TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

INVESTIGATION OF THREE DIFFERENT METHODS OF EXTENDED SPECTRUM BETA-LACTAMASES IN *E. COLI* STRAINS ISOLATED FROM VARIOUS CLINICAL SAMPLES

MONTASER MAHER YOUSEF AMRO

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

PROGRAM 2020 – NICOSIA

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ADVISOR

ASSOC. PROF. DR. MERYEM GÜVENIR

2020 – NICOSIA

The Directorate of Health Sciences Institute,

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STATEMENT (DECLARATION)

The data presented in this thesis was obtained in an experiment carried out in the microbiology laboratory/Near East University Hospital. I played a major role in the preparation and execution of the experiment, and the data analysis and interpretation are entirely my own work.

I am aware of and understand the NEAR EAST UNIVERSITY'S policy on plagiarism and I certify that this thesis is my own work I had no unethical behavior in all stages from the planning of the thesis until writing, except where indicated by the references, and the work presented in it has not been submitted in support of another degree or qualification from this or any other university or institute of learning.

MONTASER MAHER YOUSEF AMRO

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Montaser Maher Amro

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LIST OF ABBREVIATIONS AND SYMBOLS

%: PERCENT SİGN

°C: CELSİUS

ESBL: EXTENDED SPECTRUM BETA-LACTAMASES

***E. COLI*:** ESCHERICHIA COLI

ASTS: ANTİBİOTİC SUSCEPTİBİLİTY TESTS

DDST: DOUBLE-DİSC SYNERGY TEST

CDT: COMBINED DISC TEST

TDT: THREE-DIMENSIONAL TEST

EXPEC: EXTRAİNTESTİNAL PATHOGENİC STRAİNS OF *E. COLİ*

INPEC: INTESTİNAL PATHOGENİC STRAİNS OF *E. COLİ*

CFU: COLONY-FORMİNG UNİT

G: GRAM

UTI: URİNARY TRACT İNFECTİON

3RD GC: THİRD-GENERATİON CEPHALOSPORİN

MIC: MİNİMAL INHİBİTORY CONCENTRATİON

NCCLS: NATİONAL COMMİTTEE FOR CLİNİCAL LABORATORY STANDARDS

EHEC: ENTEROHEMORRHAGIC ESCHERICHIA COLI

DNA: DEOXYRIBONUCLEIC ACID

ETEC: ENTEROTOXIGENIC *E. COLI*

EPEC: ENTEROPATHOGENIC *E. COLI*

EIEC: ENTEROINVASIVE *E. COLI*

EAGGEC: ENTEROADHERENT AGGREGATIVE *E. COLI*

DAEC: DIFFUSELY-ADHERENT *E. COLI*

NAM: NUTRIENT AGAR MEDIUM

CLED: CYSTEINE LACTOSE ELECTROLYTE DEFICIENT AGAR

EMB: EOSIN METHYLENE BLUE AGAR

TSB: TRYPTIC SOY BROTH

UPEC: UROPATHOGENIC *E. COLI*

MNEC: MENINGITIS / SEPSIS

QIRS: QUIESCENT INTRACELLULAR RESERVOIRS

IBCS: BLADDER INTRACELLULAR BACTERIAL COMMUNITIES

LPS: LIPOPOLYSACCHARIDE

OMPS: OUTER MEMBRANE PROTEINS

BBB: BLOOD–BRAIN BARRIER

APEC: AVIAN PATHOGENIC *E. COLI*

DEC: DIARRHEAGENIC *E. COLI*

VTEC / STEC: TOXIN-PRODUCING VERO/SHIGA *E. COLI*

LEE: LOCUS OF ENTEROCYTE EFFACEMENT

ST: HEAT-STABLE

LT: HEAT-LABILE

CFS: COLONIZATION FACTORS

AMP: ADENOSINE MONOPHOSPHATE

GMP: GUANOSINE MONOPHOSPHATE

VT: VEROTOXIN

HC: HEMORRHAGIC COLITIS

HUS: HEMOLYTIC UREMIC SYNDROME

GB3S: GLOBOTRIAOSYLCERAMIDES

HR: HOURS

HIV: HUMAN IMMUNODEFICIENCY VIRUS

CPS: CAPSULAR POLYSACCHARIDE

SAT: SECRETED AUTOTRANSPORTER TOXIN

CDT: CYTOLETHAL DISTENDING TOXIN

VAT: VACUOLATING AUTOTRANSPORTER TOXIN

AST: ARGININE SUCCINYL TRANSFERASE

SHET-1: SHIGELLA ENTEROTOXIN-1

INOS: INDUCIBLE NITRIC OXIDE SYNTHASE

PBPS: PENICILLIN-BINDING PROTEINS

AMES: AMINOGLYCOSIDE MODIFYING ENZYMES

PBP: PENICILLIN-BINDING PROTEIN

NAG: N-ACETYL GLUCOSAMINE

NAM: N-ACETYLMURAMIC ACID

DAP: DIAMINOPIMELIC ACID

EPE: ESBL-PRODUCING ENTEROBACTERIACEAE

TEM: TEMONEIRA

SHV: SULPHYDRYLVARIABLE

OMP: OUTER MEMBRANE PROTEINS

CMY: CEPHAMYCINS

FOX: CEFOXITIN

MOX: MOXALACTAM

IMP-1: IMIPENEM-HYDROLYZING B -LACTAMASE 1

VIM: VERONA INTEGRONS-ENCODED METALLO-B-LACTAMASES

NDM-1: NEW DELHI METALLO-B-LACTAMASE 1

HPA: HEALTH PROTECTION AGENCY

ARMRL: ANTIBIOTIC RESISTANCE MONITORING AND REFERENCE LABORATORY

CTX-M: CEFOTAXIME-MUNICH

ICU: INTENSIVE CARE UNIT

WHO: WORLD HEALTH ORGANIZATION

HRI: HEALTH-RELATED INFECTIONS

CLSI: CLINICAL AND LABORATORY STANDARDS INSTITUTE

EUCAST: EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

PCR: POLYMERASE CHAIN REACTION

PFGE: PULSED-FIELD GEL ELECTROPHORESIS

LAMP: LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

TRNC: TURKISH REPUBLIC OF NORTHERN CYPRUS

CAZ: CEFTAZIDIME

CRO: CEFTRIAXONE

CTX: CEFOTAXIME

ATM: MONOCYCLIC BETA-LACTAM AZTREONAM

AMC: AMOXICILLIN-CLAVULANIC ACID

CTC: CEFOTAXIME/CLAVULANIC ACID

SD: STANDARD DEVIATION

SPSS: STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES

CDC: CENTERS FOR DISEASE CONTROL AND PREVENTION

CW: CELL WALL

ET AL.: AND OTHERS

IGA: IMMUNOGLOBULIN A

IGG: IMMUNOGLOBULIN G

IGM: IMMUNOGLOBULIN M

KG: KILOGRAM

L: LITER

LBS: A POUND

MCG /MG: MICROGRAM

MG: MILLIGRAM

MG/L: MILLIGRAMS PER LITER

MH: MUELLER-HINTON AGAR

ML: MILLILITER

MM: MILLIMETER

N: NUMBER

NO: NUMBER

PH: POTENTIAL FOR HYDROGEN

R: RESISTANT

S: SENSITIVE

U: UNIT

UT: URINARY TRACT

MG/ML: MICROGRAM PER MILLILITER

MM: MICROMETER

AMI: ANTIBODY-MEDIATED IMMUNITY

LOS: LIPOOLIGOSACCHARIDE

P-VALUE: PROBABILITY VALUE

Thesis Title: Investigation of Three Different Methods of Extended Spectrum Beta-Lactamases in *E. coli* Strains Isolated from Various Clinical Samples

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ABSTRACT

Aim: This research was performed to establish by three different methods the Extended-Spectrum Beta-Lactamases (ESBL) in *E. coli* strains obtained from multiple clinical samples and antibacterial susceptibility profile testing.

Materials and Methods: A total of 93 isolated samples were collected from inpatients identified for *E. coli* organisms and checked for Antibiotic Susceptibility Tests (ASTs) throughout the Phoenix 100 automated system. The production of ESBL was carried out using the Combined Disc Test (CDT) method, Double-Disc Synergy Test (DDST) method and the Three-Dimension Test method (TDT).

Results: Distribution of specimens were 40.9% (n: 38) isolated from males and 59.1% (n: 55) from females, and the majority of isolated specimens were urine 62.4% (n: 58). The results of ASTs were 63.4% Amoxicillin Clavulanic Acid resistant, 91.4% Ampicillin resistant, 77.4% Cefixime resistant, 82.8% Ceftazidime resistant, 81.7% Ceftriaxone resistant, 63.4% Cefuroxime Axetil resistant, 71.0% trimethoprim/sulfamethoxazole resistant, and 65.6% Ciprofloxacin resistant. In the investigation of ESBL production among Escherichia coli species, this study shows 93.5% (n: 87) positive ESBL from DDST, 78.5% (n: 73) positive ESBL from CDT, 76.3% (n: 71) positive ESBL from TDT.

Conclusion: The conspicuous prevalence of ESBL forming and multidrug-resistant of *E. coli* bacteria in our institutions provides a glimpse of potential challenges in our region of the world. Routine testing of Extended Spectrum Beta-Lactamases may be considered in the care plan.

Key Words: *Escherichia coli*, ESB , Antibiotic susceptibility test, *Enterobacteriaceae*

Tez Başlığı: Çeşitli Klinik Örneklerden İzole Edilen *E. coli* Suşlarında Genişletilmiş Spektrumlu Beta-Laktamazların Üç Farklı Yönteminin Araştırılması.

Öğrencinin Adı: Montaser Maher Yousef Amro

Danışmanı: Assoc. Prof. Dr. Meryem Güvenir.

Anabilim Dalı: Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji.

ÖZET

Amaç: Bu çalışma, çeşitli clinic örneklerden izole edilen E.coli suşlarında Genişletilmiş Spectrumlu Beta-Laktamazları (ESBL) ve antibakteriyel duyarlılık profili testini üç farklı yöntemle belirlemek için yapılmıştır.

Gereç ve Yöntem: Toplam 93 izole örnek, E. coli türleri için tanımlanan ve tam otomatik Phoenix 100 sistemi boyunca Antibiyotik Duyarlılık Testleri (AST'ler) için test edilen hastanede yatan hastalardan alınmıştır. ESBL üretimi, Çift Disk Sinerji Testi (DDST) yöntemi, Kombine ile gerçekleştirilmiştir. Disk Testi (CDT) ve Üç Boyutlu Test (TDT).

Bulgular: Örneklerin dağılımı erkeklerden% 40.9 (n: 38) ve kadınlardan% 59.1 (n: 55) izole edildi ve izole edilen örneklerin çoğu% 62.4 (n: 58) idi. AST'lerin sonuçları% 63,4 Amoksisilin Klavulanik Asit dirençli,% 91,4 Ampisiline dirençli,% 77,4 Sefiksim dirençli,% 82,8 Seftazidime dirençli,% 81,7 Seftriaksona dirençli,% 63,4 Sefuroksim Aksetil dirençli,% 71,0 trimetoprim / sülfametoksazole dirençli% 65,6 idi . *Escherichia coli* türleri arasında GSBL üretiminin araştırılmasında, bu çalışma DDST'den% 93,5 (n: 87) pozitif ESBL, CDT'den% 78,5 (n: 73) pozitif ESBL, TDT'den% 76,3 (n: 71) pozitif ESBL göstermektedir. Çalışmamızda çift disk sinerji testinin rutin disk difüzyon testiyle aynı anda uygulanabilecek, ek mali harcama ve zaman gerektirmeyen, rutin laboratuvarlarda kolaylıkla kullanılabilecek duyarlı bir test olduğu sonucuna varılmıştır. Kurumlarımızda ESBL oluşumunun ve çoklu ilaca dirençli *E. coli* bakterilerinin göze çarpan yaygınlığı, dünya bölgemizdeki potansiyel zorluklara bir bakış sağlar. Genişletilmiş Spektrumlu Beta-Laktamazların rutin izlenmesi, tedavi planında dikkate alınmalıdır.

Anahtar Kelimeler: Escherichia coli, ESBL, Antibiyotik duyarlılık testi, Enterobacteriaceae.

1. INTRODUCTION

In 1885, *Escherichia coli (E. coli)* was described as a Gram-negative rod as part of the Enterobacteriaceae family. In the intestines of vertebrates, *E. coli* have their natural habitat and are also frequently found in soil and water. As well as being the most prevalent pathogen in the enterobacterial family, it is the most prevalent species found in the warm-blooded animals and human gastrointestinal tract (J. W. Yoon & Hovde, 2008). *E. coli* mainly resides in the colon lumen and rarely causes disease in healthy people, but they can become true pathogens once the *E. coli* clones are highly adapted.In both normal hosts and those with a weakened immune system, these *E. coli* pathotypes may cause particular illnesses (Bennett et al., 2014; Forbes et al., 2007).

E.coli seems to be an opportunistic pathogen, like many other species of bacteria. This means that, under normal circumstances, it is a natural part of the human microbial flora, causing no harm. This naturally occurring strains of *E. coli* are known as commensals. However, *E. coli* may potentially cause lethal infections when transferred from the intestinal mucosa to other organs, such as the urinary tract, the gall bladder, or the bloodstream (Tenaillon et al., 2010). *E. coli* bacteria strains are classified into two main categories based on the location of infection and the illness they induce: extra intestinal pathogenic strains of *E. coli* (ExPEC) cause diseases of organs other than the intestine and, when ingested, intestinal pathogenic strains of *E. coli* (InPEC) cause gastroenteritis or colitis (T. J. Johnson & Nolan, 2009; Moriel et al., 2012). Six classes of InPEC pathotypes are known as Enterohemorrhagic *E.coli*, Enterotoxigenic *E. coli*, Enteropathogenic *E.coli*, Enteroaggregative *E. coli*, Enteroinvasive *E. coli*, and adhesive-invasive *E. coli* (Russo & Johnson, 2000).

E. coli are found to be the most widespread optional anaerobic bacteria inside the human gastrointestinal tract (109 CFU/g feces. In addition, it has the potential to colonize the intestines, and can be seen as an indication of fecal pollution of food and drinking water. Reffering to community-acquired bacteremia, *E. coli* strains was the commonest and the fifth frequent cause of nosocomial bacteremia (Friedman et al., 2002). *E. coli* is usually a

harmless microbe. Compared to non-pathogenic *E. coli*, the more virulent pathotypes also have a larger genome, Many various virulence factors have mainly been encoded within bacteriophages, genes, or plasmids (Rasko et al., 2008; Welch et al., 2002). Pathogenic E. coli serotypes and classes are characterized by lipopolysaccharide (O) and flagellar (H) antigens (Tenaillon et al., 2010).

Urinary tract infections (UTIs) are one of the most serious bacterial infections in the world. A number of pathogenic bacteria cause such infections. In addition to the ability of this organism to produce the β-lactamase enzyme, Enterobacteriaceae is the most common one, especially *E. coli*. UTI is a disease of the kidneys, ureters, bladder, and urethra of every part of the urinary system. Many infections include the lower urinary tract, the urethra, and the bladder (Mehrgan & Rahbar, 2008). Enterobacteriaceae are groups of Gram-negative bacteria. Within immunocompromised patients such as: alcoholism, chronic obstructive pulmonary disease, diabetes mellitus, malignancy and glucocorticoid therapy), the infection maybe produced refering to these species within defferent parts and a high risk. In clinical practice, the introduction of third-generation cephalosporin (3rd GC) has been very helpful in combating beta-lactamases (Paterson & Bonomo, 2005).

Resistance to these antibiotics began to emerge rapidly, however. These enzymes have been referred to as extended-spectrum β-lactamases (ESBLs) because of their increased spectrum of action, especially against 3rd GC. Mainly *E. coli* and *Klebsiella pneumonia* produce these enzymes. Other Gram-negative bacilli, such as Pseudomonas aeruginosa, Salmonella species, Proteus species and other Enterobacteriaceae, have been described. The first ESBL-producing organism was isolated in Germany in 1983 (Bradford, 2001).

Thereafter, Following outbreaks of these pathogens infectionsIn the United States, several species have been identified. ESBL enzymes are able to hydrolyze broad range cerebrosporins and monobactams, but they are not successful against cephamycins and imipenem, and they are therefore co-resistant to certain other antibiotic classes, resulting in limited therapeutic options (Astal et al., 2004). For this cause, the importance of these induced ESBL infections has been progressively documented worldwide. ESBLs have serine at their active site and have an amide bond inside the lactam ring of antibiotics that

induces their hydrolysis. Their identification is a major challenge due to the inoculum effect and substrate specificity. In the presence of clavulanic acid, two indicators of ESBLs are eight-fold reductions in MIC and potentiation of the third-generation cephalosporin inhibitor zone. For this reason, ESBLs are correlated with extended hospital visits, elevated morbidity, mortality and costs in health services by utilizing standard anti-sensitivity approaches, as well as delayed detection and documentation of gram negatives bacilli growth. Therefore, the prevalence of these species and the policy of care need to be identified and formulated (Mehrgan & Rahbar, 2008).

The National Committee for Clinical Laboratory Standards (NCCLS) has recommended that microbiology laboratories report that ESBL-producing isolates of *Klebsiella* and *E. coli* species are resistant to all cephalosporins, penicillin's (including cefepime) and aztreonam, regardless of the individual results of the in vitro test. The presence of ESBL in certain strains of *E. coli* and *Klebsiella pneumonia* poses an important concern in clinical practice as these bacteria are major sources of serious complications (Mehrgan & Rahbar, 2008).

1.2. STUDY GOALS AND OBJECTİVES

1.2.1. Aims

Distribution pattern of *E. coli* organisms isolated from separate clinical specimens and ESBL studies and antibacterial susceptibility profile testing.

1.2.2. Objectives

The study's basic objectives were to investigate:

1. Isolate and identify *E. coli* species in collected specimens.

2. Study the distribution of *E. coli* species in different clinical specimens.

3. Provide swift and correct diagnosis of *E. coli* species.

4. ESBLs and antibacterial susceptibility test patterns for *E. coli* isolates.

2. GENERAL INFORMATION

2.1. History

The German-Austrian bacteriologist and pediatrician, Theodore von Escherich, described *E. coli* in 1885. His research focused on infant gut microbes and their function in illness and digestion. In the feces of children, which he called Bacterium coli commune, he identified a fast-growing bacterium. Dr. Escherich concluded in 1988 that this microorganism may be commensal since the digestive tract activity appeared undisturbed in breastfeeding neonates. Scientists dubbed this bacterium *E. coli* in honor of its discoverer after he retired (Escherich, 1886a; J. R. Johnson & Stell, 2000).

In the beginning of the 20th century, *E. coli* has become the first bacteria for microbiology studies and laboratory selection teaching, and it was later developed as a bacterial standard for the revolution in molecular biology in the 1950s (Clermont et al., 2000). Francis's, H. C. Crick, senior scientist, H. C. Crick, who was given the Nobel Prize in Physiology in Medicine in 1962, used *E. coli* for his genetic code experiments. *E. coli* is also commonly employed in a number of areas of medicine, including commercial genetic modification, pharmaceutical research, and the biotechnology industry (Andreu et al., 2003). Many scientists had done extensive research on characterizing the isolate of *E. coli*. In 1919, Castellani and Chalmers reformed the definition of this species based on some additional characteristics.

