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

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MASTER OF SCIENCE THESIS

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SUPERVISOR

Assoc. Prof. Dr. EMRAH RUH

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Thesis Title: Determination of Intestinal Colonization Rates of Extended-Spectrum Beta-Lactamase- and AmpC Beta-Lactamase-Producing *Enterobacteriaceae* in Hospital and Community Settings.

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ABSTRACT

Aim: The present study was conducted to investigate the intestinal colonization rates of extended spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) and plasmid-mediated AmpC β-lactamase-producing *Enterobacteriaceae* (pAmpC-E) and associated risk factors in hospitalized patients and in community in Northern Cyprus

Materials and Methods: A total of 180 participants were recruited to evaluate the intestinal colonization rates of ESBL-E and pAmpC-E. The participants were grouped into two; hospitalized patients from Near East University Hospital (n=80) and community-dwellers (n=100). Stool samples were collected from each participant and a questionnaire was performed to evaluate the risk factors associated with intestinal colonization. Initial screening of resistant bacteria was done on media containing antibiotics (ceftazidime and cefotaxime at 1 μg/ml concentration) and this was followed by phenotypic confirmation for ESBL-E and pAmpC-E.

Results: Of the 180 participants, 50 (27.8%) were colonized with ESBL-E. The rates of fecal carriage were found to be 38.8% (n=31/80) and 19.0% (n=19/100) in the patient and control groups, respectively. ESBL carriage in the hospital was significantly higher than in community dwellers (p=0.003). The predominant *Enterobacteriaceae* species among the ESBL-positive isolates was *Escherichia coli* (96.0%). In this study, age (p=0.002), gender (p=0.000), educational level (p=0.001) and history of urinary tract infection (p=0.009) were found to be significant risk factors for ESBL-E colonization. In the study, six (3.3%) of 180 participants were colonized with pAmpC-E. In the patient group the rate was 6.3% (n=5/80), while in the control group the rate was 1.0% (n=1/100). All pAmpC-positive isolates were *Escherichia coli*. No significant risk factor related with intestinal colonization of pAmpC-E was detected.

Conclusion: This study shows that resistant *Enterobacteriaceae* colonize both hospitalized patients and community-dwellers. Results of the study suggest that resistance against third-generation cephalosporins in *Enterobacteriaceae* isolates should be continuously monitored in Northern Cyprus.

Key Words: Intestinal Colonization, *Enterobacteriaceae*, Extended-Spectrum Beta-Lactamase, AmpC Beta-Lactamase, Antibiotic Resistance

Tez Başlığı: Genişlemiş Spektrumlu Beta-Laktamaz ve AmpC Beta-Laktamaz Üreten *Enterobacteriaceae* Türlerinin Hastane ve Toplumdaki İntestinal Kolonizasyon Oranlarının Belirlenmesi.

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ÖZET

Amaç: Bu çalışma Kuzey Kıbrıs'ta, hastaneye yatan hastalarda ve toplumda genişlemiş spektrumlu beta-laktamaz üreten *Enterobacteriaceae* (GSBL-E) ve plazmid aracılı AmpC beta-laktamaz üreten *Enterobacteriaceae* (pAmpC-E) intestinal kolonizasyon oranlarının ve ilişkili risk faktörlerinin araştırılması amacıyla yapılmıştır.

Gereç ve Yöntem: GSBL-E ve pAmpC-E intestinal kolonizasyon oranlarının değerlendirilmesi amacıyla çalışmaya toplam 180 katılımcı dahil edilmiştir. Katılımcılar Yakın Doğu Üniversitesi Hastanesi'nde yatan hastalar (n=80) ve toplumdaki bireyler (n=100) olmak üzere iki gruba ayrılmıştır. Her katılımcıdan dışkı örneği toplanmış ve intestinal kolonizasyon ile ilişkili risk faktörlerinin değerlendirilmesi için bir anket uygulanmıştır. Dirençli bakterilerin ilk taraması antibiyotik içeren ortamlarda (1 µg/ml konsantrasyonda seftazidim ve sefotaksim içeren besiyerleri) yapılmış ve bunun ardından GSBL-E ve pAmpC-E için fenotipik doğrulama testleri uygulanmıştır.

Bulgular: 180 katılımcıdan 50 (%27,8)'sinin GSBL-E ile kolonize olduğu saptanmıştır. İntestinal kolonizasyon oranları, hasta ve kontrol grubunda sırasıyla %38,8 (n=31/80) ve %19,0 (n=19/100) olarak bulunmuştur. Hasta grubundaki GSBL-E taşıyıcılığı kontrol grubundakine göre anlamlı olarak daha yüksek bulunmuştur (p=0.003). GSBL-pozitif *Enterobacteriaceae* izolatları arasında en fazla saptanan *Escherichia coli* (%96,0) olmuştur. Bu çalışmada yaş (p=0.002), cinsiyet (p=0.000), eğitim seviyesi (p=0.001) ve idrar yolu enfeksiyonu öyküsü (p=0.009) GSBL-E kolonizasyonu için anlamlı risk faktörleri olarak saptanmıştır. Çalışmada 180 katılımcıdan 6 (%3,3)'sının pAmpC-E ile kolonize olduğu belirlenmiştir. Bu oran hasta grubunda %6,3 (n=5/80) iken, kontrol grubunda %1,0 (n=1/100) olmuştur. Tüm pAmpC-pozitif izolatlar *Escherichia coli* olarak saptanmıştır. Çalışmada pAmpC-E intestinal kolonizasyonu ile ilişkili herhangi bir risk faktörü saptanmamıştır.

Sonuç: Bu çalışma dirençli *Enterobacteriaceae* türlerinin hem hastanede yatan hastaları hem de toplumdaki bireyleri kolonize ettiğini göstermektedir. Çalışmanın sonuçları Kuzey Kıbrıs'ta *Enterobacteriaceae* izolatlarında üçüncü kuşak sefalosporinlere karşı direncin sürekli olarak takip edilmesi gerektiğine işaret etmektedir.

Anahtar Kelimeler: İntestinal Kolonizasyon, *Enterobacteriaceae*, Genişlemiş Spektrumlu Beta-Laktamaz, AmpC Beta-Laktamaz, Antibiyotik Direnci.

1. INTRODUCTION AND AIM

The discovery of antibiotics made the treatment of many bacterial infections possible; however the effectiveness of these antibacterial agents were challenged by the emergence of antibiotic resistance (Ottosson et al., 2012). The worrisome rise of antibiotic resistance has become a global health threat which is now considered pushing the world to the post-antibiotic era faster, where a simple bacterial infection can no longer be treated with the commonly used antibiotics (Da silva et al., 2007; Ventola, 2015). Effect of infections related to antibiotic resistance is projected to account for the death of over 10 million people annually in three decades to come if effective measure is not taken (De Kraker et al., 2016). This poses a threat to the global health sector and now antibiotic resistance is included in the sustainable development goal and concept of "One Health" where many fields and experts come together to fight this threat (Bousfield and Brown 2011; USAID 2016).

Antibiotic resistance refers to situation where an agent that normally effective against certain bacteria, can no longer inhibit or kill these microorganisms (Ahmed 2012; Khalifa 2016). Antibiotic resistance as a natural phenomenon that occurs through the natural ability of bacteria to become resistant, which can be by mutations or horizontally transferred (Khalifa 2016). Among the drivers of antibiotic resistance, excessive use, misuse and abuse of antibiotics in both hospital and community are considered the key contributors to the emergence and spread of antibiotic resistance (Greenwood et al., 2006; McNamara and Levy, 2016; Williamson et al., 2017; Ziad et al., 2018). Recently, it has been observed that the contribution to the emergence of resistance is multifactorial which include man's use of antibiotics as well as excessive application of the agents in farming and animal husbandry (Michael et al., 2014). In addition to the widespread use of antibiotics in human and animals treatments, the evolution of resistance is also caused by the fast growth of human population and uncontrolled stewardship of antibiotics, in addition to other ecological factors (Michael et al., 2014; Exner et. al., 2017). In a another dimension, antibiotic-resistant bacteria and

their genes are now emerging as environmental wastes which can ultimately get to humans (Michael et al., 2014; Exner et. al., 2017; Le et. al., 2016; Wang et. al., 2018).

Resistance status among *Enterobacteriaceae* is a global health problem. Of particular concern is the increase in resistance to β-lactam class of antibiotics by β-lactamase producing bacteria, especially extended-spectrum β-lactamase (ESBL), carbapenemase- and plasmid-mediated AmpC β-lactamase producing-*Enterobacteriaceae* (Baljin et al., 2016). These agents have been implicated in many hospital-associated diseases as well as community-acquired infections because they are commonly found in environments as well as in humans as commensals (Woerther et al., 2013; Sun et al., 2014). Furthermore, co-resistance to the different classes of antibiotics is possible, and this complicates the treatment of infections caused by such organisms. This is noted in ESBL-producing organisms which can also become resistant to fluoroquinolones and aminoglycosides (Woerther et al., 2013; Baljin et al., 2016).

