. Apart from this, about 25% of healthy people living in Barcelona were reported to be intestinally colonized with fluoroquinolone -resistant *E. coli (*Lavilla et al., 2008).

In another study conducted in Togo between 2013 and 2015, the distribution of fluoroquinolone-resistance gene, *qnr*, was searched among *E. coli* and *Klebsiella* species. Of the 155 bacterial strains, 107 (69%) isolates were resistant, where 61 were *E. coli* and 46 were *Klebsiella* species. The results showed that in all of the isolates tested, they were found to contain at least one type of *qnr* genes, and the distributions were as follows: 74 (47.74%) *qnr*B, 73 (47.10%) *qnr*S and 4 (2.58%) *qnr*A (Salah et al., 2019).

### 2.5. Fecal Carriage of Fluoroquinolone-Resistant *Enterobacteriaceae*

The emergence of fecal carriage of antibiotic resistant organisms, particularly carriage of fluoroquinolone-resistant *Enterobacteriaceae* is of great concern because it limits the treatment options for the infections caused by such organisms. In recent years, occurrence of fecal carriage of fluoroquinolone-resistant *Enterobacteriaceae* has been reported widely (Ovejero et al., 2017). For example, in a study conducted in Madrid which investigated the carriage of fluroquinolone-resistant *Enterobacteriaceae* showed the presence of co-resistance against both extended-spectrum cephalosporins and

fluoroquinolones (Dupouy et al., 2019). This is alarming because the sharing of resistance factors to these two important classes of antibiotics might further endanger global health sector. Moreover, high level of nalidixic acid and ciprofloxacin-resistant carriers was reported in Portugal and the United Kingdom (UK) (Leite-Martins et al., 2014).

The prevalence of fluoroquinolone-resistant *Enterobacterieaceae* is a global problem as it is detected across the world. In a recent work of Benameur et al. (2018), fluoroquinolone-resistant *Enterobacteriaceae* strains were isolated and *qnrS* in *Enterobacter cloacae* was reported. The four-year study (2010-2014) in western Algeria showed high rates of fecal carriage of fluoroquinolone-resistant *Enterobactericeae*. Of the 253 *Enterobacteriaceae* strains isolated, 233 (92.09%) were multidrug resistant. This shows that the rate of fecal carriage of fluoroquinolone-resistant *Enterobacteriaceae* is significantly at higher rate (Benameur et al. 2018).

In a recent work of Ruh et al. (2019), fecal carriage of resistant *Enterobacteriaceae* in the community was searched in Northern Cyprus. The study found the fecal carriage rate of ciprofloxacin-resistant *Enterobacteriaceae* to be 10.2% (n=51) among 500 subjects, and all of the 51 (100%) isolates were detected to be *E. coli*. In the study, the relation between higher socioeconomic status and ciprofloxacin resistance showed a significant correlation (Ruh et al., 2019).

Saksena et al. (2018) reported high colonization rate of antibiotic-resistant *Enterobacteriaceae*. A total of 343 *Enterobacteriaceae* were isolated from 100 neonates, where 58% harbored at least one member of *Enterobacteriaceae,* mostly *E. coli* and total resistance to nalidixic acid was 60%. Ciprofloxacin resistance increased significantly from 15 % (14/96) on day 1 to 38 % (50/132) on day 60. Colonization of the gut flora of the neonates might have occurred during birth. The high level of fluoroquinolone-resistant isolates showed that the emergence of resistance can occur even with less or no selection pressure because the neonates harbored fluoroquinolone-resistant *Enterobacteriaceae* isolates from day one Saksena et al., (2018).

In another study, fecal carriage of antibiotic-resistant *Enterobacteriaceae* in healthy Korean adults was investigated. Of the 109 fecal samples, *Enterobacteriaceae* isolates resistant to fluoroquinolones and other antimicrobials were detected. This suggested that healthy humans might harbor drug-resistant bacteria and transfer these microorganisms to other people (Joo et al., 2018).

# 3. MATERIALS AND METHODS

## 3.1. Design of Study and Participants

Sample collection for the study was carried out between March 2019 and July 2019. A total of 180 participants were included in this study. Hospitalized patients (n=80) were recruited from the people who stayed in the Near East University Hospital for at least 72 hours. Control group (n=100) consisted of people who did not have any history of hospitalization in the last six months before the study. The inclusion criteria for the study were being above 18 years of age and living for at least one year in Northern Cyprus. The ethics approval for this study was gained from Research Assessment Committee of the Near East University (No: YDU/2019/65-717). Written consent was taken from all participants.

## 3.2. Collection of Samples and Data

In the study, one fecal sample was obtained from each participant, hence, 180 samples were collected. During the collection of samples, a questionnaire was performed to determine the factors related with the intestinal colonization of antibiotic-resistant bacteria. Firstly, demographic and socioeconomic information (gender, age, level of education, marital status, and socioeconomic status) were obtained. Then, participants provided information on whether they had any gastrointestinal complaints during the collection of samples. Additionally, information was obtained on history of antibiotic consumption, travelling other countries, diarrhea, UTI, and hospitalization within the last six months before the study. Additionally, the patients provided information on the length of hospitalization, stay in intensive care unit, and use of antibiotics in the current hospital stay.

### 3.3. Initial Screening of Fluoroquinolone-Resistant *Enterobacteriaceae*

Suspensions of 200 mg of fecal specimens were prepared in 2 ml sterile saline. The inoculation for each sample was done on two plates (EMB media) (Merck, Germany): Control plate was used for each sample to check the bacterial growth. In order to screen fluoroquinolone resistance, a medium that contained ciprofloxacin (CIP) antibiotic (Sigma, USA) at concentration of 1 mg/L was used for each sample. In order to prepare media that contain the antibiotic, the media were brought out from autoclave and allowed to cool down at room temperature. Following this, 1 mg/L CIP was added to the media.

After an aliquot of each stool suspension was inoculated on both control and antibiotic containing media, the plates were incubated at 37$^{o}$C for 24hr. After the incubation period, the plates were evaluated in terms of bacterial growth.

Bacterial colonies that grew on antibiotic containing media were stored in stock media (nutrient broth containing 15% glycerol in microcentrifuge tubes) at -20$^{o}$C for confirmatory tests and identification**.**

### 3.4. Phenotypic Confirmatory Tests for the Fluoroquinolone-Resistant *Enterobacteriaceae* and Identification of the Resistant Isolates

Frozen samples were thawed at room temperature. These samples were inoculated on EMB and incubated at 37$^{o}$C for 24 hr. After bacterial growth, suspensions with 0.5 McFarland standard turbidity were prepared. The bacterial suspensions were inoculated on Mueller-Hinton media (Merck, Germany) for confirmation of the fluoroquinolone resistance by disc diffusion test. Each plate contained five antibiotic discs (Bioanalyse, Turkey). These were CIP (5 μg), ofloxacin (OFX, 5 μg), norfloxacin (NOR, 10 μg), levofloxacin (LVX, 5 μg), and gemifloxacin (GEM, 5 μg). Zones of inhibition of 15 mm or less for GEM and CIP; 12 mm or less for NOR and OFX; and 13 mm or less for LVX antibiotic discs were recorded as resistant (CLSI, 2018).

Identification of the fluoroquinolone-resistant *Enterobacteriaceae* isolates was done by using VITEK-2 system (bioMérieux, France).

### 3.5. Statistical Analysis

Descriptive statistics were calculated for the variables in the questionnaire. Information on frequency and percentage were given for categorical variables, while arithmetic mean, standard deviation, median, minimum and maximum were calculated for the continuous variables. Depending on the size of sample, Pearson Chi-square or Fisher's exact test was used in order to evaluate the associations between categorical variables. All of the statistical calculations were done by using IBM SPSS statistics package for Macintosh (Demo version 22.0; Armonk, NY: IBM Corp.). Significance level was accepted to be 0.05.