They combined the facts that Escherich's bacteria grow well in ordinary laboratory media, in addition to milk clotting, and do not form endospores, these are mostly optional anaerobes that can ferment glucose and lactose fully with acid and gas production. Based on all the new evidence they renamed Escherich’s bacterium to *E. coli* (Castellani & ALBERT, 1919; Escherich, 1886b). Little will they all realize that once it was established, this bacterium would play a vital role in the advancement of science and civilization

In the United States of America, These bacterial pathogens (animal and human) were first isolated and defined in 1975 by a California woman that have a sever bleeding diarrhea, 1977 Argentina livestock pathogen was found in and 1982, respectively (Fernandez, 2008). In North America when the ECCO (Escherichia coli O157:H7) outbreak was correlated to the import of infected cattle from Argentina a human infection rate was found that was nearly three times higher than those that had been found previously in North America (McMichael, 2001).

In 1982, The first E. coli contributed to the epidemic, which came to light in the USA. Oregon and Michigan in the USA. These were reported from people that developed bloody diarrhea, with severe stomach cramps, after eating hamburgers in a food chain (Besser et al., 1999; Pennington, 2010, p. 157). EHEC serotype outbreaks have mainly included uncooked ground meat products and occasionally raw milk (Abd El Tawab et al., 2015). Doyle and Schoeniin, who tested E. coli in 1987 and identified this strain as 3.7% of 164 beef, 1.5% of 264 pork, 1.5% of 263 poultry and 2.0% of 205 lamb samples, conducted the first research on the occurrence of EHEC strains in meats in 1987 (Dulo, 2014). Its fundamental cellular structure, metabolism, and genetics form the character of each organism. Together, E. coli in domain bacteria, kingdom Eubacteria, phylum Proteobacteria, class Gammaproteobacteria, order Enterobacteriales, family Enterobacteriaceae, genus Escherichia, E. coli species Escherichia are found in all these determinants.

E. coli considered being as a part of Enterobacteriaceae bacterial family. It is the commonest commensal inhabitant of human even warm-blooded gastrointestinal tracts and one of the most significant pathogens (Kaper et al., 2004b). As a commensal, it acts in a mutually advantageous relationship with hosts and seldom induces disease. However, it is also one of the most harmful human and animal pathogens, since it was responsible for a wide range of diseases. Due to the unique features of E. coli, it is a major biotechnology host organism. such as ease of processing, complete genome sequence availability, and its ability to extend both in aerobic and anaerobic conditions.

Within a wide range of industrial and medical uses. The most widely used microorganism in the field of recombinant DNA is *E. coli* (S. H. Yoon et al., 2009). As other researchers later identified the organism under several synonyms and iterations, and the term *E. coli* was not commonly accepted until 1954. More than 125 years later, in warm-blooded species, E. coli is remembered as a harmless gastrointestinal tract commensal and used as a colloquial laboratory workhorse (Cowan, 1954).

Each unique and adapted pathogen has developed a pathogen that adapts and develops forms to counter or Each unique and adapted pathogen has developed a pathogen that adapts and develops forms to counter or The pathogenicity of *E. coli* will cause a broad variety of human diseases from the gastrointestinal tract to additional intestinal locations such as the urinary tract, bloodstream and central nervous system (Kaper et al., 2004b). Some forms of E. coli enter the stomach and seldom making patients ill, unless they are sick already. Although rare, there are some cross-reactive pathogenic strains among healthy and immunocompromised individuals that can cause diarrhea or extra-intestinal diseases. Diarrheal diseases, such as rotavirus, are a serious public health issue and a main cause of mortality in infants and young children. This is particularly relevant in developing countries where E. coli bacteria might seem harmless, and only a few of its Enteropathogenic strains have been identified. Then certain serotypes of the bacterium were found to show both pathogenic and Enterotoxigenic characteristics and to contain various virulence factors. The presence of *E. coli* serotypes that can cause diarrhea was found near the end of the 1940s. When toxins similar to Vibrio cholera toxin were found in the middle of the 1950s, that caused the perspective for *E. coli* to be changed (Gomes et al., 2016).

E. coli is often recognized as several hundreds of strains and is categorized based on their particular serotypes. As an example, *E. coli O157:H7,* which appears to produce Shiga-like toxins from consuming cheese and infected meat, is a well-studied strain of bacterium. In comparison, Enteric coli can be categorized into six groups *Enterotoxigenic E. coli (ETEC),* Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli (EPEC),*

Enterohaemorrhagic. *Coli* (EHEC), Enteroadherent aggregative *E. coli* (EAggEC), and Diffusely-adherent *E. coli* (DAEC), based on its virulence properties.

Enteric bacteria have the ability to cause a variety of intestinal and extra-intestinal diseases including urinary tract infections and mastitis. The *E. coli* that resides within the digestive system can cause a number of illnesses and diseases, such as urinary tract infections and mastitis. On the other hand, it is not always an individual that can be affected by these bacteria. Most of *E. coli* it is in our intestines, helping to break down the food that we eat and to help reutilize nutrients and clean up garbage, produce vitamin K, and digest food. E. coli bacteria are not found in nature in the same way as other coliform bacteria (Palmer & Parry, 2002) (Centers for Disease Control and Prevention (U.S.) 2002). *E. coli*O157: The H7 bacterium they are Gram-negative, enterohaemorrhagic, and widespread in the food industry worldwide. The "O" in "O157" is also known as "Entero", and before the official name of the bacteria was determined as that it was incorrectly thought to be "H57," meaning it could be on all continents except Antarctica. where there is 100,000 patients and 3,000 hospitalizations with 90 deaths every year in the United States (Croxen et al., 2013).

E. coli is most often transmitted through direct or indirect contact with infected feces, through contaminated or undercooked food, near proximity to the infected person, water, animal contact, or less often by laboratory exposure. Uncooked meat like ground beef, unwashed vegetables such as lettuce or slaw, and raw dairy products accounted for an estimated 50-60% of outbreaks in the United States, with waterborne cases accounting for around 15% of outbreaks and 5% of direct animal contact (Rangel et al., 2005, 1982–2002).

Most *E. coli* O157: H7 cases infection is related to contamination of the food (Armstrong et al., 1996). An epidemiological study found that 41 % of 183 outbreaks in the USA were the result of ground beef consumption from 1982 to 2002. Transmission from person to person is also reported as a source of infection, as 50 outbreaks (out of 183) reported in the United States have spread via the fecal-oral route. Although the ingestion of infected food and/or direct contact with contaminated fecal material is still the most

frequent route for *E. coli*O157: H7 transmission, new transmission routes are a major concern(Rangeletal.,2005,1982–2002).

2.2. Morphology *E. coli*

Bacteria conducted as unicellular microorganisms that do not have chlorophyll pigments. There are no nuclei or membrane-bound organelles, so the composition of the cell is simpler than other organisms. There is a hard cell wall for all bacteria, which ensures they retain a definite cell structure. Rock-shaped bacillus *E. coli* cells which usually occurs individually and in large clumps (Barcella et al., 2016). *E. coli* was conducted as gram-negative species, Straight rod (1.1–1.5 m-2.0–6.0 m) organized in pairs or in pairs.; it's don't the ability to form spores. *E. coli* is a motile or non-motile species and peritrichous flagella is the motility organ and in some cases capsules or microcapsules could be seen (Hilmi et al., 2019). *E. coli* unlike other coliform bacteria, bacteria are not found in nature under natural conditions (Cengiz et al., 2004).

In some cultures, it can be found as small and short, like cocci, whereas it can also be found as shapes longer than normal, and even as filament shapes that also diverse as letter Y (Robinson, 2014) . It is possible for both shapes to exist together. Though it moves around itself through its lashes, its movements are slow (Cengiz et al., 2004). They can even seem non-motile. Strains of *E. coli* usually create fimbria. Fimbriae play a role in assisting the virulence factor with their characteristic to hold on cells (Elbing & Brent, 2019).

Off-white or beige, with a glossy finish is colored *E. coli* colony. It even looks like mucus on the plate's whole surface or a cloudy film. A slightly higher *E. coli* colony has a complete, established margin and a steady growth trend, which forms a focused expanding circle throughout the colony. The rings can be seen under a microscope. In the middle, older colonies are becoming darker (Shanahan & Tanner, 1948). *E. coli* has no specific dietary requirements and is readily applied to traditional media such as the Nutrient Agar Medium. The nutrient Agar Medium and MacConkey Agar Medium are widely used in *E.*

coli cultivation laboratories. The optimal *E. coli* temperature ranges between 10 and 40 °C, although the optimum temperature for most strains is 37 °C and is usually grown at this temperature in laboratories. The maximal pH is 4.5–9.5, but the highest development is detected at 7.0, i.e., neutral pH. The pH criteria often vary based on the strain of *E.coli* (*E. coli CDC*, 2020).

The laboratory utilizes a broad range of culture media: the medium of Nutrient Agar and the media of MacConkey Agar, and some like the medium of Sheen Blood Agar medium, Columbia Horse Blood Agar medium, Cysteine Red Bile Agar medium, Eosin Methylene Blue Agar Medium, Sorbitol MacConkey Agar media Medium. Apartments of Columbia Blood Agar are more commonly used in the laboratory for cultivating *E. coli*; Eosin Methylene Blue Agar is an eosin dye and methylene blue dye selective medium that inhibits the growth of several gram-positive bacteria, inhibits the growth of Shigella and salmonella species, and encourages the growth of *E. coli* and MacConkey Agar Sorbitol, that also acts as selective coli median. Blue Agar is a selective medium for coli.

Some strokes of *E. coli* display beta hemolysis in blood agar medium, particularly those which are excluded from conditions of pathology, while those who are extracted from normal people does not seem to have hemolysis on blood agar. The colonies of the MacConkey Agar are pink owing to the lactose fermentation, which is of considerable significance when it comes to separating *E. coli* from other specimen bacteria, in particular non-lactose gram and salmonella species, which gives the MacConkey agar medium colorlessly. The colonies of coli develop with a green metallic sheen in the Eosin Methylene Blue Agar medium because of the metachromatic existence of dyes and the lactose coli fermentation that shifts the pH of the medium to acidity. Render the medium more selective and simpler to identify for *E. coli* (*E. coliInfection*, 2015), (Kaper et al., 2004b) (Barrick et al., 2009) (Robinson, 2014) (Barcella et al., 2016).

E. coli is an anaerobic chemo-organic trophic microorganism. It is oxidase-negative, catalase-positive, fermentative (glucose, lactose, D-mannitol, D-sorbitol, maltose, arabinose), nitrate-lowering and galactosidase-positive. About 95% of E. The coli

strains are positive for indole and methyl red, but they are negative for Voges-Proskauer and citrate (Barnich et al., 2007; *E. coli* , n.d.) (Table 1).

Table 1. The Culture Characteristic of *E. coli* in Different Medium (Aryal, 2020)

Cultural Characteristics	**Nutrient Agar Medium**	**Eosin Methylene Blue Agar Medium**	**MacConkey Agar Medium**	**Blood Agar Medium**
Shape	Circular shape	Circular shape	Circular shape	Circular shape
Size	1-3 mm	2-3 mm	2-3 mm	1-3 mm
Elevation	Convex	Convex	Convex	Convex
Surface	Smooth; Rough (repeated subculture); mucoid (capsulated strains)	Smooth (fresh isolation); Rough (repeated subculture); mucoid (capsulated strains)	Smooth (fresh isolation); Rough (repeated subculture); mucoid (capsulated strains)	Smooth (fresh isolation); Rough (repeated subculture); mucoid (capsulated strains)
Color	Greyish white	Green metallic sheen	Pink	Greyish white
Structure	Translucent – Opaque	Opaque	Opaque	Translucent – Opaque
Hemolysis	-----	-----	-----	β-Hemolysis (in some strains)

2.3. Pathogenesis and Pathogenic Mechanisms

E. coli normally colonizes the gastrointestinal tract of human babies within a few hours of birth. *E. coli* and also its human host have been co-existing in good health and profit for decades. These commensal strains of E. coli seldom trigger disease, except in immunocompromised hosts, or where traditional gastrointestinal barriers are breached. Commensal Niche *E. coli* is the mucosal surface of the colon of mammals. The bacterium is a strongly efficient rival at this crowded position representing the most numerous facultative anaerobic of human intestinal microflora (Sweeney et al., 1996). Like most mucosal pathogens, it can be said that *E. coli* practices the necessary technique of infection of the mucosal site. Colonization, avoidance of host protection, aggregation, and harm to the host. The most common feature of Diarrheagenic *E. coli* strains is their potential to colonize the intestinal mucosal surface (Gomes et al., 2016).

Pathogenic *E. coli* strains are classified as enteral and parenteral diseases, in two groups. Both are *E. coli* extraintestinal pathogenic, and *E. coli* intestinal (diarrheagenic). *E. coli* infection is likely to occur in three general clinical syndromes: enteric/diarrheal disorder, UTIs and sepsis/meningitis. Six well-described types of intestinal pathogens include: EPEC, EHEC, ETEC, EAEC, EIEC and DAEC (Nataro, 2002).

2.4. Virulence Factors

E. coli bacillus has a complex but well-antigen structure and various types of antigen as they are similar in all movements of the intestines (Robinson, 2014). In the 1940s Kauffman suggested a serotype diagram for *E. coli* based on the somatic lipopolysaccharide which called O antigen, capsular polysaccharide that called K antigen and flagellar H (Akbar and Anal, 2011; Robinson, 2014).

2.4.1. Antigens

There are millions of lipopolysaccharide (LPS) molecules in the outer membrane of an *E. coli* organism, consisting of 1) O antigen, a polymer (1–40 units) of immunogenic repeated oligosaccharides. 2) The central area of non-repeating oligosaccharides that are phosphorylated. 3)The lipid A (endotoxin). These are antigens with a somatic lipopolysaccharide structure resistant to heat, they are resistant to boiling and alcohol, and they are not resistant to formol. This found 171 different choline O antigen (Robinson, 2014).

O antigens have cross-reaction with other related microorganisms. For example, *E. coli* O antigens make a cross-reaction on *Shigella spp*. with some O antigens. and *Salmonella spp*. Especially all O antigens (except for O antigens at some *Shigella sonnei*) react with *Shigella* (Cengiz et al., 2004). Because of this cross-reaction, many antibody-based tests that generally determine *E. coli* cause incorrect positive results to be obtained because it causes many microorganisms to cross-react with O antigens (Feng et al., 2002). *E. coli* are serologically classified into serovars with their antigens H and K according to their antigens O (Robinson, 2014).

2.4.2 H Antigens

The H antigen is a major component of flagella that is active in *E. coli's* movement and is normally encoded by the fliC gene. *E. coli* lash antigens are found in mobile origin, have a protein structure and thermolabile, are destroyed by heating at 100°C and by alcohol and proteolytic ferments, and are resistant to formol (Robinson, 2014). There are 53 H antigens known, sequentially numbered from H1 to H56, which are used to identify only around 20 of them. H antigens do not make cross-reaction with other bacteria and with H antigens (Wang et al., 2003).

2.4.3 K Antigens

Acid capsular polysaccharide is a dense and mucus-like polysaccharide that includes several pathogenic *E. coli*; there are several distinct groups of K-antigen, group I and group

II (while a minor intermediate subset (K3, K10 and K54/K96) were referred to as group III), (Group III). K antigens are a piece of polysaccharide and cell capsules found in nature. *E. coli* bacteria including these antigens do not agglutinate with O antiserums. K antigens which have been analyzed according to their agglutination characteristics are named according to the difference in their structure. Approximately 80 kinds of K antigens have been identified which are called K1, K2. These are heat resistant and can be eliminated by boiling at 100 and sometimes 120 degrees for a few hours. Antigens K88 and K99 cause diarrhea in pigs, the K99 antigen is associated with diarrhea (Don et al., 1984; Robinson, 2014).

2.4.4 Adhesion

In the attachment of E. coli to uroepithelial cells, it tends to fully overwhelm the control of urine flow; with certain pathogenic microorganisms, it is recognized as the first step of the colonization process, both the host and the *E. coli* work in this process (Bien et al., 2012; Riegman et al., 1988). For good adherence to the surface of the host cell, the capacity of UPEC to colonize depends on the expression of different Fimbrial adhesions, UPEC expresses certain adherence factors that are necessary for attachment and hence considered to be virulence factors. In a thin filamentous system called fimbriae, or pili, there are many bacterial adhesives. Fimbrial-type adhesions are essential during the attachment phase (Emody et al., 2003).

Fimbriae, it has been known as pili, which has a long hair-like projections located in surfaces of bacterial cells that commonly identify as carbohydrates particular compounds of the target host cells (Winberg, 1984), Pili is the short type of fimbriae and may be used with fimbriae interchangeably. These proteins are formed by a helical cylindrical structure that is both thinner and shorter than the flagellum. Fimbriae is composed of oligomeric pilin proteins; these protein complexes are expressed as virulence factors in Uropathogenic strains of *E. coli*. The majority of *E. coli* receptors are carbohydrates for these fimbriae, such as type 1 fimbriae, P fimbriae, and thin aggregative fimbriae (Collinson et al., 1992).

2.4.5 Toxins

Toxins secreted by certain types of *E. coli* are largely responsible for their virulence. Moreover, toxins are capable of altering the signaling pathway of host cells and modulating inflammatory responses, and these toxins often lead to inducing host cell death and releasing the required nutrients (Agarwal et al., 2012). Cytolethal distending toxin (CDT) was first reported as a virulent toxin in UPEC in 1987, and then many other toxins, including: Shigella enterotoxin (VAT), vacuolating autotransporter toxin (VAT), alpha-hemolysin (HlyA), cytolysin A, cytotoxic necrotizing factor 1 (CNF1), arginine succinyl transferase (AST), plasmid-coded toxin (PET) and secreted autotransporter toxin (SAT) were identified in UPEC (ShET-1) (Soltani et al., 2018).

2.4.6 α-hemolysin

HlyA is a very significant lipoprotein with all toxins belonging to the RTX toxin class (Eberspächer et al., 1989). HlyA is a pore-forming toxin that causes cell membrane damage and apoptosis induced by inducible nitric oxide synthase (iNOS) (Chen et al., 2003). However, by means of a process that enables UPEC to destroy host immune-effector cells and increase access to host nutrients and iron reserves, HlyA can lyse high-concentration erythrocytes and nucleated host cells. HlyA plays a major role in growing the production of IL-6 and IL-8 by inducing Ca2+ oscillations in epithelial renal cells (Cavalieri et al., 1984; Russo et al., 2005).

2.4.7 Capsule

When the capsule is shielding and defending the bacteria from external environments and the host immune response, the primary use is to shield the bacteria from different adverse conditions and the host immune response (Bien et al., 2012). The capsule also gives antimicrobial tolerance and antiserum activity defense in the host against swelling and complement-mediated bactericidal impact in the host (Bien et al., 2012; Jahandeh et al., 2015). Several capsules, such as K1 and K5, inhibit the compromised host's proper

humoral immune response by demonstrating molecular mimicry of tissue components (Bien et al., 2012). The K1 polysaccharide is a linear alpha2-8-linked sialic acid polymer, which plays a very significant role in both the production of IBC and the multiple phases of UTI pathogenesis (Nowicki et al., 1990; Olson et al., 2015).

2.4.8 Lipopolysaccharide (LPS)

Lipopolysaccharide, an important 'cell wall portion,' consists of highly conserved 'lipid A-core' subunits and repeated O-antigen subunits, which differ greatly between the strains based on 'sugar residues' and their patterns of linkage within repetitive subunits (Bower et al., 2005; Sarkar et al., 2014). LPS is well established to be present in biopsy specimens and activation of the immune system which causes the production of nitric oxide and cytokine, which enhances the immune response (Bien et al., 2012; Emody et al., 2003).