Several studies reported the presence of multidrug-resistant *Enterobacteriaceae* in hospital and community settings, and the epidemiology varies from country to country and in some countries such surveillance data have been limited or unavailable (Ibrahim et al., 2015). In a study to detect fecal carriage of multidrug-resistant *Enterobacteriaceae* among hospitalized patients in Mongolia, significant ESBL-producing *Enterobacteriaceae*, AmpC and carbapenemase-producing isolates were reported (Baljin et al., 2016). In a similar study conducted in Morocco among hospitalized patients with focus on neonates in intensive care unit, results revealed that the prevalence of fecal carriage of ESBL-producing bacteria was 58.0%, and the main isolate was *Klebsiella pneumoniae*, while the rate of carbapenemase-producing *Enterobacteriaceae* (CRE) was 1.8% (Arhounea et al., 2017). In another study, ESBL-producing *Enterobacteriaceae* and CRE were detected in non-hospitalized patients in Buenos Aires, Argentina. The result showed that 26.8% of 164 patients participated in the study harbored *K. pneumoniae*, but in contrary to the work of Arhounea et al. (2017), *E. coli* was found to be predominant in this study (Villar et al., 2013). The difference in resistance can be

explained by the sampling sites in the studies; the former was conducted in hospital while the latter was in the community.

Emergence of antibiotic-resistant *Enterobacteriaceae* is becoming a public health threat as some of the organisms are being classified under critical and high priority list of resistant-isolates according to the World Health organization (WHO), such as *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacteriaceae* (WHO, 2017; Vijayashree et al., 2018). Walsh et al. (2005) and Villar et al. (2013) reported the possibility of transferring multidrug-resistance between *Pseudomonas aeruginosa* and clinically important *Enterobacteriaceae* such as *K. pneumoniae* and *E. coli,* as the isolates have the ability to produce metallo-β-lactamase, an enzyme capable of inactivating β-lactam antibiotics. Colonization of the gastrointestinal tract by multidrug-resistant bacteria can progress to clinical symptoms, as studies suggest that such organisms can be carried by both healthy people and patients, and hence there is possibility of increase of chains of transmission between individuals living in a community and hospitalized patients (Levin 2001; Villar et al. 2013).

In the light of above facts, the present study aimed to determine the intestinal colonization rates of ESBL and AmpC β-lactamase-producing *Enterobacteriaceae* in hospital and community settings. The seconadary objective of this study was to assess the possible factors that are related to the fecal carriage of resistant *Enterobacteriaceae*.

2. GENERAL INFORMATION

2.1. General Characteristics and Taxonomy of *Enterobacteriaceae*

This family of gram-negative bacteria that is collectively called *Enterobacteriaceae* has distinct characteristics such as being facultatively anaerobic and non-spore-forming rods. Bacteria that belong to this family have the following properties; they are catalase-positive, and oxidase-negative; they can reduce nitrate to nitrite; and produce acid through fermentation of glucose (O'Hara, 2005; Ahmed et al., 2017). However, there are also some differences among the large family members, though they share similar characteristics biochemically and genetically. Analysis of 16S rDNA sequences shows that the 30 genera have at least two or more species. The sequencing of *Enterobacteriaceae* has expanded the family diversity which resulted in a large group of bacteria that contain over 180 genomes belonging to 47 species and 21 genera (Octavia and Lan, 2014).

There is a need to identify the type of the species and this process is based on the medical significance, for instance *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* requires proper culturing and ascertaining individual isolates of *Enterobacteriaceae*, especially in a mixed flora. Isolates of *Enterobacteriaceae* from different samples such as blood, cerebrospinal fluid, peritoneum and deep tissues, also need to be idenitified (Iijima et al., 2004; Brolund et al., 2010).

Swarming nature of *Proteus* spp. can be a predictor for the identification. Importantly, biochemical properties can be used as definitive identification. These include, carbohydrate utilization test and enzymes tests such as decarboxylases, urease and phenylalanine deaminase tests (Pedler, 2004).

Another way to identify this family is the use of kits, where changes in the colours determines the biochemical reactions of the organism, and the invention of automated systems enables identification as well as antimicrobial assays (Sanders et al., 2001). The advantage of such systems is the reduced time of waiting, however,

polymicrobial infections might lead to false or error in results (Ling et al., 2003; Xia et al., 2016). For better understanding of microbial identification, there is a need for molecular idenfication, for instance use of 16S rRNA gene can be used for the detection of bacteria, together with other methods like whole-genome DNA/DNA hybridization (McAvin et al., 2003).

2.2. *Escherichia coli*

Escherichia coli was first discovered by Dr. Theodore Escherich in 1885, and it is considered as the most commonly studied bacterial species. The organism is helpful in studying many processes at both molecular and cellular levels because it has been used as a model for many studies. *E. coli* is part of human gut flora and generally it is harmless but sometimes can be associated with diarrhea when contaminated water or food is ingested (De Kraker et al., 2011).

E. coli is the most famous genus of the *Enterobacteriaceae* family. It is gram-negative rod, and oxidase-negative. Other properties include being non-spore-forming and facultative anaerobic. It can be motile using flagella and ferment different types of carbohydrates which lead to production of gas and acid. Additionally, *E. coli* has the ability to ferment lactose (Abbott et al., 2003).

The gastrointestinal tract (GIT) of humans and animals is considered the main habitat of *E. coli* where they exist as harmless commensal organisms. The ubiquitous nature of *E. coli* allows it to be found in water, food and soil, which might be the result of fecal contamination. Although most *E. coli* strains are harmless, some of them can result in human diseases, and these are classified in two groups: The first group is called intestinal pathogenic *E. coli* (IPEC). Example of this member is *E. coli* that is associated with various types of diarrhoeal disease. This diversity of *E. coli* made it possible to be found as intestinal and extrainstestinal, owing to its genetic diversity (Morel et al., 2012).

The second group is associated with diseases outside intestine and known as extraintestinal pathogenic *E. coli* (ExPEC). Example of such members is *E. coli* associated with meningitis in newborn, sepsis and urinary tract infections (UTIs) (Sharif et al., 2017). The organism employs various machanisms to exert its ability to colonize and cause its pathogenicity. This is achieved mainly via genes associated with virulence factors that can be achieved by mutation (Sharif et al., 2017).

Strategies used by this organism in colonization and for subsequent evasion of host defenses during pathogenesis were already described (Johnson, 2002). These properties are easily acquired through virulence-associated genes and they adapt to changes in their environment with the help of mutations and natural selection (Johnson, 2002). Some of these genes are found on plasmids and other mobile genetic elements. Certain members of this group have unique types of pathogenecity islands which can be exchanged via mobile genetic elements horizontally. Such features are seen in enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) (Johnson, 2002).

2.2.1. Extraintestinal pathogenic *E. coli*

These types of *E. coli* can cause serious diseases when disseminated to other parts of the body. Diseases such as UTIs and meningitis in neonates are caused by uropathogenic *E. coli* (UPEC) or meningitis-associated *E. coli* respectively, and the strains contain O:K:H serotypes (Johnson and Russo, 2002; Sharif et al., 2017).

2.2.2. Urinary tract infections

E. coli that cause UTIs usually come from feces or sometimes from the periurethral flora. They usually proliferate after colonizing the periurethral area or during catheterization, and can infect hospitalized individuals. Clinical presentations include acute pyelonephritis with fever, nausea and vomiting. The strains of *E. coli* that are called UPEC are associated with acute and chronic UTIs which are different from

commensal *E. coli* and they are designated with serogroups (O1, O2, O4, O6, O7 and O75). There are certain types of genes that are encoded with the virulence factors of these types of *E. coli*. These factors are adhensins, siderophore, and other properties such as capsule and toxin production (Welch et al., 2002; Kaper et al., 2004).

The genome of *E. coli* that causes UTIs differs from *E. coli* O157:H7 and other EHEC strains, because they have no genes for type III secretion system, phage or plasmid-encoded virulence genes. However, they have pathogenic factors that help in adhension such as fimbriae. These factors are found in *Enterobacteriaceae,* however type 1 fimbriae increase the infection, especially UTIs, and result in inflammatory response (Wullt, 2003; Brolund et al., 2010).

2.2.3. *E. coli* that causes bacteremia and meningitis

Among gram-negative bacteria *E.coli* is common cause of bacteremia in humans. Isolates of *E. coli* that causes bacteremia are known to have effective virulence factors to escape from the action of immune system. The virulence factors are adhesins (P, S and M), the siderophore aerobactin and lipopolysaccharide (LPS) (De Kraker et al., 2011).

2.2.4. *E. coli* causing intestinal infection

There are six groups of intestinal pathogenic *E. coli.* These are enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAggEC) and the diffusely adherent *E. coli* (DAEC). Previously, *E. coli* strains were classified by their serotypes on the basis of somatic O antigen and flagellar H antigens and capsular K antigens. This method of classification is based on specific feature of the organism. Importantly, a strain can have the same serotype but can be grouped in different pathotypes due to the nature of pathology they cause or how they use their virulence factors (Sharif et al., 2017)

Enteroinvasive *E. coli* (EIEC):

These organisms share similar properties with *Shigella* spp., in terms of biochemical, genetical and pathogenical characteristics. Both *Shigella* and EIEC can cause bacillary dysentery, and the main mode of transmission is the fecal–oral route, by ingestion of contaminated food and water, however, person-to-person transmission is also possible (Jin *et al*., 2002; De Kraker et al., 2011).