# 4. RESULTS

## 4.1. General Characteristics of the Study Participants

The total number of participants in the study comprised of 180 participants; 80 from Near East University Hospital and 100 participants from the community setting. The mean and median age of the study group was 43.91 ± 21.42 and 39.50 (19.00-90.00). The age group ranged from 19-30 years consisted of 76 (42.2%) participants. In the age group of 31 years and above, there were 104 (57.8%) participants. There were 127 (70.6%) males and 53 (29.4%) females. Education level of the participants included one hundred and four (57.8%) for university and higher level, and 76 (42.2%) for lower than university. Marital status was also analyzed and there were 88 (48.9%) single and 92 (51.1%) married participants. Number of the participants with low and middle income was 158 (87.8%), and number of those with higher income was 22 (12.2%).

In the study population, 46 (25.6%) participants declared that they had GIS at the time of sample collection. History of antibiotic use among participants in the last six months prior to the study was 85 (47.2%) and fluoroquinolone use was noted in four (8.2%) individuals. Participants with a history of diarrhea were found to be 46 (25.6%), and number of individuals with a history of UTI was 13 (7.25%). Number of participants with travel history was 99 (55.0%). Participants who travelled to Turkey or Europe and those that travelled to Asia or Africa were recorded as 60 (60.6%) and 39 (39%), respectively.

## 4.2. Number of CIP-RE Isolates

In the study, CIP-RE intestinal colonization rate was found to be 28.9% (n=52/180). Twenty-eight (35.0%) individuals were found to be colonized with CIP-RE in the patient group, and 24 (24.0%) were colonized with CIP-RE in the control group. There was no statistically significant difference between the fecal carriage levels of the two groups ($p$=0.106) (Table 4.1).

Table 4.1. Distribution of CIP-RE among patient (n=80) and control (n=100) groups.

| Participants | CIP-RE positive n (%) | CIP-RE negative n (%) | Total n (%) |
|---|---|---|---|
| Patients | 28 (35.0) | 52 (65.0) | 80 (100.0) |
| Controls | 24 (24.0) | 76 (76.0) | 100 (100.0) |
| Total | 52 (28.9) | 128 (71.1) | 180 (100.0) |

[*] $p$=0.106

### 4.3. Identification of CIP-RE Isolates

Majority of the CIP-RE isolates (n=51/52; 98.1%) were identified as *E. coli*. One (1.9%) of 52 isolates was identified as *P. mirabilis* (Table 4.2).

Table 4.2. Distribution of bacterial species among CIP-RE isolates (n=52).

| Participants | *Escherichia coli* n/N (%) | *Proteus mirabilis* n/N (%) |
|---|---|---|
| Patients | 27/52 (51.9) | 1/52 (1.9) |
| Controls | 24/52 (46.2) | 0/52 (0.0) |
| Total | 51/52 (98.1) | 1/52 (1.9) |

### 4.4. Resistance Rates of CIP-RE Isolates Against Other Fluoroquinolones

Among CIP-resistant isolates, resistance rates to OFX, NOR, LVX and GEM were found to be 94.2% (n=49/52), 98.1% (n=51/52), 61.5% (n=32/52), and 98.1% (n=51/52), respectively (Table 4.3).

Table 4.3. Resistance patterns of CIP-resistant isolates (n=52) against other fluoroquinolones.

| | Ofloxacin | Norfloxacin | Levofloxacin | Gemifloxacin |
|---|---|---|---|---|
| Resistant | 49 (94.2) | 51 (98.1) | 32 (61.5) | 51 (98.1) |
| Intermediate | 2 (3.9) | 1 (1.9) | 11 (21.2) | 1 (1.9) |
| Susceptible | 1 (1.9) | 0 (0.0) | 9 (17.3) | 0 (0.0) |
| Total | 52 (100.0) | 52 (100.0) | 52 (100.0) | 52 (100.0) |

## 4.5. Results of the Statistical Analysis

### 4.5.1. Correlation of intestinal colonization of CIP-RE with demographic and socioeconomic factors

There was a statistical significance between the age of the study population and intestinal colonization ($p$=0.021). Also, a statistical association was found between marital status and intestinal colonization ($p$=0.035). There was no statistical association between intestinal colonization and other variables which included gender, education level, and socioeconomic status of the population (Table 4.4).

Table 4.4. Correlation of intestinal colonization of CIP-RE with demographic and socioeconomic factors in the study group (n=180).

| Risk factors | CIP-RE | |
|---|---|---|
| | Positive n/N (%) | *p* value |
| Age | | |
| 19-30 | 15/76 (19.7) | 0.021 |
| 31 and above | 37/104 (35.6) | |
| Total | 52/180 (28.9) | |
| Gender | | |
| Male | 35/127 (27.6) | 0.542 |
| Female | 17/53 (32.1) | |
| Total | 52/180 (28.9) | |
| Education | | |
| University and higher | 28/104 (26.9) | 0.496 |
| Lower then university | 24/76 (31.6) | |
| Total | 52/180 (28.9) | |
| Marital status | | |
| Single | 19/88 (21.6) | 0.035 |
| Married | 33/92 (35.9) | |
| Total | 52/180 (28.9) | |
| Socioeconomic status | | |
| Low and middle | 47/158 (29.7) | 0.496 |
| High | 5/22 (22.7) | |
| Total | 52/180 (28.9) | |

### 4.5.2. Correlation of intestinal colonization of CIP-RE with epidemiological factors

In the study, intestinal colonization of CIP-RE was also evaluated according to epidemiological factors. Among 85 participants who used antibiotic in the last six months, 36 individuals did not remember the name of the antibiotic. Therefore, assessment of fecal carriage with respect to fluoroquinolone use was done according to 49 participants who remembered the name of antibiotic. The statistical analysis showed that, a significant correlation was found between the history of UTI and intestinal colonization of CIP-RE ($p$=0.011). However, there was no statistical association between intestinal colonization of CIP-RE and other epidemiological factors (Table 4.5).

Table 4.5. Correlation of intestinal colonization of CIP-RE with epidemiological factors in the study group (n=180). (*The period covers the last six months before the study.)

| Risk factors | CIP-RE | |
|---|---|---|
| | Positive n/N (%) | *p* value |
| Presence of any GIS at the time of sample collection | | |
| Yes | 11/46 (23.9) | 0.388 |
| No | 41/134 (30.6) | |
| Total | 52/180 (28.9) | |
| History of antibiotic use* | | |
| Yes | 25/85 (29.4) | 0.884 |
| No | 27/95 (28.4) | |
| Total | 52/180 (28.9) | |
| History of fluoroquinolone use* | | |
| Yes | 2/4 (50.0) | 0.248 |
| No | 10/45 (22.2) | |
| Total | 12/49 (24.5) | |
| History of diarrhea* | | |
| Yes | 12/46 (26.1) | 0.627 |
| No | 20/134 (29.9) | |
| Total | 52/180 (28.9) | |
| History of UTI* | | |
| Yes | 8/13 (61.5) | 0.011 |
| No | 44/167(26.3) | |
| Total | 52/180 (28.9) | |
| Travel history* | | |
| Yes | 31/99 (31.3) | 0.428 |
| No | 21/81 (25.9) | |
| Total | 52/180 (28.9) | |
| Travel to Turkey or Europe* | | |
| Yes | 22/60 (36.7) | 0.154 |
| No | 9/39 (23.1) | |
| Total | 31/99 (31.3) | |
| Travel to Asia or Africa* | | |
| Yes | 9/39 (23.1) | 0.154 |
| No | 22/60 (36.7) | |
| Total | 31/99 (31.3) | |

### 4.5.3. Association of intestinal colonization of CIP-RE with hospital-related factors

There were 80 patients hospitalized for at least 72 hours. Among these, details of current hospitalization in four patients could not be obtained, therefore the statistical analysis was done according to the information of 76 patients. Eight (10.5%) of 76 patients stayed in intensive care unit (ICU). There were 27 (35.5%) patients who had surgery. Number of the patients who used urinary catheter was seven (9.2%). Fifty-six (73.7%) patients used antibiotic during hospital stay, and nine (16.4%) of these used fluoroquinolone. Statistical analysis showed that, there was a significant correlation between intestinal colonization of CIP-RE and the use of fluoroquinolone in the hospitalized patients ($p$=0.019). In the study, no significant correlation was found between intestinal colonization of CIP-RE and the other hospital-related factors (Table 4.6).

