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NEAR EAST UNIVERSITY
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DETECTION OF INTESTINAL COLONIZATION RATES OF FLUOROQUINOLONE-RESISTANT *ENTEROBACTERIACEAE* IN NORTHERN CYPRUS

Arezou FEKRAT

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAMME

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SUPERVISOR
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ABSTRACT

Fekrat, A. Detection of Intestinal Colonization Rates of Fluoroquinolone-Resistant *Enterobacteriaceae* in Northern Cyprus. Near East University Institute of Health Sciences, M.Sc. Thesis in Medical Microbiology and Clinical Microbiology Programme, Nicosia, 2018.

Fluoroquinolones are one of the most important synthetic antimicrobial agents, which are used worldwide. In the last few years, the resistance to fluoroquinolones has increased globally. In bacteria, DNA gyrase is known as the primary target for fluoroquinolones. *Enterobacteriaceae* are one of the most important groups whose members are significantly becoming resistant to antibiotics. In *Enterobacteriaceae,* the emergence and spread of resistance cause the problems in the treatment of serious nosocomial infections. In the present study 500 stool samples and fecal swabs were collected and subjected to bacteriological analysis to identify *Enterobacteriaceae* and to determine their resistance patterns to ciprofloxacin by disk diffusion method. In the study, bacterial isolates that were resistant and intermediate-resistant to ciprofloxacin were also analysed in terms of demographic, socio-economic and epidemiological characteristics of the participants. The statistical analysis revealed that fecal carriage of ciprofloxacin-intermediate and -resistant *Enterobacteriaceae* was not significantly affected by any of the demographic, socio-economic and epidemiological factors ($p > 0.05$). Yet, the present study suggests that the antimicrobial resistance cannot be ignored, and the test results should be routinely monitored in Northern Cyprus.

Key words: *Enterobacteriaceae*, Fluoroquinolones, Ciprofloxacin, Intestinal Colonization, Northern Cyprus

Supported by Near East University

ÖZET

Fekrat, A. Kuzey Kıbrıs'ta Florokinolon Dirençli *Enterobacteriaceae* Türlerinin İntestinal Kolonizasyon Oranlarının Saptanması. Yakın Doğu Üniversitesi Sağlık Bilimleri Enstitüsü, Tibbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı Yüksek Lisans Tezi, Lefkoşa, 2018.

Florokinolonlar dünya çapında kullanılan en önemli sentetik antimikrobiyal ajanlardan biridir. Son birkaç yılda florokinolonlara karşı direnç küresel olarak artmıştır. Bakterilerde DNA giraz, florokinolonların birincil hedefi olarak bilinmektedir. *Enterobacteriaceae* üyeleri antibiyotiklere karşı önemli derecede direnç gösteren en önemli gruplardan biridir. *Enterobacteriaceae* türlerinde direncin ortaya çıkması ve yayılması ciddi nozokomiyal enfeksiyonların tedavisinde sorunlara neden olmaktadır. Bu çalışmada 500 dışkı örneği ve rektal sürüntü toplanmıştır. *Enterobacteriaceae* türlerinin tanımlanması ve bunların disk difüzyon yöntemi ile siprofloksasin direnç profillerinin belirlenmesi amacıyla bakteriyolojik analizler uygulanmıştır. Çalışmada, siprofloksasine dirençli ve orta derecede dirençli bakteri izolatları katılımcıların demografik, sosyo-ekonomik ve epidemiyolojik özellikleri açısından analiz edilmiştir. İstatistiksel analiz sonucunda siprofloksasine dirençli ve orta derecede dirençli *Enterobacteriaceae* türlerinin dışkıda taşıyıcılığının demografik, sosyo-ekonomik ve epidemiyolojik faktörlerden etkilenmediği sonucuna varılmıştır ($p > 0.05$). Bununla birlikte, bu çalışma, Kuzey Kıbrıs'ta antimikrobiyal direncin gözardı edilmemesi, ve test sonuçlarının rutin olarak taranması gerektiğine işaret etmektedir.

Anahtar Kelimeler: *Enterobacteriaceae*, Florokinolonlar, Siprofloksasin, İntestinal Kolonizasyon, Kuzey Kıbrıs

Destekleyen Kurum: Yakın Doğu Üniversitesi

TABLE OF CONTENTS

SYMBOLS AND ABBREVIATIONS

CIP	Ciprofloxacin
DNA	Deoxyribonucleic acid
DAEC	Diffusely adherent *Escherichia coli*
EAggEC	Enteroaggregative *Escherichia coli*
EHEC	Enterohemolytic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
Hep-2	Human epithelial type 2
HUS	Hemolytic uremic syndrome
STEC	Shiga toxin-producing *Escherichia coli*
Stx	Shiga toxin
UTI	Urinary tract infection
VTEC	Verocyto toxin-producing *Escherichia coli*

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Since the discovery of penicillin by Alexander Fleming in 1928 (Fleming, 1929), antibiotics have revolutionized the clinical treatment of bacterial diseases. However, the fast emergence and dissemination of resistant bacterial strains have reduced the powerful therapeutically benefits of nearly all antibiotics that have been developed (Ventola, 2015). Currently, bacterial antibiotic resistance constitutes a global public health problem with death approximately ranging from 23000 in the US, 25000 in the EU, to 58000 in India (Chaudhary, 2016).

The causes of antibiotic resistance are largely attributed to the continuing misuse and overuse of antibiotics. When incorrectly prescribed, sub-inhibitory antibiotic concentrations can promote resistance through genetic alterations and mutagenesis. Moreover, epidemiological studies have shown a direct link among antibiotic consumption and the appearance of resistant bacterial strains. The overuse of antibiotics increases the risk of spontaneous mutations responsible for resistance.

The horizontal gene transfer between bacteria aggravates the problem allowing the transfer of resistance-causing plasmids between different bacterial species (Ventola, 2015). Fluoroquinolones are one example of broad-spectrum antibacterial agents, which are becoming increasingly inefficient in treating emerging resistant gram-negative *Enterobacteriaceae* infections (Dalhoff, 2012). These infections cause a wide range of diseases including urinary tract infection, septicemia, gastroenteritis, and hospital-acquired infections (Irving et al., 2005). *Enterobacteriaceae* are mostly gut commensals, which can, under some circumstances such as the case of immunocompromised individuals, become pathogenic and provoke a disease (Irving et al., 2015). Many epidemiological studies are increasingly suggesting that intestinal carriage of resistant *Enterobacteriaceae* is indeed a reservoir for infections, permitting person to person transmission and recurrent infections with resistant bacteria (Zerr et al., 2014).

The present study aimed to determine the intestinal colonization rates of ciprofloxacin-resistant *Enterobacteriaceae* in Northern Cyprus. The other objective of this study was to evaluate the association between the fecal carriage of

ciprofloxacin-intermediate and -resistant *Enterobacteriaceae* with demographic, socio-economic and epidemiological factors.

2. GENERAL INFORMATION

2.1. *Enterobacteriaceae*

Enterobacteriaceae are a ubiquitous and heterogeneous family of gram-negative, non-spore forming bacteria found in a wide range of environments such as soil, water, fruits, plants, vertebrate and invertebrate animals (Kayser et al., 2005).

Some *Enterobacteriaceae* such as *Escherichia coli*, *Enterobacter aerogenes* or *Klebsiella* have a commensal relation with mammals and are present in the normal gut flora of humans and animals (Guentzel et al., 1996).

Under specific conditions of imbalance between the host resistance and the bacterial strains, commensal species may become infective and cause a disease (Samapaio, 2016). Other *Enterobacteiaceae* species such as *Salmonella, Shigella* and *Yersinia* are exclusive pathogens (Giannella, 1996).

Enterobacteriaceae are characterized with a straight rod shape and a length of 1-3 μm. They grow well on MacConkey agar. They are catalase-positive, oxidase-negative, and are facultatively anaerobes (Samapaio et al., 2016).

Enterobacteriaceae group consists of more than 176 known species and 44 genera (Ibrahim and Hameed, 2015) and can be classified according to their lactose fermentation ability.

Three groups of *Enterobacteriaceae* can be distinguished:

i) Lactose fermenters which include *Escherichia coli* and *Enterobacter* spp.

ii) Late lactose fermenters including *Citrobacter* spp.and *Serratia* spp.

iii) Lactose non-fermenters such as *Proteus* spp., *Salmonella* spp., and *Shigella* spp.

2.2. Antigenic Structure of *Enterobacteriaceae*

Enterobacteriaceae presents a complex antigenic structure with three major antigens: H, K, and O.