Enterotoxigenic *E. coli* (ETEC):

The members of ETEC release two types of enterotoxins. The heat-labile toxin is also classified into type l and type ll. The type l is encoded on plasmid, but the type ll is located on chromosome (Lencer and Tsai, 2003; Sandvig and Van Deurs 2005). Human infections are mainly due to heat-stable enterotoxin as reported by many studies (Donnenberg, 2002; Kaper et al., 2004; Sharif et al., 2017). Futhermore, ETEC have capacity to firmly attach to intestinal epithelium using adhesive fimbriae (Elsinghorst, 2002). ETEC are prevalent in developing and tropical countries, and are associated with travellers' diarrhea (Brolund et al., 2010).

Enteroaggregative *E. coli* (EaggEC):

These strains are particularly seen in developing countries and they lead to diarrhea in infants as well as diarrheal disease in adults across the world (Kaper et al., 2004). Nataro and Steiner (2002) suggested that the toxins produced by EAggEC are heat-stable in nature, thus they are referred to as enteroaggregative heat-stable toxin-1 (EAST-1). The other type that can be affected by heat is called plasmid-encoded toxin (Vila et al., 2000; Sheikh et al., 2002).

Diffusely Adherent *E. coli* (DAEC):

These strains have the classical feature to adhere and the ability to firmly attach to different type of mucosal cells and it presents in diffuse manners (Peiffer et al., 2000; Nataro and Steiner, 2002). The phenotypes that show such properties are said to be achieved by the help of fimbriae. The clinical presentation of DAEC is watery diarrhea (Kyaw et al., 2003; Bétis et al., 2003; Kumarasamy et al., 2012).

Enteropathogenic *E. coli* (EPEC):

EPEC is considered as the most commonly studied pathogenic *E. coli* together with EHEC. The first stage of pathogenesis is the attachment of the organism to the epithelium of intestine via bundle-forming pilus (BFP) found on plasmid (Kaper et al., 2004). Humans are typical reservoir of EPEC but they can also be isolated from animals. They have the capacity to adehere firmly to the epithelial cells which results in many forms of signal transduction pathways (Trung et al., 2016).

Enterohaemorrhagic *E. coli* (EHEC):

The other names of EHEC are VTEC (verocytotoxin-producing *E. coli*) and STEC (shiga toxin-producing *E. coli*). These are considered as a great challenge to human health, and some strains of EHECs such as O157:H7 are used as a model in studying phylogenetic profiles (Kaper, Nataro and Mobley, 2000). They are associated with hemorrhagic colitis and hemolytic uremic syndrome in humans (Mellmann et al., 2011).

2.3. *Shigella* spp.

Shigellae spp. are small gram-negative rods that are non-lactose fermenters. They are non-motile and can grow on some selective media like deoxycholate citrate

agar (DCA), MacConkey, *Salmonella–Shigella* (SS) or xylose–lysine–deoxycholate (XLD) agar (Pazhani et al., 2004; Teneja 2007).

Shigella was classified into four species which include *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei.* This classification is based on their O-polysaccharide antigens (Prabhurajeshwar and Kelmani, 2018). In the family of *Enterobactericeae,* it is closely related to *E. coli*, hence, they are phylogenetically indistinguishable (Lan and Reeves, 2002; Jin et al., 2002; Wei et al., 2003).

Shigella spp. are one of the agents that cause bloody diarrhea (Pazhani et al., 2004; Teneja 2007). *Shigella* spp. mainly cause infection in the colon but also they are implicated in intestinal perforation (Sakaguchi et al., 2002; Taneja et al., 2012).

Virulence factors of the organism which help in the invasion are located on the plasmid (Jennison and Verma 2004). Also there is an outer membrane protein, called IcsA, which help to the passage into epithelial cells (Purdy et al., 2002; Jennison and Verma 2004). These species are known to prouduce type 1 exotoxin (Stx) which increases local vascular damage. *Shigellae* toxin are similar to Shiga-like toxins of *E. coli* in terms of structure (Cherla et al., 2003; Kwak et al., 2015).

2.4. *Salmonella* spp.

Salmonella spp. cause infections that are of concern for the general public worldwide. They also cause a food-borne disease called salmonellosis. Moreover, the typhoid fever still remains alarming rate of death in developing countries. They are known for their 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).

Salmonella spp. cause gastrointestinal tract infections, but can also cause systemic infections and life threatening conditions due to their ability to multiply in the reticuloendothelial system. The processs of escaping host immune system by the organism involves inhibiting signal transduction pathways. One of the mechanisms used

to escape the intracellular environment of the host is the microorganism's ability to invade macrophages. M cells of the Peyer's patches are destroyed by the translocation process (Eriksson et al., 2003; Parkhill et al., 2001; Harshey and Partridge, 2015; Le Hello et al., 2013; Nata and Cucunawangsih 2017).

The clinical features include salmonellosis, and additionally the infection can further be invasive and results in bacteremia and causes systemic effect such as fever. The disease has an incubation period of six hours and results in symptoms within 24 hours of ingestion of the pathogen. The symptoms are fever and abdominal pain. In addition, when the bacterium enters into the blood stream, it can cause other conditions such as absecess formation and osteomyelitis (Katsenos et al., 2008).

2.5. *Klebsiella* spp.

The genus *Klebsiella* was originated from the work of Edwin Klebs who lived between 1834 and 1913. The most important members are *K. pneumoniae, K. ozaenae, K. rhinoscleromatis, K. aerogenes* and *K. oxytoca* (Kyungwon et al., 2011). *Klebsiella* spp. are members of normal gut flora in healthy people. However, they are also associated with hospital infections worldwide (El et al., 2016).

Klebsiella spp. are gram-negative rods, and their size range from 0.3–1 μm to 0.6–6 μm. They are not motile and they possess thick polysaccharide capsule and fimbriae. They are facultative anaerobes and they can ferment carbohydrates (Kyungwon et al. 2011). In addition, they can hydrolyze other substances such as urea, citrate and glucose. However, there is a difference between *K. oxytoca* and *K. pneumoniae* as the former being indole-positive while the latter being idole-negative (Barguigua et al., 2011; Lamine et al., 2013).

K. pneumoniae is associated with nosocomial and opportunistic infections; however, it is also known to cause chronic infections of upper airways called rhinoscleroma (Hart and Rao, 2000). Especially intestinal tract of hospitalized patients are the main reservoir, although these bacteria can be found in other areas such as

oropharynx, skin and vagina. The main virulence factor that contributes to the development of disease is the fimbriae. Adhesion of *Klebsillea* is achieved by the presence of fimbriae which can be type 1 or type 3 and can be strongly attached to epithelial cells (Struve et al., 2015). Another factor is the capsule which makes bacteria resistant to opsonization and killing by the complement cascade. Furthermore, lipopolysaccharide (LPS) also helps bacteria resist against action of host immune system (El et al., 2016).

2.6. *Citrobacter* spp.

Citrobacter spp. are gram-negative rods and they contain flagella which help them to be motile. Their appearance on media can be smooth or rough and biochemically they resemble *Salmonella*. Their habitat is mainly intestinal tract of humans and other animals and they are also found in environment. They cause intestinal tract infection and the transmission is usually through hands (Townsend et al., 2003). Important species in this genus include *C. freundii, C. koseri,* and *C. amalonaticus* (Wang et al., 2000).

2.7. *Enterobacter* spp.

Species in the genus include *E. cloacae*, *E. aerogenes*, *E. agglomerans,* and *E. gergoviae*. Main infections caused by *Enterobacter* spp. are UTIs and opportunistic infections in immunocompromised patients (Davin-Regli, 2015).

The genus has 22 families and mostly found in environment and intestine of mammals, hence they can cause opportunistic infections (Harada et al., 2016). In a comparative study, *E. clocae* was shown to be more related to *Klebsiella* among the *Enterobacteriacea* (Liu et al., 2013). Since it was described as an opportunistic pathogen found in the hospital, it is complicated in outbreaks in intensive care units, affecting the most vulnerable patients with long term hospitalization (Schroder et al., 2007; Liu et al., 2013).

2.8. *Serratia* spp.

These bacteria are flagellated gram-negative rods that are facultatively anaerobes. Most species are indole-negative with the exception of *S. odorifera* strains, they are citrate-positive and most strains have the ability to produce DNase and hydrolyze gelatin (Berlanga and Viñas 2000). The main species that are associated with human infections are *S. marcescens* and *S. rubidaea* as well as *S. liquefaciens* (Hu and Zhao, 2009).

2.9. *Proteus* spp.

Proteus spp. are gram-negative rods and they are mostly found in the human intestinal tract as part of normal flora (Armbruster et al., 2018). The main habitats of this genus are, long-term care facilities, hospitals, and also soil, water and sewage (Adeolu et al., 2016). There are five species in this genus which include *P. mirabilis, P. vulgaris, P. penneri, P. hauseri* and *P. myxofaciens.* The last species, *P. myxofaciens*, has less importance as a human pathogen (Kalra et al., 2011).