Table 4.6. Association of intestinal colonization of CIP-RE with hospital-related factors among hospitalized patients (n=80).

| Risk factors | CIP-RE | |
|---|---|---|
| | Positive n/N (%) | *p* value |
| Stay at Intensive Care Unit* | | |
| Yes | 2/8 (25.0) | 1.000 |
| No | 23/68 (23.8) | |
| Total | 25/76 (32.9) | |
| Surgery* | | |
| Yes | 6/27 (22.2) | 0.142 |
| No | 19/49 (38.8) | |
| Total | 25/76 (32.9) | |
| Urinary catheter* | | |
| Yes | 3/7 (42.9) | 0.678 |
| No | 22/69 (31.9) | |
| Total | 25/76 (32.9) | |
| Antibiotic use* | | |
| Yes | 17/56 (30.4) | 0.431 |
| No | 8/20 (40.0) | |
| Total | 25/76 (32.9) | |
| Fluoroquinolone use* | | |
| Yes | 6/9 (66.7) | 0.019 |
| No | 11/46 (23.9) | |
| Total | 17/55 (30.9) | |

*Indicates the current hospitalization.

# 5. DISCUSSION

Resistance rates of *Enterobacteriaceae* to different groups of antibiotics is well documented (Kotb et al., 2019; Nakano et al., 2019; Trautner, 2018), however, intestinal colonization rates of fluoroquinolone-resistant *Enterobacteriaceae* among hospitalized patients is not clear in Northern Cyprus. Therefore, it is necessary to study the resistance rates of *Enterobacteriaceae* against different antibiotics, especially fluoroquinolones which is an important class of antibiotics used to treat high priority pathogens (Breijyeh et al., 2020).

Resistance of infective organisms to antibiotics particularly has become a global problem with serious effects on the treatment of infectious diseases (Shaikh et al., 2015). This is a result of persistent use and misuse of drugs in treatment of bacterial infections (Rather et al., 2017). Due to sort of several studies from both community and hospital with different results of the risk factors and their association with the resistance, there is need to report a study that comprises the results of both hospitalized and community settings.

This study searched the fecal carriage rates of CIP-RE in both hospital and community settings. A total of 180 individuals were involved in the study. Eighty participants were hospitalized patients, and 100 participants were community-dwellers. Stool specimens were collected from the participants and tested for CIP-RE isolates.

The bacterial species among CIP-RE isolates were identified to be *E. coli* (98.1%) and *P. mirabilis* (1.9%) (Table 4.2). Taha et al. (2019) reported 37% *E. coli* isolates among CIP-resistant *Enterobacteriaceae*, while Schulz et al. (2016) reported 52% *E. coli* isolates in CIP-RE. The rates documented by the two studies are lower than the obtained percentage in our study.

The resistance rates of CIP-RE isolates to OFX, NOR, LVX and GEM were 94.2%, 98.1%, 61.5%, and 98.1%, respectively. Susceptibility rate was higher for LVX (17.3%) and this was followed by OFX (1.9%). There was no susceptible isolate to NOR

(0.0%) and GEM (0.0%) (Table 4.3). Higher susceptibility of LVX when compared to OFX and NOR may be due to the improved efficacy as a 3$^{rd}$ generation fluoroquinolone (Idowu and Schweizer, 2017).

In a hospital-based study in Northern Cyprus, Ruh et al. (2016) reported the prevalence of antibiotic resistance rate of certain gram-negative bacteria, which is quite significant. The authors reported lower resistance rates compared to other studies in some regions. This could be due to variability in resistance according to geographical location.

In order to evaluate the potential risk factors related with fecal carriage of CIP-RE, a survey was obtained from the study participants. Age has been used in several studies to assess the correlation with antibiotic resistance. Two variables (age and marital status) showed statistical association with intestinal colonization of CIP-RE ($p$=0.021 and $p$=0.035, respectively) (Table 4.4). This finding is consistent with the study of Duplessis et al. (2012) which found a statistical correlation between age and fluoroquinolone-resistance; and also, suggested that age might be a predictive factor in revealing the history of antibiotic usage and hospitalization. Furthermore, Sadigov et al. (2017) reported a high correlation of older age with antibiotic resistance in a community-based study in Azerbaijan.

In addition, study of de Lastours et al. (2014) showed that gender was significantly associated with fluoroquinolone-resistance. In another study, the pattern of antibiotic resistance (e.g. penicillin and quinolone) was noted to be similar in both males and females; and very high resistance was found in *E. coli* (Singh et al., 2018). But some studies reported that females tend to have more resistance to antibiotic than the male participants. This could be the result of some infections that affect females more than males, such as UTI (Dolk et al., 2018; Smith et al., 2018). Singh et al. (2018) in addition, showed that there was a statistical correlation between the education levels of the participants with antibiotic resistance.

In the present study, there was no significant association between education level of the participants and intestinal colonization of CIP-RE. This result contradicts the

study of Luvsansharav et al. (2012) that found a statistical correlation between the education level and CTX-M type ESBL fecal carriage in a countryside setting.

Socioeconomic status did not have any statistical association with antibiotic resistance in our study. However, in another study, Ruh et al. (2019) showed a significant correlation between socioeconomic status and intestinal colonization with CIP-RE.

In this study, existence of any GIS at the time of stool sample collection was not significantly related with the fecal carriage of CIP-RE. This is also contrary to the recent study of Abdallah et al. (2017) and Reuland et al. (2016) that showed statistical significance of community-onset gastrointestinal complaints.

Use of antibiotics is known as one of the most common causes of antibiotic resistance along with other factors (Ventola, 2015). However, Caudell et al. (2018) reported no association between antibiotic use and antimicrobial resistance in humans and livestock in Tanzania. In another study conducted by Williamson et al. (2018) there was a decline in antibiotic resistance at certain time in Australia; but later there was an increase in the prevalence. This might be due to the continuous use of the antibiotics among the population.

Exposure to antibiotics is a factor for selecting antibiotic resistance mostly in community setting, and fluoroquinolones have been used as potential drugs to treat UTI. Exposure to fluoroquinolones was found to reduce *Enterobacteriaceae* significantly, however at the same time, this led to colonization with CIP-RE in patients with UTI (Stewardson et al., 2018).

In this study, history of UTI within the last six months had a significant correlation with CIP-RE ($p$=0.011) (Table 4.5). This is consistent with the study of Stewardson et al. (2018) and Zhu et al. (2020) which also found a significant correlation between UTI with CIP-RE. Martín-Gutiérrez et al. (2016) also reported UTI as a risk factor for the occurrence of CIP-resistance in *E. coli*.

In a recent study, use of antibiotics, diarrhea, and travel history (India and South Africa) have been found to be associated with fecal carriage of antibiotic-resistant *E. coli* (Hu et al., 2020). Likewise, some studies reported significant association of resistance rate with travel history; mostly to endemic regions in the world (Dandachi et al., 2019; Dash et al., 2018; Kuskucu et al., 2018). Travel history was detected to be a risk factor for the prevalence of ESBL-producing *Enterobacteriaceae* in a community setting in Netherlands (Arcilla et al., 2020; Reuland et al., 2016) and also in Korea (Park et al., 2019). In our study, there was no correlation between fecal carriage and general travel history, and also travel to Turkey, Europe, Asia or Africa ($p>0.05$) (Table 4.5). This finding is consistent with the recent study of Ruh et al. (2019) which has also reported no statistical correlation between the journey destination and fecal carriage of resistant bacteria.