A combination of these antigens permits a further classification of *Enterobacteriaceae* into different serotypes which are associated with the virulence degree and the habitat which the strains prefer (Guentzel *et al*., 1996).

O antigens are located at the external part of the cell envelope. They are characterized by layers of polysaccharides. O antigens have been linked to many diseases such as diarrhea and urinary tract infections (UTIs) (Mamza *et al*., 2010).

K antigens are either capsular polysaccharides or proteins. They have been linked to upper urinary tract infections. K antigens are characterized by their capacity to inhibit the phagocytosis of bacterial cells (Todar, 2004).

H antigens are located on the flagella and are common in mobile *Enterobacteriaceae*. They contain flagellins which are flagella proteins, and they are heat-labile (Dauda, 2014).

2.3. Clinically Important *Enterobacteriaceae*

2.3.1. *Escherichia coli*

Escherichia coli is a gram-negative, non-spore forming rod. It is part of the normal microbial flora in the gut. *E. coli* appears soon after birth in the gastrointestinal tract. After acquiring plasmid DNA encoding enterotoxins, some strains become virulent and pathogenic (Moriel *et al*., 2010).

E. coli plays an important role in maintaining a balance in the functions and activities of the intestinal organs. *E. coli* was regarded as a harmless commensal in feces, beneficial to the host by preventing gut colonization with other potential pathogens. The emergence of clinical manifestations such as severe diarrhea prompted its classification as an opportunistic pathogen (Rivas et al., 2015). *E. coli* can colonize the mucosal surface of the intestinal organs, or cause invasive infections (Evans and Evans, 1996).

Different strains of *E. coli* have been associated with diarrhea which is a major public health problem. Over two million deaths related to *E. coli* infection occur each year (Deborah Chen and Frankel, 2005).

The pathotypes of *E. coli* that cause intestinal diseases have been divided into six groups, which are enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (Evans and Evans, 1996).

Enteropathogenic *E. coli* (EPEC)

EPEC is non-invasive and non-toxigenic, it causes diarrhea by disrupting the absorption-secretion balance in the small intestine. Symptoms include fever, malaise, vomiting and diarrhea (Brooks et al., 2014).

EPEC affects mostly children and infants and is common where there is a low-quality sanitation (Evans and Evans, 1996)

Outbreaks mainly occur in pediatric wards, day care centers, and nursing houses (Ochoa and Contreras, 2011).

EPEC is a major cause of infant diarrhea in developing countries, with a mortality rate of 30% (Deborah Chen and Frankel, 2005).

Persistent diarrhea caused by EPEC is most probably polymicrobial and is usually associated with parasitic infections such as *Cryptosporidium* and *Giardia* (Ochoa and Contreras, 2011).

Enterohemorrhagic *E. coli* (EHEC)

EHEC is a human pathogenic *E. coli* responsible for hemorrhagic colitis (bloody diarrhea) which might develop into hemolytic uremic syndrome (HUS). HUS is a severe complication of an EHEC infection and a life-threatening disease that can cause kidney damage. EHEC belongs to the Shiga toxin-producing *E. coli* (STEC). The STEC are also known as Verocyto toxin-producing *E. coli* (VTEC) (Mellmann et al., 2011).

EHEC accounts for various food-borne infections and its pathogenicity results mainly from its production of Shiga toxin (Shiga toxin 1 [Stx1] and/or Stx2) (Kintz et al., 2017).

EHEC is transmitted through the fecal-oral route, and can spread between animals by direct contact or via water troughs, shared food, and contaminated pastures (Mellmann et al., 2011).

Humans become infected with EHEC O157:H7 by ingesting contaminated food and water, or after contact with ruminant animals, feces and contaminated soil (Kintz et al., 2017).

The infectious dose for humans is estimated to be from 10 to less than 100 microorganisms. EHEC O157:H7 is implicated in food-borne outbreaks involving undercooked meat or unpasteurized milk and cheese.

Contaminated irrigation water is an important source of EHEC O157:H7 in vegetables (Kintz et al., 2017).

Enteroinvasive *E. coli* (EIEC)

EIEC is a diarrheagenic group of *E. coli*. EIEC is known to cause shigellosis-like symptoms in adults and children. It is biochemically and genetically related to *Shigella* spp. (Vieira *et al.*, 2007).

EIEC infections cause dysentery (bloody and mucoid diarrhea). EIEC invades the epithelial cells of the colon and damages the intestine causing inflammation and ulceration (Van den Beld and Reubsaet, 2012).

Enterotoxigenic *E. coli* (ETEC)

ETEC is the most common cause of travelers' diarrhea in developing countries (Kayser et al., 2005).

The pathogenicity of ETEC is determined by specific virulence factors including enterotoxins and colonization factors, differentiating it from other

diarrheagenic *E. coli*. The colonization factors allow ETEC to effectively adhere and colonize the small intestine and then cause diarrhea (Evans and Evans, 1996).

Transmission of ETEC occurs by the fecal-oral route. Bacteria colonize the intestinal epithelia with their pili (fimbriae) (Kayser et al., 2005).

ETEC cytotoxic enterotoxins are encoded on plasmids or bacteriophage DNA and are responsible for provoking the watery diarrhea symptom (Evans and Evans, 1996).

The cytotoxic enterotoxins, either plasmid- or bacteriophage-encoded, induce tissue damage. The plasmid-encoded invasion factors cause the invasion of the mucosa (Evans and Evans, 1996).

Enteroaggregative *E. coli* (EAggEC)

EAggEC is responsible for persistent diarrhea in children and adults especially in developing countries (Okhuysen and DuPont, 2010).

The pathogenesis of EAggEC infections is not yet understood even though a number of virulence factors have been reported (Abbasi et al., 2015). However, the pathogenesis of EAggEC is determined by its adherence to intestinal cells, its production of enterotoxins and cytotoxins, and induction of inflammation (Scheutz et et al., 2011).

EAggEC is able to aggregate intimately with each other, to human HEp-2 cells, and to non-living surfaces when grown in vitro (Okhuysen and DuPont, 2010).

Diffusely adherent *E. coli* (DAEC)

DAEC is a group of diarrheagenic *E. coli* strains capable of adhering to HEp-2 and HeLa cells, which allows the bacteria to attach themselves evenly to the entire surface of the cell. DAEC causes diarrhea in children. DAEC comprises a heterogeneous group of *E. coli*, with different enteropathogenicity grades (Patzi-Vargas et al., 2013). Some strains of DAEC have been reported to cause bloody diarrhea (Ochoa and Contreras, 2011).

2.3.2. *Klebsiella*

Klebsiella spp. are ubiquitous bacterial strains found in the environment, such as water surface, sewage, soil, and on plants. They can also be found on mucosal surfaces of mammals including humans and animals. The most clinically important species is *Klebsiella pneumoniae*. Although it is responsible for a small number of pneumonia cases, it causes important damage. Furthermore, the rate of mortality resulting from *K. pneumoniae* infections is high (Guentzel *et al.*, 1996).

K. pneumoniae is a gram-negative bacterium. It ferments lactose, and it is characterized with a rod shape and a capsule (Holt et al., 2015).

K. pneumoniae is an opportunistic pathogen being carried asymptomatically in the intestinal tract, skin, nose, and throat by healthy individuals (Holt et al., 2015). Opportunistic *K. pneumoniae* mostly affects immunocompromised subjects who are weakened by other underlying infections (Li et al., 2014).

K. pneumoniae is a major cause of neonatal sepsis and nosocomial infections in hospitalized patients. It is responsible for pneumonia, UTIs, wound and soft tissue infections (Li et al., 2014).

K. pneumoniae is also responsible for community-acquired infections, including pneumonia, meningitis and pyogenic liver abscess (Holt *et al*., 2015). Opportunistic *K. pneumoniae* infections begin with the colonization of the gastrointestinal tract, and this is followed by the development of invasive nosocomial infections (Holt et al., 2015).

In hospitals, medical devices such as catheters and endotracheal tubes can get contaminated and become a significant source of infection for hospitalized patients (Lery et al., 2014).

Nosocomial infections caused by *K. pneumoniae* are usually chronic. *K. pneumoniae* can form a biofilm which protects the microorganism from the host immune response and antibiotics (Laminet et al., 2013).

A major health problem is the multidrug resistance observed in the nosocomial infections with *K. pneumoniae*. In fact, most strains of *K. pneumoniae* carry plasmids encoding extended-spectrum beta-lactamases (ESBLs) and

carbapenemases, making most antibiotic treatments ineffective and difficult (Holt et al., 2015).

The capsule polysaccharide is the main part responsible for the virulence of *K. pneumoniae*. Hypervirulent *K. pneumoniae* has increased production of capsule polysaccharide responsible for causing life threatening community-acquired infections, such as severe pneumonia, pyogenic liver abscess, meningitis, necrotizing fasciitis and endophthalmitis (Li et al., 2014).