Proteus spp. are opportunistic bacterial pathogens that can cause UTIs, cystitis, and acute pyelonephritis. Also there are unusual cases of bacteremia associated with *Proteus* spp. (Kalra et al 2011). Other diseases are meningitis in neonates or infants and rheumatoid arthritis, endocarditis and brain abscesses (Fujihara et al., 2011; Rozalski et al., 2012; Ghaidaa et al., 2016). The organism is oxidade-negative and non-lactose fermenting bacterium and it has the swarming mortility character (Gibbs et al., 2008). The virulence factors of these species include fimbriae (Coker et al., 2000). Armbruster et al. (2018) demonstrated that adherence of the bacterium to the epithelium and catheter surfaces is achieved by 17 different fimbriae.

2.10. *Yersinia* spp.

There are three main species in this genus that cause human infections but the most common one is *Y pestis* which causes bubonic plague. *Y. pseudotuberculosis* and *Y. enterocolitica* cause gastrointeritis that can lead to serious complications under special circumstances (Imataki et al., 2014). *Yersinia* spp. are gram-negative and non-spor forming coccobacillli. *Y. pestis* is non-motile while other species are motile at 25^{o}C, and non-motile at 37^{o}C (Armbruster et al., 2018).

Some of the important factors associated with *Y. pestis* are plasmid-encoded virulence factors which help for the flea colonization. There is also a polypeptide that helps the bacterium evade the action of phagocytic cells such as macrophages (Kawahara et al., 2002).

2.11. Antibiotics and the Mechanisms of Action

An antibiotic refers to the substance that has the ability to kill or inhibit growth of bacteria. The action of antibiotics can either be bactericidal or bacteriostatic. Bactericidal antibiotics are the antibiotics that kill bacteria, while bacteriostatic antibiotics inhibit bacterial growth (Elsalabi, 2013; Larsson, 2014). Antibiotics saved millions of lives of not only humans but also animals; hence there is a need for preserving the effectiveness of these agents (Kohanski et al., 2010; Kumarasamy et al., 2010; Voolaid, 2014).

There are five main antibiotic targets; bacterial cell wall, cell membrane, protein synthesis, nucleic acids and metabolic pathway (Elsalabi, 2013). The main focus will be the inhibitors of cell wall synthesis.

2.12. Antibiotics that Inhibit the Bacterial Cell Wall Synthesis

2.12.1. β-Lactam antibiotics

The main classes of agents that interfere with cell wall are β-lactam and glycopeptide antibiotics. These agents can affect cell size and shape which lead to release of cell contents and eventually lysis. β-Lactam antibiotics are important agents that are used orally or parenterally in many clinical cases (Finch et al., 2010).

β-Lactam antibiotics such as penicillins and cephalosporins inhibit the peptide bond formation that is catalyzed by transpeptidase. They block the peptidoglycan cross-linking (Kohanski et al., 2010). Inhibitors of cell wall synthesis are β-lactams, glycopeptides, fosfomycin, cycloserine, and bacitracin. These antibiotics selectively inhibit different stages of cell wall synthesis (Finch et al., 2010).

Glycopeptides such as vancomycin and teicoplanin, block binding of N-acetlyglucosamine (NAG) during muramyl pentapeptide formation to the acyl-D-alanine tail, hence transglycosylation is prevented. Importantly, glycopeptides are effective only against gram-positive bacteria. Due to their large molecular size, they cannot pass through the cell wall of gram-negative bacteria (Finch et al., 2010; Kohanski et al., 2010).

The building block molecules need to be transported to the membrane using undecaprenyl pyrophosphate as a carrier, and further dephosphorylation is required to allow addition of phosphate group. However, bacitracin can block this step, and prevent further cell wall synthesis. Importantly, the major side effect of this drug is its toxicity to a similar step in humans (Finch et al. 2010). The final step in cell wall synthesis, transpeptidation, is prevented by β-lactam antibiotics. It is well established that cross-link of peptidoglycan is responsible for the cell wall rigidity (Josephine et al., 2004).

β-Lactam antibiotics are classified as penicillins, cephalosporins, carbapenems and monobactams. This class of antibiotics contains a β-lactam ring in their structure

which allows them to block the synthesis of the peptidoglycan layer of cell wall (Josephine et al., 2004).

2.12.2. Penicillins

Penicillins are used to combat various bacterial diseases worldwide, due to their efficacy, relatively low price, and easy route of administration (Meneksedag et al., 2013).

Penicillin antibiotics contain members such as:

- Natural penicillins: penicillin G and penicillin V
- Penicillinase-resistant penicillins: Methicillin, nafcillin, oxacillin, cloxacillin and dicloxacillin.
- Broad-spectrum penicillins: Aminopenicillins (ampicillin, amoxicillin), carboxypenicillins (carbenicillin, ticarcillin), and ureidopenicillins (piperacillin).
- Penicillins with β-lactamase inhibitor: Ampicillin-sulbactam, amoxicillin-clavulanate, ticarcillin-clavulanate and piperacillin-tazobactam (Zhang and Hao, 2011).

2.12.3. Cephalosporins

This group of antibiotics is closely related to penicillins in terms of their structure and action. Cephalosporins are divided into five generations:

- 1^{st} generation cephalosporins such as cefazolin
- 2^{nd} generation cephalosporins such as cefoxitin
- 3^{rd} generation cephalosporins such as cefotaxime and ceftazidime

- 4th generation cephalosporins such as cefepime
- 5th generation cephalosporins such as ceftaroline (Etebu and Arikekpar, 2016).

2.12.4. Carbapenems

Carbapenems are the antibiotics of last resort (Gelband et al., 2015). Examples of carbapenems are imipenem, meropenem, ertapenem and doripenem. There are various types of carbapenemase enzymes according to their ability to hydrolyze carbapenem antibiotics. Carbapenemase enzymes belong to the molecular groups A (KPC), B (MBL) and D (OXA) (Thomson, 2010).

2.12.5. Monobactams

Monobactams are monocyclic β-lactam antibiotics. Spectrum of monobactams is limited to gram-negative bacteria. They are used in combination with other antibiotics (Etebu and Arikekpar, 2016).

2.13. General Mechanisms of Antibiotic Resistance

The efficacies of antibiotics are challenged by the emergence of resistance. The ability of microorganisms to overcome action of antimicrobials and multiply in the presence of an agent that would normally inhibit or kill the bacteria can be described as antibiotic resistance (Toma and Dayno, 2015). As a survival strategy for bacteria, the resistance could be naturally possessed or acquired. This stratergy gives them the ability to overcome other microorganisms in a microbial community. There are four mechanisms of resistance. These are modification of target site, efflux pumps, enzyme inactivation, and alteration of membrane permeability (Toma and Dayno, 2015; Vijayashree et al., 2018).

2.14. Resistance to β-Lactam Antibiotics

Penicillin is a class of β-lactam antibiotic used in the treatment of bacterial infections. This important antibiotic becomes ineffective because of the resistance. Bacteria use two mechanisms to confer resistance to penicillin either by enzyme inactivation or mutation of the target sites (Adekunle, 2012). Production of β-lactamase enzymes is considered the main mechanism of resistance due to the global dissemination. Metallo-β-lactamases particularly New Delhi metallo-β-lactamase (NDM-1) and ESBLs such TEM and SHV type enzymes totally resist the action of penicillins and the threat rapidly spreads globally (Pages et al., 2009; Guo et al., 2011; Adekunle, 2012).

Different enzymes are used for inactivating different beta-lactam antibiotics. For instance, *E. coli* species are able to produce β-lactamase enzymes. AmpC β-lactamase is made by bacteria like *Pseudomonas aeruginosa* and *Enterobacter* spp., and hydrolyzes both penicillins and cephalosporin antibiotics. Carbapenem is hydrolyzed by carbapenemase and metallo-β-lactamase but is resistant to hydrolysis by penicillinases (Guo et al., 2011).

2.14.1. β-Lactamase Enzymes

β-Lactamases are group of enzymes that hydrolyse β-lactam antibiotics by opening the β-lactam ring and therefore make the antibiotic non-functional (Zhang and Hoa, 2011). There are two types of β-lactamase classifications: (i) Ambler classification system, which is based on the structure of amino acid sequence and separate β-lactamases into class A, B, C and D enzymes, and (ii) Bush-Jacoby-Medeiros system, known as functional classification scheme, with groups 1 to 4 (Drawz and Bonomo 2010).

Antibiotics that are hydrolyzed by AmpC β-lactamases include cephalosporins, aztreonam and penicillins. Gram-negative bacteria such as *Enterobacter*, *Salmonella*,

Citrobacter and *Proteus* are associated with the production of this enzyme. In addition, *E. coli* and *Klebsiella* spp. produce this enzyme at low levels (Barnaud, et al., 2001; Thomson et al., 2001). AmpC β-lactamase genes can be carried on plasmids or they can be chromosomally encoded (Jacoby 2009).