Intensive care unit (ICU) is believed to be a source for many infections due to the continuous number of cases. In our study, there was no statistical correlation between ICU stay and CIP-RE ($p>0.05$) (Table 4.6). This contradicts several studies such as Tran et al. (2017), Karam et al. (2018), and Gorrie et al. (2017) where a significant association was found between ICU stay and antimicrobial resistance.

In this study, there was no significant association between surgery and CIP-RE (Table 4.6). This contradicts the findings of Gorrie et al. (2017) which reported a correlation between surgery and antimicrobial resistance.

In a study conducted by Yousefipour et al. (2019), use of urinary catheter was reported to be associated with ESBL-producing bacteria in hospitalized patients. The authors also found that *Klebsiella* showed the highest rate of ESBL. In the current study, there was no significant correlation between the use of urinary catheter and CIP-RE ($p=0.678$).

Steensels et al. (2012) reported that use of fluoroquinolones among the last six months prior to biopsy was a risk factor for the increased fluoroquinolone-resistance in *E. coli* strains. Yamamoto et al. (2017) also found a significant correlation between antibiotic-resistance and increased exposure to antibiotics. In our study, there was no

statistical correlation between antibiotic use in general and the rate of resistance. However, a significant association was found between the use of fluoroquinolones in the current hospitalization and fecal carriage of CIP-RE isolates ($p$=0.019) (Table 4.6).

# 6. CONCLUSION

In this study, fecal carriage rate of CIP-RE was found to be 28.9% (n=52/180). Majority of the CIP-RE isolates (n=51/52; 98.1%) were identified as *E. coli*. One (1.9%) of 52 isolates was identified as *P. mirabilis*. Resistance rates of CIP-RE isolates to OFX, NOR, LVX and GEM were 94.2%, 98.1%, 61.5%, and 98.1%, respectively. Statistical analysis showed that, age ($p$=0.021) and marital status ($p$=0.035) of the participants were significantly associated with the fecal carriage of CIP-RE. Also, a statistical correlation was found between the history of urinary tract infection and intestinal colonization of CIP-RE ($p$=0.011). Moreover, the use of fluoroquinolones in the current hospitalization was found to be a significant factor for the fecal carriage of CIP-RE isolates ($p$=0.019).

Our study provided information about the fecal carriage rates of CIP-RE isolates in both hospital and community settings. Also, the risk factors for CIP-RE colonization were indicated. Results of the study suggest that fluoroquinolone resistance in *Enterobacteriaceae* should be continuously monitored in Northern Cyprus.

# REFERENCES


Abbas, Hisham A., and Wael AH Hegazy. "Targeting the virulence factors of Serratia marcescens by ambroxol. Rom Arch Microbiol Immunol.2017; 76.2: 27-32.

Abdallah HM, Alneima N, Reuland EA, Winterman BB, Koek A, Abdelwahab AM, Samy A, Abdelsalam KW, Vandenbrouke-Grauls CMJE. Fecal carriage of extended spectrum b-lactamase and carbapenemase-producing enterobacteriaceae in Egypt patients with community-onset gastrointestinal complaints: a hospital-based cross-sectional study. Antimicrob Resist Infect Control. 2017; 6:62:1-7.

Adegoke AA , Faleye AC , Singh G , Stenströ TA. Antibiotic Resistant Superbugs: Assessment of the Interrelationship of Occurrence in Clinical Settings and Environmental Niches (Molecules).Molecules. 2017; 22: 29:2-17

Adeleke, S. I., Asani, M. O., Belonwu, R. O., & Ihesiulor, G. U. Urinary tract pathogens and antimicrobial sensitivity patterns in childhood urinary tract infection, Kano Nigeria. Ann Niger Med.2005; 1(2), 14-16.

Adeolu, M., Alnajar, S., Naushad, S., & Gupta, R. S. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. Int J Syst Evol Micr .2016; 66(12), 5575-5599.

Akingbade, O., Balogun, S., Ojo, D., Akinduti, P., Okerentugba, P. O., Nwanze, J. C., & Okonko, I. O. Resistant plasmid profile analysis of multidrug resistant Escherichia coli isolated from urinary tract infections in Abeokuta, Nigeria Afr Health Sci.2014; 14(4), 821-828.

Aldred, Katie J., Robert J. Kerns, and Neil Osheroff. "Mechanism of quinolone action and resistance. Biochemistry. 2014; 53.10: 1565-1574.

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol. 2010;8:251-259

Arcilla, Maris S., Jarne M. van Hattem, Manon R. Haverkate, Martin CJ Bootsma, Perry JJ van Genderen, Abraham Goorhuis, Martin P. Grobusch et al. "Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study." Lancet Infect Dis.2017; 17, no. 1 : 78-85.

Armbruster, Chelsie E., Harry LT Mobley, and Melanie M. Pearson. "Pathogenesis of Proteus mirabilis infection. EcoSal Plus.2018; 8.1.

Arnold, H. M., Hollands, J. M., Skrupky, L. P., Smith, J. R., Juang, P. H., Hampton, N. B., ... & Micek, S. T. Prolonged infusion antibiotics for suspected gram-negative infections in the ICU: a before-after study. Ann Pharmacother.2013; 47(2) , 170-180.

Arnold, R. S., Thom, K. A., Sharma, S., Phillips, M., Johnson, J. K., & Morgan, D. J. Emergence of Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria. South Med J. 2011; 104(1), 40.

Benameur, Q., Tali-Maamar, H., Assaous, F., Guettou, B., Benklaouz, M. B., Rahal, K., & Ben-Mahdi, M. H. Characterization of quinolone-resistant Enterobacteriaceae strains isolated from poultry in Western Algeria: First report of qnrS in an Enterobacter cloacae. Vet. World.2018; 11(4), 469.

Berlanga M, Viñas M. Salicylate induction of phenotypic resistance to quinolones in *Serratia marcescens*. J Antimicrob Chemother. 2000; 46:279-82.

Bhattacharya, D., Sugunan, A.P., Bhattacharjee, H., Thamizhmani, R., Sayi, D.S., Thanasekaran, K., Manimunda, S.P., Ghosh, A.R., Bharadwaj, A.P., Singhania, M. and Roy, S., Antimicrobial resistance in Shigella-rapid increase & widening of spectrum in Andaman Islands, India. Indian J Med Res.2012; 135(3), p.365.

Bradford, Patricia A. "Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev.2001; 14.4: 933-951.

Breijyeh, Zeinab, Buthaina Jubeh, and Rafik Karaman. "Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It." Molecules.2020; 25.6: 1340.

Brolund, A., Sundqvist, M., Kahlmeter, G., & Grape, M. Molecular characterisation of trimethoprim resistance in Escherichia coli and Klebsiella pneumoniae during a two year intervention on trimethoprim use. PLoS One.2010; 5(2).

Campbell, E. A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A., & Darst, S. A. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell.2001; 104(6), 901-912.

Caudell, Mark A., Colette Mair, Murugan Subbiah, Louise Matthews, Robert J. Quinlan, Marsha B. Quinlan, Ruth Zadoks, Julius Keyyu, and Douglas R. Call. "Identification of risk factors associated with carriage of resistant Escherichia coli in three culturally diverse ethnic groups in Tanzania: a biological and socioeconomic analysis. Lancet Planet Health.2018; 2, no. 11 e489-e497.

Chang, S. C., Fang, C. T., Hsueh, P. R., Chen, Y. C., & Luh, K. T. Klebsiella pneumoniae isolates causing liver abscess in Taiwan. Diagn Micr Infec Dis.2000; *37*(4), 279-284.

Cheesbrough, Monica. District laboratory practice in tropical countries. Cambridge university press, 2006.

Clinical and Laboratory Standards Institute. M100. Performance standards for antimicrobial susceptibility testing. 28th ed. USA: Wayne, PA; 2018.

Costanzo, Simon D., John Murby, and John Bates. "Ecosystem response to antibiotics entering the aquatic environment." Mar Pollut Bull. 2005; 51.1-4: 218-223.

