ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Mariam HASSAN MOHAMED

INVESTIGATION OF OCCURENCE OF AUTOTRANSPORTER GENES IN *Escherichia coli* ISOLATED FROM CLINICAL AND FOOD SOURCES AND IDENTIFICATION OF PHYLOGENETIC RELATIONSHIP OF *E.coli* STRAINS BY USING PFGE METHOD

DEPARTMENT OF BIOTECHNOLOGY

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We certify that the thesis titled above was reviewed and approved for the award of the degree of the Master of Science by the board of jury on20/06/2018.

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ABSTRACT

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The autotransporter proteins constitute one of the most important cell components responsible for pathogenicity in bacteria and are frequently transferred as mobile genetic elements from different bacteria and allow the host cell to transfer new virulence genes. The purpose of this study was to identify the presence, distribution and virulence potential of autotransporter genes that are effective in the protection of the bacteria against host defence systems. Therefore, autotransporter genes have also been questioned the prevalence in the surveillance of *E.coli* strains and in the distribution of food among the people of the ExPEC strains, such as UPEC or whether or not there were porter roles. Sixty-five *E.coli* strains isolated from non-intestinal system clinical specimens and thirty-five *E.coli* strains isolated from the food sources. A total of 100 *E.coli* isolates were used in this study. The prevalence of the autotransporter-related genes was determined in the *E.coli* strains by using the PCR method which was as follows: Agn43 15%; Tsh 1%; UpaJ 8% and UpaC 6%. While that other genes such as Sat, Vat and UpaB genes could not be detected. It was used PFGE method for the detection of clonal relationships and was found in 6 (6.8%) out of 89 isolates of human and food isolates had a clonal relationship. In conclusion, *E.coli* strains isolated from clinical specimens were found to have higher prevalence autotransporter genes than *E.coli* strains isolated from food samples. However, the specific distribution of autotransporter among *E.coli* strains has not established yet.

Key words: *Escherichia coli*, Autotransporter, clinical, food, PCR, PFGE.

ÖZ

YÜKSEK LİSANS TEZİ

GIDA KAYNAKLI VE KLINIK ÖRNEKLERDEN İZOLE EDILEN *ESCHERICHIA COLI* SUŞLARINDA OTOTRANSPORTER PROTEINLERIN OLUŞUMUNUN ARAŞTIRILMASI VE FILOGENETIK İLIŞKILERININ PFGE ILE TESPITI

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Ototransporter sistemler, bakterilerde patojeniteden sorumlu en önemli hücre elemenlarından birisi olup, sıklıkla farklı bakterilerden mobil genetik elemanlar olarak transfer edilirler ve konak hücrenın yeni virülans genlerini transfer edebilmesine imkân sağlarlar. Bu çalışma, *E.coli* suşlarına gerek metabolik faliyetlere katılan gerekse konak savunma sistemlerine karşı bakterinin korunmasında rol olan ototransporter genlerinin varlığı, dağılımı ve virülanspotensiyel katkılarınıortaya çıkartma amacı ile planlanmıştır. Böylece ototransporter genlerinin, *E.coli* suşlarının süreveyansındaki önemi ve gıdaların UPEC gibi ExIEC suşlarının insanlar arasında dağılımında rezervuar veya portör rollerinin olup olmadığıda sorgulanmıştır. Çalışmada intestinal sistem dışı klinik örneklerden izole edilmiş altmış beş ve gida örneklerindenizole edilmiş otuz beş *E.coli* suşu olmak üzere toplam 100 *E.coli* izolatıörnek olarak kullanılmıştır. PCR yöntemi ile yapılan bu genlerin prevalansı;Agn43 için %15,Tsh için % 1, UpaC için %6 ve UpaJ icin %8 olarak tespit edilmiştir. Buna karşılık Sat, Vat ve UpaB genlerine ait hedef gendizilerinin varlığı gösterilememiştir. *E.coli*suşları arasındaki genotipik ilişkinin gösterilmesi için PFGE metodu kullanılmıştır. İnsan ve gıda kaynaklı 89 izolatın 6'sında (%6. 8) klonal anlamlı bir ilişki bulunmadığı görülmüştür. Sonuç olarak klinik örneklerden izole edilen *E.coli* suşlarının gıda örneklerinden elde edilen suşlara göre daha yüksek prevalansa sahip olduğu belirlenmiştir. Bununla birlikte bazı ototransporterlerin. *E.coli* suşlarının çeşitliliği arasındaki spesifik dağılımı henüz belirlenmemiştir. Ototransporterin virülans faktörü ile olan ilişkisini tanımlamak ve anlamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: *E.coli*, ototransporter, gida, klinik, PCR, PFGE