NDM is a broad-spectrum β-lactamase and it has the ability to resist β-lactamase inhibitors (Guo et al., 2011). This type of β-lactamase is isolated in *Enterobacteriaceae* such as *K. pneumoniae* and *E. coli*, and it became a global issue because it is not only restricted to Indian region where it was originated but reported worldwide (Poirel et al., 2011). Kumarasamy et al. (2010) reported *Enterobacteriaceae* isolates with NDM-1 as *K. pneumoniae*, *E. coli*, *Enterobacter*, *Morganella morganii*, and *Citrobacter freundii* in the UK. This was directly linked to recent travel to Indian subcontinent or the patients referred from the region. This made UK to be the pioneer European country to harbour NDM-1 which hydrolyzes many penicillins, carbapenams and other β-lactams. Poirel et al. (2011) reported the spread of NDM-1 in Morocco particularly in *K. pneumoniae* which indicated that production of β-lactamases is the major mechanism of resistance to penicillins. The authors demonstrated that the isolates contained bla_{NDM}, a gene containing class B metallo β-lactamase. Furthermore, the authors detected other β-lactamases such as SHV-1, OXA-1, OXA-2 and TEM-1, which indicated that *K. pneumoniae* can use wide range of enzymes as a major mechanism of resistance to penicillin. Oberal et al. (2013) demonstrated data of combined effect of many β-lactamases to confer resistance in gram-negative bacterial isolates of intensive care unit in India. Of the 273 isolates, the production of β-lactamase was reported in 193 strains among which 96 (35.13%) strains were ESBL, 30 (10.98%) were MBL and 15 (5.4%) were AmpC producers. These data reported *E. coli* as producer of ESBL, followed by *P. aeruginosa*, and *K. pneumoniae* (Oberal et al., 2013). The study further stated that AmpC was greatly seen in *E. coli*, while *K. pneumoniae* was the dominating MBL producer. Production of more than one type of enzymes indicated that acquiring enzymatic resistance mechanism is more common than any mechanisms especially in gram-negative bacteria (Oberal et al., 2013).

2.14.2. ESBL-producing *Enterobacteriaceae*

Dissemination of ESBL among *Enterobacteriaceae* especially *E. coli* presents a significant human health challenge. Emergence of β-lactam-resistant *Enterobacteriaceae* is mostly due to the production of β-lactamases. These types of enzymes are referred to as ESBL, and they are mostly plasmid-encoded. Examples of ESBL include TEM-1,2 and SHV-1 (Gniadkowski, 2001). *E. coli* is known to produce CTX-M as seen in the UK (Woodford et al., 2004) and elsewhere (Munday et al., 2004).

There are reports on the increasing number of ESBL-producing members of *Enterobacteriaceae* across many countries, and carbapenems are used as the first priority agents in infections that are caused by ESBL producers. GIT is considered as a main reservoir for carbapenem-resistant *Enterobacteriaceae,* it causes cross transmission in the hospital setting, and hence the people that are at high risk are the hospitalized patients. Therefore it is necessary to implement preventive measures in hospital and community settings (Macpherson and Harris, 2004; Kothari et al., 2013; Walsh et al., 2013). Thus, there is a need for surveillance of carriage of these resistant-organisms in both hospital and community and determine the risk factors related with the colonization of such organisms.

2.15. Fecal Carriage of ESBL-E and AmpC β-lactamase-Producing *Enterobacteriaceae*

Infections caused by ESBL-E and AmpC β-lactamase-producing *Enterobacteriaceae* (AmpC-E) have become alarming. Organisms that produce ESBL and AmpC have showed significant problems for the treatment (Hazirolan et al., 2018). Importanly, plasmid-mediated AmpC (pAmpC) is less frequent than ESBL, however it has a wider capacity of hydrolyzing more β-lactam antibiotics than ESBL (Ahmed et al., 2014; Hazirolan et al., 2018). Both ESBL and AmpC can be transferred on the same plasmid; therefore they constitute high prevalence and threat (Hazirolan et al., 2018).

Fecal carriage of ESBL- and plasmid-mediated AmpC-producing *Enterobacteriaceae* have been widely reported (Nordmann et al., 2012; Yamamoto et

al., 2017; Ruh et al., 2019). The co-production of ESBL-E and AmpC-E are emerging threat to public health in both hospital and community. In a recent research of Mandal et al. (2020) in Nepal, 260 isolates were detected among 190 participants which included both outpatients and healthy volunteers. The results showed the rates 30.92%, 18.4% and 13.81% for ESBL, AmpC and co-producer (ESBL+AmpC β-lactamase) respectively. This indicated that the prevalence of ESBL-E and AmpC-E are not only restricted to hospital, but they are also found in the community.

In a similar study in India, a total of 792 samples were investigated for carriage of ESBL-E and AmpC-E in hospitalized patients and healthy individuals in the community (Rashid et al., 2015). The results indicated the prevalence of ESBL-E of 9.3% and 4.4% for hospitalized patients and community-dwellers, respectively. Furthermore, the prevalence rates of AmpC-E were at 0.5% for individuals in the community, and 1.7% for hospitalized patients. This study showed similar rates with the study of Hazirolan et al. (2018) which reported the rate of ESBLs to be higher than AmpC-E. In general, the rate of fecal carriage of both ESBL-E and AmpC-E is higher in hospitalized patients than in community-dwellers (Hazirolan et al., 2018)

In a study conducted in Spain, ESBL-E was reported to be higher in hospitalized patients than in the community at the rate of 7.5% and 3.7%, respectively, in a total of 108 subjects (Kumar and Babu, 2012). In another study, fecal carriage of AmpC-E was reported in healthy volunteers in Japan, and in the study, the subjects were not exposed to antibiotics in last one month, yet they harbored AmpC-E (Kaneko et al., 2005).

In a recent study of Ruh et al. (2019), fecal carriage rates of antibiotic-resistant *Enterobacteriaceae* were evaluated in Northern Cyprus. Among 500 participants, fecal carriage rates were found to be 21.4% and 3.0%, for ESBL-E and plasmid-mediated AmpC β-lactamase producing *Enterobacteriaceae* (pAmpC-E), respectively. The authors indicated that the history of antibiotic use and travelling abroad were risk factors for ESBL-E and pAmpC-E, respectively.

3. MATERIALS AND METHODS

3.1. Study Design and Participants

In the study, samples were collected between March 2019 and July 2019 from two groups. The first group was the patients (n=80) who were hospitalized in the Near East University Hospital for at least 72 hours. The second group was the controls (n=100) who did not have any history of hospitalization within the last six months before the study. The inclusion criteria for the study were being above 18 years of age and living in Northern Cyprus for a minimum of one year. The ethics approval of this study was obtained from Research Assessment Committee of the Near East University (No: YDU/2019/65-717). Written informed consent was obtained from all of the participants.

3.2. Collection of the Samples and Data

In this study, one stool specimen was collected from each individual, therefore, 180 samples were obtained. During the collection of fecal specimens, a questionnaire was performed to evaluate the factors related with the carriage of antibiotic-resistant bacteria. After demographic and socioeconomic information (gender, age, education level, marital status, and socioeconomic status) were noted, participants provided information on presence of any gastrointestinal complaints during the sample collection. Additionally, information was obtained on antibiotic consumption history, travelling to other countries, diarrhea, UTI, and hospitalization within the last six months before the study. Also, the patients provided information on the duration of hospitalization, stay in intensive care unit, and use of antibiotics in the current hospital stay.

3.3. Preparation of Antibiotics for the Media

Ceftazidime (CAZ) powder (Sigma, USA) was prepared in 0.1 N of sodium hydroxide (NaOH) by following the manufacturer's protocol. The final concentration was adjusted to be 1 μg/ml CAZ in Eosin Methylene Blue (EMB) agar (Merck, Germany).

Cefotaxime (CTX) powder (Sigma, USA) was dissolved in distilled water according to the manufacturer's instructions with the target of 1 μg/ml CTX in EMB agar (Merck, Germany).

3.4. Initial Screening of ESBL-E and/or pAmpC-E

Stool samples were suspended in 2 ml normal saline and an aliquot was inoculated onto the CTX and CAZ containing media. After the incubation at 37^{o}C for 24 hours, the culture plates were evaluated. Bacterial colonies that were purified on the antibiotic containing media were tested further for confirmation of the presence of ESBL and/or AmpC.

3.5. Phenotypic Confirmatory Tests for ESBL-E and/or pAmpC-E

For the confirmatory tests, suspensions with 0.5 McFarland standard turbidity were prepared, and the bacterial suspensions were inoculated on Mueller-Hinton media (Merck, Germany).

3.5.1. Confirmation of ESBL-producing isolates

CAZ (30 μg) and CTX (30 μg) discs were used alone and with clavulonic acid (CLA) (10 μg) (Bioanalyse, Turkey). In case there was a difference of 5 mm or more in the CAZ/CLA or CTX/CLA zone diameter compared to the antibiotic discs without clavulonic acid, this indicated the presence of ESBL production (CLSI, 2018).