2.3.3. *Salmonella*

Salmonella spp. are gram-negative, rod-shaped and facultative anaerobic bacteria that contain flagella (Giannella, 1996).

Salmonella infect humans and animals causing salmonellosis which manifests as a self-limiting gastroenteritis in humans. Occasionally salmonellosis evolves into a systemic infection and enteric fever (Giannella, 1996).

Gastroenteritis and diarrhea are caused by the inflammatory response of the host leading to ulcerations and the destruction of the mucosa. Virulent strains are capable of intracellular multiplication after invading the intestinal epithelium and spread afterwards through systemic circulation to the lymph nodes and the rest of body. In most of the cases extraintestinal organisms are destroyed and the infection is limited with the intestine (Brooks et al., 2014).

Animals especially chicken, turkey, and pigs are the main reservoir for *Salmonella* with transmission occurring mainly through contaminated food. *Salmonella* can survive for long periods in uncooked meat. Ingestion of undercooked contaminated meat is a source for transmission (Cui et al., 2008).

Salmonella species causing typhoid fever usually spread from person to person since they lack animals as a main reservoir. Transmission occurs through contaminated human feces and water (Giannella, 1996).

2.3.4. *Shigella*

Shigella are gram-negative rod-shaped and non-motile bacilli. They are very closely related to *E. coli* and they can only be differentiated from one another based on their virulence grade and their lactose fermentation, *Shigella* not being able to ferment lactose (Hale and Keusch, 1996).

Shigellosis, the disease caused by *Shigella*, is characterized by bloody and watery diarrhea, with or without mucus. Abdominal cramps and rectal tenesmus are common. Fever, dehydration and vomiting can also be present (Hale and Keusch, 1996).

Shigella is transmitted by direct fecal-oral contact, and humans are the main host. Other primates may be infected as well. Poor sanitation and crowded housing constitute the main cause for its spread. This is the case of most developing countries where 50% of children's diarrhea can be attributed to *Shigella* (Hale and Keusch, 1996).

In some cases, flies (*Musca domestica*) can transmit *Shigella* from feces to food and then to humans through the ingestion of contaminated food or water (Temu et al., 2007).

In developed countries, outbreaks may involve child care facilities, public camps and parks with shared sanitation and water sources. *S. dysenteriae* and *S. flexneri* are mostly found in tropical countries, while *S. sonnei* is predominant in developed countries (Temu et al., 2007).

After the ingestion of *Shigella*, infection occurs, and the diarrhea is the earliest symptom. However, complications follow and are due to the invasion of the colonic epithelium by the bacteria, causing colitis and dysentery. Presence of blood and neutrophils in stools is a sign of shigellosis (Hale and Keusch, 1996).

Shiga toxins which are cytotoxins encoded on plasmids are the major cause for virulence and for the severe gastrointestinal disease and dysentery observed in infections with *Shigella* (Brooks et al., 2008).

2.3.5. *Yersinia*

Yersinia are gram-negative, catalase-positive, oxidase-negative, and facultative anaerobic rods. Animals are the main reservoir for *Yersinia* (Savin et al., 2014).

Yersinia includes 17 known species (Savin *et al*., 2014). Bubonic plague is caused by *Yersinia pestis* and is transmitted by rat fleas. It is a fatal disease manifesting with black lymph nodes followed by septicemia and hemorragic pneumonia. Transmission can occur through respiratory droplets from person to person (Cocolin and Comi., 2005).

Two other *Yersinia* species, *Y. enterocolitica* and *Y. pseudoturberculosis*, cause severe diarrhea and severe enterocolitis respectively (Collins, 1996).

Yersinia spp. are able to grow at low temperatures and can be therefore transmitted through refrigerated and not thoroughly cooked food (Cocolin and Comi, 2005).

After the initial colonization of the intestinal tract, ulceration follows, and leukocytes appear in feces. Symptoms include fever, abdominal pain and watery to bloody diarrhea (Galindo et al., 2011).

2.3.6. *Enterobacter*

Enterobacter are rod-shaped, gram-negative, non-spore-forming and facultative anaerobic bacteria. They are the major cause of respiratory tract and urinary tract infections. Some species can cause opportunistic infections especially in immunocompromised patients (Davin-Regli, 2015).

2.4. Fecal Carriage of Resistant *Enterobacteriaceae*

Enterobacteriaceae are gut commensals, potentially pathogenic, originating from the digestive tract which acts as a reservoir for an important number of bacterial infections.

Infection may occur when large amounts of pathogenic bacteria reach the gut from the hands, nasal and pharyngeal secretions, and from the ingestion of food and drinks. Under normal conditions with the stable gut flora of healthy individuals, ingested pathogens are easily cleared.

However, the misuse and overuse of wide-spectrum antibiotics in particular, have the capacity to disrupt the gut microbiome, killing bacteria and anaerobic microorganisms which act as a defence barrier in the digestive tract.

Even after the successful treatment of pathogens with wide-spectrum antibiotics such as fluoroquinolones, resistance to antibiotics can occur in commensal flora in the gut, including *Enterobacteriaceae*.

This is mainly explained by the horizontal gene transfer between different bacterial strains. *Enterobacteriaceae*, other pathogens, and the gut flora exchange genetic information including antibiotic resistance encoding plasmids.

Additionally, frequent antibiotic treatments can result in the selection of resistant bacterial strains that were initially present in small amounts in the gut.

The presence of resistant *Enterobacteriaceae* strains in high amounts in the gut, therefore in the rectum and feces, increases the risk of cross-transmission.

Their transmission to and between even healthy individuals can occur through the environmental contamination of food and drinks, and the lack of hygiene measures in hospitals and the community (Carlet, 2012).

2.5. Fluoroquinolones

Fluoroquinolones are synthetic antibiotics used in the treatment of urinary tract, respiratory, gastrointestinal, and skin infections in hospitalized and outpatients (Dalhoff, 2012). Fluoroquinolones are orally active, broad-spectrum, and heat-stable antibiotics, making them the treatment of choice for patients with salmonellosis, cholera, severe gastroenteritis, and enteric fever (Chattaway et al., 2016).

2.5.1. First-Generation Quinolones

The first quinolone to be used in the 1960's for the oral treatment of simple urinary tract infections was nalidixic acid (Dalhoff, 2012). It was generated from an antimalarial agent, chloroquine. However, bacterial resistance to nalidixic acid developed shortly after its production. Furthermore, its high toxicity to the host cell limits its application (Sharma et al., 2009).

2.5.2. Second-Generation Fluoroquinolones

Based on the molecule of nalidixic acid, second-generation quinolones including ciprofloxacin, enoxacin, norfloxacin and ofloxacin were developed. They have an increased activity against some gram-negative and gram-positive bacteria. They are administered for the treatment of complicated UTIs, pyelonephritis, sexually transmitted diseases, skin infections and some pulmonary infections. Ciprofloxacin and ofloxacin exist as oral and intravenous formulations, and are therefore the most widely used second-generation quinolones (King et al., 2000).

2.5.3. Third-Generation Fluoroquinolones

Third-generation quinolones include levofloxacin, gatifloxacin and sparfloxacin. Similar to the second-generation quinolones, they have a broad-spectrum activity against gram-negative bacteria. Additionally, they have an expanding coverage of gram-positive bacteria such as penicillin resistant *Streptococcus pneumoniae* and some pathogenic organisms such as *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*. Their expanded antimicrobial spectrum makes them useful in treating community-acquired pneumonia, acute sinusitis and acute bronchitis. Moreover, gatifloxacin is indicated for the treatment of UTIs and gonorrhea (King et al., 2000).

2.5.4. Fourth-Generation Fluoroquinolones

One of the fourth-generation quinolones is known to be moxifloxacin. Moxifloxacin is mainly used as prophylaxis for treating endophthalmitis. Recently it has been found that the intraocular penetration of this antibiotic is not suitable for bacterial endophthalmitis after operation. Based on the research cytotoxic activity was recognized for moxifloxacin. This antibiotic has shown a great influence against anaerobes. Other antibiotic which is classified in this group is gemifloxacin (Idowu, 2017).

2.6. Mechanism of Action of Fluoroquinolones

Fluoroquinolones are bactericidal agents which act by inhibiting bacterial DNA synthesis. They target two DNA topoisomerases: DNA gyrase and topoisomerase IV. In gram-negative bacteria such as *Enterobacteriaceae*, DNA gyrase is the primary target, while in gram-positive bacteria fluoroquinolones tend to inhibit DNA topoisomerase IV (Drlica et al., 2007).