As well, in the study of this new substance, it was noted that it induces the production of specific antibodies and helps in the development of an immune-adjuvant effect, allowing for a quicker immune response. Results have shown that the pathogen may also possess the property of being able to withstand human serum that normally helps to destroy pathogens (Cirl et al., 2008). Acute renal dysfunction due to LPS is entirely contingent on the systemic reaction to LPS and does not rely on the renal expression of the functional LPS receptor, TLR4. Although it is unknown if LPS plays a part in the development of rising UTIs and resulting renal failure, it is believed to be a source of acute allograft injury (Bien et al., 2012).

2.4.9 Motility

The bacterium produces flagella and maybe its way to get around and also plays a role in the forming of biofilm in the formation of the initial adhesion (Bien et al., 2012; Ong et al., 2008). In research, it has been found that the bacteria are "moving" down towards the kidneys after they pass into the bladder (Lane et al., 2007). About 70–90% of all urinary tract infections arise because of flagellated Beta-Hemolytic uropathogens, and

oral bacteria touch and epithelial cells of the urinary tract are a contributing factor throughout the pathogenesis (Bien et al., 2012). Since flagellar motility is a way for bacteria to adapt, it greatly improves the bacteria's capacity to react to some attractive or repellent environmental stimuli (Emody et al., 2003). Table 2 show the list of actions of the virulence factors of *E. coli* strains.

Table 2. The Virulence Factors Specific to *E. coli* strains

Virulence Factor	Strain	Effect
P Fimbria, AFAI and AFAII, Dradesin, Type 1 battery	Uropathogenic strains	Holding on target cell
S battery	Strains that cause meningitis	Holding on target cell
EspA	EPEC strains	Holding on intestinal epithelial
CFA/I and CFA/II	ETEC strains	Bonding on small intestinal microvillus
Shiga Toxin (Verotoxin)	EHEC strains	Inhibition of protein synthesis
Intimin	EPEC and some other strains	Triggering of disruption of absorption at intestine
LT	ETEC	Development of diarrhea because of cAMP formation
ST	ETEC	Development of diarrhea because of cGMP formation
Hemolysin	ETEC strains	Lysis of erythrocytes

2.5. Treatment, Prevention and Control

Treatment of *E. coli*O157: H7 infections with antibiotics may worsen the disease, the use of antibiotics may lead to the breaking up of the bacteria that increase Shiga toxin production and secretion (Hiko et al., 2008). Data have shown that *E. coli*O157: H7 induces Shiga toxin production by ciprofloxacin or sub-inhibitory concentrations of trimethoprim-sulfamethoxazole. Therefore, diagnosis is mainly helpful to reduce the duration of symptoms and preventing systemic complications (Lim et al., 2010, p. 157). For people with diarrhea, clear liquids are recommended to prevent dehydration and loss of electrolytes (Dulo, 2014). To accomplish the overall aim of avoiding and managing the spread of E-coli, we should concentrate on ensuring access to reliable sources of water, protecting our food supply from pollution, and informing the public to deter the spread of the pathogen (Mielke, 2010; Organization, 2001).

Measures to protect food products against infection involve sufficient temperatures for storage and preparation, food irradiation technologies can be used in elevated risk products to significantly reduce the bacterial load (Seib et al., 2012). In order to prevent the spread of multi-resistant pathogens in the hospital, there needs to be a strict focus and prevention of cross-contamination in the hospital environment, and the use of antimicrobial drugs needs to be completely regulated and managed (Mielke, 2010). Infections can spread easily whenever workers touch a contaminated surface with a contaminated hand. To prevent this, it is important to always wipe your hands and sterilize your work environment through the use of proper hand hygiene.

Antibiotics play a key function in both avoiding and managing human and animal (animal) Escherichia coli infections (De Vrese et al., 2008). As more and more antibiotics are misused, and over-prescribed, we have seen a rise in antibiotic resistant pathogens and a decrease in normal human bacterial flora (Van Duijn et al., 2011). Based on clinical proof, utilizing aerophagies in a manner that are healthy and reasonable should be a requirement for avoiding the production and spread of antibiotic resistant bacteria. The probiotics can be a choice to prophylaxis over several E. coli infections, especially those involving the

gastrointestinal tract; probiotics are viable and stable microorganisms, primarily of the subgenus Lactobacillus and Bifidobacterium, that could infect the intestinal tract even thus interact with infections bacteria (Gillings, 2013). Probiotics were used for the prevention of infectious diarrhea. This has had positive benefits in reducing amounts of diarrhea (Preidis et al., 2011). According to the report, the usage of fermented milk and yogurt with the microorganism Lactobacillus in stable humans as part of the microbial environment decrease the probability of UTI and vaginal infections (Amdekar et al., 2011).

2.6. Resistance

We as human beings are becoming immune to the medicinal properties of antimicrobial agents. Antibiotic resistance has been a significant concern. Many bacteria had responded to the usage of antibiotics before the use of penicillin was introduced and their ability to evolve and expand antimicrobial susceptibility to other species. Many antibiotics are being obtained at an increased rate and are being used improperly, which led to these species being more resistant to these medications. Going along with disease, the social (travel) activities of moving between countries as well as travel by international tourists and businessmen has an effect on the likelihood of the spread of multidrug-resistant strains of disease (van der Bij & Pitout, 2012).

Antimicrobial tolerance was also observed in animals, and is when antimicrobials are sprayed, ointments are added, or administered to cure infections and other medical issues (Szmolka & Nagy, 2013). While in humans, the development of antimicrobial agents in pathogenic bacteria and endogenous bacteria was related to the increased frequency of drug resistance. The bacteria from animals could specifically reach through the human skin and food items, as well as indirectly through animal products. Resistance to multiple antifungals is characterized as resistance over than three antibiotic classes that may not have the intrinsic potential of the bacterium or are immune to it (Magiorakos et al., 2012). It is alarming that multi-resistant strains of bacteria are on the rapid increase across the Earth. The reason for this is that these strains are acquired for many pathogenic bacteria,

as well as multiple other microorganisms, by the multiple methods of zoonosis, such as infection via contaminated water or food. Therefore, it is imperative that the global community work to prevent the propagation of these bacterial strains. There are carriers of these genes which have the ability resist the all-common classes of antibiotics. In addition, this combination often leads to species of bacteria that are resistant to all major classes of antibiotics (Johnson & Nolan, 2009; Kaper et al., 2004a).

E. coli was naturally resistant to the therapeutic doses of penicillin G, the first β-lactam antibiotic put through clinical practice, referring to the outer membrane barrier. E-coli is also able to resist a lot types of antibiotics and has a unique sort of resistance mechanism (Erb et al., 2007; Johnson et al., 2012). The creation of the β-lactamase for the narrow-spectrum of the β-lactams is the most important roll initiate the resistance of the broad-spectrum of the β-lactams. It is proven that bacterial β-lactamases is found in pathogenic and commensal bacteria. Investigators have found at least 30 distinct phenotypic and genetic patterns among Escherichia coli. However, the types and degrees of plasmid-encoded β-lactamase genes in *E. coli* are unknown. Samples of pathogenic and commensal E. coli from 11 different clinical sources were characterized.

DNA from clinical isolates was extracted and amplified using nested forward and reverse primers specific for each β-lactamase. Xenografts from a chronic pancreatitis survivor were examined in a clinical laboratory setting to establish colony types that harbor identified β-lactamase genes and to screen clinical isolates for additional plasmid-encoded β-lactamase genes following a 2-month exposure. Thermostability criteria were utilized to identified enzymes in a recombinant, plasmid-encoded β-lactamase system (Poirel et al., 2012). β-lactamases confer penicillin and cephalosporin resistance and are an emerging cause of multidrug resistance in Gram-negative bacteria (Poirel et al., 2012). It alludes to a systemic that looks to decode conditions of β-lactamases, ESBLs and their species that likely enhance conformity to cephalosporins and monobactams of the third and fourth centuries (Cantón & Coque, 2006). The most prevalent type of cluster variant is the CTX-M-1, though the change of the CTX-M-15 is one that is rapidly increasing in the world.

There are several types of CTX-M-14 even for CTX-M-15 which mostly found on humans (Ewers et al., 2012).

2.6.1. Beta-Lactam Antibiotics

β-lactam antibiotics are one of modern science and technology's most strong and successful achievements. Among the antibiotics medicines listed, penicillins and cephalosporins are the world's largest biotechnology products with worldwide revenues of $15 billion USD or 65% of the global antibiotics industry (Elander, 2003). Since their discovery by Fleming in 1928 and their introduction as antibacterial agents in the early 1950s, β-lactam antibiotics have been the most effective medication for treating bacterial infections. Today's most used β-lactam medicines stem from the initial discovery and production of natural products from microorganisms such as penicillin, cephalosporin, and other β-lactam dependent antibiotics.

These are a class of antibiotics that includes all those antibiotics agents whose molecular structure carries beta-lactam, and this is necessary for all the antibiotics in this group to function. This antibiotic inhibits bacterial cell wall formation by interfering with the protein necessary for cell wall formation where later bacteria are either killed or inhibited from growth, some bacterial enzymes called penicillin-binding protein (PBP) considered as a specific role in the synthesis of peptidoglycan, and these antibiotics bind to these PBP which later leads to lysis and death of cells. The antibiotics of B-lactam are primarily divided into four groups: Penicillins, Cephalosporins, Monobactams, Beta-lactamase inhibitors and Carbapenems (Etebu & Arikekpar, 2016).

2.6.2. Chromosomal- Beta Lactam Antibiotics

Perhaps a main reason for the inhibitory effect of β-lactams is that they bind to PBPs, which are important proteins involved in cross-linking of peptidoglycan. Peptidoglycan is part of the prokaryote cell wall, and inhibition of peptidoglycan cross-linking can weaken cell integrity as the cell grows and eventually lyse the cell. For example, cephalosporins in the cell envelope might trigger autolytic enzymes, causing lysis of the cells. In addition, β

lactams have a different activity range, as some are broad-spectrum antibiotics with specific ability against both Gram-positive and negative bacteria, while others have a narrow activity spectrum, such as monobactams (which only affects aerobic Gram-negative). Third and later generation cephalosporins have an extended activity continuum, indicating that they have an even larger variety (Kaspersen, 2015).

Resistance to beta-lactams is usually assumed to be induced by a structural alteration in the penicillin-binding proteins (leading to lower drug affinity) or bacterial development of enzymes that forms the beta-lactam ring (which may make the beta-lactam resistant). Some other way to reduce permeability is by shutting off the active transference pumps (Mandell, 2005).

2.6.2.1. Penicillins

In 1928 a doctor in London happened to spot the spores of a genus of fungus called Penicillium on an injury, and he removed the final active component from it and named it as penicillin (Fleming, 1929). Until 1940, when Howard Florey and Ernst Chain carried out extensive penicillin experiments in Oxford, no more breakthroughs were made (Florey et al., 1949). They showed their chemotherapeutic action in mice and reported their lack of toxicity, the manufacturing of penicillin by the strain of Penicillium notatum was quite sluggish and it took a long time until the first effective clinical human experiments could be conducted, the need for large-scale production was inevitable, but due to World War II this could not be achieved in England. Penicillin G (benzylpenicillin) was first used to treat combat casualties effectively in 1943. Since then, several various penicillins have been identified and used in the pharmacy. In 1945, Fleming was given the Nobel Prize for the discovery and development of penicillin by Florey and Chain (Mandell, 2005).

Based on their antimicrobial spectrum and resistance against penicillinases, penicillins are classified into subgroups and can be administered orally or parenterally (Etebu & Arikekpar, 2016). The most excreted penicillins are not metabolized by the kidneys, so urine concentration can reach high levels. This class includes beta-lactam compounds with a centering of 6 amino-penipenicillanic acid rings and other rings inside chains. The

penicillin gets its potency from the nucleus of 6-aminopenipenicillanic acid, which acts primarily against gram-positive bacteria (Berendsen et al., 2013).

2.6.2.2. Cephalosporins

Cephalosporins are also bicyclic, or cephemes, they did not become commercially available until the 1960s although discovered earlier. Both in structure and function, this class is like penicillin. It contains in the nucleus 7- aminocephalosporanic acid in the nucleus and 3,6 dihydro-2 H-1,3 thiazane rings (Mandell, 2005). Noteworthy is the ability of resistance to emerge when treating Enterobacter spp., Citrobacter spp., Serratia spp., Morganella spp., and Providencia spp. This is because the production of naturally occurring beta-lactamases is either naturally mutated or up-regulated (Jacoby, 2009; Jones et al., 1997).

Patients who are taking cephalosporins might become exposed to it through oral, intramuscular, or intravenous administration. They are sometimes prescribed empirically in accordance with an aminoglycoside or metronidazole (depending on presumed etiology) to treat severe infections such as acute pneumonia, intraabdominal infections, septicemia, or in patients with febrile neutropenia, but whether combination therapy is successful or not, it has been widely debated (Micek et al., 2010; Safdar et al., 2004). The cephalosporins are called the first generation of drugs. The newest generations of antibiotics are more active against gram-negative bacteria (Pegler & Healy, 2007).

2.6.2.3. Monobactams

These medications are monocyclic chemicals, and aztreonam is the only commercially usable one. It is also used against one specific spectrum of antibiotics (Chambers & Neu, 2005). These antibiotics are part of the beta-lactam compounds but are different from other beta-lactams, whereas the beta-lactam ring in Monobactams stays on one side and is not attached to any ring (Etebu & Arikekpar, 2016). The treatment was for a Gram-negative bacterial infection and was injected. Symptoms of regulation 141/2000/EC includes

gonorrhea and some severe urinary/respiratory tract infections. While quite unlikely, certain harmful consequences that may arise.

2.6.2.4. Beta-lactamase inhibitors

Beta-lactamase inhibitors demonstrate a poor antibacterial activity, but it works as a suicide substrate (both in conjunction with penicillins) to treat MRSA. They are capable of forming stable intermediates and thus can collect and deactivate beta-lactamases. These drug-producing organisms, penicillinases, chromosomal cephalosporins, and ESBLs are resistant to this medication (Chambers & Neu, 2005).

Amoxicillin-clavulanic acid seems to as a combined of antimicrobial agent whose antibacterial abilities include gram-positive and gram-negative bacteria, aerobic and anaerobic bacteria, and contained in numerous locations, from the skin, to the respiratory tract, to the saliva. It even works on parts of the Enterobacteriaceae bacterium. This is a medication that is formulated to cure unthreatening pneumonia and upper respiratory tract diseases, UTIs and bite wounds

Piperacillin-tazobactam seems to be a broad-spectrum class of antibiotic which is used in the treatment of various infections like nosocomial pneumonia, intra-abdominal infections, or febrile neutropenia. The action of this drug works against germs and respiratory system-causing bacteria like Pseudomonas. and many anaerobic bacteria.

2.6.2.5. Carbapenems

A group of compounds with high molecular weight that came out in the 80's. Carbapenems have the property of a wide spectrum of antibiotics that gives this antibiotic strength to fight ESBL and Metallo beta lactamase (Francis & Eric, 2017). Therefore, this antibiotic is considered to be trustworthy and with an increase in resistance to this antimicrobial agent is considered to be the main health-related problems for people. Due to its anti-bacterial influence, induces adverse effects to both Gram-positive bacteria and gram-negative bacteria as well as anaerobic bacteria due to the large range care (Chambers & Neu, 2005).

2.6.3. Beta-lactam Enzymes

The ß-lactamases are the collective names for enzymes that open the carbapenem ring by introducing a water molecule to the carbapenem ring, which stops the carbapenem from working, for example, they convert the carbapenem to penicillin. For the first time in 1940, this hydrolysis was detected by Chain and Abraham, a lethal bacterial strain of *E. coli* (penicillinase) (Abraham & Chain, 1940). However, as of the 1950s, when the first ß-lactam-resistant Staphylococcus aureus was discovered. There emerged isobutyric acid-isolated isolates in hospitals; the clinical effect of such hydrolyzing formations undetected (Jacoby, 2017; Kirby, 1944).

Since that, the number of β-lactam antibiotics has also increased the selection pressures on bacteria, promoting the survival of various β-lactamases in organisms. (Bush & Jacoby, 2010; Massova & Mobashery, 1998). More than 850 β-lactamases have been identified and it is speculated that high frequency of mutation, rapid recombination, and replication rates are responsible for the adaptation of bacteria to novel β-lactams by the evolution of these β-lactams (Perez, 2007). Early βlactamases were referred to as penicillinases or cephalosporinases due to their ability to hydrolyze penicillins or cephalosporins. They were lauded for their efficacy against highly resistant pathogens such as *Pseudomonas spp* when semisynthetic penicillins were introduced in the 1970s as less β-lactamase susceptible alternatives to natural product penicillins (Bodey et al., 1971; Paterson & Bonomo, 2005)

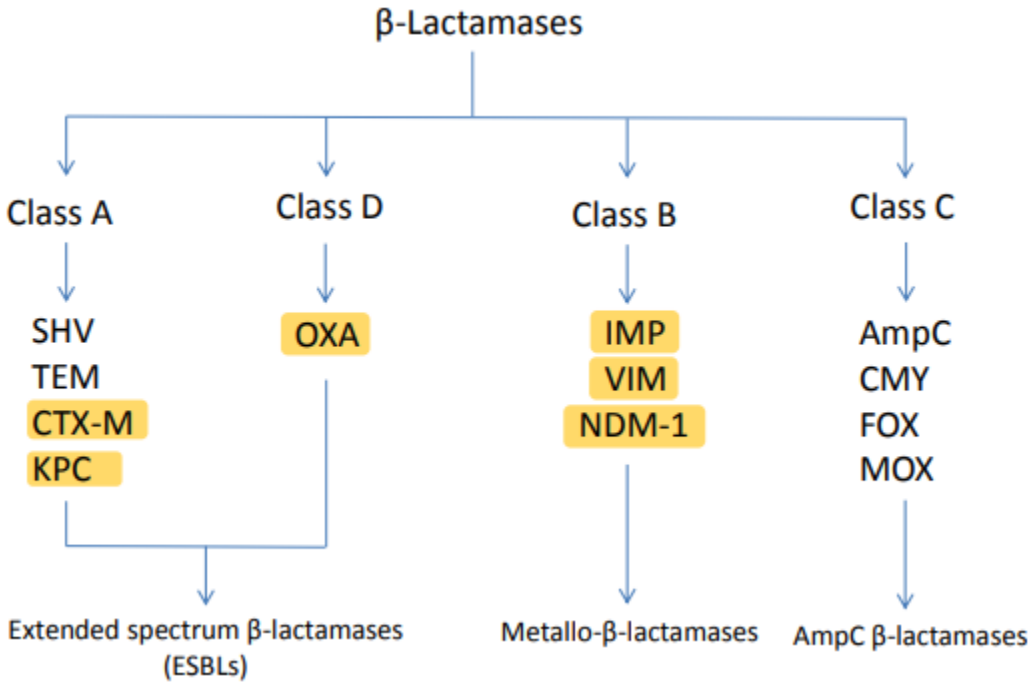
The ß-lactamases in S. aureus are located in chromosomes and are mostly inducible, whereas the first plasmid-mediated ß-lactamases were discovered in gram-negative bacteria were found in Greece in the 1960s, and TEM was named for the patient (Temoneira) who bore the pathogen (N. Datta & Kontomichalou, 1965). TEM-1 is the most abundant β-lactamase in Gram-negative bacteria and can hydrolyze penicillin (ampicillin). ß-lactamases spread rapidly to other bacteria, and shortly after shifts in either one or a few amino acids, these enzymes were able to hydrolyze narrow-spectrum cephalosporins and were found in Enterobacteriaceae, Neisseria gonorrhoeae and Hemophilus influenza. In the biochemical

system, sulfhydryl ß-lactamases (SHV) are similar relative to TEMs, but are more common in Klebsiella spp. Cephalosporins of the third century were secured from hydrolysis by the initial TEMs and SHVs (Brunton et al., 1986).