GENİŞLETİLMİŞ ÖZET

Gıda üretim teknolojilerinde son 30 yılda üretim alanından sofraya kadar yaşanan değişiklikler, yeni gen kazanımları ile virülansları artmış, yeniden önem kazanan, birçok mikroorganizmanın üretimin farklı aşamalarında gıda kaynaklarını kontamine ederek gıda-kaynaklı enfeksiyonlara yol açabilmesine sebep olmuştur. Gıdaları kontamine eden bu mikroorganizmalar, bir taraftan besinler içerisinde üreme esnasında ürettikleri adezinler ile gıdaların kalite ve raf ömrünü olumsuz yönde etkilerken diğer taraftan da oluşturdukları ciddi enfeksiyonlar ile tüketici ve halk sağlığı için büyük tehdit oluşturmaktadır. *E.coli* intestinal florada yer alan majör gram negatif basildir. Barsaklarda kolay gen kazanabilen *E.coli,* gıda kökenli enfeksiyonlar içinde önemli bir tehdittir. Bu virülan türlerden bazılarının kendi kendisini sonlandırabilen basit sekretuvar tip ishallelerin yanı sıra, hemorajik kolit ve toksik mega kolon gibi daha ağır patolojilere ve hemolitik üremik sendrom gibi intestinal sistem dışı komplikasyonlara yol açabildikleri gösterilmiştir. *E.coli* türlerinin hastalık oluşturabilme özellikleri; transpozanlar gibi mobil genetik elemanlar ile taşınabilen adezinleri kodlayan genleri mikro-çevreden kazabilme yeteneklerine bağlıdır.

Günümüzde artan nüfusun beslenmesi, yerel kaynak ve kültürel farklılıklardan kaynaklanan gıda üretim çeşitliliğinin yanı sıra yeni beslenme zevk ve alışkanlıklarına da cevap verecek düzeyde kompleks üretim teknolojilerinin yaratılmasını gerekli kılmıştır. Özellikle endüstrileşmiş ülkelerin gıda ihtiyaçlarının önemli bir bölümünü maliyetlerdeki düşüklük sebebi ile gelişmekte olan ülkelerdeki üretim kaynaklarından karşılama çabaları, gıda güvenliği ve gıda yolu ile bulaşan hastalıklar konusunda üretimin kontrolü kadar, patojen tanımı ve bulaş yollarının araştırılması konusunda da çaba sarf edilmesi gerekliliğini ortaya koymuştur (Perna ve ark,. 2001).Bu problemin çözümü, gıda mikrobiyologları da dahil gıda üreticisinden işletmeciye kadar gıda işletme zinciri içerisinde yer alanların sorumluluk duygusu içerisinde çalışmaları ile kontrol edilebilir (Delicato