Fluoroquinolones form a drug-topoisomerase-DNA complex leading to the generation of breaks in the double-stranded DNA, which blocks the progress of the DNA replication. This process ultimately results in DNA damage and bacterial cell death (Drlica et al., 2007). Mechanism of action of fluoroquinolones was shown in the Figure 2.1.

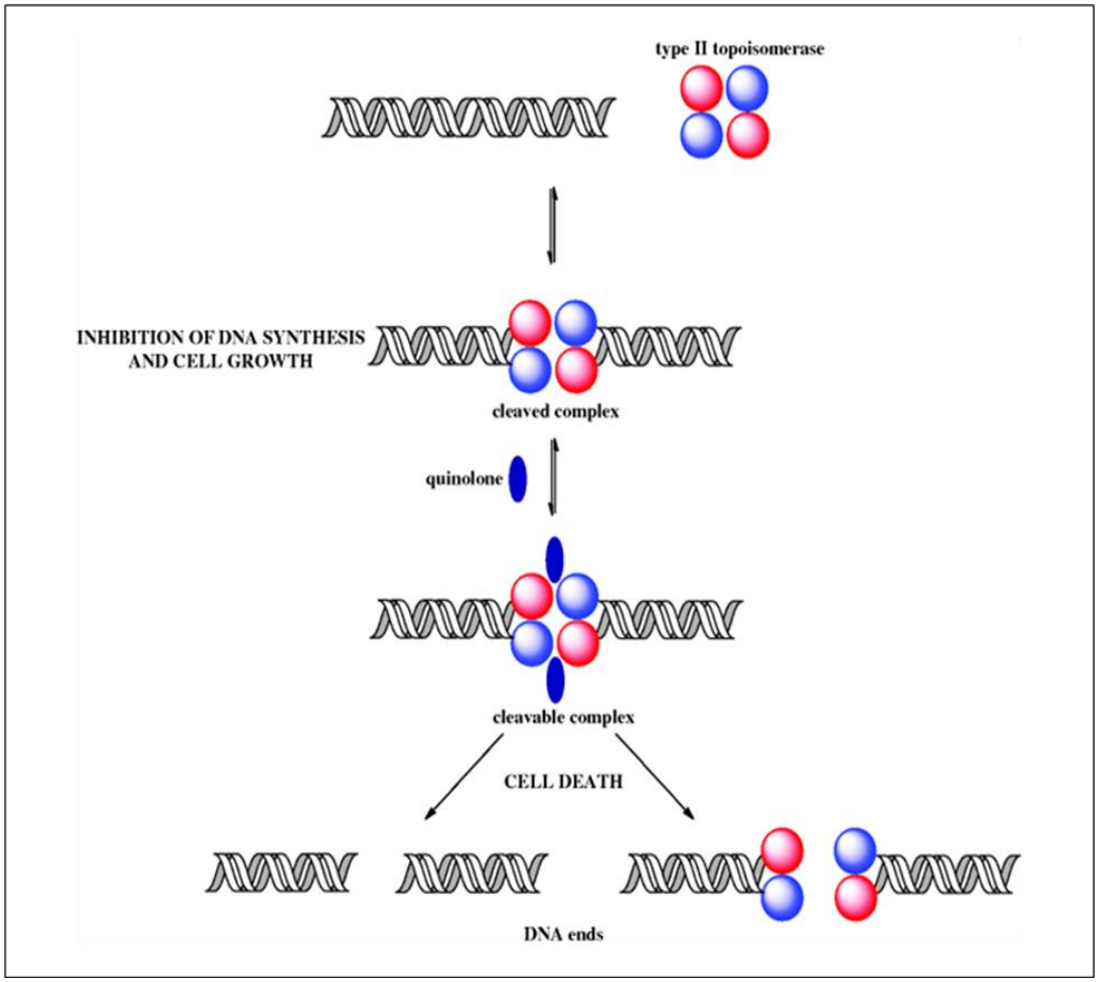

Figure 2.1. Mechanism of action of fluoroquinolones.
(Source: https://basicmedicalkey.com/quinolone-antibacterial-agents/)

2.7. General Mechanisms of Antibiotic Resistance

Bacterial resistance to antibiotics has evolved from ancient mechanisms through evolution and can therefore be described as either intrinsic or acquired (Iredell et al., 2016).

2.7.1. Intrinsic Resistance

Intrinsic resistance is a natural occurring phenomenon. Some bacteria exhibit antibiotic resistance without any prior antibiotic exposure, and without any horizontal gene transfer (Cox and Wright, 2013).

Such bacteria are able to resist an antibiotic through its inherent functional or structural characteristics. Since most antibiotics are natural molecules produced in the environment, many co-existing bacteria were able to evolve mechanisms to resist theses antimicrobial agents, making them intrinsically resistant (Cox and Wright, 2013).

2.7.2. Acquired Resistance

Antibiotic resistance is considered acquired when mutations in genes affecting the activity of an antibiotic are developed in a subset of bacterial cells derived from an antibiotic susceptible population. The non-mutated susceptible bacteria will be eliminated by the antibiotic, and only the resistant bacteria carrying the mutation remain (Munita and Arias, 2016).

Resistance can also be acquired through horizontal gene transfer (HGT), which is one of the main reasons of the development of bacterial resistance. Bacteria can acquire external genetic material by tansformation, transduction and conjugation (Iredell et al., 2016).

Conjugation is the most frequent mechanism of acquiring resistance-encoding genes. It involves contact between the cells and most probably occurs at high rate in the gastrointestinal tract of humans under antibiotic treatment. During conjugation, plasmids are exchanged and are therefore responsible for the dissemination of antimicrobial resistance (Munita and Arias, 2016).

The main mechanisms of acquired antibiotic resistance are:

- Enzymatic inactivation of the drug
- Alteration of the membrane permeability
- Active efflux pumps
- Mutation of the target site (Munita and Arias ,2016).

<u>Enzymatic Inactivation of the Drug</u>

Bacteria can produce enzymes which are capable of inactivating the antibiotics. Chemical alterations to the antibiotic most frequently include acetylation,

adenylation, and phosphorylation. Gram-negative as well as gram-positive bacteria exhibit this principle of acquired resistance (Munita and Arias, 2016). An example is the production of aminoglycoside modifying enzymes (AMEs) by bacteria. They are harboured in mobile genetic elements and confer resistance to aminoglycosides. The destruction of the antibiotic molecule is mostly illustrated by the example of β-lactamases. These enzymes destroy the amide bond of β-lactam antibiotics rendering them ineffective (Munita and Arias, 2016).

Alteration of the Membrane Permeability

In order to be effective against bacteria, many antibiotics must reach an intracellular target or the cytoplasmic membrane. Resistant bacteria are capable in this case of decreasing the uptake of the antimicrobial agent. Gram-negative bacteria are particularly active in limiting the influx of antibiotics such as β-lactams, tetracycline, and fluoroquinolones (Toma and Deyno, 2015).

Active Efflux Pumps

Many classes of efflux pumps have already been characterized in both gram-negative and gram-positive bacteria. Efflux pumps can be substrate-specific or present a wide specificity. Fluoroquinolones, β-lactams, tetracyclines, and carbapenems are some of the antibiotics affected by this mechanism of resistance. The mutated genes can be chromosomal, or located on mobile genetic elements (Toma and Deyno, 2015).

Mutation of the Target Site

Bacterial resistance can also occur when antibiotics are unable to reach their target. This is achieved by the means of two main mechanisms: target protection, or target site alteration resulting in its decreased affinity to the antibiotic. Genes for the proteins involved in target protection are mostly encoded on plasmids (Toma and Deyno, 2015).

The quinolone resistance protein Qnr, encoded on a plasmid, is frequently found in clinical isolates. Qnr acts as a homologue competing with the DNA binding site of the DNA topoisomerases II, decreasing the chance of quinolone's binding with the DNA-topoisomerase II complex. Target changes can consist of:

- Alteration of the binding site by enzymes
- Mutations in the genes encoding the target
- Replacement of the original target (Adegboye et al., 2012).

Fluoroquinolone resistance is an example for the target site alteration resistance mechanism. Mutations within the subunits encoding bacterial gyrase and DNA topoisomerase IV decrease the binding capacity of fluoroquinolone to the DNA topoisomerase complex (Adegboye et al., 2012).