There are four ways how bacteria can become resistant to β lactam antibiotics: by producing βlactamase, by producing altered PBPs, by reducing external membrane protein (OMP) expression, and by producing efflux pumps. The most popular and efficient resistance method of bacteria to β-lactam antibiotics is the production of β-lactamases. These enzymes that certain bacteria generate have tolerance to β-lactam antibiotics (Pfaendler & Golz, 2016).

However, Ambler's molecular classification tends to be generally recognized instead of Bush's phenotype classification owing to its simplicity and phylogenetic association between the enzymes. Classic and extended-spectrum β-lactamases (ESBLs) are present in Class A and Class D, consisting primarily of TEM, SHV, CTX-M, and OXA enzymes, Class B Metallo-β-lactamases, and finally Class C AmpC β-lactamases. With the exception of Class B Metallo enzymes, β-lactamases belong to the serine reactive hydrolase family. β-lactamases have been subdivided into β-lactamases of the AmpC type, Metallo-β-lactamases, classic β-lactamases, and β-lactamases of the expanded spectrum (Figure 1) (Ambler, 1980; Rawlings et al., 2016).

Figure 1. The classification scheme of a part of the β-lactamases.



2.7. Extended -Spectrum Beta Lactamases

Beta-lactamases are enzymes that hydrolyze beta-lactam antibiotics that break down the nitrogen-carbonyl bond in the beta-lactam ring and are the most prevalent mode of action in Gram-negative bacteria for beta-lactam resistance (Walsh, 2003). The incidence of β-lactamases has grown steadily in the treatment of infectious diseases in recent years and has become an increasing concern. Excessive use of extended-spectrum β-lactam antibiotics, primarily 3rd-generation cephalosporins, results in the development of ESBLs by Gram-negative rods of the Enterobacteriaceae family, such as *E. Coli* and *Pneumoniae Klebsiella* (Jacoby & Munoz-Price, 2005).

Carboxypenicillins, amino-ureido and penicillin, 1st, 2nd, and 3rd generation cephalosporins and aztreonam are broken down by ESBL enzymes. The term ESBL refers to the mostly plasmid-encoded enzymes of molecular class A that can be inhibited by clavulanic acid and tazobactam. Carbapenems are the only β-lactam antibiotics that work effectively on ESBL-producing bacteria (Giske et al., 2009). The genes that encode ESBLs

are usually found on plasmids and are transmitted by bacteria, plasmids or other transferable DNA between individuals, institutions, and countries. The risks associated with antibiotic resistance caused by ESBL-production have been identified in previous studies. A comparison of the cases of sepsis caused by ESBL-producing and non-ESBL-producing *E. coli* and *Klebsiella* has shown that the delay incorrect antibiotic treatment is five times more frequent (Schwaber and Carmeli, 2007). Death is almost three times more common, and the duration of stay is significantly longer when bacteria producing ESBL are involved (Melzer and Petersen, 2007; Schwaber et al., 2006).

In the 1980s, the first ESBLs were discovered and were derivatives of SHV and TEM β-lactamases that break down older β-lactam antibiotics with more narrow activity than cephalosporins of the third generation. Since then, over 200 β-lactamases of the TEM type and nearly 200 enzymes of the SHV type have been described; almost half of the former and one fourth of the latter have ESBL activity.

2.8. Spread of ESBL

ESBLs were identified in 1983 and were described in Enterobacteriaceae and Pseudomonadaceae, mainly in *Klebsiella pneumonia* and *Escherichia coli*, in different regions of the world. The plurality of ESBLs contained in the clinical sample such as TEM and SHV forms that originated from narrow-spectrum beta-lactamases such as TEM-1, TEM-2, and SHV-1. The CTX-M enzyme, reported in Enterobacteriaceae and reported from Asia, Africa, Europe, South America, and North America, originated from *Kluyvera spp* (Pitout et al., 2005).

The ESBL producers were mainly found in the hospital environment and mainly in the ICU (Intensive Care Unit) during the 80s and 90s, and the responsible hosts for the development of this enzyme were *Klebsiella* and *Enterobacter spp*. One of the surveys found that the percentage of *E. coli* associated with ESBL production is increasing, rising to 3.6 percent in 2005 and 4.8 percent in 2008. This number is mostly found in the USA and Canada, but in Europe, it is less (Schoevaerdts et al., 2011). With the passing of time, hospital-acquired infections are growing and becoming the world's top-ranked problem,

and *Klebsiella spp*., which produces ESBLs, is the responsible pathogen. This is the main concern because there are few antibiotic numbers for its treatment and its rate of transmission to other Gram-negative bacilli or Enterobacter is also high (Bellíssimo-Rodrigues et al., 2006).

Until the 1990s, two forms of ESBL were globally active, namely, Temoniera (TEM) and Sulfhydrylvariable (SHV), mainly associated with hospital outbreaks, and *Klebsiella pneumoniae* was predominantly the bacteria that produced this enzyme. But another form of ESBL enzyme named Cefotaxime Munich (CTX-M) erupted after 2000, and the bacteria responsible for this enzyme was mainly Escherichia coli. Some of the studies were also done in the hospital's clinical samples and found that the ability to produce CTX M enzymes other than *E. coli, K. oxytoca,* and *K. pneumoniae* has also been found and resistance has occurred mainly due to gram-negative bacteria. Therefore, one of the ways in which ESBL is transmitted to humans is either by physical contact or by taking contaminated food infected with strains producing ESBL. Food of animal origin have higher chances of accumulating ESBL, and the wide spectrum antibiotic Cephalosporin inactivated because of enzymes produced by these bacteria (Vásquez-Jaramillo et al., 2017).

The WHO (World Health Organization) has a statement in 2011 mentioned that healthcare-related infections are more common in low and middle-income countries and it is 10.1 % whereas its number is less in high-income countries and is 7.6%. When the number of staying days in the hospital is more, it is directly linked to more antibiotic-resistant, more chance of increased Health-Related Infections (HRI) (Hendrik et al., 2015). E. coli and CTX-M enzymes are not uncommon in patients today. Furthermore, with the introduction of carbapenemases such as OXA-48, first found in Turkey, the tolerance demonstrated by K.pneumoniae has achieved a higher degree of resistance (Aktaş et al., 2008).

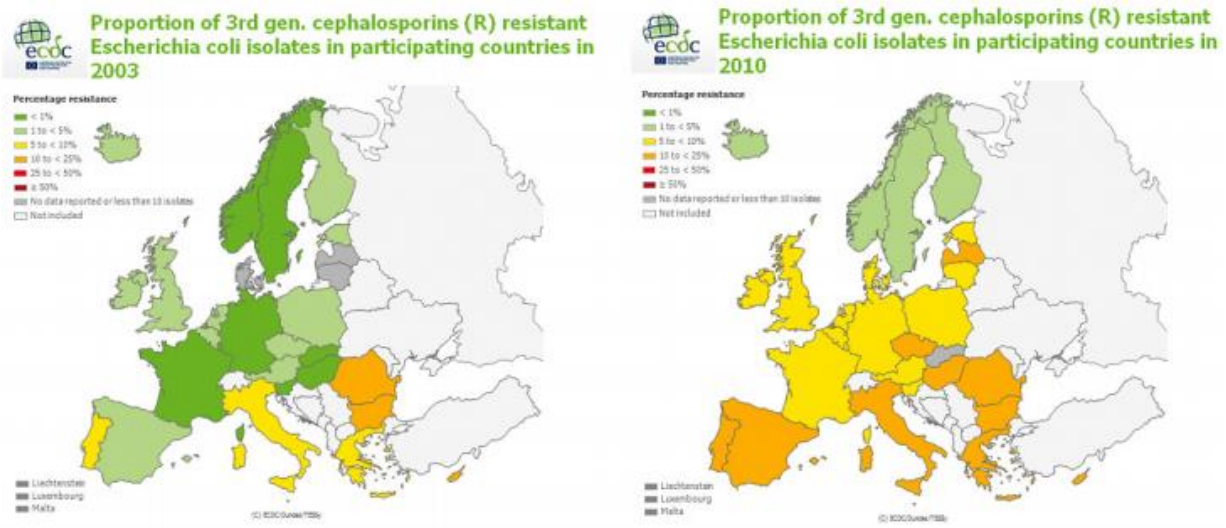


Figure 2. *E. coli* resistance to third generation cephalosporins from 2003 and 2010 (ECDC)

2.9. Laboratory Methods of Extended -Spectrum Beta Lactamases

Phenotypic tests based on sensitivity to β-lactam agents and synergy between them and clavulanic acid detect the ESBL activity of bacteria. These tests are generally used to check and confirm the presence of ESBLs in *K. pneumoniae* and *E. coli*, and the results assist doctors in selecting the best treatment. These tests are standardized, and the results can also be compared with other countries' test results. The major guidelines used in North America and Europe are the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Resistance Research (EUCAST) (Clinical and Institute, 2017; EUCAST, 2015).

Various methods to the characterization of ESBLs have been implemented due to the rise in antimicrobial tolerance in enterobacteriaceae worldwide (Taneja *and* Sharma, 2008). Techniques that include any form of phenotype (phenotype) (such as visual analysis, disk diffusion, double disk synergy test, E-test strips, Vitek 2 and Micro-Scan Walk Away 96) and/or genotype (such as DNA, PCR, and sequencing) may all be categorized under the proper word (Goyal et al., 2008; Teresa Spanu et al., 2006; Taneja *and* Sharma, 2008).

Studies on various methods have produced contradictory findings in terms of their sensitivity and specificity. A short description of the methods used to classify blood containing ESBL-producing bacteria is given below (Anderson et al., 2007; Goyal et al., 2008).

2.9.1. Screening Tests

According to EUCAST, the recommended strategy for detecting ESBLs in Enterobacteriaceae is based on non-susceptibility to the indicator of oxyimino-cephalosporin followed by phenotypic and in some cases genotypic confirmation tests. Broth dilution, agar dilution, or disk diffusion are the recommended methods for ESBL screening in group 1 Enterobacteriaceae. Both cefotaxime (or ceftriaxone) and ceftazidime are used as indicators of cephalosporins, as there may be significant differences between the minimum inhibitory concentrations (MICs) of cefotaxime (or ceftriaxone) and ceftazidime for the different isolates producing ESBL. ESBL confirmation is done through various phenotypic tests after screening (Gekenidis et al., 2018).

The double-disk synergy test is a simple way used to detect presence ESBL bacteria (T. Spanu et al., 2002). In this procedure, 30µg of cefotaxime, 30µg of aztreonam, 30µg of ceftriaxone, 30µg of ceftazidime, and 30µg of cefepime comprising amoxicillin (20µg) plus clavulanic acid (10µg) are put around the disk (Drieux et al., 2008; T. Spanu et al., 2002). Demonstrating synergy or a term called "keyhole" between 3rd or 4th generation cephalosporin disks and amoxicillin-clavulanic acid disks is considered to be positive among isolates (Hsueh et al., 2010).

It is possible to distinguish ESBL phenotypes using E-test ESBL strips which generate a cefepime concentration gradient on one end and at the other end produces a cefepime concentration gradient plus clavulanic acid (Lee et al., 2010; Stürenburg et al., 2004). The ESBL-production shows fewer than three two-fold dilutions of clavulanic acid or the existence of a phantom inhibition region. Antibiotic susceptibility testing and identification using the Vitek method system is a quick and semi-automatic method that can be used to

detect ESBL in enterobacteriaceae (David L. Paterson *and* Bonomo, 2005; Sanders et al., 1996; Taneja *and* Sharma, 2008).

ESBL-Production in *Escherichia coli* and *Klebsiella pneumoniae* detected by the Vitek method displayed 99.5 per cent sensitivity and 100 per cent accuracy, according to Sanders et al. (Sanders et al., 1996). The sensitivity and specificity of the E-test system ranged between 87-100 percent and 95-100 percent respectively, with the double-disk test providing a sensitivity of 79-97 percent and a specificity of 94-100 percent, respectively (Rawat & Nair, 2010).

2.9.2. Verification Tests

While the Vitek 2 technique was effective, real-time PCR is still the favored approach for E. coli screening. Combining the standard disk diffusion approach with one of the other methods listed above, such as E-test ESBL, is the best method of confirmation. This is because different test's sensitivity and specificity does not meet 100 percent. Which testing method is used ultimately depends on the tester's requirements and the availability of chemicals for testing (Drieux et al., 2008).

2.9.3. Biochemical and Molecular Tests

For the detection of clinical isolates, the API20E method (BioMerieuxTM, USA) is used and is a consumer package developed for the identification of Enterobacteriaceae and other non-fastidious Gram-negative bacteria species. The test strips consist of twenty mini-test tubes from which the variations between the different species can be seen, based on the ability of the test organism. O-nitrophenyl-β-D-galactosidase (ONPG), arginine dihydrolase (ADH), lysine and ornithine decarboxylase (LOD), citrate utilization, hydrogen sulfide (H2S), urease (Ure), tryptophan deaminase (TPD), indole, and Voges-Proskauer (acetoin) were applied to the wells checked. The suspension was prepared from the measurement of the colony count of 2-3 bacteria. Viral suspension was applied to the

wells in the slides, and each slide was then incubated aerobically at 37 °C for 24 hours. After 24 hours, the color reactions were read and the species of the research isolate was calculated.

A technique used to amplify certain genes that can identify the presence or absence of individual genes (PCR). It utilizes multiple gene-specific DNA sequences to produce a large number of copies of the target sequence so that gel electrophoresis can be easily detected (Garibyan & Avashia, 2013). PCR provides a positive and negative result and is the most used approach for identifying a wide range of ESBL genes (Naas et al., 2010). The benefit of quantifying the synthesized substance is quantitative real-time PCR (qRT-PCR), which provides greater throughput and a quicker process for evaluating gene quality. However, PCR techniques do not determine whether or not a gene is functioning, and assistance from prior sequence data is needed to design primers (Garibyan & Avashia, 2013). Any of the best approaches for the detection of different classes of identified β-lactamases in extended-spectrum β-lactamase-producing isolates have been evaluated and have shown 100% precision (Pitout et al., 2004).

Finally, the molecular typing of bacterial organisms is achieved by utilizing a technology like gel electrophoresis (PFGE) (Durmaz et al., 2009). Different size fragments are removed using an electrical field that easily changes path when a gel matrix is formed during the DNA digestion with a restriction enzyme (Durmaz et al., 2009; Schwartz & Cantor, 1984). When this method is used, 98% sensitivity is achieved and 89% specificity is achieved when it is applied to provide rapid identification of multidrug-resistant Salmonella spp. Isolates (Fontana et al., 2003).

The PCR technique was used to identify the types of *E. coli* the bacteria carried. To resuspend bacterial cells in 200 µl of distilled water and raise the temperature to 95˚C for 10 minutes, DNA was prepared. The efficacy of the primer sets to detect the amount of blaCTX-M, blaSHV, blaTEM, and blaOXA genes has been checked. Per reaction tube produced ten milliliters of master mixture, four milliliters of primers, and one milliliter of DNA. A total of 20 milliliters of distilled water was used. The PCR response parameters

were a denaturation time of 10 minutes at 95-C accompanied by 12 cycles at 94-C, 90s at 62-C and 60s at 72-C, with a final extension step of 30 minutes at 72-C (Fang et al., 2008).

MALDI-TOF: The technique of molecular detection like Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) detects the genes responsible for the activity of ESBL, and its limiting factor is that all ESBL encoding genes cannot be detected and are expensive. A kit named Rapid ESBL Screen Kit 98022 is available commercially and gives the result within 2 hours, but also has some limitations (Poirel et al., 2016).

3. MATERIAL AND METHODS

3.1 Design of Study

The research was performed in the MICROBIOLOGY LABORATORY of the NEAR EAST UNIVERSITY HOSPITAL in the Turkish Republic of Northern Cyprus (TRNC). A total of 93 samples for the study was executed from different clinical specimens of hospitalized patients from various hospital departments. The study protocol was accepted by the Near East University Research Assessment Committee. *E. coli* organisms obtained from separate clinical specimens were used for testing and repeated isolates were removed from the same clinical specimen of the same patient.

3.2. Specimens Collection

In the microbiology laboratory, 93 clinical specimens of *E. coli* were collected between July 2012 and October 2020. The isolated strains were kept in storage tubes for bacteria (OR-BAK, Ankara, Turkey) until they were used at -80°C.

3.3. Culture

The stored samples of *E. coli* isolates were inoculated on Blood Agar (Merck KGaA, Darmstadt, Germany) to get pure colonies. Blood Agar was prepared as per the manufacturer's directions as follows:

i. First, I weighed 40.0 grams in 1000 ml of purified water from the base of agar blood.

ii. Using fire in order to heat up the medium until it can dissolve, and then blend it entirely.

iii. It must be sterilized by autoclaving at 15 lbs. for (121°Celsius) for 15 minutes.

iv. When the agar had cooled to 45-50 ° C, I added 5% of the sterile fiber debris that I put heating it to room temperature and mixing it gently.

v. Mix together well and pour into clean petri plates

After a 24-48-hour incubation period at 37 ° C, *E. coli* colonies were produced. Colonies include Circular, Convex, Smooth (fresh isolation); Rough (repeated subculture); Mucoid and Greyish white (capsulated strains). After that, on EMB agar, we do subculture (Becton Dickinson, Sparks, MD 211 52 USA). EMB Agar was prepared as per the manufacturer's directions as follows:

i. First, I weighed 73.4 grams in 1000 ml of purified water from the base of EMB agar.

ii. Using fire in order to heat up the medium until it can dissolve, and then blend it entirely.

iii. It must be sterilized by autoclaving at 15 lbs. for (121°Celsius) for 15 minutes.

iv. Mix together well and pour into clean petri plates

After an incubation time of 24-48 hours at 37°C, colonies were produced. Rough (repeated subculture); mucoid (capsulated strains) and Greyish white are circular, convex, smooth (fresh isolation) colonies. As in Figure 3, as shown. For different detection methods of *E. coli*, these colonies were used.

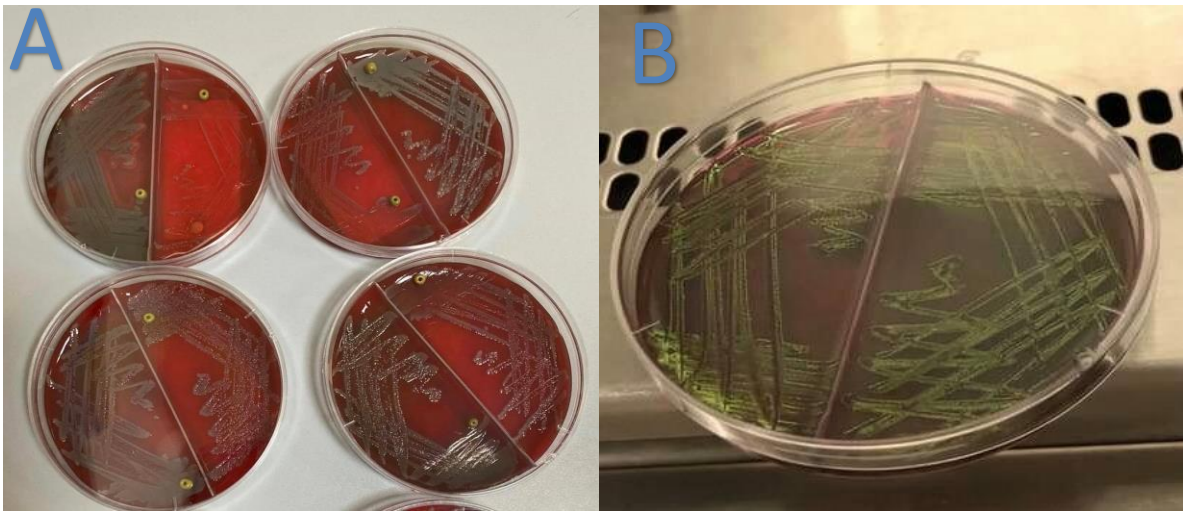


Figure 3. (a) *E. coli* Colonies on Blood Agar. (b) *E. coli* Colonies on EMB Agar.