Da Silva MF, Vaz-Moreira I, Gonzalez-Pajuelo M, Nunes OC, Manaia CM. Antimicrobial resistance patterns in *Enterobacteriaceae* isolated from an urban wastewater treatment plant. FEMS Microbiology Ecology. 2007; 60:1:166–176.

Dalhoff, Axel. "Global fluoroquinolone resistance epidemiology and implictions for clinical use. Interdiscip Perspect Infect Dis. 2012 .

Dandachi, I., Chaddad, A., Hanna, J., Matta, J., & Daoud, Z. Understanding the Epidemiology of Multi-Drug Resistant Gram-Negative Bacilli in the Middle East Using a One Health Approach. Front Microbiol.2019; 10, 1941.

Dash, N., Al-zarouni, M., Al-Kous, N., Al-Shehhi, F., Al-Najjar, J., Senok, A., & Panigrahi, D. Distribution and resistance trends of community associated urinary tract pathogens in Sharjah, UAE. Microbiol Insights.2008; 1, MBI-S780.

Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. Microbiol Mol Biol Rev. 2010;74:3: 417-433.

de Lastours, V., Chau, F., Roy, C., Larroque, B., & Fantin, B. Emergence of quinolone resistance in the microbiota of hospitalized patients treated or not with a fluoroquinolone. J Antimicrob Chemother.2014; 69(12), 3393-3400.

Dolk, F. C. K., Pouwels, K. B., Smith, D. R., Robotham, J. V., & Smieszek, T. Antibiotics in primary care in England: which antibiotics are prescribed and for which conditions?. J Antimicrob Chemother.2018; 73(suppl_2), ii2-ii10.

Dorman, Charles J., and Colin P. Corcoran. "Bacterial DNA topology and infectious disease. Nucleic Acids Res Spec Publ.2009; 37.3 : 672-678.

Drlica K, Malik M, Kerns RJ, Zhao X. Quinolone mediated death cell. Antimicrob Agents Chemother. 2008;52:2:385-392.

Drlica, Karl, and Xilin Zhao. "DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev.1997; 61.3: 377-392.

Dsouza, Roshan, Naina Adren Pinto, InSik Hwang, YoungLag Cho, Dongeun Yong, Jongrak Choi, Kyungwon Lee, and Yunsop Chong. "Panel strain of Klebsiella pneumoniae for beta-lactam antibiotic evaluation: their phenotypic and genotypic characterization. PeerJ.2017; 5 : e2896.

Duplessis, Christopher A., Mary Bavaro, Mark P. Simons, Charles Marguet, Michael Santomauro, Brian Auge, Daniel A. Collard, Joshua Fierer, and James Lesperance. "Rectal cultures before transrectal ultrasound-guided prostate biopsy reduce post-prostatic biopsy infection rates." Urology.2012; 79, no. 3 : 556-563.

Dupouy, Véronique Michèle, Mouni Abdelli, Gabriel Moyano, Nathalie Arpaillange, Delphine Bibbal, Marie Christine Cadiergues, Diego Lopez-Polin et al. "Prevalence of beta-lactam and quinolone/fluoroquinolone resistance in Enterobacteriaceae from dogs in France and Spain-Characterization of ESBL/pAmpC isolates, genes and conjugative plasmids." Front Vet Sci.2019; 6 : 279.

Finch R, Greenwood D, Whitley R, Norrby SR. Antibiotic and Chemotherapy 9th Edition. Saunders. 2011; 12-24.

Fraga, Mario F., and Manel Esteller. "Epigenetics and aging: the targets and the marks. Trends Genet.2007; 23.8 : 413-418.

Fraser, G. M., Claret, L., Furness, R., Gupta, S., & Hughes, C. Swarming-coupled expression of the Proteus mirabilis hpmBA haemolysin operon. Microbiology (Reading, Engl.2002; 148(Pt 7), 2191.

Fujihara, M., Obara, H., Watanabe, Y., Ono, H. K., Sasaki, J., Goryo, M., & Harasawa, R. Acidic environments induce differentiation of Proteus mirabilis into swarmer morphotypes. Microbiol Immunol.2011; *55*(7), 489-493.

Fuzi, Miklos, Jesus Rodriguez Baño, and Akos Toth. "Global Evolution of Pathogenic Bacteria With Extensive Use of Fluoroquinolone Agents. Front Microbiol.2020; 11: 271.

Gibbs, K. A., Wenren, L. M., & Greenberg, E. P. Identity gene expression in Proteus mirabilis. J Bacteriol Res.2011; 193(13), 3286-3292.

Gomez, Lucia, Javier Garau, Cristina Estrada, Montserrat Marquez, David Dalmau, Mariona Xercavins, Josep Maria Martí, and Cristina Estany. "Ciprofloxacin prophylaxis in patients with acute leukemia and granulocytopenia in an area with a high prevalence of ciprofloxacin-resistant Escherichia coli." Cancer.2003; 97, no. 2 : 419-424.

Gorrie, Claire L., et al. "Antimicrobial-resistant Klebsiella pneumoniae carriage and infection in specialized geriatric care wards linked to acquisition in the referring hospital. Arch Clin Infect Dis.2018; 67.2 : 161-170.

Hardjo, NP Lugito. "Antimicrobial Resistance of Salmonella enterica Serovars Typhi and Paratyphi Isolates from a General Hospital in Karawaci, Tangerang, Indonesia: A Five-Year Review." Int J Microbiol. 2017;  6215136-6215136.

Heeb, Stephan, et al. "Quinolones: from antibiotics to autoinducers. FEMS Microbiol Rev.2011; 35.2 : 247-274.

Hong, Sang Sook, et al. "Multiplex PCR for rapid detection of genes encoding class A carbapenemases. Ann Lab Med.2012; 32.5 : 359-361.

Hooper DC. Mechanisms of Action of Antimicrobials: Focus on Fluoroquinolones. Arch Clin Infect Dis.2001; 32:1:9–15.

Hu, Y., Matsui, Y., & Riley, L. W. Risk factors for fecal carriage of drug-resistant Escherichia coli: a systematic review and meta-analysis. Antimicrob Resist In.2020; 9(1), 31.

Hu, Zhuting, and Wei-Hua Zhao. "Identification of plasmid-and integron-borne blaIMP-1 and blaIMP-10 in clinical isolates of Serratia marcescens. J Med Microbiol.2009; 58.2 : 217-221.

Huang HI, Lee WY, Cheng MF. Fecal carriage of multidrug resistant Escherichia coli by community children I southern Taiwan. BMC Gastroenterology. 2018; 18:86:1-6.

Huang Y, Ogutu JO, Gu J, Fengshu D, You Y, Huo Y, Zhao H, Li W, Zhang Z, Zhang W, Chen X, Fu Y, and Zhang F. Comparative analysis of quinolone resistance in clinical isolates of Klebsiella pneumonia and Escherichia coli from Chinese children and Adults. Biomed Res Int. 2015;168292:1-6.

Idowu, Temilolu, and Frank Schweizer. "Ubiquitous nature of fluoroquinolones: the oscillation between antibacterial and anticancer activities." Antibiotics.2017; 6.4 : 26.

Jacobsen, S. M., et al. "Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev.2008; 21.1 : 26-59.

Jasovský D, Littmann J, Zorzet A, Cars O. Antimicrobial resistance-a threat to the world's sustainable development. Upsala J Med Sci. 2016; 121:3: 159-164.

Joo, Eun-Jeong, et al. "Fecal carriage of antimicrobial-resistant Enterobacteriaceae in healthy Korean adults. J Microbiol Biotechn .2018; 28 : 1178-84.

Josephine HR, Kumar I, Pratt RF . The perfect penicillin? Inhibition of a bacterial DD-peptidase by peptidoglycan-mimetic beta-lactams. J Am Chem Soc. 2004; 126:26:8122-8123.

Kalra, A., Cooley, C., & Tsigrelis, C. Treatment of endocarditis due to Proteus species: a literature review. Int J Infect Dis.2011; 15(4), e222-e225.