2.8. Autoinduction of Fluoroquinolone Resistance

Fluoroquinolone's inhibition of bacterial DNA gyrase and topoisomerase IV leads to the activation of the SOS gene network in the bacterial cell, thus triggering the synthesis of specific proteins involved in bacterial repair mechanisms. Two proteins, RecA and LexA, control the SOS system. RecA's effect is to induce the activity of the SOS response, while LexA is a repressor (Dalhoff, 2012). The presence in the bacterial cell of an increased level of DNA damage in the form of single-stranded DNA activates the autocleavage of LexA. LexA presents the ability to bind to the SOS box and to derepress the SOS genes (Qin et al., 2015).

The SOS network comprises more than 40 genes including polymerases. The upregulation of the SOS gene products increases bacterial mutation ability, giving the bacteria an active role in the mutation of their own genomes (Qin et al., 2015).

Furthermore, the SOS response plays a direct role in target site resistant mechanisms, triggering the gene *qnrB*-mediated quinolone resistance. The SOS network promotes *qnrB* gene expression. The product QnrB peptide provides the bacteria with a low-level quinolone resistance by protecting bacterial DNA topoisomerases from the quinolone inhibition (Dalhoff, 2012).

2.9. Mechanisms of Fluoroquinolone Resistance

Plasmid-encoded fluoroquinolone resistance can be transferable between bacterial strains and species. Qnr belongs to the pentapeptide repeat (PPR) family of proteins. Qnr prevents fluoroquinolone inhibition of bacterial DNA topoisomerase IV and DNA gyrase by interacting with the latter. Qnr provokes nalidixic acid resistance and confer the bacteria with a low-level fluoroquinolone resistance (Dalhoff, 2012). A variant of the *aac(6′)-Ib* gene encoding an aminoglycoside acetyltransferase is another plasmid gene responsible for fluoroquinolone resistance. The enzyme can acetylate ciprofloxacin and norfloxacin, reducing their activity by fourfold (Dalhoff, 2012). The quinolone efflux pumps OqxAB and Qep constitute the third type of plasmid-mediated quinolone resistance. The efflux pumps extrude hydrophylic fluoroquinolones causing a 32- to 64-fold increase in minimal inhibitory concentrations (Dalhoff, 2012).

Permeation barriers and efflux pumps, either in association with target modifications or on their own, can affect fluoroquinolones. Many studies have shown that the increase in the expression of efflux pumps provokes an increase in fluoroquinolone resistance through cross-resistance between fluoroquinolones and other antibacterials of chemically unrelated drug classes. This is mostly explained by the limited substrate specificity of bacterial efflux pumps. This mechanism of resistance can be associated with target site mutations as well as acquired plasmid mutations, indicating the complexity of fluoroquinolone resistance (Dalhoff, 2012).

2.10. Fluoroquinolone Resistance in *Enterobacteriaceae*

Enterobacteriaceae may cause different diseases, from UTIs, to lower respiratory tract, intra-abdominal, and skin infections. Until recent years, the rate of *Enterobacteriaceae* which are resistant to fluoroquinolones was still quite low; the percentage varied between 1% and 3% in the community in 2008. However, quinolone resistance in *Enterobacteriaceae* in the community has reached alarming rates with 10% to 30% in the United States, and more than 50% in different countries recently. (Spellberg and Doi, 2015).

Recent studies have shown increased carriage of potentially pathogenic and highly resistant *E. coli* strains by healthy individuals, including those without any prior exposure to antibiotics. The spread of these quinolone-resistant strains is believed to be the result of person-to-person transmission. This can occur in the community such as day care and school settings, and in the hospital's contaminated environment (Spellberg and Doi, 2015).

3. MATERIALS AND METHODS

3.1. Study Design and Sample Collection

A total of 500 stool and fecal swap samples were collected between September and December 2017 from participants on consecutive laboratory admission in hospitals which are located in different cities in Northern Cyprus. These hospitals were Near East University Hospital, Nicosia Dr. Burhan Nalbantoglu State Hospital, Kyrenia Dr. Akçiçek Hospital and Famagusta State Hospital. For further phenotypic screening, the samples were sent to the microbiology laboratory in Near East University.

The ethical approval for the study was obtained from the Near East University Research Assessment Committee (Project no: YDU/2016/37-296). Written informed consent was collected from each participant.

3.2. Data Collection

A questionnaire was applied to each participant. The questionnaire contained two sections:

I. Demographic and socio-economic data
   i. Age
   ii. Gender
   iii. Marital status
   iv. Educational level
   v. Socio-economic level

II. Epidemiological information
   i. Antibiotic use within the past six months
   ii. Diarrheal experience within the past six months
   iii. UTI experience within the past six months
   iv. Traveling out of Northern Cyprus within the past six months
   v. Source of drinking water
   vi. Eating habits
   vii. Close contact with animals

3.3. Initial Screening of Ciprofloxacin-Resistant Bacteria

The specimens were suspended in 2 ml normal saline and an aliquot was inoculated into 1 μg/ml ciprofloxacin (CIP) containing EMB agar and the control media (EMB agar without antibiotic). The media were incubated at 37°C for 24 hours. Bacterial colonies that grew on the antibiotic containing media were regarded as potential ciprofloxacin-resistant isolates and purified for further confirmatory tests.

3.4. Determination of Antibiotic Susceptibility Patterns

Antibiotic susceptibility test was carried out by using the disc diffusion method (CLSI, 2016). For each potential ciprofloxacin-resistant isolate, a 0.5 McFarland standard suspension was prepared and inoculated into Mueller-Hinton agar. Following this, ciprofloxacin, ofloxacin, norfloxacin, levofloxacin and gemifloxacin discs were placed on Mueller-Hinton agar. After the incubation period, the inhibition zones were measured and interpreted according to CLSI guidelines (2016).

3.5. Identification of Bacterial Isolates

After the disc diffusion test, ciprofloxacin-intermediate and -resistant isolates were used for identification. The identification was done by using the BD Phoenix 100 system (software version 6.01A).

3.6. Statistical Analysis

For the descriptive statistics, frequency and percentage were calculated for categorical variables. Statistical analysis was performed with either Pearson Chi-square test or Fisher's Exact Test, depending on the expected counts. All statistical calculations and analysis were done by using SPSS software package (Demo Version 18.0). Level of significance was accepted to be 0.05.

4. RESULTS

4.1. Demographic and Socio-Economic Characteristics of the Study Participants

In 500 participants, 180 (36%) were female and 320 (64%) were male. Among the participants, 243 (48%) were single and 257 (51.4%) were married. They were divided into three groups based on age which included 238 (48.2%) individuals for age 19-30; 154 (31.2%) for age 31-40; and 102 (20.6%) for the age group of 41 and above.

The educational status of the participants was grouped as follows: Participants without education: 8 (1.6%); elementary school: 53 (10.6%); middle school: 117 (23.4%); high school: 204 (40.8%); university education: 96 (19.2%); and postgraduate education: 22 (4.4%). In this study the participants were grouped in three socio-economic classes. Socio-economic status of the participants were: Low-income: 229 (59.8%), middle-income: 189 (38.3%), and high-income: 5 (1%).

4.2. Epidemiological Characteristics of the Study Participants

In the study, 45 (9%) participants declared that they had diarrhea within the last six months prior to the study. Number of the participants who traveled out of Northern Cyprus in the last six months was 92 (18.4%). A total of 124 (24.8%) participants declared that they used antibiotics within the last six months. Number of the individuals that had contact with animals was 70 (14.1%). Of the 500 participants, 328 (65.7%) stated that they ate regularly in restaurants. Number of the participants using bottled water was 483 (96.6%). Furthermore, 19 (3.8%) participants indicated that they had UTI in the past six months.

4.3. Identification of *Enterobacteriaceae* Isolates that were Intermediate or Resistant to Ciprofloxacin

In this study initially 113 isolates grew in the ciprofloxacin-containing plates. Among these, 54 isolates were CIP-resistant; 41 isolates were CIP-intermediate; and 18 isolates were CIP-susceptible. The isolates that were intermediate or resistant to

ciprofloxacin (n: 95; 19.0%) were identified as *E. coli* (n: 92; 18.4%), *K. pneumoniae* (n: 1; 0.2%), and *E. cloacae* (n: 2; 0.4%) (Table 4.1).

Table 4.1. Distribution of ciprofloxacin-intermediate or -resistant *Enterobacteriaceae* isolates among 500 samples.

Isolate	n (%)
E. coli	92 (18.4)
K. pneumoniae	1 (0.2)
E. cloacae	2 (0.4)
Total	95 (19.0)

4.4. Association of Ciprofloxacin-Intermediate or -Resistant *Enterobacteriaceae* with Possible Risk Factors

In the study, no statistical correlation was found between the intestinal colonization of ciprofloxacin-intermediate or -resistant *Enterobacteriaceae* and demographic and socio-economic characteristics of the study participants ($p > 0.05$).