3.4 Identification and Antibiotic Susceptibility Test (AST)

Bacterial identification and ASTs were performed by full automated system Phoenix 100 (Becton Dickinson, Sparks, MD, USA) in line with the manufacturer's recommendations (0.5 McFarland) and results were evaluated according to the European Committee on Antimicrobial Susceptibility Testing criteria (EUCAST).

3.5 Double-Disc Synergy Test (DDST)

The Double-Disk Synergy Test (DDST) is a test for the detection of ESBL production in bacteria. It has been checked in compliance with the EUCAST guidelines. The bacterial suspension was prepared in accordance with the manufacturer's normal density (0.5 McFarland) and then applied to the Mueller-Hinton Agar plate (MHA) (Merck, KgaA, Germany).

Mueller-Hinton Agar was prepared as per the manufacturer's directions as follows:

i. First, I weighed 38.0 grams in 1000 ml of purified water from the Mueller-Hinton Agar.

ii. Using fire in order to heat up the medium until it can dissolve, and then blend it entirely.

iii. It must be sterilized by autoclaving at 15 lbs. for (121°Celsius) for 15 minutes.

iv. Mix together well and pour into clean petri plates.

The Antibiotics Discs that were used in Double-Disc Synergy Test (Bioanalyse, Ankara, Turkey)

Antibiotics Discs	Catalog No.	Symbol	µg
Amoxicillin/Clavulanic Acid	ASD00700 ASD00420	AMC	20/10
Ceftazidime	ASD02101	CAZ	30
Aztreonam	ASD00700	ATM	30
Ceftriaxone	ASD02300	CRO	30
Cefotaxime	ASD01810	CTX	30

The Disks containing cephalosporin Cefotaxime (CTX 30µg), Ceftriaxone (CRO 30µg), Ceftazidime (CAZ 30µg), and Monocyclic Beta-Lactam Aztreonam (ATM 30µg) are placed in the middle of the plates containing Clavulanic Acid (Amoxicillin-Clavulanic Acid (AMC 20/10 µg) at a distance of 30 mm (center to center).

Preparation of Inoculum

i. The bacterial cells are collected and suspended in 3-5 ml (0,85% NaCl) of sterile saline solution from the primary isolation medium by five colonies showing identical morphology either by direct colony suspension method and suspended using a flamed loop or by using a sterile cotton swab. For 15 seconds, mix the cell suspension using a vortex mixer.
ii. Once the turbidity is visible, the suspension density is adjusted by the use of a spectrophotometer. The turbidity needs to be balanced to 0,08 to 0,10 at an absorbance of 625 nm for the 0,5 McFarland standard. It needs to be used about an hour after the standard suspension has been prepared.

Inoculation into Agar Plates

i. Mix well the prepared bacterial suspension with a sterile cotton swab and the excess fluid of the swab is removed by gently pushing and spinning the swab within the tube above the fluid level.

ii. To achieve even inoculation, streak the whole agar surface of a Muller Hinton plate three times, turning the plate 60 ° between streaks.
iii. For preventing the excessive moisture of the medium, allow Petri dishes to dry for 3 to 5 minutes, maximum of 15 minutes, at room temperature.

Application of Antibiotics discs to Inoculated Agar Plates and Incubation

i. The cartridge is opened under the flame and then discs are discharged from the cartridge onto a clean Petri dish with the help of a flamed and cooled forceps.
ii. The discs must be distributed into the agar plates no closer than 24 mm from the center to the center and 12 mm away from the edge of the agar plate within 15 minutes.

iii. Once in touch with the surface of the agar, do not move the disk.
iv. Incubate all plates for 20 to 24 hours.

Reading Inhibition Zones and Interpretation of Results

The expansion of the inhibition region around the cephalosporin or ATM to the AMC disc after incubation at 35 °C overnight or the presence of a synergy area in which bacteria reproduce indicates the presence of ESBL.

3.6. Combined Disc Test (CDT)

The Combined Disc Test (CDT) is a test for the identification of the production of ESBL in bacteria. It has been tested according to the EUCAST guidelines. And the bacterial suspension was prepared in accordance to the manufacturer's standard density (0.5 McFarland) then spread on Mueller-Hinton Agar (MHA) (Merck, KgaA, Germany) plate.

The Antibiotics Discs that were used in Combined Disc Test (CDT). Antibiotics Discs (Bioanalyse, Ankara, Turkey)

Antibiotics Discs	Catalog No.	Symbol	µg
Cefotaxime	ASD01810	CTX	30
Cefotaxime/Clavulanic Acid	ASD01820	CTC	30/10

In the combined disc method, Cefotaxime (CTX 30µg) discs with Cefotaxime/Clavulanic Acid (CTC 40µg) were placed on Mueller-Hinton Agar (MHA) plates. It was left to incubate at 35 ° C overnight. Inhibition zones around discs with and without Cefotaxime/Clavulanic Acid were measured and compared. Inhibition zone around the combination discs. Inhibition zones were measured and compared. If the difference between discs containing and without Cefotaxime/Clavulanic Acid was greater than or equal to 5 mm, ESBL was considered positive.

3.7 Three-dimensional Test (TDT)

The Three-dimensional Test (TDT) is a test for the identification of the production of ESBL in bacteria. It has been tested according to the EUCAST guidelines. And the bacterial suspension was prepared in accordance to the manufacturer's standard density (0.5 McFarland) then spread on Mueller-Hinton Agar (MHA) (Merck, KgaA, Germany) plate.

The Antibiotics Discs that were used in Three-dimensional Test (TDT).

Antibiotics Discs	Catalog No.	Symbol	µg
Cefotaxime	ASD01810	CTX	30
Aztreonam	ASD00700	ATM	30
Ceftazidime	ASD02101	CAZ	30
Ceftriaxone	ASD02300	CRO	30

On the surface of the Mueller-Hinton Agar (MHA) the prepared bacterial suspension with a density of 0.5 McFarland was smeared. In the middle of the petri dish, and 3 mm away from the antibiotic discs used, the medium was cut into circles. The medium line formed was filled with a liquid medium with which it produced the microorganism to be evaluated. Ceftazidime (CAZ 30µg), Ceftriaxone (CRO 30µg), Cefotaxime (CTX 30µg) and Aztreonam (ATM 30µg) disks were applied after inoculation. At 37°C for 16-18 hours, the plate was then incubated.

When the inhibition areas around each of the cephalosporin disks are raised in the direction of the clavulanic acid disk. ESBL was evaluated as positive.

3.8 Statistical Analysis

Qualitative and quantitative data values along with the percentage and mean ±standard deviation (SD) is represented as frequency. The Chi-square test is tested as appropriate on the association between two or more variables. Pictorial explanations of the major results of the study were rendered using an appropriate statistical graph. All statistical analysis was conducted using SPSS version 25,00 statistical packages (SPSS Inc. Chicago, IL, USA). Significance level was accepted to be 0.05.

4. RESULTS

4.1. Antimicrobial Susceptibility Testing Results

Antibiotic susceptibility tests were conducted in this research using the completely automated Phoenix 100 method, the results as shown in (Table 3) were described the sensitivity and the resistance of several important antibiotics as follow: Amikacin the sensitive were n=93 (100%), Amoxicillin Clavulanic Acid the sensitive were n=34 (36.6%) and the resistant were n=59 (63.4%), Ampicillin the sensitive were n=8 (8.6%) and the resistant were n=85 (91.4%), Cefixime the sensitive were n=21 (22.6%) and the resistant were n=72 (77.4%), Ceftazidime the sensitive were n=16 (17.2%) and the resistant were n=77 (82.8%), Ceftriaxone the sensitive were n=17 (18.3%) and the resistant were n=76 (81.7%),Cefuroxime the sensitive were n=34 (36.6%) and the resistant were n=59 (63.4%), Ciprofloxacin the sensitive were n=32 (34.4%) and the resistant were n=61 (65.6%), Ertapenem the sensitive were n=93 (100%), Fosfomycin the sensitive were n=89 (95.7%) and the resistant were n=4 (4.3%), gentamicin the sensitive were n=70 (76.3%) and the resistant were n=22 (23.7%), imipenem the sensitive were n=93 (100%), Meropenem the sensitive were n=93 (100%), Nitrofurantoin the sensitive were n=91 (97.8%) and the resistant were n=2 (2.2%), Piperacillin/Tazobactam the sensitive were n=65 (69.9%) and the resistant were n=28 (30.1%), and Trimethoprim/sulfamethoxazole the sensitive were n=27 (29.0%) and the resistant were n=66 (71.0%).

Table 3. Pattern of antimicrobial resistance of an *E. coli* species.

Antibiotic Name	**Sensitive/Resistance**	**n (%)**
Amikacin	Sensitive	93 (100%)
	Resistant	0 (0%)
Amoxicillin Clavulanic Acid	Sensitive	34 (36.6%)
	Resistant	59 (63.4%)
Ampicillin	Sensitive	8 (8.6%)
	Resistant	85 (91.4%)
Cefixime	Sensitive	21 (22.6%)
	Resistant	72 (77.4%)
Ceftazidime	Sensitive	16 (17.2%)
	Resistant	77 (82.8%)
Ceftriaxone	Sensitive	17 (18.3%)
	Resistant	76 (81.7%)
Cefuroxime	Sensitive	34 (36.6%)
	Resistant	59 (63.4%)
Ciprofloxacin	Sensitive	32 (34.4%)
	Resistant	61 (65.6%)
Ertapenem	Sensitive	93 (100%)
	Resistant	0 (0%)
Fosfomycin	Sensitive	89 (95.7%)
	Resistant	4 (4.3%)
Gentamicin	Sensitive	71 (76.3%)
	Resistant	22 (23.7%)
Imipenem	Sensitive	93 (100%)
	Resistant	0 (0%)

Meropenem	Sensitive	93 (100%)
	Resistant	0 (0%)
Nitrofurantoin	Sensitive	91 (97.8%)
	Resistant	2 (2.2%)
Piperacillin/ Tazobactam	Sensitive	65 (69.9%)
	Resistant	28 (30.1%)
Trimethoprim Sulfamethoxazole	Sensitive	27 (29.0%)
	Resistant	66 (71.0%)

4.2. Detection of ESBL on CDT Method

In this study positive ESBL production was found 78.5% (n: 73) among all samples, and negative ESBL production was 21.5% (n: 20) as shown in Table 4 and Figure 4.

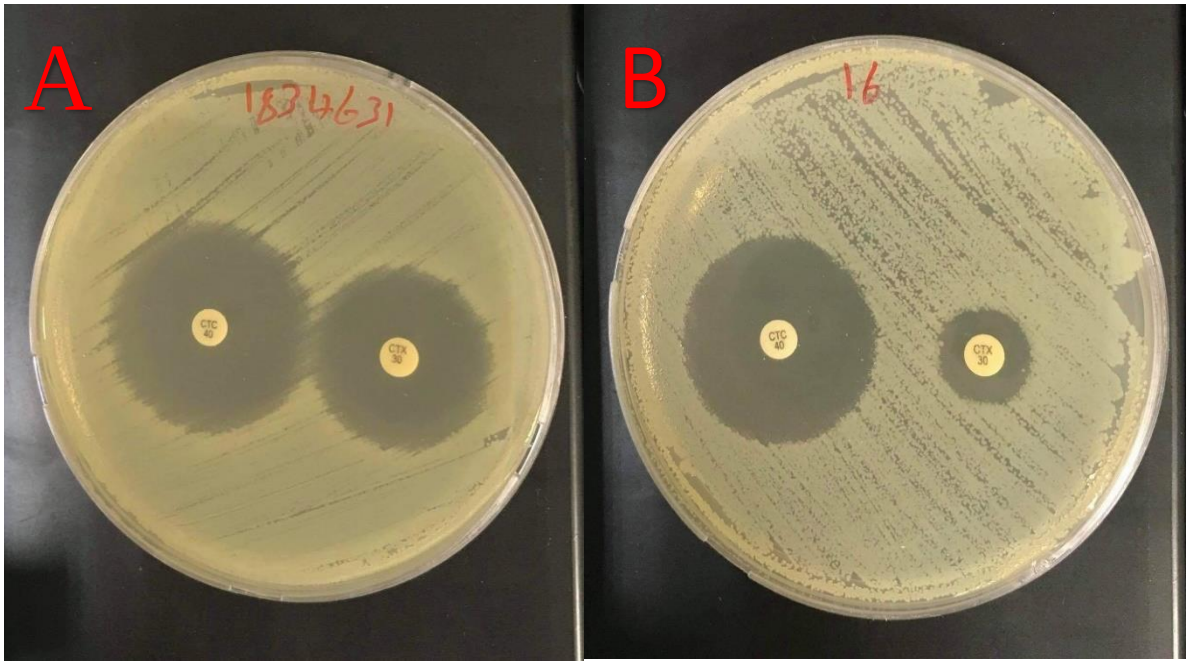


Figure 4. According to the figure 4 that shows the Positive ESBL production in (A) and Negative ESBL production in (B) from CDT.

Table 4. Distribution of ESBL form CDT

Distribution of ESBL form CDT			Total
ESBL	**Negative**	**Count**	20
		% of Total	21.5%
	Positive	**Count**	73
		% of Total	78.5%
Total		**Count**	93
		% of Total	100%

4.3. Detection of ESBL on DDST Method

In this study positive ESBL production was found 93.5% (n: 87) among all samples, and negative ESBL production was 6.5% (n: 6) as shown in Table 6 and Figure 5.

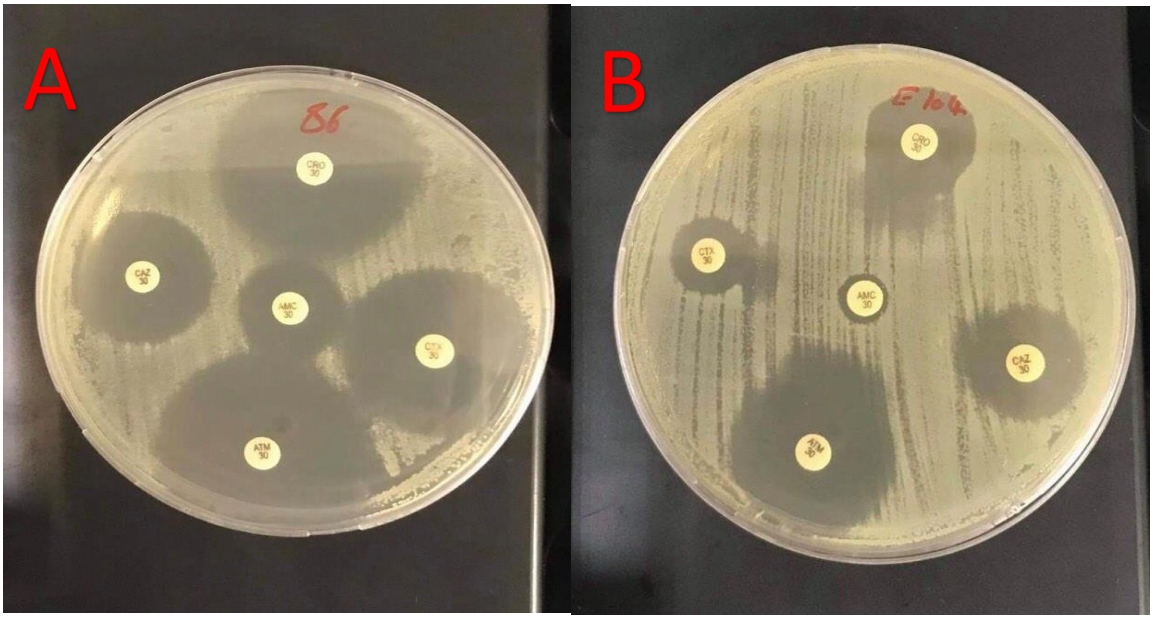


Figure 5. This figure shows the Positive ESBL production in (A) is reported when the areas of inhibition are augmented in the direction of the clavulanic acid disc around each of the cephalosporin disks. The distance between the disks is important, and for cephalosporin 30 µg disks, 20 mm center to center was found to be ideal and a Negative ESBL production in (B).

Table 5. Distribution of ESBL from DDST Test.

ESBL from DDST Test			Total
ESBL	**Negative**	**Count**	6
		% of Total	6.5%
	Positive	**Count**	87
		% of Total	93.5%
Total		**Count**	93
		% of Total	100%

4.4. Detection of ESBL on TDT Method

In this study positive ESBL production was found 76.3% (n: 71) among all samples, and negative ESBL production was 23.7% (n: 22) as shown in Table 8 and Figure 6.

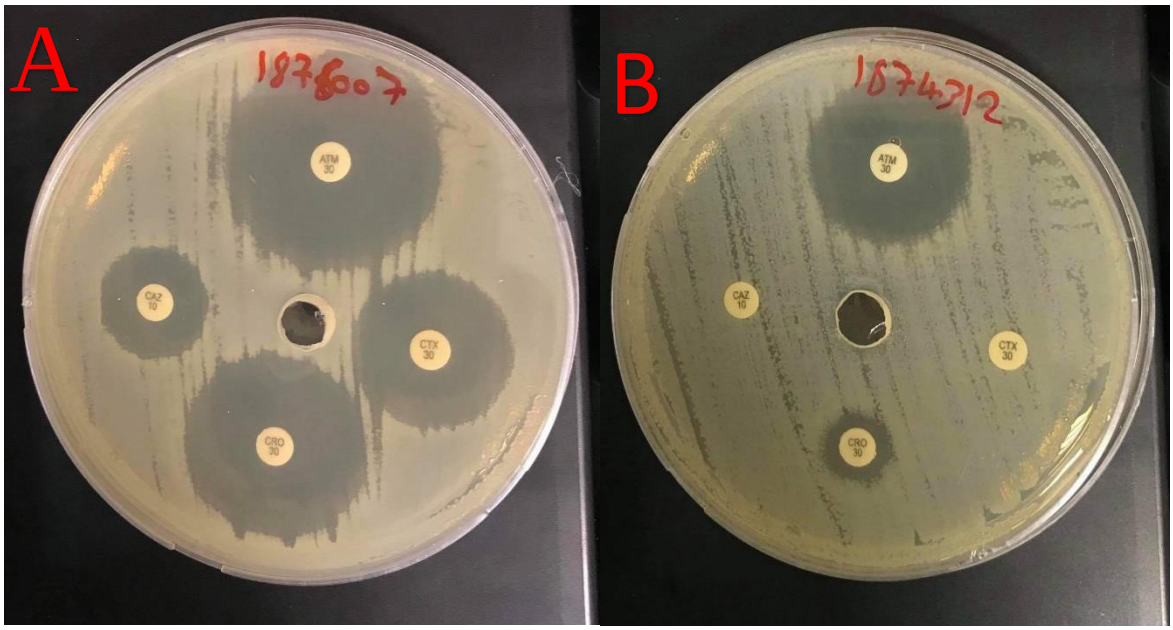


Figure 6. A) Show the Positive ESBL from TDT is reported when the zones of inhibition around any of the cephalosporin disks are augmented in the direction of the clavulanic acid disc. B) Show Negative ESBL.