Kao, Cheng-Yen, et al. "Plasmid-mediated quinolone resistance determinants in quinolone-resistant Escherichia coli isolated from patients with bacteremia in a university hospital in Taiwan, 2001–2015. Sci Rep.2016; 6 : 32281.

Kaper, James B., James P. Nataro, and Harry LT Mobley. "Pathogenic escherichia coli." Nat Rev Microbiol.2004; 2.2: 123-140.

Karam, M., Brault, I., Van Durme, T., & Macq, J. Comparing interprofessional and interorganizational collaboration in healthcare: A systematic review of the qualitative research. Int J Nurs Stud.2018; 79, 70-83.

Katz L, Ashley GW. Translation and protein synthesis: Macrolides. Chem Rev. 2005; 105:2:499-528.

Kim, Jung Beom, Seung-Hak Cho, Yong Bae Park, Jung Bok Lee, Jong Chan Kim, Bok Kwon Lee, Hae Kyung Lee, and Hiun Suk Chae. "Surveillance of stool samples for the presence of Enterobacter sakazakii among Korean people." Yonsei Med J.2008; 49, no. 6 : 1017-1022.

Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol. 2010; 8: 6:423-435.

Kotb, D. N., Mahdy, W. K., Mahmoud, M. S., & Khairy, R. M. Impact of co-existence of PMQR genes and QRDR mutations on fluoroquinolones resistance in Enterobacteriaceae strains isolated from community and hospital acquired UTIs. BMC Infect Dis.2019; 19(1), 979.

Kumarasamy, Karthikeyan, and Aravindan Kalyanasundaram. "Emergence of Klebsiella pneumoniae isolate co-producing NDM-1 with KPC-2 from India." J Antimicrob Chemother.2012; 67.1 : 243-244.

Kumari, R., Dey, J. B., Jana, A., Ghosh, T., & Tudu, N. K. Citrobacter Emerging as a Common Uropathogen in Pediatric Population.

Kümmerer, Klaus. "Antibiotics in the aquatic environment–a review–part I." Chemosphere.2009; 75.4 : 417-434.

Kurz, C. L., Chauvet, S., Andrès, E., Aurouze, M., Vallet, I., Michel, G. P., ... & Steinmetz, I. Virulence factors of the human opportunistic pathogen Serratia marcescens identified by in vivo screening. The EMBO journal.2003; *22*(7), 1451-1460.

Kuskucu, M. A., Karakullukcu, A., Ailiken, M., Otlu, B., Mete, B., & Aygun, G. Investigation of carbapenem resistance and the first identification of Klebsiella pneumoniae carbapenemase (KPC) enzyme among Escherichia coli isolates in Turkey: A prospective study. Travel Med Infect Di. 2016; 14(6), 572-576.

Larsson DG. Antibiotics in the environment. Ups J Med Sci. 2014;119:2:108-112.

Lautenbach E, Harris A, Perencevich E, Nachamkin I, Tolomeo P, Metlay JP. Test characteristics of perirectal and rectal swab compared to stool sample for detection of fluoroquinolone-resistant *Escherichia coli* in the gastrointestinal tract. Antimicrob Agents Chemother.2005;49:798–800

Lavilla, S., J. J. Gonzalez-Lopez, M. Sabate, A. Garcia-Fernandez, M. N. Larrosa, R. M. Bartolome, A. Carattoli, and G. Prats. "Prevalence of qnr genes among extended-spectrum β-lactamase-producing enterobacterial isolates in Barcelona, Spain." J Antimicrob Chemother.2008; 61, no. 2 : 291-295.

Leite-Martins, Liliana R., Maria IM Mahú, Ana L. Costa, Ângelo Mendes, Elisabete Lopes, Denisa MV Mendonça, João JR Niza-Ribeiro, Augusto JF de Matos, and Paulo Martins da Costa. "Prevalence of antimicrobial resistance in enteric Escherichia coli from domestic pets and assessment of associated risk markers using a generalized linear mixed model." Prev Vet Med. 2014; 117, no. 1 : 28-39.

Leski, Tomasz A., et al. "High prevalence of multidrug resistant Enterobacteriaceae isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone. BMC Infect Dis.2016; 16.1: 167.

Lin, Jung-Chung, Tse Hsien Koh, Nelson Lee, Chang-Phone Fung, Feng-Yee Chang, Yu-Kuo Tsai, Margaret Ip, and L. Kristopher Siu. "Genotypes and virulence in serotype K2 Klebsiella pneumoniae from liver abscess and non-infectious carriers in Hong Kong, Singapore and Taiwan." Gut Pathog. 2014; 6, no. 1 : 21.

Livermore, David M. "Bacterial resistance: origins, epidemiology, and impact." Clin Infect Dis.2003; 36.Supplement_1 : S11-S23.

Lukac PJ, Bonomo RA, Logan LK. Extended spectrum b-lactamase-producing enterobacteriaceae in children:Old foe, emerging threat. Clin Infect Dis. 2015; 5:1-7

Luvsansharav, Ulzii-Orshikh, Itaru Hirai, Arisa Nakata, Kaori Imura, Kou Yamauchi, Marie Niki, Chalit Komalamisra, Teera Kusolsuk, and Yoshimasa Yamamoto. "Prevalence of and risk factors associated with faecal carriage of CTX-M β-lactamase-producing *Enterobacteriaceae* in rural Thai communities." J Antimicrob Chemother. 2012; 67, no. 7 : 1769-1774.

Martín-Gutiérrez, G., Rodríguez-Martínez, J. M., Pascual, Á., Rodríguez-Beltrán, J., & Blázquez, J. Plasmidic qnr genes confer clinical resistance to ciprofloxacin under urinary tract physiological conditions. Antimicrob Agents Ch.2017; 61(4), e02615-16.

Maslow JN, Lautenbach E, Glaze T, Bilker WB, Johnson JR. Colonization with extraintestinal pathogenic *Escherichia coli* among nursing home residents and its relationship to fluoroquinolone resistance. Antimicrob Agents Chemother. 2004;48:3618–3620

Maslow, Joel N., Betsy Lee, and Ebbing Lautenbach. "Fluoroquinolone-resistant Escherichia coli carriage in long-term care facility." Emerg Infect Dis.2005; 11.6 : 889.

Mitscher, Lester A. "Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents. Chem. Rev.2005; 105.2 : 559-592.

Mohammed, Ghaidaa Jihadi, Mohanad Jawad Kadhim, and Imad Hadi Hameed. "Proteus species: Characterization and herbal antibacterial: A review. Int J Pharmacogn Phytochem Res.2016; 8.11 : 1844-1854.

Mukhtar, Tariq A., and Gerard D. Wright. "Streptogramins, oxazolidinones, and other inhibitors of bacterial protein synthesis. Chem Rev.2005; 105.2 : 529-542.

Nakano, Ryuichi, Akiyo Nakano, Michiko Abe, Noriyuki Nagano, Miwa Asahara, Taiji Furukawa, Yasuo Ono, Hisakazu Yano, and Ryoichi Okamoto. "Prevalence and mechanism of fluoroquinolone resistance in clinical isolates of Proteus mirabilis in Japan." Heliyon.2019; 5, no. 3 : e01291.

Ovejero, C. M., J. F. Delgado-Blas, W. Calero-Caceres, M. Muniesa, and B. Gonzalez-Zorn. "Spread of mcr-1-carrying Enterobacteriaceae in sewage water from Spain." J Antimicrob Chemother. 2017;72, no. 4 : 1050-1053.

Owens Jr, Robert C., and Paul G. Ambrose. "Clinical use of the fluoroquinolones. Med Clin N Am.2000; 84.6 : 1447-1469.

Park, Ae Kyung, Eunkyung Shin, Soojin Kim, Jungsun Park, Hyun Ju Jeong, Jeong-Hoon Chun, Kyu Jam Hwang, and Junyoung Kim. "The Traveler-associated High-level Ciprofloxacin-resistant Salmonella enterica Serovar Kentucky in the Republic of Korea." J Glob Antimicrob Re .2019.