3.5.2. Confirmation of pAmpC-producing isolates

Bacterial colonies that grew on at least one of the CAZ or CTX containing media were also tested for pAmpC production. After cefoxitin (FOX) (30 μg) (Bioanalyse, Turkey) was placed, the plates were incubated at 37^{o}C for 24 hours. The isolates that had a zone diameter of less then 18 mm for FOX (CLSI, 2018) were evaluated further by combined disc test. CAZ (30 μg) with CAZ/cloxacillin (CZX) (30 μg/750 μg) and CTX (30 μg) with CTX/cloxacillin (CEX) (30 μg/750 μg) (Bioanalyse, Turkey) were placed on Muller-Hinton agar, and the plates were incubated at 37ºC for 24 hours. The difference in the inhibition zone between CTX and CEX and/or CAZ and CZX that appeared to be ≥5mm was recorded as positive for pAmpC (Reuland et al., 2015).

Identification of the ESBL-E and/or pAmpC-E isolates was done by using VITEK-2 system (bioMérieux, France).

3.6. Statistical Analysis

For the variables in the questionnaire, descriptive statistics were calculated. 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 sample sizes, Pearson Chi-square or Fisher's exact test was applied in order to evaluate the associations between categorical variables. All of the statistical calculations were performed by using IBM SPSS statistics package for Macintosh (Demo version 22.0; Armonk, NY: IBM Corp.). Level of significance was set at 0.05.

4. RESULTS

4.1. The Study Population

One hundred and eighty volunteers were included in this study, which contained 80 patients from Near East University Hospital and 100 participants from the community setting. Stool specimens that were obtained from the participants were subjected to microbiological analysis. Of the total participants, 127 (70.6%), and 53 (29.4%) were male and female respectively. The mean and median age was 43.91 ± 21.42 and 39.50 (19.00 – 90.00), respectively. The age groups of the study population were represented as follows; age 19-30 were 76 (42.2%), while age group 31 and above were 104 (57.8%). On educational background, 104 (57.8%) had university degree and higher, while 76 (42.2%) were below university degree. Among the study population, 92 (51.1%) were married, while 88 (48.9%) were single. One hundred and fifty eight (87.8%) were low and middle class participants.

Of the study population, 46 (25.6%) participants confirmed that they had at least one GIS when they provided specimen for the study. Eighty five (47.2%) individuals confirmed use of antibiotics in the past six months before the study; however, 36 participants could not remember the name of the antibiotic used. Of the 49 individuals that remembered the name of antibiotic, 40 (81.6%) confirmed use of beta-lactam. Of the 180 participants, 46 (25.6%) had a history of diarrhea, while 13 (7.2%) individuals had UTI within the last six months prior to the study. Of the 180 individuals, 99 (55.0%) had history of travel outside Northern Cyprus. Sixty (60.6%) participants travelled to Turkey or Europe, while 39 (39.4%) travelled to Asia or Africa in the last six months before the study.

A total of 80 patients were hospitalized for at least three days, however, detail of hospitalization of four patients could not be obtained. Of the 76 patients, eight (10.5%) individuals stayed at intensive care unit (ICU), 27 (35.5%) patients underwent surgery, and seven (9.2%) patients had urinary catheter. Among the hospitalized patients, 56

(73.7%) used antibiotics during hospital stay, and among these 48 (87.3%) used beta-lactam antibiotic.

4.2. Gastrointestinal Colonization with ESBL-E

In the current study, of the 180 participants, 50 (27.8%) individuals were colonized with ESBL-E. The rates of fecal carriage were found to be 38.8% (n=31/80) and 19.0% (n=19/100) in the patient and control groups, respectively. The result was was found to be statistically significant. The *p* value was 0.003 (Table 4.1).

Table 4.1. Distribution of ESBL-E among patient (n=80) and control (n=100) groups*.

Participants	ESBL-E positive n (%)	ESBL-E negative N (%)	Total n (%)
Patients	31 (38.8)	49 (61.3)	80 (100.0)
Controls	19 (19.0)	81 (81.0)	100 (100.0)
Total	50 (27.8)	130 (72.2)	180 (100.0)

*p=0.003

4.3. Distribution of ESBL-E Isolates

Among the ESBL-positive isolates, *E. coli* was detected at the highest rate (n=48/50; 96.0%). Apart from this, one *K. pneumoniae* (2.0%) and one *E. cloacae* (2.0%) isolate were identified as ESBL-producing bacterial species (Table 4.2).

Table 4.2. Distribution of *Enterobacteriaceae* species among ESBL-positive isolates (n=50).

Participants	*Escherichia coli* n/N (%)	*Klebsiella pneumoniae* n/N (%)	*Enterobacter cloacae* n/N (%)
Patients	29/50 (58.0)	1/50 (2.0)	1/50 (2.0)
Controls	19/50 (38.0)	0/50 (0.0)	0/50 (0.0)
Total	48/50 (96.0)	1/50 (2.0)	1/50 (2.0)

4.4. Fecal Carriage of ESBL-E and Correlation with Risk Factors

4.4.1. Association of ESBL-E fecal carriage with demographic and socioeconomic factors

Fecal carriage rate was significantly higher (n=38/104; 36.5%) in the age group of 31 and above (p=0.002). Also, fecal carriage rate of ESBL among the gender showed a significantly higher in females (n=25/53; 47.2%) than males (n=25/127; 19.7%) (p=0.000). ESBL-E carriage rate was not significantly affected by the marital status (p=0.070), and also the statistical analysis revealed that there was no statistical association between socioeconomic status and carriage of ESBL-E (p=0.652) (Table 4.3).

Table 4.3. Association of fecal carriage of ESBL-E with demographic and socioeconomic factors in the study group (n=180).

Risk factors	ESBL-E	
	Positive n/N (%)	*p* value
Age		
19-30	12/76 (15.8)	0.002
31 and above	38/104 (36.5)	
Total	50/180 (27.8)	
Gender		
Male	25/127 (19.7)	0.000
Female	25/53 (47.2)	
Total	50/180 (27.8)	
Education		
University and higher	19/104 (18.3)	0.001
Lower then university	31/76 (40.8)	
Total	50/180 (27.8)	
Marital status		
Single	19/88 (21.6)	0.070
Married	31/92 (33.7)	
Total	50/180 (27.8)	
Socioeconomic status		
Low and middle	43/158 (27.2)	0.652
High	7/22 (31.8)	
Total	50/180 (27.8)	

4.4.2 Association of fecal carriage of ESBL-E with epidemiological data

ESBL-E carriage rate was significantly higher in the participants with history of UTI in the last six months before the study (n=8/13; 61.5%) (p=0.009). The remaining epidemiological factors did not significantly affect the fecal carriage of ESBL-E. The P-value was greater than 0.05 as indicated in Table 4.4.

Table 4.4 Association of ESBL-E fecal carriage with epidemiological factors in the study group (n=180). (*Indicates the last 6 months before the study)

Risk factors	ESBL-E	
	Positive n/N (%)	*p* value
Presence of any GIS when the specimen was collected		
Yes	15/46 (32.6)	0.397
No	35/134 (26.1)	
Total	50/180 (27.8)	
History of antibiotic use*		
Yes	27/85 (31.8)	0.259
No	23/95 (24.2)	
Total	50/180 (27.8)	
History of beta-lactam antibiotic use*		
Yes	13/40 (32.5)	0.702
No	2/9 (22.2)	
Total	15/49 (30.6)	
History of diarrhea*		
Yes	16/46 (34.8)	0.219
No	34/134 (25.4)	
Total	50/180 (27.8)	
History of UTI*		
Yes	8/13 (61.5)	0.009
No	42/167(25.1)	
Total	50/180 (27.8)	
Travel history*		
Yes	25/99 (25.3)	0.403
No	25/81 (30.9)	
Total	50/180 (27.8)	
Travel to Turkey or Europe*		
Yes	18/60 (30.0)	0.177
No	7/39 (17.9)	
Total	25/99 (25.3)	
Travel to Asia or Africa*		
Yes	7/39 (17.9)	0.177
No	18/60 (30.0)	
Total	25/99 (25.3)	

4.4.3 Association of ESBL-E fecal carriage with hospital related factors

Based on the statistical analysis, no statistical association was found between the ESBL-E carriage and hospital related factors (Table 4.5).

Table 4.5. Association of ESBL-E fecal carriage with hospital-related factors among hospitalized patients (n=80).

Risk factors	ESBL-E	
	Positive n/N (%)	*p* value
Stay at Intensive Care Unit*		
Yes	1/8 (12.5)	0.145
No	28/68 (41.2)	
Total	29/76 (38.2)	
Surgery*		
Yes	10/27 (37.0)	0.881
No	19/49 (38.8)	
Total	29/76 (38.2)	
Urinary catheter*		
Yes	4/7 (57.1)	0.417
No	25/69 (36.2)	
Total	29/76 (38.2)	
Antibiotic use*		
Yes	22/56 (39.3)	0.735
No	7/20 (35.0)	
Total	29/76 (38.2)	
Beta-lactam antibiotic use*		
Yes	20/48 (41.7)	0.689
No	2/7 (28.6)	
Total	22/55 (40.0)	

*Indicates the current hospitalization.

4.5. Fecal carriage of pAmpC-E

In this study, pAmpC-E colonization rate was detected to be 3.3% (n=6/180). Five (6.3%) of the 80 patients were pAmpC-E positive, while in the control group (n=100), one (1.0%) participant was detected positive for pAmpC-E. The result was not found to be statistically significant (p=0.09) (Table 4.6).