Table 6. Distribution of ESBL from TDL Test.

Distribution of ESBL from TDT Test.			Total
ESBL	**Negative**	**Count**	22
		% of Total	23.7%
	Positive	**Count**	71
		% of Total	76.3%
Total		**Count**	93
		% of Total	100%

4.5. Comparison of the methods used for detection ESBL

According to this study, Double disk synergy, Combined disk diffusion test, and Three-dimensional test were used as phenotypic screening tests for ESBL detection. A Combined disk diffusion test is recommended by EUCAST for ESBL confirmation. With the DDT 87 (93.5%) strains of *E. coli* were positive and 6 (6.5%) negative, CDT 73 (78.5%) were positive and 20 (21.5%) negative, TDT 71 (76.3%) were positive and 22 (23.7%) negatives. In this thesis, according to method of ESBL production. Comparison of the methods used for detection ESBL results were significant as shown in Table 7.

Table 7. Combined Disc Diffusion, Double Disc Synergy, Three-Dimensional Test Method Results.

Method to detect ESBL			**ESBL in *E. coli***
Double Disk Synergy Test	**Positive**	**Number**	87
		Percent (%)	93.5%
	Negative	**Number**	6
		Percent (%)	6.5%
Combined Disc Test	**Positive**	**Number**	73
		Percent (%)	78.5%
	Negative	**Number**	20
		Percent (%)	21.5%
Three-Dimensional Test	**Positive**	**Number**	71
		Percent (%)	76.3%
	Negative	**Number**	22
		Percent (%)	23.7%

According to statistical analysis:

- P Value between production of ESBL in *E. coli* spp DDST and CDT is p=0.223.
- P Value between production of ESBL in *E. coli* spp DDST and TDT is p=0.188.
- P Value between production of ESBL in *E. coli* spp CDT and TDT is p=0.000.

5. DISCUSSION

Infections of *E. coli* species have intensified and become more complicated to manage owing to the rise of immunogenic diseases, misuse of broad-spectrum antibiotics, increasing usage of immunosuppressive drugs, malnutrition, endocrine diseases, extensive use of medical devices, aging, and an increase in the number of patients. In addition, in the last two decades, the whole evolution of infectious diseases has undergone significant changes. In patients that are immunocompromised and chronically ill, bacteria that were previously deemed nonpathogenic or less virulent are now recognized as a major cause of morbidity and mortality.

Beta-lactamase activity is the most significant and widespread cause of resistance to beta-lactam antibiotics. The effectiveness of broad-spectrum cephalosporins against the Enterobacteriaceae family is challenged by newly emerging enzymes and innovations in the production of old enzymes. Especially in the enzyme group called ESBL. It is responsible for the development of resistance to beta-lactams such as aztreonam, ceftazidime, ceftriaxone, and cefotaxime. In Europe, these enzymes were first identified, but it was known that they appeared in several different countries, such as the United States of America and Japan. This has been associated with the increased use of third-generation cephalosporins in particular (Livermore, 1995; Paterson & Bonomo, 2005). Epidemiological studies of the presence of ESBL in E.coli strains are more common and are important factors for public and hospital infections, especially in urinary tract infections. (Henshke-Bar-Meir et al., 2006).

Worldwide, ESBL-positive isolates of Klebsiella and *E. coli* have been published, but there are few systematic studies exploring how or why these species predominate in multiple settings. The burden or cost of the transition of resistance genes to the organism is likely to be one of the most important factors influencing the level of their isolation and the degree of resistance distributed through the microbial community (Pope et al., 2010; Pränting, 2010).

Several reports have been performed on this issue; for example, according to evidence from the National Nosocomial Infections Surveillance System (NNIS), there was an improvement in the frequency of gram-negative bacterial nosocomial infections, while the prevalence of gram-negative nosocomial infection was 67.8% in 1975 and 73% in 2003 (Rosenthal et al., 2010).

Due to their bactericidal effects and low side effects, beta-lactam antibiotics are mostly preferred during treatment. The rate of resistance to antibiotics in this group has increased in direct proportion to the increase in their clinical use. Although microorganisms that produce ESBL are detected worldwide, the nature and type of enzymes vary from region to region, country to country, and even from hospital to hospital. *K. pneumonia* and *E. coli* are the microorganisms that produce the most ESBL among the members of the family Enterobacteriaceae (Babini & Livermore, 2000, pp. 1997–1998; Bell et al., 2002).

This study investigates the Extended Spectrum Beta Lactamase (ESBL) properties of strains of Escherichia coli species obtained from several Near East Hospital clinical samples, according to the distribution of specimens in this study 93 Escherichia coli specimens that collected from different site of the body, in this study, most of the isolates were obtained from urine 62.4% (n=58), aspirate 15.1% (n=14), sputum 16.1% (n=15) and wound and Pus 6.5% (n=6). Also found that most specimens were isolated from the Child Health and Diseases department with 14.0% and n=13, Urology department with 12.9% and n=12, Urgent department with 10.8% and n=10.

In the investigation of ESBL production among *E. coli*, this study shows 93.5% (n=87) positive ESBL, and 6.5% (n=6) negative ESBL by Double Disk Synergy Test, 78.5% (n=73) positive ESBL, and 21.5% (n=20) negative ESBL by Combined Disc Test, 76.3% (n=71) positive ESBL, and 23.7% (n=22) negative ESBL by Three-Dimensional Test. By reference to recent studies that were detected from Kumari et al. 2020 noted that ESBL positive were 5,98%, Sharma et al. in 2019 noted that ESBL positive were 31%, Dumaru et al. in 2019 revealed that ESBL positive were 15,87%, Alkasaby and El Sayed Zaki in 2017 noted that ESBL positive were 2,1%, Shamsuzzaman in 2017 noted that 9% were ESBL positive, and Coskun et al. in 2019 in turkey noted only 2% were positive to ESBL.

In this study, the relationship among ESBL and antibiotic resistance was detected to be statistically significant for all antibiotics. (Alkasaby & El Sayed Zaki, 2017; Coskun et al., 2019; Dumaru et al., 2019).

The percentage of ESBL positive in *E. coli* isolates ranged from 0 to 35 in studies conducted between 1998-1999 and in South Africa and the Asia Pacific in the SENTRY antimicrobial surveillance program report. No ESBL positive strain was found in a study conducted in Australia, and ESBL production of more than 20% was observed in *K. pneumonia* strains in studies conducted in South Africa, China, and Japan. In the same report, China GSBL production was reported at 35% (Bell et al., 2002; Winokur et al., 2001).

Studies conducted between 1998-2005 in Turkey show a difference in the production of ESBL. ESBL production was detected in *E. coli* strains at 0-63% and in *K. pneumonia* strains 15-77% (AKÇAM et al., 2004). From Kacmaz et al. The rate of ESBL positive strains of *K. pneumonia* and *E. coli* was found to be 77% and 63 %, respectively, in 2005 (KAÇMAZ et al., 2005; Rota et al., 2000). In a study conducted by Bülüç et al. In 2003, the Istanbul University Faculty of Medicine reported that the rate of production of ESBL was 48% for strains of *K. pneumoniae* and 14% for strains of *E. coli* (Bülüç et al., 2003).

ESBL concentrations indicate differences in studies conducted abroad. While ESBL is found in 20-25% of Klebsiella strains isolated in Europe from intensive care units, this rate in France goes up to 30-40% (Jacoby & Medeiros, 1991; Nordmann, 1998). In a study conducted in Finland, while cefuroxime and cephalosporins of the third generation were used more intensively than in other Scandinavian countries, none of the *E. coli* and Klebsiella strains examined produced ESBL (AYGÜN & DERİN, n.d.). Jacoby and Han identified ESBL in 141 of 169 *K. pneumoniae* and 41 of 111 E. coli they obtained from 29 hospitals in the United States (Jacoby & Han, 1996). It was observed that 373 (80.2%) of 465 *K. pneumoniae* strains, most of which were isolated from urine in Scotland, produced ESBL (Hobson et al., 1996).

In a study conducted with materials taken from patients coming from Acıbadem Hospital in Istanbul with UTI examination, 1456 urine samples were collected, 1100 of them were identified as *E. coli*, and 356 as *Klebsiella spp* isolates. The ESBL rates of the isolated isolates were 12% (n: 132) for *E. coli* and 12% (n: 41) for *Klebsiella spp*. In ESBL (+) *E. coli strains*, amikacin 3%, nitrofurantoin 3%, cefoxitin 5.3%, piperacillin-tazobactam 21.2%, gentamicin 34.8%, trimethoprim / sulfamethoxazole 68.9%, tobramycin 75.9%, ciprofloxacin and norfloxacin 80.3%; Klebsiella strains were resistant to amikacin 2.4%, nitrofurantoin 85.4%, cefoxitin 5.3%, piperacillin-tazobactam 58.5%, gentamicin 41.5%, trimethoprim / sulfamethoxazole 58%, tobramycin 56.1%, ciprofloxacin and norfloxacin 21.9%. Resistance to Fosfomycin was observed at a rate of 4.9 percent in Klebsiella isolates. Ampicillin, Ceftazidime, Cefepime, Cefazoline, Aztreonam, and ceftriaxone were shown to be resistant to all ESBL (+) strains (Akyar, 2008).

ESBL rates were found to be 40 percent for *E. coli* and 49 percent for *Klebsiella pneumoniae* in the assessment of 459 *E. coli* and 226 *Klebsiella pneumoniae* isolates isolated in blood culture using the disk diffusion method. In addition, high resistance rates were found to other antibiotics in this study, and no carbapenem resistance was found. ESBL was detected at a rate of 4.5% in 255 *E. coli* isolates and 47% in 44 Klebsiella isolates previously produced in urine samples sent from the clinic and polyclinics of the Department of Urology in 2001 (Koksal et al., 2009)

In the Aygün & Derin et al. study, ESBL was detected in 26/135 *E. coli* (19.2%) and 4/24 Klebsiella (16.6%) strains. It is noteworthy that while ESBL increases in *E. coli* isolates, it decreases in Klebsiella isolates. For *E. coli*, the increase is an expected phenomenon that is often associated with antibiotic use. The decrease in the ESBL rate in Klebsiella isolates can be explained by an epidemic in the first study and the low number in the second study (AYGÜN & DERİN, n.d.). In the study conducted by Akata et al., They found the rate of ESBL in enteric bacteria isolated in the hospital as 11.8% (Akata et al., 2003). In a study conducted on *E. coli* strains in 1998, ESBL was found in 7.8% of outpatient isolates and 9% of hospital isolates. In 1997, ESBL production was detected in

69 (21.5%) of 320 *E. coli* strains isolated as the cause of hospital infection and 71 (49.3%) of 144 *K. pneumoniae* strains (Tünger et al., 1998).

By Mehli et al. The presence of Double Disk Synergy Extended Spectrum Beta-Lactamase in *E. coli* isolates obtained from different clinical samples has been investigated. ESBL was shown to be positive for 212 isolates of *E. coli* (49%). In the study performed by Muhtaseb and Kaygusuz, ESBL was studied with 59 *E. coli* isolates and ESBL was positive in 20 samples (34%) (Al-Muhtaseb, 2008).

In 1992, three-dimensional test ESBL production was found to be positive in 79% of Enterobacteriaceae strains confirmed in their study by Thomson et al (Thomson & Sanders, 1992). In some studies, carried out in Turkey Akyıldız et al ESBL production by 13% using the three-dimensional test, while Hoşgör et al found that 35% positive (Akyıldız et al., 1998). Studies in the literature have reported that cefotaxime and ceftriaxone discs are more sensitive than ceftazidime in three-dimensional testing (P. Datta et al., 2004; MacKenzie et al., 2002).

In a study conducted at Eskişehir Osmangazi University, 82 clinical samples were evaluated, 49 of them were reported as ESBL (+) *E. coli* isolates, and 33 as ESBL (+) Klebsiella pneumoniae isolates. In both isolate and resistance levels, strong resistance rates were found; ciprofloxacin 50.9%, cefepime 81.7%, trimoxazole 47.4%, tetracycline 75.0%, and gentamicin 48.7%. Meropenem and imipenem were 100% susceptible and in *K. pneumoniae* isolates, ertapenem was 100% susceptible and in *E. coli* isolates, 95.9%. Just two *E. coli* isolates were confirmed to be ertapenem-resistant (Zafar et al., 2014).

In a study investigating the risk factors in nosocomial UTI at Trakya University Faculty of Medicine, 91 samples were collected and *E. coli* (n: 48) isolates were grown in 40.8% of these samples and *Klebsiella pneumoniae* (n: 8) isolates in 6.8 %. ESBL was detected in 27% of *E. coli* isolates and 25% of *K. pneumoniae* isolates. The highest sensitivity in *E. coli* isolates was imipenem and nitrofurantoin with a rate of 100% and amikacin with a rate of 97.7%; The lowest sensitivity was found to be ampicillin with a rate of 26.7% and amoxicillin-clavulanic acid with a rate of 44.4% (AYGÜN & DERİN, n.d.).

60 ESBL (+) E. coli and Klebsiella pneumoniae isolates were determined in a study investigating the risk factors in UTI at Uludağ University, and the use of quinolone or cephalosporin in the last 3 months for any infection was reported as risk factors (Yilmaz et al., 2008).

In a study of risk factors in *Escherichia coli* and *Klebsiella pneumoniae* isolates at the Osmangazi University Faculty of Medicine, 100 *Klebsiella pneumoniae* and 100 *Escherichia coli* isolates were used in the analysis and ESBL positive values calculated by the disk diffusion method were 12% for *Escherichia coli* isolates and 12% for *K. pneumoniae* isolates. The established prevalence of ESBL enzyme generating species in Greek hospitals is 47% and 50% of ESBL positive isolates have been identified in ICUs, 36.1% in inpatients and 13.3% in outpatients. Trigger factors for ESBL (+) are the isolates of *K. pneumoniae* and *Escherichia coli*; Foley catheter, intravenous catheter, venous catheter, intubation and mechanical ventilation. (Hosoglu et al., 2007).

6. CONCLUSION

In this study, several research groups found that not all E. coli strains tested were sensitive to almost all antimicrobials that were studied. that make this nosocomial pathogen one of the most important microbial challenges to be managed in the future. The conspicuous prevalence of ESBL producing and multidrug-resistant bacteria in our institutions presents an impression of potential challenges in our region of the world. Routine observation of beta-lactamases; thus, along with stringent enforcement of infection management and prevention practices may be advised in clinical laboratories.

References

Abd El Tawab, A. A., El-Hofy, F. I., Nada, S. M., & Deiab, R. A. (2015). Detection of virulence genes of enterohaemorrhagic E. coli isolated from some meat products by polymerase chain reaction. *Benha Veterinary Medical Journal*, *29*(1), 45–52.

Abraham, E. P., & Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. *Nature*, *146*(3713), 837–837.

Agarwal, J., Srivastava, S., & Singh, M. (2012). Pathogenomics of uropathogenic Escherichia coli. *Indian Journal of Medical Microbiology*, *30*(2), 141.

Akata, F., Tatman-Otkun, M., Ozkan, E., Tansel, Ö., Otkun, M., & Tugrul, M. (2003). Prevalence of extended-spectrum beta-lactamases produced by nosocomial isolates of Enterobacteriaceae in Trakya University Hospital, Turkey. *The New Microbiologica*, *26*(3), 257–262.

Akbar, A., & Anal, A. K. (2011). Food safety concerns and food-borne pathogens, Salmonella, Escherichia coli and Campylobacter. *FUUAST Journal of Biology*, *1*(1 June), 5–17.

AKÇAM, F., Gönen, İ., Onur, K., & YAYLI, G. (2004). Hastane infeksiyonu etkeni enterobakterilerde beta-laktam antibiyotiklere duyarlılık ve ESBL sıklığının araştırılması. *SDÜ Tıp Fakültesi Dergisi*, *11*(1).

Aktaş, Z., Kayacan, Ç. B., Schneider, I., Can, B., Midilli, K., & Bauernfeind, A. (2008). Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in Klebsiella pneumoniae in Istanbul, Turkey. *Chemotherapy*, *54*(2), 101–106.

Akyar, I. (2008). Antibiotic resistance rates of extended spectrum beta-lactamase producing Escherichia coli and Klebsiella spp. Strains isolated from urinary tract infections in a private hospital. *Mikrobiyoloji Bulteni*, *42*(4), 713–715.

Akyıldız, R., Özsoy, M. F., Altunay, H., Koçak, N., Çavuşlu, Ş., & Yenen, O. (1998). Klebsiella pneumonia suşlarında genişlemiş spektrumlu beta-laktamaz sıklığı ve beta laktam antibiyotik direncinin araştırılması. *Klimik Derg*, *11*(2), 53–58.

Alkasaby, N. M., & El Sayed Zaki, M. (2017). Molecular study of Acinetobacter baumannii isolates for Metallo-β-lactamases and extended-spectrum-β-lactamases genes in intensive care unit, Mansoura University Hospital, Egypt. *International Journal of Microbiology*, *2017*.

Al-Muhtaseb, M. (2008). Kaygusuz A. Kan kültürlerinden izole edilen Escherichia coli ve Klebsiella pneumoniae suşlarında genişlemiş spektrumlu beta-laktamaz (GSBL) sıklığı. *Ankem Derg*, *22*(4), 175–82.

Ambler, R. P. (1980). The structure of β-lactamases. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *289*(1036), 321–331.

Amdekar, S., Singh, V., & Singh, D. D. (2011). Probiotic therapy: Immunomodulating approach toward urinary tract infection. *Current Microbiology*, *63*(5), 484.

Anderson, K. F., Lonsway, D. R., Rasheed, J. K., Biddle, J., Jensen, B., McDougal, L. K., Carey, R. B., Thompson, A., Stocker, S., & Limbago, B. (2007). Evaluation of methods to identify the Klebsiella pneumoniae carbapenemase in Enterobacteriaceae. *Journal of Clinical Microbiology*, *45*(8), 2723–2725.

Andreu, A., Sanfeliu, I., Viñas, L., Barranco, M., Bosch, J., Dopico, E., Guardia, C., Juncosa, T., Lite, J., Matas, L., Sánchez, F., & Sierra, M. (2003). Declive de la incidencia de la sepsis perinatal por estreptococo del grupo B (Barcelona 1994-2001). Relación con las políticas profilácticas. *Enfermedades Infecciosas y Microbiología Clínica*, *21*(4), 174–179. https://doi.org/10.1016/S0213-005X(03)72913-9

Armstrong, G. L., Hollingsworth, J., & Morris Jr, J. G. (1996). *Emerging foodborne pathogens: Escherichia coli O157: H7 as a model of entry of a new pathogen into the food supply of the developed world*.

Aryal, S. (2020, January 3). Cultural Characteristics of E. coli. *Microbe Notes*. https://microbenotes.com/cultural-characteristics-of-e-coli/

Astal, Z., Sharif, F. A., Abdallah, S. A., & Fahd, M. I. (2004). Extended spectrum beta-lactamases in Eschericia coli isolated from community-acquired urinary tract infections in the Gaza Strip, Palestine. *Annals of Saudi Medicine*, *24*(1), 55–57.

AYGÜN, D. D. G., & DERİN, E. (n.d.). *ÜROLOJİ KLİNİĞİNDE ÜRİNER İNFEKSİYON ETKENİ OLAN KLEBSİELLA SPP VE ESCHERİCHİA COLİ İZOLATLARINDA ANTİBİYOTİK DİRENCİ VE KLONAL*.