Furthermore, there was no statistical association between the fecal carriage of ciprofloxacin-intermediate or -resistant *Enterobacteriaceae* and the epidemiological factors ($p > 0.05$) (Table 4.2).

Table 4.2. Distribution of ciprofloxacin-intermediate or -resistant *Enterobacteriaceae* (n: 95) isolates according to the epidemiological factors.

Epidemiological Factors	**Ciprofloxacin-intermediate or -resistant *Enterobacteriaceae***	**Ciprofloxacin susceptible isolates**	***p* value**
	n (%)	**n (%)**	
Diarrhea:			
Yes (n: 45)	9 (20)	36 (80)	0.858
No (n: 455)	86 (18.9)	369 (81.1)	
Total (n: 500)	95 (19)	405 (81)	
Travel:			
Yes (n: 92)	20 (21.7)	72 (78.3)	0.458
No (n: 408)	75 (18.4)	333 (81.6)	
Total (n: 500)	95 (19)	405 (81.8)	
Antibiotic use:			
Yes (n: 124)	25 (20.2)	99 (79.8)	0.704
No (n: 376)	70 (18.6)	306 (81.4)	
Total (n: 500)	95 (19)	405 (81)	
Animal contact:			
Yes (n: 70)	12 (17.1)	58 (82.9)	0.657
No (n: 428)	83 (19.4)	345 (80.6)	
Total (n: 498)	95 (19.1)	403 (80.9)	
Eating in restaurant:			
Yes (n: 328)	61 (18.6)	267 (81.4)	0.728
No (n: 171)	34 (19.9)	137 (80.9)	
Total (n: 499)	95 (19)	404 (81)	
Source of water:			
Bottled water (n: 483)	92 (96.8)	391 (96.5)	0.987
Tap water (n: 11)	2 (2.1)	9 (2.2)	
Other source (n: 6)	1 (1.1)	5(12)	
Total (n: 500)	95 (19)	405 (81)	
UTI:			
Yes (n: 19)	4 (4.2)	15 (3.7)	0.816
No (n: 481)	91 (95.8)	390 (96.3)	
Total (n: 500)	95 (19)	405 (81)	

4.5. Susceptibility Patterns of the Isolates to Different Fluoroquinolones

In this study, the 113 isolates that grew in ciprofloxacin containing EMB agar were also evaluated in terms of susceptibility to norfloxacin, ofloxacin, levofloxacin and gemifloxacin. The results were shown in the Table 4.3.

Table 4.3. Susceptibility patterns of the isolates (n: 113) against norfloxacin, ofloxacin, levofloxacin and gemifloxacin.

Fluoroquinolone	R		I		S		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
Norfloxacin	49	(43.4)	41	(36.3)	23	(20.3)	113	(100)
Ofloxacin	52	(46.0)	40	(35.4)	21	(18.6)	113	(100)
Levofloxacin	40	(35.4)	46	(40.7)	27	(23.9)	113	(100)
Gemifloxacin	38	(33.6)	42	(37.2)	33	(29.2)	113	(100)

5. DISCUSSION

The emergence and distribution of resistance in *Enterobacteriaceae* pose a serious threat to the cure of some nosocomial infections which are very critical. Resistant bacteria can be identified from different sources such as environmental source or clinical samples. In this study, bacteriological analysis was done to identify members of *Enterobacteriaceae* in stool samples and to assess their resistance patterns to fluoroquinolones. This study also highlights the variations in susceptibility to ciprofloxacin in *E. coli* and other members of *Enterobacteriaceae* isolates and therefore amplify previous studies where a connection existed between many *E. coli* and possible drivers of resistance (Bukh et al., 2009; Sahuquillo-Arce, et al., 2011).

A previous study reported ciprofloxacin resistance profile in *Enterobacteriaceae* in a period of 10 years. In the study, the result showed variability of resistance by *Enterobacteriaceae* to ciprofloxacin. It was reported that resistance in *Enterobacteriaceae* to quinolone was usually because of chromosomal mutations leading to frequency in drug accumulation or in target enzymes (Peterson, 2006).

Similarly, a twelve-year period reaserch was conducted to ascertain the outbreak of resistance of *Enterobacteriaceae* to ciprofloxacin (Lautenbagh et al., 2004). In this study, the authors compared the resistance with the admission of patient to hospitals, and the result showed high resistance among inpatients than outpatients. This could be due to high ciprofloxacin consumption in hospitals than community. In our study, other factors such as, antibiotic use and travel history were considered.

Among 500 samples, 113 isolates grew in the ciprofloxacin-containing plates in this study. These isolates were further evaluated by disc diffusion test. Of 113 bacterial isolates, 54 were ciprofloxacin-resistant, 41 were ciprofloxacin-intermediate, while 18 strains were susceptible to ciprofloxacin. The isolates that were intermediate or resistant to ciprofloxacin (n: 95; 19.0%) were identified as *E. coli* (n: 92; 18.4%), *K. pneumoniae* (n: 1; 0.2%), and *E. cloacae* (n: 2; 0.4%) (Table 4.1). The isolates (n: 113) showed different susceptibility patterns to norfloxacin, ofloxacin, levofloxacin and gemifloxacin (Table 4.3).

The bias in the resistance pattern based on the marital status is insignificant on the distribution. The driving factors could be due to indiscriminate use of the drug but not marital status. Studies indicated fluoroquinolone use increased in the recent times, which could be contributing factor to the emergence of resistance to this important class of antibiotics (Reacher et al., 2000; Livermore, 2002).

An interesting factor viewed in this study was the correlation between traveling out of study area and the prevalence and distribution of resistance pattern of *Enterobacteriaceae* to ciprofloxacin. Studies indicated that international travel contributed to distribution of resistance as more and more people are travelling for work, holidays or having medical tourism (Memish et al., 2003; Johnning et al., 2011; van der Bij and Pitout 2012; Kuenzli 2016). According to Kuenzli (2016), in the last decade, it was marked that travellers who were coming back from subtropical countries were colonized with antibiotic-resistant *Enterobacteriaceae.* Depending on the location visited, colonization rates differ between different geographical regions, with southern Africa and Indian subcontinent having higher rates than other parts of the world. This could be due to possible uncontrolled used of antibiotics. Cosmopolitan travel is known to make changes in the human gut microbiome. Changes in the gut microbiome may prepare the basis for the attainment of resistant bacteria. Bengtsson-Palme et al. (2015) stated several human actions that increased the prevalence of antibiotic resistance, such as use of antimicrobials inappropriately, lack of infection control strategies in the hospitals, poor control of environmental pollution by antibiotics, and food trade between the countries. Travelers' diarrhea and taking antibiotics while traveling are found to be a linked with a high risk of colonization by ciprofloxacin-resistant strains. While these two factors lead to changes in the gut microbiome, more comprehensive information about the duty of the human microbiome in obtaining and destroying resistant bacteria is necessary. Such information may contribute to the expantion of new probiotics to be used for hamper colonization (Bengtsson-Palme et al., 2015; Kuenzli, 2016).

Fluoroquinolone-resistant *Enterobacteriaceae* have also been reported in many studies (Albinu et. al., 2007; Chattaway et. al., 2016; Juma et. al., 2016). Fluoroquinolone resistance has predominantly been reported in *E. coli, Enterobacter, Citrobacter freundii, Klebsiella pneumoniae, Salmonella typhi, Salmonella enterica,*

Shigella flexneri and *Vibro cholerae* in descending order (Nordmann and Poirel, 2005; Rasool et al., 2010; Baker et al., 2013; Juma et al., 2016).

Juma et. al. (2016) described the diarrhea caused by members of *Enterobacteriaceae* as the most important cause of childhood mortality and morbidity. Most of these enteropathogenic species show a pattern towards increase in microbial resistance to quinolones, and the common resistant species are identified as *E. coli* and *Shigella*. There is an increase in fluoroquinolone and ciprofloxacin resistance in *Enterobacteriacea* and this increase leads to a major challenge in current treatment strategies. Diarrhea caused by *Enterobacteriacea* species are usually related with drinking contaminated water or eating uncooked food (Juma et al, 2016). In this study no correlation was found between eating habits and ciprofloxacin-intermediate or -resistant *Enterobacteriaceae* (Table 4.2).