Table 4.6. Distribution of pAmpC-E among patient (n=80) and control (n=100) groups*.

Participants	pAmpC-E positive n (%)	pAmpC-E negative n (%)	Total N (%)
Patients	5 (6.3)	75 (93.8)	80 (100.0)
Controls	1 (1.0)	99 (99.0)	100 (100.0)
Total	6 (3.3)	174 (96.7)	180 (100.0)

*p=0.09

In the study, all pAmpC-positive isolates (patients, n=5; controls, n=1) were detected to be *E. coli*.

4.6. Correlation of Fecal Carriage of pAmpC-E with Risk Factors

4.6.1. Correlation of fecal carriage of pAmpC-E with demographic and socioeconomic factors

The statistical analysis indicated that age group, marital status, gender, educational level, and socioeconomic level were not significant factors for the colonization of pAmpC-E (p>0.05) (Table 4.7).

Table. 4.7. Correlation of fecal carriage of pAmpC-E with demographic and socioeconomic factors in the study group (n=180).

Risk factors	pAmpC-E	
	Positive n/N (%)	*p* value
Age		
19-30	1/76 (1.3)	0.403
31 and above	5/104 (4.8)	
Total	6/180 (3.3)	
Gender		
Male	2/127 (1.6)	0.063
Female	4/53 (7.5)	
Total	6/180 (3.3)	
Education		
University and higher	1/104 (1.0)	0.084
Lower then university	5/76 (6.6)	
Total	6/180 (3.3)	
Marital status		
Single	4/88 (4.5)	0.436
Married	2/92(2.2)	
Total	6/180 (3.3)	
Socioeconomic status		
Low and middle	6/158 (3.8)	1.000
High	0/22 (0.0)	
Total	6/180 (3.3)	

4.6.2. Correlation of fecal carriage of pAmpC-E with epidemiological factors

The statistical analysis showed that presence of GIS during the sample collection was not statistically significant for intestinal colonization with pAmpC-E (p>0.05). In addition, history of antibiotic consumption in general, use of beta-lactam antibiotic, history of diarrhea, UTI, travel history to Turkey, Europe, Asia or Africa within the last 6 months before the study were not significantly associated with pAmpC-E colonization (p>0.05) (Table 4.8).

Table 4.8. Association of fecal carriage of pAmpC-E with epidemiological factors in the study group (n=180). (*Indicates the last 6 months before the study.)

Risk factors	pAmpC-E	
	Positive n/N (%)	*p* value
Presence of any GIS when the specimen was collected		
Yes	2/46 (4.3)	0.646
No	4/134(3.0)	
Total	6/180 (3.3)	
History of antibiotic use*		
Yes	3/85 (3.5)	1.000
No	3/95 (3.2)	
Total	6/180 (3.3)	
History of beta-lactam antibiotic use*		
Yes	2/40 (5.0)	0.464
No	1/9 (11.1)	
Total	3/49 (6.1)	
History of diarrhea*		
Yes	2/46 (4.3)	0.646
No	4/134 (3.0)	
Total	6/180 (3.3)	
History of UTI*		
Yes	0/13 (0.0)	1.000
No	6/167(3.6)	
Total	6/180 (3.3)	
Travel history*		
Yes	1/99 (1.0)	0.092
No	5/81 (6.2)	
Total	6/180 (3.3)	
Travel to Turkey or Europe*		
Yes	1/60 (1.7)	1.000
No	0/39 (0.0)	
Total	1/99 (1.0)	
Travel to Asia or Africa*		
Yes	0/39 (0.0)	1.000
No	1/60 (1.7)	
Total	1/99 (1.0)	

4.6.3. Correlation of fecal carriage of pAmpC-E with hospital-related factors

Statistical analysis showed that stay at ICU, surgery, use of urinary catheter, antibiotic consumption in general, and use of beta-lactam antibiotic during hospitalization did not significantly affect the intestinal colonization with pAmpC (p>0.05) (Table 4.9).

Table 4.9. Correlation of fecal carriage of pAmpC-E with hospital-related factors among hospitalized patients (n=80).

Risk factors	pAmpC-E	
	Positive n/N (%)	*p* value
Stay at Intensive Care Unit*		
Yes	1/8 (12.5)	0.365
No	3/68 (4.4)	
Total	4/76 (5.3)	
Surgery*		
Yes	2/27 (7.4)	0.612
No	2/49 (4.1)	
Total	4/76 (5.3)	
Urinary catheter*		
Yes	0/7(0.0)	1.000
No	4/69 (5.8)	
Total	4/76 (5.3)	
Antibiotic use*		
Yes	2/56 (3.6)	0.282
No	2/20 (10.0)	
Total	4/76 (5.3)	
Beta-lactam antibiotic use*		
Yes	1/48 (2.1)	1.000
No	0/7 (0.0)	
Total	1/55 (1.8)	

*Indicates the current hospitalization.

5. DISCUSSION

Determination of the profiles of resistant bacteria is important in both hospital and community settings, as they can disseminate rapidly and colonize both healthy and hospitalized patients (Ruh et al., 2019). This spread of ESBL-E and pAmpc-E complicated treatment of infections (Khalil et al., 2017). Thus, the present study aimed to detect intestinal colonization rates of ESBL- and AmpC β-lactamase-producing *Enterobacteriaceae* in the hospital and community settings in Northern Cyprus. Also, in this study, factors that increase the risk of ESBL-E and pAmpC-E carriage were evaluated.

In this study, 50 (27.8%) of 180 participants were colonized with ESBL-E (Table 4.1). In a recent study, Ruh et al. (2019) reported the prevalence of ESBL to be 21.4% which was lower than the present study. The current result of ESBL-E fecal carriage is higher than in previous studies which reported 12.7% of colonization rate of ESBL-E (Hagel et al., 2019). There are also studies that reported low prevalence of ESBL-E around the world (Al-gamy et al., 2016; Bassyouni et al., 2015; Reuland et al., 2015; Hu et al., 2019; Founou et al., 2019). In contrast to our study, Andrew et al. (2017) reported high prevalence of ESBL-E in Sudan (45.1%), and also, the rate of ESBL-E was high (62.1%) in Lebanon (Shaikh et al., 2015).

In this study, *E. coli* was reported to be dominant *Enterobacteriaceae* species (Table 4.2). The prevalence of *E. coli* among ESBL-E isolates was 96.0%. This rate is similar with the report of Ruh et al. (2019) which revealed that 94.4% of ESBL-E species were *E. coli*. In the present study, *K. pneumoniae* (2.0%) and *E. cloacae* (2.0%) were found at lower rates among ESBL-E; this is similar with the report of Ruh et al. (2019). Numerous studies reported *E. coli* as the predominant species of ESBL-E intestinal colonization (Karami et al., 2017; Mandal et al., 2017; Xu et al., 2018). In a report from India, the distribution of ESBL-E showed that *E. coli* was the dominant species (32.2%) (Mandal et al., 2017). In Iran, ESBL-producing *E. coli* (79.7%) was reported to be at high level (Karami et al., 2017).

In the current study, a total of 180 participants were recruited to assess the presence of ESBL-E and pAmpC-E and possible factors that increase the chance of occurrence of resistance. In this study, firstly, demographic information and socioeconomic data were analyzed. The present study found that females had higher rate and this was found to be statistically significant (Table 4.3). In another study, ESBL-E was reported to be higher in females than males (Rahamatullah et al., 2019). In contrary, another study showed the rate was not statistically significant as reported by Sanneh et al., (2018) in a study conducted in Gambia.

Another demographic data considered in this study was age group; occurrence of ESBL-E fecal carriage was significantly higher in 31 and above age group than the 19-30 age group (p=0.002) (Table 4.3). Hassuna et al. (2020) reported higher rate of ESBL-E in Egypt which is in agreement with the current study.

Increased fecal carriage of ESBL-E was found in those that did not have university degree (n=31/76; 40.8%) (p=0.001) compared to the ESBL-E prevalence among those that attended university or higher education (n=19/104; 18.3%) (Table 4.3). This is demonstrating the role of education and lifestyle in the harboring of resistant *Enterobacteriaceae*.

This study found that marital status was not a significant factor in fecal carriage of ESBL-E (Table 4.3). However, Liakopoulos et al. (2018) found strong correlation between high rate of intrafamilial ESBL-E fecal carriage within the family members, indicating co-colonization between family members. In a study in France, the results revealed that fecal carriage of ESBL-E in a family member might increase the risk for others (Blanc et al., 2014).

The data of socioeconomic factors in the present study showed that there was no statistical correlation (Table 4.3). Sanneh et al. (2018) reported the association between economic status with ESBL-E fecal carriage in Gambia, which occurred largely in high and cosmopolitan individuals while less in those having low economy.

Several studies reported GIS as the increasing chance for development of both ESBL-E and pAmpC-E occurrence. In this study, 32.6% of the participants that had GIS during sample collection harbored ESBL-E and this was not statistically significant (Table 4.4). GIS was found to be associated with fecal carriage of ESBL-E in many studies (Kantele et al., 2015; Lubbert et al., 2015; Ruppe et al., 2015). Kantele et al. (2015) showed that 67% (n=288/430) had GIS and were colonized by ESBL-E which was higher than the present study. Also, Lubbert et al. (2015) found the association of ESBL-E with GIS to be significant (p=0.011).