ve ark.,2002).Gıda kaynaklarının patojen mikroorganizmalar ile kontaminasyonu ve kontaminant mikroorganizmaların besinler içerisinde/üzerinde kolonize olarak çoğalması esnasında ürettikleri toksik metabolitler ve toksinler, bir taraftan gıdanın kalitesini etkilerken diğer taraftan tüketici ve halk sağlığı için büyük tehdit oluşturmaktadır (Kaper ve ark.,2004).Gıda yolu ile bulaşan hastalıklar ekonomik büyümenin önündeki en önemli sebeplerden birisi olup gelişmişlik farkı gözetmeksizin bütün toplumları etkileyen uluslararası bir problemdir. Mesela ABD'de (2005) her yıl yaklaşık olarak 76 milyon kişi gıda kökenli enfeksiyonlardan etkilenmekte ve bu sebeple 325,000 kişi hastanelerde takip edilmekte ve bu hastalardan da 5.200'ü kaybedilmektedir. Avrupa birliği ülkelerinde enfeksiyon insidansı 320.000 olarak verilmiş ancak gerçek rakamın bu sayının kat kat fazlası olduğu ileri sürülmüştür (Anon.,2011). Gıda kökenli enfeksiyonların yine ABD için yıllık maliyetinin 7 milyar dolar civarında olduğu aynı araştırmada bildirilmiştir. Bu rakam sadece tanısı konmuş etiyolojisi tespit edilebilen *Salmonella, Campylobacter, Bacillus cereus, E. coli* ve *Listeria* gibi 5 patojene bağlı gıda kökenli enfeksiyonlara aittir. Genel olarak tanı zorluğu sebebi ile hastaların ve hastalıklara bağlı kayıpların %50 kadarı "sebebi bilinmeyenler" olarak kaydedilmektedir .

Escherichia coli (*E. coli*) insan ve hayvanların intestinal florasında yer alan majör gram negatif, fermentatif, oksidaz negatif basildir (Patel, ve ark., 2004). Bu sebeple *E. coli*, gaita kontaminasyonunun önemli olduğu sanitasyon uygulamalarında indikatör mikroorganizmadır ve gaita ile bulaşması mümkün olan bir çok patojen mikroorganizmanın bulunabileceğinin göstergesidir (Leyton ve ark., 2012). Bununla birlikte özel şartlarda bazı özel *E. coli* suşları yüksek morbidite ve mortalitenin de sebebidir. Çoğu barsaklarda kommensal veya simbiotik olarak yaşayan yaklaşık olarak 700 den fazla serotipi olan *E. coli* suşları, antijenik özelliklerinin yanı sıra klinik-patolojik özellikleri ile de sınıflandırılmıştır (Ieva ve ark, 2009) . Pato-tipleme olarak da tanımlanan bu sınıflandırmada *E. coli* suşları, kommensalller, intestinal patojenler (InPEC) ve intestinal sistem dışı