Furthermore, the present study has investigated the use of antibiotics as important cause of resistance, in which the patients were asked if they had used antibiotics over period of six months. It was found that the participants who used antibiotics showed 20.2% resistance, and for the individuals who didn't use antibiotics, the percentage was 18.6%. No statistical association was found between antibiotic use within the last six months and the fecal carriage of ciprofloxacin-intermediate or -resistant bacteria (Table 4.2). Previous studies reported the raise of antibiotic resistance in the community due to the transmission of mobile genetic elements (Weinstein, 2001; Goossens et. al., 2005). Not surprisingly, improper use of antibiotics in medicine and even massive use of antibiotics in agriculture as growth factors lead to increase in the resistance. Massive animal production involves giving livestock animal great amount of antibiotics to boost growth and hinder infection. Such use of antibiotics promotes the antibiotic resistance in bacterial crowed. The resistant bacteria may be transmitted to humans from agricultural environments, which they cause disease that cannot be treated by conventional antibiotics (Khachatourians, 1998; Evan den Borgaard and Stobberingh, 2000).

Another possible risk factor that was investigated in this study was the animal contact. However, there was no statistical correlation between animal contact and intestinal colonization of ciprofloxacin-intermediate or -resistant *Enterobacteriaceae*

(Table 4.2). Previously, it was stated that there could be exchange of possible resistant isolates and the pets could serve as a source for resistance genes (Wang et al., 2009).

In the present study, no statistical association was found between eating in restaurants and fecal carriage of ciprofloxacin-intermediate or -resistant bacteria (Table 4.2). In the study source of drinking water as possible driver of resistance has also been investigated as previously described by other authors (Wang et al., 2009; Su et al., 2008). This could be attributed to the possible failure of proper disinfection process of water from main source or during distribution. This result is in accordance with the study of Su et al. (2008), which observed high resistance pattern among people drinking tap water.

6. CONCLUSION

The importance of antibiotics in the treatment of infectious diseases should not be underestimated. However, great breakthrough of discovery of antibiotics has been threatening by the development and increase in the resistance. The present study is important to detect the level of resistance of *Enterobacteriaceae* to ciprofloxacin.

This investigation provides some evidence to the ciprofloxacin resistance pattern of *Enterobacteriaceae* isolated from stool. According to the study, the total ciprofloxacin resistance rate of the participants was found to be 19%.

The present study will lead to increase in the knowledge of resistance profiles of *Enterobacteriaceae* to ciprofloxacin in Northern Cyprus.

From the result obtained, it is recommended that more studies and surveillance programs that would monitor trends in antibiotic resistance in different hospital and environments are needed in Northern Cyprus. Furthermore, molecular studies should be employed to determine the specific resistant genes related with ciprofloxacin resistance encoded by *Enterobactericeae*.

REFERENCES

Abbasi, P., Kargar, M., Doosti, A., Mardaneh, J., Ghorbani-Dalini, S. Ali Dehyadegari, M. (2014). Enteroaggregative *Escherichia coli* (EAEC): An Emerging Enteric Pathogen in South of Iran. *J Med Bacteriol* vol.3 40-46.

Adegboye, M.F., Babalola, O.O., and Akinpelu, D.A. (2012). Issues of resistance of pathogens to antimicrobial agents. *Scientific Research and Essays*,7(41),3468-3478.

Baker, S. J., Hernandez, V., Crepin, T., Palencia, A., Cusack, S., Akama, T. (2013) Discovery of a Novel Class of Boron-Based Antibacterials with Activity against Gram-negative Bacteria, *Antimicrobaial Agents and Chemotherapy*, 62. *1394-1403.*

Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S.A., and Mietzner, T.A. (2014). *Microbiologia Medica de Jawets,Melnick and Adelberg*-26.AMGH Editora.

Bukh, A. S., Schonheyder, H. C., Emmersen, J. M. G., Sogaard, M., Bastholm, and Roslev,S. (2009). *Escherichia coli* phylogenetic groups are associated with site of infection and level of antibiotic resistance in community-acquired bacteraemia: a 10 year population-based study in Denmark, *J. Antimicrob. Chemother,* (*64), 163-68.*

Carlet, J. (2012). The gut is the epicentre of antibiotic resistance, *Antimicrob Resist Infect Contro1 (1)39.*

Chattaway, M. A., Aboderin, A. O., Fashae, K., Okoro, C. K., Opintan, J. A. and Okeke, I. N. (2016). Fluoroquinolone-Resistant Enteric Bacteria in Sub-Saharan Africa: Clones, *Implications and Research Needs. Front Microbiol*. (7), 558

Chaudhary, A. S. (2016). A review of global initiatives to fight antibiotic resistance and recent antibiotics′ discovery, *Acta Pharmaceutica Sinica, 6(6):552-6.*

CLSI (2016). Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100S. Wayne, Pennsylvania, USA.

Cocolin, L. and Comi, G. (2005). Use of a culture-independent molecular method to study the ecology of *Yersinia* spp. in food. *Int J Food Microbiol*, 105(1):71-82. Epub 2005/08/09.

Collins, F. M. (1996). *Pasteurella*, *Yersinia*, and *Francisella*. In: Baron S, Medical Microbiology. 4th edition. Chapter 29.

Cox G, Wright GD. (2013). Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. Int J Med Microbiol.; 303(6-7):287-92. Epub 2013/03/19.

Cui,S,.Li,J,.Sun,Z.,Hu,C,.Jin,S,.Li,F,.et al.(2008).Characterization of Salmonella enterica isolates from infants and toddlers in Wuhan,China, *Journal of antimicrobial chemotherapy*,63(1),87-94.

Dalhoff A. (2012) Global Fluoroquinolone Resistance Epidemiology and Implictions for Clinical Use. *Interdiscip Perspect Infect Dis*

Davine-Regli,A. (2015). *Enterobacter aerogenes* and *Enterobacter cloacae*;versatile bacterial pathogens confronting antibiotic treatment.*Frontiers in microbiology*,6,392.

Deborah Chen, H. and Frankel, G. (2005). Enteropathogenic *Escherichia coli*: unravelling pathogenesis. *FEMS Microbiology Reviews*. 29: 83–98.

Drlica, K., Malik, M., Kerns, R. J. and Zhao, X. (2008) .Quinolone-Mediated Bacterial Death, *Antimicrob Agents Chemother*, 52:385-92.

Evans DJ Jr.and Evans DG. (1996). Escherichia *Coli* in Diarrheal Disease. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 25.

Fleming A. (1980). Classics in infectious diseases: on the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae by Alexander Fleming, Reprinted from the British Journal of Experimental Pathology 10:226-236, 1929. *Rev Infect Dis*, 2(1):129-39.

Galindo CL, Rosenzweig JA, Kirtley ML, Chopra AK. (2011). Pathogenesis of *Y. enterocolitica* and *Y. pseudotuberculosis* in Human Yersiniosis. *J Pathog.* :182051. Epub 2011/01/01.

Giannella RA. (1996). *Salmonella*. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 21.

Goosens, H., Ferech, M, Sticele, R. V. and Elseviers, M. (2005). Outpatient Antibiotic Use in Europe And Association With Resistance: A Cross-National Database Study, *The Lancet*, 365(9459):579-58.

Guentzel MN. (1996) Escherichia, *Klebsiella*, Enterobacter, Serratia, Citrobacter, and Proteus. In: Baron S, editor. *Medical Microbiology*. 4th edition . Chapter 26.

Hale TL and Keusch GT. (1996) *Shigella*. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston;.Chapter 22.

Holt, K. E., Wertheim, H., Zadoks, R. N., Baker, S., Whitehouse, C. A. and Dance, D. (2015) Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health., *Proceedings of the National Academy of Sciences 112(27):E3574.*

Idowu,T and Scheweizer, F. (2017). Ubiquitous Nature of Fluoroquinolones:The Oscillation between Antibacterial and Anticancer Activities. *Antibiotics* 6(4), 26.

Iredell, J., Brown, J., and Tagg, K. (2016). Antibiotic resistance in *Enterobacteriaceae*:mechanism and clinical implication. *BMJ*, 352,H6420.

Irving, P., Ubeda, J. M., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., Hoffmann, J. A., Hetru, C. and Meister, M. (2005) New Insights Into Drosophila Larval Haemocyte Functions Through Genome-Wide Analysis, *Cell Microbiol*, 7(3): 335-50.

Johnning,A., Kristiansson, E., Angelin, M, Marathe, N., Shouche, Y. S., Johansson, A. and Larsson, D. G. J. (2015) Quinolone Resistance Mutations in te Faecal Microbiota of Swedish Travellers to India, *BMC Microbiology* 15:235.

Juma J., Liu J., Platts-Mills J.A., Kabir F., Nkeze J., Okoi C., Operario D.J. (2016), Houpt E.R. Use of Quantitative Molecular Diagnostic Methods to Identify Causes of

Diarrhoea In Children: A Reanalysis of The GEMS Case-Control Study, *The Lancet 388(10051): 1291-1301.*

Kayser, F.H., Bienz, K.A., Eckert, J., and Zinkernagel, R.M. (2005). *Medical microbiology. Georg Thieme Verlage*

Khachatourians, G. G. (1998) Agricultural Use Of Antibiotics And The Evolution And Transfer Of Antibiotic-Resistant Bacteria, *Canadian Medical Association*, 159(9):1129-36.