In this study, antibiotic use in general was not statistically correlated with ESBL-E fecal carriage (p>0.05) (Table 4.4), and this result is not consistent with previous study conducted by Ruh et al. (2019) where use of antibiotic in the past six month was found to be a risk factor for ESBL-E (p=0.031). Also, Hu et al. (2019) has shown that recent use of antibiotic use was associated with ESBL-E fecal carriage in a systemic review involving 20 countries. In a similar study, antibiotic use was shown to be an important factor in development of ESBL-E which was observed 60 days prior to the study conducted in USA (Zerr et al., 2016).

Many studies also indicated that exposure to antibiotic was associated with increased colonization rate of ESBL-E (Alyamani et al., 2015; Aldrazi, 2019; Somily et al., 2015). Overuse of antibiotics has been linked to the emergence of ESBL-E, which was suggested by Laplente et al. (2017) and Rabaan et al. (2017).

Association of antibiotic use was reported to increase chance of ESBL-E in both community and hospital settings by Goyal et al. (2019). The authors suggested that exposure to antibiotic for three months increased the risk for ESBL-E fecal carriage. A recent study by van den Bunt et al. (2019) in Netherlands has indicated the link between fecal carriage of ESBL-E and antibiotic exposure. This was also consistent with the study of Reuland et al. (2016). A study conducted in Tanzania that is in agreement with our study found no correlation between antibiotic use and fecal carriage of ESBL-E (Moremi et al., 2017). Similarly, Ny et al. (2016) showed no correlation between exposure to antibiotics in the past 6 months prior to the study with ESBL fecal carriage.

The present study showed that there was no relationship between use of beta-lactam antibiotic within six months before the study and ESBL-E fecal carriage (p=0.702) (Table 4.4). Another study that did not find the previous use of beta-lactam as a risk factor for the emergence of ESBL-E include Moghnieh et al. (2015) in patients from Lebanon. Similarly, a study from China reported that use of beta-lactam antibiotic was not associated with the fecal carriage of ESBL-E (Xu et al., 2018). Arcilla et al. (2020) reported that use of beta-lactam was a strong predictor for ESBL-E which contradicts this study.

In this study, history of diarrhea in the past six months was not considered as a risk factor for fecal carriage of ESBL-E (p=0.219) (Table 4.4). A study conducted in Sweden did not find relationship between diarrhea and colonization rate of ESBL-E (Ljungquist et al., 2019), and this was also consistent with Papst et al., (2015). Significant association of diarrhea with ESBL-E was shown in many studies, such as Karanika et al., (2016), Woerther et al. (2017) and Nepal et al. (2017). These studies considered diarrhea as a risk factor for the intestinal colonization of ESBL.

The statistical analysis in the current study showed that UTI was significantly associated with fecal carriage of ESBL-E (p=0.009) (Table 4.4). In the study of Goyal et al. (2019), it was revealed that ESBL-E was significantly associated with history of UTIs (p<0.05). There are also other studies which reported the history of UTI as factor that increased the chance of carriage of ESBL-E. Koksal et al. (2019) showed that presence of UTI was an important risk factor for producing ESBL-E (p=0.04) in a study conducted in Turkey. Another study in Palestine showed that individuals with history of UTI had five times higher risk of carriage of ESBL-producing *Enterobacteriaceae* (Taha et al., 2018).

Another risk factor evaluated in this study was travel history outside Northern Cyprus in the past six months before the study conducted. In the current study, statistical analysis showed that the travel history did not affect the ESBL-E carriage (p=0.403) (Table 4.4). This is consistent with the recent study of Ruh et al. (2019) which revealed that travel history did not affect ESBL-E colonization. There are studies that showed

travel to Southeast Asia and Africa was a major factor for ESBL-E colonization (Kanika et al., 2019; Hu et al., 2020). These are inconsistent with the present study which found that travel history to Turkey or Europe and travel to Asia or Africa were not significantly associated with ESBL-E carriage. Travel-associated fecal carriage of ESBL-E was reported to be significant risk factor in developing countries of Africa and Asia (Hassing et al. 2015).

Fecal carriage of pAmpC-E was also evaluated in this study. Here, the pAmpC-E colonization rate (3.3%) was observed to be less than ESBL-E (Table 4.6). This is consistent with the recent report of Ruh et al. (2019) in the same study area. This is also consistent with previous studies which reported lower rate of pAmpC-E compared to ESBL-E (van Hoek et al., 2015; Hazirolan et al., 2018). In contrast, reports of pAmpC carriage from Libya showed higher rate of pAmpC-E (n=13/22; 59%) than ESBL-E (n=5/24; 20.8%) (Ahmad et al., 2019). In our study, pAmpC-E rate was higher among hospitalized patients (6.3%) than the control group (1.0%), and this was not statistically significant (p=0.09) (Table 4.6). In a community-based study from Turkey, the rate of pAmpC-E was documented as 5.3%, which was much lower than ESBL-E (34.3%) (Hazirolan et al., 2018). This is consistent with the findings in this study and previous report of Ruh et al. (2019) in Northern Cyprus.

In the present study, *E. coli* was detected in all pAmpC-E isolates in both patient and control samples. Ruh et al. (2019) reported the rate of *E. coli* among pAmpC-E positive isolates as 86.7%. In an Egyptian study, pAmpC-E was detected in 5.6% of hospitalized patients; in addition, pAmpC-E occurred together with ESBL-E in half of the isolates (Rensing et al., 2019). In some North African countries, the prevalence of pAmpC-E varied; in Libya this was 6.7%, and in Algeria the rate was 2.3% (Abdulhamid et al., 2017; Rensing et al., 2019). Rates of pAmpC-producing *Enterobacteriaceae* in Europe tend to be lower than the present study. Reports from Netherland and Spain showed the percentage as low as 0.6%. Also much lower rates were reported from Denmark, France and Czech Republic as 0.06%, 0.09% and 1.3%, respectively (van Hoek et al., 2015; Reuland et al., 2015).

In this study, statistical analysis showed that age group, gender, marital status, socioeconomic status and educational level were not statistically significant factors for the pAmpC-E carriage (p>0.05) (Table 4.7). These findings are consistent with the report of Ruh et al. (2019). However, there are reports that showed statistical correlation between fecal carriage of pAmpC-E with some of these variables. Canen et al. (2015) reported that there was a correlation between age and fecal carriage of pAmpC-E, where most of the carriers were elderly. In another study, Reuland et al. (2016) showed that women with underlying condition tend to be at higher risk for pAmpC-E colonization.

In the present study, association of epidemiological factors with fecal carriage of pAmpC-E was also evaluated. The statistical analysis indicated that history of diarrhea, use of beta-lactam antibiotic, history of GIS, UTI, and travel to Turkey, Europe, Asia or Africa were not risk factors for pAmpC fecal carriage (p>0.05) (Table 4.8). Previously, Reuland et al. (2016) demonstrated that UTI was associated with pAmpC-E colonization. Also, Canen et al. (2015) reported a statistical relation between history of antibiotic use in two months before the study and fecal carriage of pAmpC-E. Our findings are inconsistent with those studies. Moreover, hospital-related factors among hospitalized patients in this study were not significantly associated with fecal carriage of pAmpC-E (p>0.05) (Table 4.9).

6. CONCLUSION

In this study, 50 (27.8%) of 180 participants were colonized with ESBL-E. The rates of ESBL-E fecal carriage were 38.8% (n=31/80) and 19.0% (n=19/100) in patient and control groups, respectively. The difference of ESBL-E rates in patient and control groups was found to be statistically significant (p=0.003). *E. coli* was noted to be the predominant (96.0%) ESBL-producing *Enterobacteriaceae* species, followed by *K. pneumoniae* (2.0%) and *E. cloacae* (2.0%). Evaluation of possible risk factors related with fecal carriage of ESBL-E revealed that age (p=0.002), gender (p=0.000) and educational level (p=0.001) were the significant factors, while marital status and socioeconomic status were not considered to be risk factors (p>0.05). Among the epidemiological factors in the study, only history of UTI was found to be significantly associated with fecal carriage of ESBL-E (p=0.009). The hospital-related factors were not significantly related with the ESBL-E carriage (p>0.05).

In the study, overall pAmpC-E rate was found to be 3.3% (n=6/180). In the patient group the rate was 6.3% (n=5/80), while in the control group the rate was 1.0% (n=1/100). *E. coli* was detected in all pAmpC-positive isolates in this study. Statistical analysis showed that demographic, socioeconomic, epidemiological and hospital-related factors were not significantly associated with intestinal colonization of pAmpC-E (p>0.05).

Results of this study provided information about the gastrointestinal colonization rates of ESBL-E and pAmpC-E isolates in both hospital and community settings. Also, the factors that are related with the colonization of ESBL-E were indicated. Although ESBL-E colonization rates are particularly high, presence of pAmpC-E should not be ignored. Results of the study suggest that resistance against third-generation cephalosporins in *Enterobacteriaceae* isolates should be continuously monitored in Northern Cyprus.

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