patojenler (ExPEC) olmak üzere 3 grupta toplanmıştır (Al-Hasani ve ark.,2001). Klinik patolojik özelliklere dayalı bu sınıflamada *E. coli* suşları için belirleyici olan, sahip oldukları ve kazandıkları virülans faktörlerinin tür ve sayısıdır. *E. coli'*nin farklı serotipleri, EPEC ve EHEC gibi InPEC ve ExPEC suşlarının insanlardaki kolonizasyonu ile ilişkili moleküler mekanizmaları tam olarak aydınlatılamamıştır. InPEC patotipi içerisinde yer alan ve son yıllarda adı gıda kökenli enfeksiyonlar ile en sık anılan Shiga toksijenik *E. coli* suşları, shiga-like toxin 1 (stx1), shiga-like toxin 2 (stx2) olarak tanımlanan ve insanlarda intestinal sistem patolojilerine yol açan potent bir toksin geni taşırlar (Parham ve ark., 2004) . Bu suşlar ishalle seyreden intestinal patolojilerin yanı sıra hemorajik kolit ve toksik mega kolon gibi ağır intestinal sistem ve hemolitik üremik sendrom gibi intestinal sistem dışı komplikasyonlara yol açarlar. Epidemiyolojik çalışmalar HUS vakalarının %5-10'undan EHEC suşlarının sorumlu olduğunu göstermiştir. Bu suşlar intestinal toksin genlerinin yanı sıra patojeniteden sorumlu hücre kısımlarında kodlanmış, enterosit effacement lokusu (LEE) ve ototransporterlar gibi etkili olabilen virülans proteinlerinin mikroçevre ve/veya hedef hücre sitozolüne gönderilmesinde etkili olan salgı sistemlerine sahiptir. Ototransporter sistemler, yani patojeniteden sorumlu hücre kısımlarında,mobil genetik elemanlar olup ,diger mikroganizmalardan transfer edilirler ve konak hücrenın yeni virülans genlerini transfer edebililer (Ruiz-Perez ve ark., 2011). Bu sebeple bu sekresyon veya transport sistemleri *E.coli* suşları da dâhil birçok mikroorganizma için virülans belirleyicisidir. *E.coli* O157:H7'nin rezervuarı büyük ve küçükbaş hayvanlardır. Ancak kanatlılar, geyikler ve domuzlar da kontaminasyon kaynağıdır. Etler sıklıkla kesim esnasında karkasın enfekte hayvan gaitası ile teması sonucu kontamine olur. Diğer EPEC, ETEC, ve EIEC gibi diarejenik *E. coli* ve O157:H7 dışı EHEC suşlarının gıda kontaminasyonları da benzer şekilde yani enfekte insan ve/veya hayvan gaitası ile temas sonucu şekillenir (Maroncle ve ark.,2006) .

Gıda kökenli enfeksiyonların en önemli etkenleri olan EPEC ve EHEC gibi InPEC ve ExPEC suşlarının insanlardaki kolonizasyon ile ilişkili moleküler mekanizmalar tam olarak aydınlatılamamıştır. EPEC ve EHEC suşlarında kolonizasyon ve virülans ile kuvvetli ilişkisi olduğu düşünülen LEE, bir T3SS'de kodlanmaktadır. Ancak bu iki suşa ait toksini fizyolojik olarak farklı kılan belirleyici proteinlerin yapısı ve önemleri bilinmemektedir.

Son yıllarda birçok gram negatif bakteriyel patojenin hücre yüzeyinde tanımlanmış olan Tip 5 ototransporter sistem proteinlerinin ekzotoksinlerle sıkı şekilde bağımlı mekanizmalar olduğu ve hedef hücre sitozolüne veya mikroçevreye transferindeki rolleri tartışılmaya başlanmıştır. Literatürde ototransporter proteinleri (AT)fonksiyonel olarak 3 büyük grup içerisinde toplanmışlardır. Bunlar; *Enterobacterecea* serin proteaz AT proteinleri (SPATEs), AIDA-1 tip AT proteinleri ve Trimerik AT adezin proteinleridir ((Anderson ve ark., 2003).

Çalışmamızda, et, sebze-meyve ve tavuk gibi gıda kaynaklarından izole edilen toplam otuz beş (35) *E. coli* suşu ve intestinal sistem dışı klinik örnekleri olarakkan, idarörneklerden izole edilmiş altmış beş (65) *E.coli* suşuolmak üzere toplamda 100 *E.coli* izolatı konak hücreye adezyon invazyonu arttırarak virülansa katkı sağlayan ototransporter adeziv proteinleri açısından değerlendirilmek üzere kullanılmıştır. PCR yöntemi ile yapılan bu genlerin prevalansı; Agn43 için %15,Tsh için % 1, UpaC için %6 ve UpaJ icin %8 olarak tespit edilmiştir. Buna karşılık Sat, Vat ve UpaB genlerine ait hedef gen dizilerinin varlığı gösterilememiştir. *E. coli* suşları arasındaki genotipik ilişkinin gösterilmesi için PFGE metodu kullanılmıştır. İnsan ve gıda kaynaklı 89 izolatın 6'sında (%6.8) klonal anlamlı bir ilişki bulunmadığı görülmüştür. Sonuç olarak klinik örneklerden izole edilen *E. coli* suşlarının gıda örneklerinden elde edilen suşlara göre daha yüksek prevalansa sahip olduğu belirlenmiştir. Bununla birlikte bazı ototransporterlerin. *E. coli* suşlarının çeşitliliği arasındaki spesifik dağılımı henüz belirlenmemiştir. Ototransporterin virülans faktörü ile olan ilişkisini tanımlamak ve anlamak için daha fazla çalışmaya ihtiyaç vardır.