King DE, Malone R, Lilley SH. (2002). New classification and update on the quinolone antibiotics. *Am Fam Physician* 61(9):2741-8. Epub 2000/05/23.

Kintz E, Brainard J, Hooper L, Hunter P. (2017) Transmission pathways for sporadic Shiga-toxin producing *E. coli* infections: A systematic review and meta-analysis. *International Journal of Hygiene and Environmental Health.* 220(1):57-67.

Kuenzli, E. (2016) Antibiotic Resistance and International Travel: Causes And Consequences, *Travel Medicine Journal* 16(6): 595-98.

Lamba K, Nelson JA, Kimura AC, et al. (2016) Shiga Toxin 1–Producing *Shigella* sonnei Infections, California, United States, 2014–2015. *Emerging Infectious Diseases.* 22(4):679-686.

Lery, L.M., Frangeul, L., Tomas, A., Passet, V., Almedia, A.S., Bialek-Davent, S., et al. (2014). Comparative analysis of *Klebsiella pneumoniae* genomes identifies a phospholipase D family protein as a novel virulence factor. *BMC biology,* 12(1), 41.

Li W, Sun G, Yu Y, Li N, Chen M, Jin R, et al. (2014) Increasing occurrence of antimicrobial-resistant hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* isolates in China. *Clin Infect Dis*, 58(2):225-32. Epub 2013/10/09.

Livermore, D. M. (2002). Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? *Clin. Infect. Dis, 34(5): 634:40.*

Mamza, S. A., Egwu, G. O. and Mshelia, G. D. (2010) Beta-Lactamase *Escherichia coli* and *Staphylococcus aureus* Isolated From Chickens In Nigeria, *Vet Italy* , 46(2):155-65.

Mellmann, A., Bielaszewska, M., Kock, R., Friedrich, A.W., Fruth, A., Middendorf, B., et al. (2008). Analysis of collection of haemolytic uremic syndrome-associated enterohemorrhagic *Escherichia coli. Emerging infectious diseases*,14(8),1287.

Mellmann, A., Harmsen, D., Cummings, C.A., Zentz, E.B., Leopold, S.R., Rico, A., et al. (2011). Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. *PloS* one, 6(7), e22751.

Memish, Z. A. and Almuneef, M. (2003). Effective Medical Waste Management: It Can Be Done, *American Journal of Infection Control*. 31(3): 188-92.

Moriel DG, Rosini R, Seib KL, Serino L, Pizza M, Rappuoli R. (2012). *Escherichia coli:* great diversity around a common core. *M Bio*, 3(3).

Munita JM, Arias CA. (2016) Mechanisms of Antibiotic Resistance. *Microbiol Spectr*, 4(2).

Nordmann, P. and Poirel, L. (2005). Emergence of Plasmid-mediated Resistance to Quinolones in *Enterobacteriaceae*, *Journal of Antimicrobial Chemotherapy*, 56(3): 463-69.

Ochoa TJ and Contreras CA. (2011). Enteropathogenic escherichia *coli* infection in children. *Curr Opin Infect Dis*. 24(5):478-83. Epub 2011/08/23.

Okhuysen, P. C. and Dupont, H. L. (2010). Enteroaggregative *Escherichia Coli* (EAEC): A Cause Of Acute And Persistent Diarrhea Of Worldwide Importance, *J Infect Dis.* 202(4):503-5.

Patzi-Vargas S, Zaidi MB, Perez-Martinez I, León–Cen M, Michel-Ayala A, Chaussabel D, et al. (2015). Diarrheagenic *Escherichia coli* Carrying Supplementary Virulence Genes Are an Important Cause of Moderate to Severe Diarrhoeal Disease in Mexico. *PLoS Neglected Tropical Diseases*, 9(3).

Qin, T.T., Kang, H.Q., Ma, P., Li, P.P., Huang, L.Y., Gu, B. (2015). SOS response and its regulation on the fluoroquinolone resistance. *Annals of Translational Medicine*, 3(22): 358.

Rasool, F., Ahmad, M., Kan, H. M. S., Akhtar, N. And Murtaza, G. (2010). The Effect of Binders on the Bioavailability of Ofloxacin Tablets in Animal Model, *Acta Polonaie Pharmaceutica-Drug Research,* 67(2): 185-89.

Reacher, M. H., Shah, A., Livermore, D. M., Wale, M. C. J., Graham, C., Johnson, A. P. George, R. C. (2000). Bacteraemia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998: trend analysis, *BMJ : British Medical Journal,* 320(7229), 213–216.

Rivas, L., Mellor, G.E., Gobius, K., Fegan, N. (2015). Detection and Typing Strategies for Pathogenic *Escherichia coli*.

Sahuquillo-Arce, J. M., Tatay, A. I., Luna, M. L., Boix, Y. S., Deltell, J. G., Barberá, E. G., Heras, J. B. and Serrano, M. G. (2011) Estudio In Vitro De Las Propiedades Antimicrobianas De Una Espuma De Poliuretano Que Libera Iones De Plata, *Cirugía Española* 89(8): 532-538.

Savin C, Martin L, Bouchier C, Filali S, Chenau J, Zhou Z, et al. (2014). The *Yersinia* pseudotuberculosis complex: characterization and delineation of a new species, *Yersinia* wautersii. *Int J Med Microbiol*. 304(3-4):452-63. Epub 2014/03/07.

Scheutz , F., Nielsen, E.M., Frimodt-Moller, J., Boisen, N., Morabito, S., Tozzoli, R. et al. (2011). Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uremic syndrome in Germany, May to June 2011. *Euro surveill*, 16(24), 19889.

Sharma PC, Jain A, Jain S. (2009). Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects. *Acta Pol Pharm*, 66(6):587-604.

Spellberg, B. and Doi, Y. (2015 The Rise of Fluoroquinolone-Resistant *Escherichia coli* in the Community: Scarier Than We Thought, *The Journal of Infectious Diseases* Volume 212, Issue 12, 15, Pages 1853–1855.

Su, S.C., Siu, L.K., Ma, L., Yeh, K.M., Fung, C.P., Lin, J.C., et al. (2008). Community-acquired liver abscess caused by serotype K1 *Klebsiella pneumoniae* with CTX-M-15-type extended-spectrum beta-lactamase. *Antimicrobial Agents and Chemotherapy,* 52(2): 804-5.

Temu MM, Kaatano GM, Miyaye ND, Buhalata SN, Shushu ML, Kishamawe C, et al. (2007). Antimicrobial susceptibility of *Shigella flexneri* and *S. dysenteriae* isolated from stool specimens of patients with bloody diarrhoea in Mwanza, Tanzania. *Tanzan Health Res Bull,* 9(3):186-9.

Todar. Todar's(2004) online textbook of bacteriology. Available at: http://ltc-ead.nutes.ufrj.br/constructore/objetos/Todar-microbiology.pdf

Toma, A., and Deyno, S. (2015). Overview on Mechanism of antibacterial Resistance. IJRPB,2(1),27.

Van den Beld MJ, Reubsaet FA. (2012) Differentiation between *Shigella* , enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli*. *Eur J Clin Microbiol Infect Dis*. 31(6):899-904.

Van der Bij, A. K. and Pitout, J. D. (2012) The Role Of International Travel In The Worldwide Spread Of Multiresistant *Enterobacteriaceae*, *J Antimicrob Chemother,* 67(9):2090-100.

Ventola CL. (2015). The Antibiotic Resistance Crisis. Part 1: Causes and Threats. *Pharmacy and Therapeutics*, 40(4): 277-83.

Vieira N, Bates SJ, Solberg OD, Ponce K, Howsmon R, Cevallos W, et al. (2007). High prevalence of enteroinvasive *Escherichia coli* isolated in a remote region of northern coastal Ecuador. *Am J Trop Med Hyg*, 76(3): 528-33. Epub 2007/03/16.

Wang, Y., Xu, N., Xi, A., Ahmed, Z., Zhang, B. and Bai X. (2009). Effects of *Lactobacillus plantarum* MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. *Applied Microbiology and Biotechnology*. 84(2):341-347.

Weinstein, R. A., Neuauser, M. M. And Rydman, R. (2003) Antibiotic Resistance Among Gram-Negatice Bacili in US Intensive Care Units: Implications for Fluoroquinolone Use, *JAMA*,289(7):885-88.