Paul, M., Gafter-Gvili, A., Fraser, A., & Leibovici, L. The anti-cancer effects of quinolone antibiotics?. Eur J Clin Microbiol.2007; 26(11), 825-831.

Pommier, Yves, Elisabetta Leo, HongLiang Zhang, and Christophe Marchand. "DNA topoisomerases and their poisoning by anticancer and antibacterial drugs." Chem. Biol. 2010; 17, no. 5 : 421-433.

Prabhurajeshwar, C., and C. R. Kelmani. "Shigellosis: Its Prevention and Management Issues. Con Dai & Vet Sci. 2018; 1 (5)- CDVS. MS. ID 121.

Ranjan, K. P., and Neelima Ranjan. "Citrobacter: An emerging health care associated urinary pathogen. Urol. Ann.2013; 5.4 : 313.

Rather, Irfan A., Byung-Chun Kim, Vivek K. Bajpai, and Yong-Ha Park. "Self-medication and antibiotic resistance: Crisis, current challenges, and prevention." Saudi J Biol Sci 2017; 24, no. 4 : 808-812.

Renau, Thomas E., Roger Léger, Rose Yen, Miles W. She, Eric M. Flamme, Joan Sangalang, Carla L. Gannon, Suzanne Chamberland, Olga Lomovskaya, and Ving J. Lee. "Peptidomimetics of efflux pump inhibitors potentiate the activity of levofloxacin in Pseudomonas aeruginosa." Bioorg Med Chem Lett. 2002; 12, no. 5 : 763-766.

Reuland, E. A., N. Al Naiemi, A. M. Kaiser, M. Heck, J. A. J. W. Kluytmans, P. H. M. Savelkoul, P. J. M. Elders, and C. M. J. E. Vandenbroucke-Grauls. "Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam." J Antimicrob Chemother. 2016; 71, no. 4 : 1076-1082.

Rodríguez-Martínez, José Manuel, María Eliecer Cano, Carmen Velasco, Luis Martínez-Martínez, and Alvaro Pascual. "Plasmid-mediated quinolone resistance: an update." J Infect Chemother.2011; 17, no. 2 : 149-182.

Ronald, Allan. "The etiology of urinary tract infection: traditional and emerging pathogens. Am J Med Sci.2002; 113.1 : 14-19.

Różalski, A., Torzewska, A., Moryl, M., Kwil, I., Maszewska, A., Ostrowska, K., ... & Stączek, P. Proteus sp.–an opportunistic bacterial pathogen–classification, swarming growth, clinical significance and virulence factors. Folia Biol-Prague.2012; 8(1), 1-17.

Ruh, E., Gazi, U., Guvenir, M., Suer, K., Çakır, N. Antibiotic resistance rates of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated from a university-affiliated hospital in North Cyprus. Turk Hij Den Biyol Derg. 2016; 73:333-344.

Ruh, E., Zakka, J., Hoti, K., Fekrat, A., Guler, E., Gazi, U., Erdogmus, Z., Suer, K. Extended-spectrum β-lactamase, plasmid-mediated AmpC β-lactamase, fluoroquinolone resistance, and decreased susceptibility to carbapenems in Enterobacteriaceae: fecal carriage rates and associated risk factors in the community of Northern Cyprus. Antimicrob Resist Infect Control. 2019; 8:98.

Ruiz, J., M.J. Pons, and C. Gomes, Transferable mechanisms of quinolone resistance. Int J Antimicrob Agents. 2012;40:3:196-203.

Sadigov, Alizamin, and Kamala Agayeva. "Risk Factors Associated With Antibiotic-Resistant Pathogens in Community-Acquired Pneumonia in Azerbaijan Republic." Chest.2017; 152.4 : A123.

Saksena, Rushika, Rajni Gaind, Anju Sinha, Charu Kothari, Harish Chellani, and Manorama Deb. "High prevalence of fluoroquinolone resistance amongst commensal flora of antibiotic naïve neonates: a study from India." J Med Microbiol. 2018;67, no. 4 : 481-488.

Salabi, El, and Allaaeddin Ali. Characterisation of antibiotic resistance mechanisms in Gram-negative bacteria from Tripoli and Benghazi, Libya. Cardiff University, 2011.

Salah, Fortune Djimabi, Serge Théophile Soubeiga, Abdoul Karim Ouattara, Adodo Yao Sadji, Amana Metuor-Dabire, Dorcas Obiri-Yeboah, Abiba Banla-Kere, Simplice Karou, and Jacques Simpore. "Distribution of quinolone resistance gene (qnr) in ESBL-producing Escherichia coli and Klebsiella spp. in Lomé, Togo." Antimicrob Resist In.2019; 8, no. 1 : 104.

Schulz, Jochen, Inga Ruddat, Jörg Hartung, Gerd Hamscher, Nicole Kemper, and Christa Ewers. "Antimicrobial-resistant Escherichia coli survived in dust samples for more than 20 years." Front Microbiol.2016; 7 : 866.

Sekyere, John Osei, and Daniel Gyamfi Amoako. "Genomic and phenotypic characterisation of fluoroquinolone resistance mechanisms in Enterobacteriaceae in Durban, South Africa." PLoS One.2017; 12.6 .

Shaikh, Sibhghatulla, Jamale Fatima, Shazi Shakil, Syed Mohd Danish Rizvi, and Mohammad Amjad Kamal. "Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment." Saudi J Biol Sci. 2015;22, no. 1 : 90-101.

Sharif, N. Mohammad, B. Sreedevi, R. K. Chaitanya, and D. Sreenivasulu. "Beta-lactamase antimicrobial resistance in Klebsiella and Enterobacter species isolated from healthy and diarrheic dogs in Andhra Pradesh, India." Vet World .2017; 10, no. 8 : 950.

Singh, Ashish Kumar, Saurav Das, Samer Singh, Varsha Rani Gajamer, Nilu Pradhan, Yangchen Doma Lepcha, and Hare Krishna Tiwari. "Prevalence of antibiotic resistance in commensal Escherichia coli among the children in rural hill communities of Northeast India." PloS one .2018;13, no. 6 .

Smith, David RM, F. Christiaan K. Dolk, Timo Smieszek, Julie V. Robotham, and Koen B. Pouwels. "Understanding the gender gap in antibiotic prescribing: a cross-sectional analysis of English primary care." BMJ open .2018; 8, no. 2 : e020203.

Steensels, D., K. Slabbaert, Liesbeth De Wever, Pieter Vermeersch, Hendrik Van Poppel, and Jan Verhaegen. "Fluoroquinolone-resistant E. coli in intestinal flora of patients undergoing transrectal ultrasound-guided prostate biopsy-should we reassess our practices for antibiotic prophylaxis?." Arch Clin Infect Dis.2012; 18, no. 6 : 575-581.

Stewardson, Andrew J., J. Vervoort, N. Adriaenssens, S. Coenen, M. Godycki-Cwirko, A. Kowalczyk, B. D. Huttner et al. "Effect of outpatient antibiotics for urinary tract infections on antimicrobial resistance among commensal Enterobacteriaceae: a

multinational prospective cohort study." Clin Microbiol Infect. 2018; 24, no. 9 : 972-979.

Strahilevitz, Jacob, George A. Jacoby, David C. Hooper, and Ari Robicsek. "Plasmid-mediated quinolone resistance: a multifaceted threat." Clin Microbiol Rev.2009; 22, no. 4 : 664-689.

Taha, Samaa A., and Hanan Hassan Omar. "Characterization of plasmid-mediated qnrA and qnrB genes among Enterobacteriaceae strains: quinolone resistance and ESBL production in Ismailia, Egypt. Egypt. J Med Hum Genet.2019; 20.1 : 26.