Babini, G. S., & Livermore, D. M. (2000). Antimicrobial resistance amongst Klebsiella spp. Collected from intensive care units in Southern and Western Europe in 1997–1998. *Journal of Antimicrobial Chemotherapy*, *45*(2), 183–189.

Barcella, L., Barbaro, A. P., & Rogolino, S. B. (2016). Colonial morphology of Escherichia coli: Impact of detection in clinical specimens. *Microbiologia Medica*, *31*(2). https://doi.org/10.4081/mm.2016.5636

Barnich, N., Carvalho, F. A., Glasser, A.-L., Darcha, C., Jantscheff, P., Allez, M., Peeters, H., Bommelaer, G., Desreumaux, P., & Colombel, J.-F. (2007). CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn disease. *The Journal of Clinical Investigation*, *117*(6), 1566–1574.

Barrick, J. E., Yu, D. S., Yoon, S. H., Jeong, H., Oh, T. K., Schneider, D., Lenski, R. E., & Kim, J. F. (2009). Genome evolution and adaptation in a long-term experiment with Escherichia coli. *Nature*, *461*(7268), 1243–1247.

Bell, J. M., Turnidge, J. D., Gales, A. C., Pfaller, M. A., Jones, R. N., & Group, S. A. S. (2002). Prevalence of extended spectrum β-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: Regional results from SENTRY Antimicrobial Surveillance Program (1998–99). *Diagnostic Microbiology and Infectious Disease*, *42*(3), 193–198.

Bellíssimo-Rodrigues, F., Gomes, A. C. F., Passos, A. D. C., Achcar, J. A., Perdoná, G. da S. C., & Martinez, R. (2006). Clinical outcome and risk factors related to extended-spectrum beta-lactamase-producing Klebsiella spp. Infection among hospitalized patients. *Memorias Do Instituto Oswaldo Cruz*, *101*(4), 415–421.

Bennett, J. E., Dolin, R., & Blaser, M. J. (2014). *Mandell, douglas, and bennett's principles and practice of infectious diseases: 2-volume set* (Vol. 2). Elsevier Health Sciences.

Berendsen, B. J., Gerritsen, H. W., Wegh, R. S., Lameris, S., van Sebille, R., Stolker, A. A., & Nielen, M. W. (2013). Comprehensive analysis of ß-lactam antibiotics including penicillins, cephalosporins, and carbapenems in poultry muscle using liquid chromatography coupled to tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *405*(24), 7859–7874.

Besser, R. E., Griffin, P. M., & Slutsker, L. (1999). Escherichia coli O157: H7 gastroenteritis and the hemolytic uremic syndrome: an emerging infectious disease. *Annual Review of Medicine*, *50*(1), 355–367.

Bien, J., Sokolova, O., & Bozko, P. (2012). Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage. *International Journal of Nephrology*, *2012*.

Bodey, G. P., Whitecar, J. P., Middleman, E., & Rodriguez, V. (1971). Carbenicillin therapy for Pseudomonas infections. *JAMA*, *218*(1), 62–66.

Bower, J. M., Eto, D. S., & Mulvey, M. A. (2005). Covert operations of uropathogenic Escherichia coli within the urinary tract. *Traffic*, *6*(1), 18–31.

Bradford, P. A. (2001). Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*, *14*(4), 933–951.

Brunton, J., Clare, D., & Meier, M. A. (1986). Molecular epidemiology of antibiotic resistance plasmids of Haemophilus species and Neisseria gonorrhoeae. *Reviews of Infectious Diseases*, *8*(5), 713–724.

Bülüç, M., Gürol, Y., & Bal, Ç. (2003). Genişlemiş spektrumlu beta-laktamaz oranları: 2000-2002. *Türk Mikrobiyol Cem Derg*, *33*, 31–4.

Bush, K., & Jacoby, G. A. (2010). Updated functional classification of β-lactamases. *Antimicrobial Agents and Chemotherapy*, *54*(3), 969–976.

Cantón, R., & Coque, T. M. (2006). The CTX-M β-lactamase pandemic. *Current Opinion in Microbiology*, *9*(5), 466–475.

Castellani, A., & ALBERT, J. (1919). CHALMERS: Manual of Tropical Medicine. *Ed*, *2*, 1018–1026.

Cavalieri, S. J., Bohach, G. A., & Snyder, I. S. (1984). Escherichia coli alpha-hemolysin: Characteristics and probable role in pathogenicity. *Microbiological Reviews*, *48*(4), 326.

Cengiz, A. T., Mısırlıgil, A., & Aydın, M. (2004). Tıp ve diş hekimliğinde genel ve özel mikrobiyoloji. *Ankara: Güneş Kitapevi*.

Centers for Disease Control and Prevention (U.S.), & Centers for Disease Control and Prevention (U.S.) (Eds.). (2002). *Foodborne disease outbreak investigation, epidemiologic case study. A multistate outbreak of E. coli 0157:H7 infection* (Student's version). U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Center for Infectious Diseases, Epidemiology Program Office, Public Health Practice Program Office.

Chambers, H. F., & Neu, H. C. (2005). Other beta-lactam antibiotics. *Principles and Practice of Infectious Diseases. Philadelphia, PA: Elsevier*, 311–318.

Chen, M., Jahnukainen, T., Bao, W., Daré, E., Ceccatelli, S., & Celsi, G. (2003). Uropathogenic Escherichia coli toxins induce caspase-independent apoptosis in renal proximal tubular cells via ERK signaling. *American Journal of Nephrology*, *23*(3), 140–151.

Cirl, C., Wieser, A., Yadav, M., Duerr, S., Schubert, S., Fischer, H., Stappert, D., Wantia, N., Rodriguez, N., & Wagner, H. (2008). Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain–containing proteins. *Nature Medicine*, *14*(4), 399–406.

Clermont, O., Bonacorsi, S., & Bingen, E. (2000). Rapid and Simple Determination of theEscherichia coli Phylogenetic Group. *Applied and Environmental Microbiology*, *66*(10), 4555–4558. https://doi.org/10.1128/AEM.66.10.4555-4558.2000

Clinical, & Institute, L. S. (2017). Performance standards for antimicrobial susceptibility testing. In *CLSI supplement M100*. Clinical and Laboratory Standards Institute Wayne, PA.

Collinson, S. K., Emödy, L., & Kay, W. W. (1992). Thin aggregative fimbriae from diarrheagenic Escherichia coli. *Journal of Bacteriology*, *174*(13), 4490–4495.

Coskun, U. S. S., Caliskan, E., Cicek, A. C., Turumtay, H., & Sandalli, C. (2019). β-lactamase genes in carbapenem resistance Acinetobacter baumannii isolates from a Turkish university hospital. *The Journal of Infection in Developing Countries*, *13*(01), 50–55.

Cowan, S. T. (1954). A review of names for coliform organisms. *International Journal of Systematic and Evolutionary Microbiology*, *4*(2), 119–124.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent Advances in Understanding Enteric Pathogenic Escherichia coli. *Clinical Microbiology Reviews*, *26*(4), 822–880. https://doi.org/10.1128/CMR.00022-13

Datta, N., & Kontomichalou, P. (1965). Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. *Nature*, *208*(5007), 239–241.

Datta, P., Thakur, A., Mishra, B., & Gupta, V. (2004). Prevalence of clinical strains resistant to various beta-lactams in a tertiary care hospital in India. *Japanese Journal of Infectious Diseases*, *57*(4), 146–149.

De Vrese, M., Schrezenmeir, & J. (2008). Probiotics, prebiotics, and synbiotics. In *Food biotechnology* (pp. 1–66). Springer.

Don, J. B., Noel, R. K., & James, T. S. (1984). *George M. Garrity, ed. The Gammaproteobacteria. Bergey's Manual of Systematic Bacteriology 2B*. NewYork: Springer (2005).

Drieux, L., Brossier, F., Sougakoff, W., & Jarlier, V. (2008). Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: Review and bench guide. *Clinical Microbiology and Infection*, *14*, 90–103. https://doi.org/10.1111/j.1469-0691.2007.01846.x

Dulo, F. (2014). *Prevalence and antimicrobial resistance profile of Escherichia coli O157: H7 in goat slaughtered in dire dawa municipal abattoir as well as food safety knowledge, attitude and hygiene practice assessment among slaughter staff, Ethiopia* [PhD Thesis]. Addis Ababa University.

Dumaru, R., Baral, R., & Shrestha, L. B. (2019). Study of biofilm formation and antibiotic resistance pattern of gram-negative Bacilli among the clinical isolates at BPKIHS, Dharan. *BMC Research Notes*, *12*(1), 38.

Durmaz, R., Otlu, B., Koksal, F., Hosoglu, S., Ozturk, R., Ersoy, Y., Aktas, E., Gursoy, N. C., & Caliskan, A. (2009). The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of Acinetobacter baumannii, Escherichia coli and Klebsiella spp. *Jpn J Infect Dis*, *62*(5), 372–7.

E. coli (Escherichia coli) | E. coli | CDC. (2020, February 26). https://www.cdc.gov/ecoli/index.html

E. Coli Infection: Causes, Symptoms, Prevention, Risks & More. (2015, October 15). Healthline. https://www.healthline.com/health/e-coli-infection

Eberspächer, B., Hugo, F., & Bhakdi, S. (1989). Quantitative study of the binding and hemolytic efficiency of Escherichia coli hemolysin. *Infection and Immunity*, *57*(3), 983–988.

Elander, R. P. (2003). Industrial production of β-lactam antibiotics. *Applied Microbiology and Biotechnology*, *61*(5–6), 385–392.

Elbing, K. L., & Brent, R. (2019). Recipes and Tools for Culture of *Escherichia coli*. *Current Protocols in Molecular Biology*, *125*(1), e83. https://doi.org/10.1002/cpmb.83

Emody, L., Kerenyi, M., & Nagy, G. (2003). Virulence factors of uropathogenic Escherichia coli. *International Journal of Antimicrobial Agents*, *22*, 29–33.

Erb, A., Stürmer, T., Marre, R., & Brenner, H. (2007). Prevalence of antibiotic resistance in Escherichia coli: Overview of geographical, temporal, and methodological variations. *European Journal of Clinical Microbiology & Infectious Diseases*, *26*(2), 83–90.

Escherich, T. (1886a). *Die darmbakterien des säuglings und ihre beziehungen zur physiologie der Verdauung*. F. Enke.

Escherich, T. (1886b). *Die darmbakterien des säuglings und ihre beziehungen zur physiologie der Verdauung*. F. Enke.

Escherichia coli: Video, Anatomy, Definition & Function. (n.d.). Osmosis. Retrieved August 16, 2020, from http://www.osmosis.org

Etebu, E., & Arikekpar, I. (2016). Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. *Int. J. Appl. Microbiol. Biotechnol. Res*, *4*(2016), 90–101.

EUCAST, T. (2015). European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs and zone diameters. In *Version 5.0, 2015*. European Society of Clinical Microbiology and Infectious Diseases Basel ….

Ewers, C., Bethe, A., Semmler, T., Guenther, S., & Wieler, L. H. (2012). Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and

their putative impact on public health: A global perspective. *Clinical Microbiology and Infection*, *18*(7), 646–655.

Fang, H., Ataker, F., Hedin, G., & Dornbusch, K. (2008). Molecular epidemiology of extended-spectrum β-lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *Journal of Clinical Microbiology*, *46*(2), 707–712.

Feng, P., Weagant, S. D., Grant, M. A., Burkhardt, W., Shellfish, M., & Water, B. (2002). BAM: Enumeration of Escherichia coli and the Coliform Bacteria. *Bacteriological Analytical Manual*, *13*.

Fernandez, T. F. (2008). E. coli O157: H7. *Veterinary World*, *1*(3), 83.

Fleming, A. (1929). On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *British Journal of Experimental Pathology*, *10*(3), 226.

Florey, H. W., Chain, E. B., Heatley, N. G., Jennings, M. A., Sanders, A. G., Abraham, E. P., & Florey, M. E. (1949). *Antibiotics) London*. Oxford University Press.

Fontana, J., Stout, A., Bolstorff, B., & Timperi, R. (2003). Automated ribotyping and pulsed-field gel electrophoresis for rapid identification of multidrug-resistant Salmonella serotype Newport. *Emerging Infectious Diseases*, *9*(4), 496.

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). *Diagnostic microbiology*. Mosby St Louis.

Francis, C., & Eric, D. (2017). Carbapenem resistance: A review. *Medical Sciences*, *6*(1), 1.

Friedman, N. D., Kaye, K. S., Stout, J. E., McGarry, S. A., Trivette, S. L., Briggs, J. P., Lamm, W., Clark, C., MacFarquhar, J., & Walton, A. L. (2002). Health care–associated bloodstream infections in adults: A reason to change the accepted definition of community-acquired infections. *Annals of Internal Medicine*, *137*(10), 791–797.

Garibyan, L., & Avashia, N. (2013). Research techniques made simple: Polymerase chain reaction (PCR). *The Journal of Investigative Dermatology*, *133*(3), e6.

Gekenidis, M.-T., Qi, W., Hummerjohann, J., Zbinden, R., Walsh, F., & Drissner, D. (2018). Antibiotic-resistant indicator bacteria in irrigation water: High prevalence of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli. *PloS One*, *13*(11), e0207857.

Gillings, M. R. (2013). Evolutionary consequences of antibiotic use for the resistome, mobilome and microbial pangenome. *Frontiers in Microbiology*, *4*, 4.

Giske, C. G., Sundsfjord, A. S., Kahlmeter, G., Woodford, N., Nordmann, P., Paterson, D. L., Canton, R., & Walsh, T. R. (2009). Redefining extended-spectrum β-lactamases: Balancing science and clinical need. *Journal of Antimicrobial Chemotherapy*, *63*(1), 1–4.

Gomes, T. A. T., Elias, W. P., Scaletsky, I. C. A., Guth, B. E. C., Rodrigues, J. F., Piazza, R. M. F., Ferreira, L. C. S., & Martinez, M. B. (2016). Diarrheagenic Escherichia coli. *Brazilian Journal of Microbiology*, *47*, 3–30. https://doi.org/10.1016/j.bjm.2016.10.015

Goyal, A., Prasad, A., Ghoshal, U., & Prasad, K. N. (2008). Comparison of disk diffusion, disk potentiation & double disk synergy methods for detection of extended spectrum beta lactamases in Enterobacteriaceae. *Indian Journal of Medical Research*, *128*(2), 209–212.

Hendrik, T. C., Voor, A. F., & Vos, M. C. (2015). Clinical and molecular epidemiology of extended-spectrum beta-lactamase-producing Klebsiella spp.: A systematic review and meta-analyses. *PLoS One*, *10*(10), e0140754.

Henshke-Bar-Meir, R., Yinnon, A. M., Rudensky, B., Attias, D., Schlesinger, Y., & Raveh, D. (2006). Assessment of the clinical significance of production of extended-spectrum β-lactamases (ESBL) by Enterobacteriaceae. *Infection*, *34*(2), 66–74.

Hiko, A., Asrat, D., & Zewde, G. (2008). Occurrence of Escherichia coli O157: H7 in retail raw meat products in Ethiopia. *The Journal of Infection in Developing Countries*, *2*(05), 389–393.

Hilmi, B., Bustami, Y., Trongsatitkul, T., & Hamid, Z. A. A. (2019). The Effect of Natural Antimicrobial Agents on Staphylococcus aureus and Escherichia coli Growth. *Journal of Physical Science*, *30*.

Hobson, R. P., MacKenzie, F. M., & Gould, I. M. (1996). An outbreak of multiply-resistant Klebsiella pneumoniae in the Grampian region of Scotland. *Journal of Hospital Infection*, *33*(4), 249–262.

Hosoglu, S., Gundes, S., Kolayli, F., Karadenizli, A., Demirdag, K., Gunaydin, M., Altindis, M., Caylan, R., & Ucmak, H. (2007). Extended-spectrum beta-lactamases in ceftazidime-resistant Escherichia coli and Klebsiella pneumoniae isolates in Turkish hospitals. *Indian Journal of Medical Microbiology*, *25*(4), 346.

Hsueh, P.-R., Ko, W.-C., Wu, J.-J., Lu, J.-J., Wang, F.-D., Wu, H.-Y., Wu, T.-L., & Teng, L.-J. (2010). Consensus statement on the adherence to Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing Guidelines (CLSI-2010 and CLSI-2010-update) for Enterobacteriaceae in clinical microbiology laboratories in Taiwan. *Journal of Microbiology, Immunology and Infection*, *43*(5), 452–455.

Jacoby, G. A. (2009). AmpC β-lactamases. *Clinical Microbiology Reviews*, *22*(1), 161–182.

Jacoby, G. A. (2017). History of drug-resistant microbes. In *Antimicrobial drug resistance* (pp. 3–8). Springer.

Jacoby, G. A., & Han, P. (1996). Detection of extended-spectrum beta-lactamases in clinical isolates of Klebsiella pneumoniae and Escherichia coli. *Journal of Clinical Microbiology*, *34*(4), 908–911.

Jacoby, G. A., & Medeiros, A. A. (1991). More extended-spectrum beta-lactamases. *Antimicrobial Agents and Chemotherapy*, *35*(9), 1697.

Jacoby, G. A., & Munoz-Price, L. S. (2005). The new β-lactamases. *New England Journal of Medicine*, *352*(4), 380–391.

Jahandeh, N., Ranjbar, R., Behzadi, P., & Behzadi, E. (2015). Uropathogenic Escherichia coli virulence genes: Invaluable approaches for designing DNA microarray probes. *Central European Journal of Urology*, *68*(4), 452.

Johnson, J. R., & Stell, A. L. (2000). Extended Virulence Genotypes of *Escherichia coli* Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. *The Journal of Infectious Diseases*, *181*(1), 261–272. https://doi.org/10.1086/315217

Johnson, T. J., Logue, C. M., Johnson, J. R., Kuskowski, M. A., Sherwood, J. S., Barnes, H. J., DebRoy, C., Wannemuehler, Y. M., Obata-Yasuoka, M., & Spanjaard, L. (2012). Associations between multidrug resistance, plasmid content, and virulence potential among extraintestinal pathogenic and commensal Escherichia coli from humans and poultry. *Foodborne Pathogens and Disease*, *9*(1), 37–46.

Johnson, T. J., & Nolan, L. K. (2009). Pathogenomics of the virulence plasmids of Escherichia coli. *Microbiology and Molecular Biology Reviews*, *73*(4), 750–774.

Jones, R. N., Baquero, F., Privitera, G., Inoue, M., & Wiedemann, B. (1997). Inducible β-lactamase-mediated resistance to third-generation cephalosporins. *Clinical Microbiology and Infection*, *3*, s7–s20.

KAÇMAZ, B., ÇAKIR, Ö. F., & AKSOY, A. (2005). Hastane kaynaklı infeksiyonlardan izole edilen Escherichia coli, Klebsiella pneumoniae ve Klebsiella oxytoca türlerinde genişletilmiş spektrumlu beta-laktamaz saptanması. *Ankem Dergisi*, *19*(3), 125–129.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004a). Pathogenic escherichia coli. *Nature Reviews Microbiology*, *2*(2), 123–140.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004b). Pathogenic Escherichia coli. *Nature Reviews Microbiology*, *2*(2), 123–140. https://doi.org/10.1038/nrmicro818

Kaspersen, H. (2015). *Molecular characterization of extended-spectrum beta lactamase producing Klebsiella pneumoniae from the Stavanger region between 2003 and 2012* [Master's Thesis]. University of Stavanger, Norway.