Taneja, Neelam, Abhishek Mewara, Ajay Kumar, Garima Verma, and Meera Sharma. "Cephalosporin-resistant Shigella flexneri over 9 years (2001–09) in India." J Antimicrob Chemother.2012; 67, no. 6 : 1347-1353.

Taneja, Neelam. "Changing epidemiology of shigellosis and emergence of ciprofloxacin-resistant Shigellae in India. J Clin Microbiol.2007; 45.2 : 678-679.

Tang, Y., Shen, P., Liang, W., Jin, J., & Jiang, X. A putative multi-replicon plasmid co-harboring beta-lactamase genes blaKPC-2, blaCTX-M-14 and blaTEM-1 and trimethoprim resistance gene dfrA25 from a Klebsiella pneumoniae sequence type (ST) 11 strain in China. PloS one.2017; 12(2).

Tran, Giang M., Thao P. Ho-Le, Duc T. Ha, Chau H. Tran-Nguyen, Tuyet SM Nguyen, Thao TN Pham, Tuyet A. Nguyen et al. "Patterns of antimicrobial resistance in intensive care unit patients: a study in Vietnam." BMC Infect Dis. 2017; 17, no. 1 : 429.

Trautner, Barbara W. "Fluoroquinolones for urinary tract infection and within-household spread of resistant Enterobacteriaceae: the smoking gun. Clin Microbiol Infect.2018; 24.9 : 929-930.

Turton, J. F., Baklan, H., Siu, L. K., Kaufmann, M. E., & Pitt, T. L. Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in Klebsiella sp. and

comparison of isolates within these serotypes. FEMS Microbiol Lett.2008; 284(2), 247-252.

Turton, J. F., Perry, C., Elgohari, S., & Hampton, C. V. PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. J Med Microbiol.2010; 59(5), 541-547.

Ventola CL. The antibiotic resistance crisis. Pharmacy and Therapeutics. 2015;40:4:277-283.

Walker, Kristy J., Young R. Lee, and Amanda R. Klar. "Clinical Outcomes of Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae Infections with Susceptibilities among Levofloxacin, Cefepime, and Carbapenems." Can J Infect Dis Med. 2018 .

Williamson, Deborah A., Courtney R. Lane, Marion Easton, Mary Valcanis, Janet Strachan, Mark G. Veitch, Martyn D. Kirk, and Benjamin P. Howden. "Increasing antimicrobial resistance in nontyphoidal Salmonella isolates in Australia from 1979 to 2015." Antimicrob Agents Ch.2018; 62, no. 2 : e02012-17.

Xia, J., Gao, J., Tang, W., Nosocomial infection and its molecular mechanisms of antibiotic resistance. Biosci Trends.2016; 10, 14–21.

Yagci, D., F. Yoruk, A. Azap, and O. Memikoglu. "Prevalence and risk factors for selection of quinolone-resistant Escherichia coli strains in fecal flora of patients receiving quinolone therapy." Antimicrob Agents Chemother. 2009; 53, no. 3 : 1287-1289.

Yamamoto, Norihisa, Ryuji Kawahara, Yukihiro Akeda, Rathina Kumar Shanmugakani, Hisao Yoshida, Hideharu Hagiya, Naohiro Hara et al. "Development of selective medium for IMP-type carbapenemase-producing Enterobacteriaceae in stool specimens." BMC Infect Dis. 2017; 17, no. 1 (2017): 229.

Yang, Hee Young, You Sun Nam, and Hee Joo Lee. "Prevalence of plasmid-mediated quinolone resistance genes among ciprofloxacin-nonsusceptible Escherichia coli and Klebsiella pneumoniae isolated from blood cultures in Korea. Can J Infect Dis Med.2014; 25.3 : 163-169.

Yousefipour, Mehdi, Mehrnaz Rasoulinejad, Azar Hadadi, Negin Esmailpour, Alireza Abdollahi, Sirous Jafari, and Atieh Khorsand. "Bacteria Producing Extended Spectrum β-lactamases (ESBLs) in Hospitalized Patients: Prevalence, Antimicrobial Resistance Pattern and its Main Determinants." Iran J Pathol.2019; 14, no. 1 : 61.

Zhu, Dong-Mei, Qiu-Hong Li, Yan Shen, and Qin Zhang. "Risk factors for quinolone-resistant Escherichia coli infection: a systematic review and meta-analysis." Antimicrob Resist Infect Control. 2020; 9:11.

# CURRICULUM VITAE

| Personal Information | |
|---|---|
| **Name and surname:** | Abdallah Suleiman Ahmad Abuzaid |
| **Date of birth:** | 1987-11-26 |
| **Marital status:** | Married |
| **E-mail:** | abduallahabuzaid78@gmail.com |
| **Phone** | +90 5488490520 or +962 788715887 |
| **Address:** | Amman – Jordan |

| Education | |
|---|---|
| **Master of Medical Microbiology and Clinical Microbiology** | Near East University, Northern Cyprus, 2020 |
| **Bachelor of Medical Analysis** | Al Zarq'a University, Jordan, Jordan, 2011. |

| Training Courses |
|---|
| • Medical Lab Technician Trainee Certificate (Bachelor degree requirement) - Islamic Hospital. |
| • Medical Lab Technician Trainee Certificate (Bachelor degree requirement) - Prince Hamza Hospital. |
| • Medical Lab Technician Trainee Certificate - The Specialty Hospital |
| • Medical Lab Technician Trainee Certificate - Al-Salam Medical Lab – Wehdat district. |

| Work Experience |
|---|
| Worked as a Medical representative – Omar Abuzaid Pharmaceutical Warehouse 2012 – 2016 |

# ACKNOWLEDGEMENTS

My profound gratitude to Allah the God of the universe for giving me the opportunity to complete my thesis successfully.

To my supervisor, Assoc. Prof. Emrah Ruh, who has guided and supervised this thesis, thank you so much.

My special gratitude is also to Prof. Nedim Çakır (Head of Department of Medical Microbiology and Clinical Microbiology) for his intense guide throughout the process of this research.

I am also grateful to Prof. Hüseyin Kaya Süer and Assist. Prof. Özgür Tosun for their support and study guidance throughout my postgraduate study.

I am thankful to Near East University and Faculty of Medicine for supporting my thesis work.

I have special gratitude to the clinicians and nurses in the Near East University Hospital, Dr. Nagat Balaman and MSc. Emrah Guler for their support and advice.

To my parents, I owe you a deep gratitude.

Finally, to my wife Eman Ghazal and the entire family, thank you so much for your support and prayers through my study.

# TABLE OF CONTENTS

# LIST OF TABLES

Page No

# LIST OF ABBREVIATIONS AND SYMBOLS

**CIP**: Ciprofloxacin

**CIP-RE**: Ciprofloxacin-resistant *Enterobacteriaceae*

**CTX**: Cefotaxime

**EHEC**: Enterohemorrhagic *Escherichia coli*

**EIEC**: Enteroinvasive *Escherichia coli*

**EMB**: Eosin methylene blue

**EPEC**: Enteropathogenic *Escherichia coli*

**ESBL**: Extended-spectrum beta-lactamase

**ETEC**: Enterotoxigenic *Escherichia coli*

**GEM**: Gemifloxacin

**GIS**: Gastrointestinal symptom

**GIT**: Gastrointestinal tract

**ICU**: Intensive care unit

**LVX**: Levofloxacin

**mg/L**: Milligrams per liter

**mg**: Milligram

**ml**: Milliliter

**mm**: Millimeter

**MS-HA**: Mannose-sensitive hemaglutinin

**NAG**: N-acetylglucosamine

**NAM**: N-acetylmuramic acid

**NOR**: Norfloxacin

**OFX**: Ofloxacin

**PMQR**: Plasmid-mediated fluoroquinolone resistance

**QRDR**: Quinolone resistance-determining region

**UCA**: Uroepithelial cell adhesin

**UTI**: Urinary tract infections

**XLD**: Xylose Lysine Deoxycholate

**µg**: Microgram