Kirby, W. M. (1944). Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. *Science*, *99*(2579), 452–453.

Koksal, F., Ak, K., Kucukbasmaci, O., & Samasti, M. (2009). Prevalence and antimicrobial resistance patterns of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolated from blood cultures in an Istanbul University Hospital. *Chemotherapy*, *55*(4), 293–297.

Lane, M. C., Alteri, C. J., Smith, S. N., & Mobley, H. L. (2007). Expression of flagella is coincident with uropathogenic Escherichia coli ascension to the upper urinary tract. *Proceedings of the National Academy of Sciences*, *104*(42), 16669–16674.

Lee, C.-C., Lee, N.-Y., Yan, J.-J., Lee, H.-C., Chen, P.-L., Chang, C.-M., Wu, C.-J., Ko, N.-Y., Wang, L.-R., & Chi, C.-H. (2010). Bacteremia due to extended-spectrum-β-lactamase-producing Enterobacter cloacae: Role of carbapenem therapy. *Antimicrobial Agents and Chemotherapy*, *54*(9), 3551–3556.

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A brief overview of Escherichia coli O157: H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*, *20*(1), 5.

Livermore, D. M. (1995). Beta-Lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews*, *8*(4), 557–584.

MacKenzie, F. M., Miller, C. A., & Gould, I. M. (2002). Comparison of screening methods for TEM-and SHV-derived extended-spectrum β-lactamase detection. *Clinical Microbiology and Infection*, *8*(11), 715–724.

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., & Olsson-Liljequist, B. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268–281.

Mandell, D. (2005). *Bennett's principles and practice of infectious diseases. Mandell GL, Bennett JE, Dolin R*. Elsevier Churchill Livingstone.

Massova, I., & Mobashery, S. (1998). Kinship and diversification of bacterial penicillin-binding proteins and β-lactamases. *Antimicrobial Agents and Chemotherapy*, *42*(1), 1–17.

McMichael, A. J. (2001). Human culture, ecological change, and infectious disease: Are we experiencing history's fourth great transition? *Ecosystem Health*, *7*(2), 107–115.

Mehrgan, H., & Rahbar, M. (2008). Prevalence of extended-spectrum β-lactamase-producing Escherichia coli in a tertiary care hospital in Tehran, Iran. *International Journal of Antimicrobial Agents*, *31*(2), 147–151.

Melzer, M., & Petersen, I. (2007). Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing E. coli compared to non-ESBL producing E. coli. *Journal of Infection*, *55*(3), 254–259.

Micek, S. T., Welch, E. C., Khan, J., Pervez, M., Doherty, J. A., Reichley, R. M., & Kollef, M. H. (2010). Empiric combination antibiotic therapy is associated with improved outcome against sepsis due to Gram-negative bacteria: A retrospective analysis. *Antimicrobial Agents and Chemotherapy*, *54*(5), 1742–1748.

Mielke, M. (2010). Prevention and control of nosocomial infections and resistance to antibiotics in Europe–Primum non-nocere: Elements of successful prevention and control of healthcare-associated infections. *International Journal of Medical Microbiology*, *300*(6), 346–350.

Moriel, D. G., Rosini, R., Seib, K. L., Serino, L., Pizza, M., & Rappuoli, R. (2012). Escherichia coli: Great diversity around a common core. *MBio*, *3*(3).

Naas, T., Cuzon, G., Truong, H., Bernabeu, S., & Nordmann, P. (2010). Evaluation of a DNA microarray, the Check-Points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-

spectrum β-lactamases and KPC carbapenemases. *Antimicrobial Agents and Chemotherapy*, *54*(8), 3086–3092.

Nataro, J. P. (2002). Diarrhoeagenic Escherichia coli. In *Molecular medical microbiology* (pp. 1463–1504). Elsevier.

Nordmann, P. (1998). Trends in β-lactam resistance among Enterobacteriaceae. *Clinical Infectious Diseases*, *27*(Supplement_1), S100–S106.

Nowicki, B., Labigne, A., Moseley, S., Hull, R., Hull, S., & Moulds, J. (1990). The Dr hemagglutinin, afimbrial adhesins AFA-I and AFA-III, and F1845 fimbriae of uropathogenic and diarrhea-associated Escherichia coli belong to a family of hemagglutinins with Dr receptor recognition. *Infection and Immunity*, *58*(1), 279–281.

Olson, P. D., Justice, S. S., & Hunstad, D. (2015). *Escherichia coli in urinary tract infections. Molecular Medical Microbiology*. London: Academic Press.

Ong, C.-L. Y., Ulett, G. C., Mabbett, A. N., Beatson, S. A., Webb, R. I., Monaghan, W., Nimmo, G. R., Looke, D. F., McEwan, A. G., & Schembri, M. A. (2008). Identification of type 3 fimbriae in uropathogenic Escherichia coli reveals a role in biofilm formation. *Journal of Bacteriology*, *190*(3), 1054–1063.

Organization, W. H. (2001). *Global strategy for containment of antimicrobial resistance. Geneva: WHO; 2001*. WHO/CDS/CSR/DRS.

Palmer, S., & Parry, S. (2002). *E.coli: Environmental Health Issues of VTEC 0157*. Routledge.

Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: A clinical update. *Clinical Microbiology Reviews*, *18*(4), 657–686.

Pegler, S., & Healy, B. (2007). In patients allergic to penicillin, consider second and third generation cephalosporins for life threatening infections. *Bmj*, *335*(7627), 991–991.

Pennington, H. (2010). Escherichia coli O157. *The Lancet*, *376*(9750), 1428–1435.

Perez, F. (2007). endimiani a, Hujer Km, Bonomo Ra. The continuing challenge of eSBls. *Curr Opin Pharmacol*, *7*(5), 459–469.

Pfaendler, H. R., & Golz, G. (2016). *Fluorescent carbapenems*. Google Patents.

Pitout, J. D., Hossain, A., & Hanson, N. D. (2004). Phenotypic and molecular detection of CTX-M-β-lactamases produced by Escherichia coli and Klebsiella spp. *Journal of Clinical Microbiology*, *42*(12), 5715–5721.

Pitout, J. D., Nordmann, P., Laupland, K. B., & Poirel, L. (2005). Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy*, *56*(1), 52–59.

Poirel, L., Bonnin, R. A., & Nordmann, P. (2012). Genetic support and diversity of acquired extended-spectrum β-lactamases in Gram-negative rods. *Infection, Genetics and Evolution*, *12*(5), 883–893.

Poirel, L., Fernández, J., & Nordmann, P. (2016). Comparison of three biochemical tests for rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae. *Journal of Clinical Microbiology*, *54*(2), 423–427.

Pope, C. F., McHugh, T. D., & Gillespie, S. H. (2010). Methods to determine fitness in bacteria. In *Antibiotic Resistance Protocols* (pp. 113–121). Springer.

Pränting, M. (2010). *Bacterial Resistance to Antimicrobial Peptides: Rates, Mechanisms and Fitness Effects* [PhD Thesis]. Acta Universitatis Upsaliensis.

Preidis, G. A., Hill, C., Guerrant, R. L., Ramakrishna, B. S., Tannock, G. W., & Versalovic, J. (2011). Probiotics, enteric and diarrheal diseases, and global health. *Gastroenterology*, *140*(1), 8.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of Escherichia coli O157: H7 outbreaks, united states, 1982–2002. *Emerging Infectious Diseases*, *11*(4), 603.

Rasko, D. A., Rosovitz, M. J., Myers, G. S., Mongodin, E. F., Fricke, W. F., Gajer, P., Crabtree, J., Sebaihia, M., Thomson, N. R., & Chaudhuri, R. (2008). The pangenome structure of Escherichia coli: Comparative genomic analysis of E. coli commensal and pathogenic isolates. *Journal of Bacteriology*, *190*(20), 6881–6893.

Rawat, D., & Nair, D. (2010). Extended-spectrum β-lactamases in Gram Negative Bacteria. *Journal of Global Infectious Diseases*, *2*(3), 263.

Rawlings, N. D., Barrett, A. J., & Finn, R. (2016). Twenty years of the MEROPS database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Research*, *44*(D1), D343–D350.

Riegman, N., Van Die, I., Leunissen, J., Hoekstra, W., & Bergmans, H. (1988). Biogenesis of F71 and F72 fimbriae of uropathogenic Escherichia coli: Influence of the FsoF and FstFG proteins and localization of the Fso/FstE protein. *Molecular Microbiology*, *2*(1), 73–80.

Robinson, R. K. (2014). *Encyclopedia of food microbiology*. Academic press.

Rosenthal, V. D., Maki, D. G., Jamulitrat, S., Medeiros, E. A., Todi, S. K., Gomez, D. Y., Leblebicioglu, H., Khader, I. A., Novales, M. G. M., & Berba, R. (2010). International nosocomial infection control consortium (INICC) report, data summary for 2003-2008, issued June 2009. *American Journal of Infection Control*, *38*(2), 95–104.

Rota, S., Yalınay-Çırak, M., Kalcancı, A., Gökdal, İ., & Kuştİmur, S. (2000). Comparison of inhibitor potentiated disk diffusion test with double disk synergy test for the detection of extended spectrum beta-lactamases in clinical isolates of Escherichia coli. *İnfeksiyon Dergisi= Turkish Journal of Infection*, *14*(4), 495–498.

Russo, T. A., Davidson, B. A., Genagon, S. A., Warholic, N. M., MacDonald, U., Pawlicki, P. D., Beanan, J. M., Olson, R., Holm, B. A., & Knight III, P. R. (2005). E. coli virulence factor hemolysin induces neutrophil apoptosis and necrosis/lysis in vitro and necrosis/lysis and lung injury in a rat

pneumonia model. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *289*(2), L207–L216.

Russo, T. A., & Johnson, J. R. (2000). Proposal for a new inclusive designation for extraintestinal pathogenic isolates of Escherichia coli: ExPEC. *The Journal of Infectious Diseases*, *181*(5), 1753–1754.

Safdar, N., Handelsman, J., & Maki, D. G. (2004). Does combination antimicrobial therapy reduce mortality in Gram-negative bacteraemia? A meta-analysis. *The Lancet Infectious Diseases*, *4*(8), 519–527.

Sanders, C. C., Barry, A. L., Washington, J. A., Shubert, C., Moland, E. S., Traczewski, M. M., Knapp, C., & Mulder, R. (1996). Detection of extended-spectrum-beta-lactamase-producing members of the family Enterobacteriaceae with Vitek ESBL test. *Journal of Clinical Microbiology*, *34*(12), 2997–3001.

Sarkar, S., Ulett, G. C., Totsika, M., Phan, M.-D., & Schembri, M. A. (2014). Role of capsule and O antigen in the virulence of uropathogenic Escherichia coli. *PloS One*, *9*(4), e94786.

Schoevaerdts, D., Bogaerts, P., Grimmelprez, A., de Saint-Hubert, M., Delaere, B., Jamart, J., Swine, C., & Glupczynski, Y. (2011). Clinical profiles of patients colonized or infected with extended-spectrum beta-lactamase producing Enterobacteriaceae isolates: A 20 month retrospective study at a Belgian University Hospital. *BMC Infectious Diseases*, *11*(1), 12.

Schwaber, M. J., & Carmeli, Y. (2007). Mortality and delay in effective therapy associated with extended-spectrum β-lactamase production in Enterobacteriaceae bacteraemia: A systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy*, *60*(5), 913–920.

Schwaber, M. J., Navon-Venezia, S., Kaye, K. S., Ben-Ami, R., Schwartz, D., & Carmeli, Y. (2006). Clinical and economic impact of bacteremia with extended-spectrum-β-lactamase-producing Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*, *50*(4), 1257–1262.

Schwartz, D. C., & Cantor, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell*, *37*(1), 67–75.

Seib, K. L., Zhao, X., & Rappuoli, R. (2012). Developing vaccines in the era of genomics: A decade of reverse vaccinology. *Clinical Microbiology and Infection*, *18*, 109–116.

Shanahan, A. J., & Tanner, F. W. (1948). Further Studies on the Morphology of Escherichia coli Exposed to Penicillin. *Journal of Bacteriology*, *55*(4), 537–544.

Soltani, S., Emamie, A. D., Dastranj, M., Farahani, A., Davoodabadi, A., & Mohajeri, P. (2018). Role of toxins of uropathogenic Escherichia coli in development of urinary tract infection. *Journal of Pharmaceutical Research International*, 1–11.

Spanu, T., Luzzaro, F., Perilli, M., Amicosante, G., Toniolo, A., Fadda, G., & Group, I. E. S. (2002). Occurrence of extended-spectrum β-lactamases in members of the family Enterobacteriaceae in Italy: Implications for resistance to β-lactams and other antimicrobial drugs. *Antimicrobial Agents and Chemotherapy*, *46*(1), 196–202.

Spanu, Teresa, Sanguinetti, M., Tumbarello, M., D'Inzeo, T., Fiori, B., Posteraro, B., Santangelo, R., Cauda, R., & Fadda, G. (2006). Evaluation of the new VITEK 2 extended-spectrum beta-lactamase (ESBL) test for rapid detection of ESBL production in Enterobacteriaceae isolates. *Journal of Clinical Microbiology*, *44*(9), 3257–3262.

Stürenburg, E., Sobottka, I., Noor, D., Laufs, R., & Mack, D. (2004). Evaluation of a new cefepime–clavulanate ESBL Etest to detect extended-spectrum β-lactamases in an Enterobacteriaceae strain collection. *Journal of Antimicrobial Chemotherapy*, *54*(1), 134–138.

Sweeney, N. J., Klemm, P., McCormick, B. A., Moller-Nielsen, E., Utley, M., Schembri, M. A., Laux, D. C., & Cohen, P. S. (1996). The Escherichia coli K-12 gntP gene allows E. coli F-18 to occupy a distinct nutritional niche in the streptomycin-treated mouse large intestine. *Infection and Immunity*, *64*(9), 3497–3503.

Szmolka, A., & Nagy, B. (2013). Multidrug resistant commensal Escherichia coli in animals and its impact for public health. *Frontiers in Microbiology*, *4*, 258.

Taneja, N., & Sharma, M. (2008). ESBLs detection in clinical microbiology: Why & how? *Indian Journal of Medical Research*, *127*(4), 297.

Tenaillon, O., Skurnik, D., Picard, B., & Denamur, E. (2010). The population genetics of commensal Escherichia coli. *Nature Reviews Microbiology*, *8*(3), 207–217.

Thomson, K. S., & Sanders, C. C. (1992). Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: Comparison of the double-disk and three-dimensional tests. *Antimicrobial Agents and Chemotherapy*, *36*(9), 1877–1882.

Tünger, A., Hilmioğlu, S., Dibek, M. A., Çavuşoğlu, C., Aktaş, L., Özkan, F., & Özinel, M. A. (1998). Hastane infeksiyonu etkeni olarak soyutlanan Klebsiella pneumoniae ve Escherichia coli kökenlerinde genişlemiş spektrumlu beta-laktamaz sıklığı. *İnfeksiyon Derg*, *12*, 165–8.

van der Bij, A. K., & Pitout, J. D. (2012). The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, *67*(9), 2090–2100.

Van Duijn, P. J., Dautzenberg, M. J., & Oostdijk, E. A. (2011). Recent trends in antibiotic resistance in European ICUs. *Current Opinion in Critical Care*, *17*(6), 658–665.

Vásquez-Jaramillo, L., Ramírez, N. F., Akineden, Ö., & Fernández-Silva, J. A. (2017). Presence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in bulk-tank milk of bovine dairy farms in Antioquia, Colombia. *Revista Colombiana de Ciencias Pecuarias*, *30*(2), 85–100.

Walsh, C. (2003). *Antibiotics: Actions, origins, resistance.* American Society for Microbiology (ASM).

Wang, L., Rothemund, D., Curd, H., & Reeves, P. R. (2003). Species-wide variation in the Escherichia coli flagellin (H-antigen) gene. *Journal of Bacteriology*, *185*(9), 2936–2943.

Welch, R. A., Burland, V., Plunkett, G., Redford, P., Roesch, P., Rasko, D., Buckles, E. L., Liou, S.-R., Boutin, A., & Hackett, J. (2002). Extensive mosaic structure revealed by the complete genome sequence of uropathogenic Escherichia coli. *Proceedings of the National Academy of Sciences*, *99*(26), 17020–17024.

Winberg, J. (1984). P-fimbriae, bacterial adhesion, and pyelonephritis. *Archives of Disease in Childhood*, *59*(2), 180.

Winokur, P. L., Canton, R., Casellas, J.-M., & Legakis, N. (2001). Variations in the prevalence of strains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. *Clinical Infectious Diseases*, *32*(Supplement_2), S94–S103.

Yilmaz, E., Akalin, H., Özbey, S., Kordan, Y., Sinirtaş, M., Gürcüoglu, E., Özakin, C., Heper, Y., Mistik, R., & Helvaci, S. (2008). Risk factors in community-acquired/onset urinary tract infections due to extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae. *Journal of Chemotherapy*, *20*(5), 581–585.

Yoon, J. W., & Hovde, C. J. (2008). All blood, no stool: Enterohemorrhagic Escherichia coli O157: H7 infection. *Journal of Veterinary Science*, *9*(3), 219–231.

Yoon, S. H., Jeong, H., Kwon, S.-K., & Kim, J. F. (2009). Genomics, Biological Features, and Biotechnological Applications of Escherichia coli B: “Is B for better?!” In S. Y. Lee (Ed.), *Systems Biology and Biotechnology of Escherichia coli* (pp. 1–17). Springer Netherlands. https://doi.org/10.1007/978-1-4020-9394-4_1

Zafar, S., Naqvi, S. B. S., Abbass, T., Qazi, F., & Sheikh, R. (2014). Resistance pattern in clinical isolates of Eschericia coli against four groups of antibiotics. *International Research Journal of Pharmaceutical and Applied Sciences*, *4*(1), 100–106.

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Educational Level

	Name of the Institution where was graduated	**Graduation year**
Postgraduate/Specialization	Near East University, Northern Cyprus.	2020
Masters	Near East University, Northern Cyprus.	2020
Undergraduate	Jordan University of Science & Technology (JUST), Irbid-Jordan.	2019
High school	Majid Abu Sharar School, Hebron-Palestine.	2014

Masters Thesis	
Title:	Investigation of Three Different Methods of Extended Spectrum Beta-Lactamases in *E. coli* Strains Isolated from Various Clinical Samples.
Supervisor:	Assoc. Prof. Dr. Meryem GÜVENIR

Job Experience

Duty	**Institution**	**Duration (Year - Year)**
Medical Laboratory technician	Prince Rashed Bin Al Hasan Military Hospital, Irbid- Jordan	Feb 2019 – July 2019
Medical Laboratory technician	Ibn Al-Nafis hospital, Irbid-Jordan	For Six months

Courses and Certificate

Name	Name of the Institution where take place	year
Medical Lab Technician Trainee Certificate	King Hussein Medical Center, Jordan	2019
Mathematical Modeling in Health Certificate (Attendance)	DESAM Institute, Near East University, Nicosia	2019
Parasitology Academic Course: Essential and Application	Turkish Microbiology Society, TMC-KKTC Microbiology Platform, Nicosia	2020

Foreign Languages	Reading comprehension	Speaking *	Writing*
English	Excellent	Excellent	Excellent

Foreign Language Examination Grade

YDS	ÜDS	IELTS	TOEFL IBT	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE
•			•	•				

Computer Knowledge

Program	Use proficiency
SPSS	Excellent
Python Programming Language	Excellent
Common Computer Programs and Skills	Excellent