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LIST OF SYMBOLS AND ABBREVIATONS

AT	: Autotransporter
Agn43	: Antigen 43
°C	: Degree Celcius
DNA	: Deoxyribonucleic acid
E. coli	: *Escherichia coli*
EDTA	: Etilendiamin tetraasetik asit
EHEC	: Enterohemorrhagic *E. coli*
EIEC	: Enteroinvasive *E. coli*
EMB	: Eosin Methylene Blue
EPEC	: Enteropathogenic *E. coli*
ETEC	: Enterotoxigenic *E. coli*
ExPEC	: Extra-intestinal pathogenic *E. coli*
FLSB	: Fluorocult Lauryl Slfate Broth
IMVIC	: Indole, Methyl red, Voges-Proskauer and Citrate
MPN	: Most probable number
NCBI	: The National Center for Biotechnological Information
PCR	: Polymerase Chain Reaction
PFGE	: Pulse field gel electrophoresis
SAT	: Serine auto-transporter toxin
SPATE	: Serine Protease Autotransporters from Enterobacteriaceae
TA	: Trimeric Autotransporters
TBE	: Trismabase, Boric Acid, EDTA
TE	: Tris-HCL, EDTA
Tsh	: temperature sensitive hemagglutinin
UPEC	: Uropathogenic *E. coli*
VAT	: Vacuolating autotransporter toxin

1. INTRODUCTION

Diseases transmitted through food is an international problem, that affects all societies regardless of disparities and it is one of the most important cause in front of economic growth. According to the research aetiology diagnosed with *Salmonella, Campylobacter, Bacillus cereus, E.coli and Listeria* belong to foodborne infections such as 5 pathogen depends(Rodriguez-Siek et al., 2005). Bacteria, viruses, parasites, and toxins can be main cause more than 250 known foodborne diseases because of food contamination (Figure 1. 1) and this continues to be a public health problem across the World(Ahmed and Hall, 2014). Overall diagnostic difficulties caused by up to 50% of losses due to patients and diseases "of unknown cause" to be recorded (Scallan, et al. 2011).

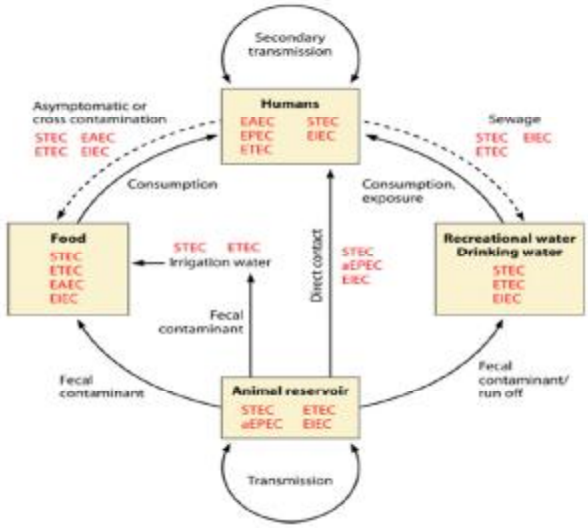

Figure 1.1. Reservoirs and Transmission modes of *E.coli* (Croxen, et al., 2013).

In a various animal, reservoirs can be found Pathogenic *E. coli* strains that can distribute from animal reservoirs and other animals. It can contaminate food, irrigation water or drinking water. Humans can become exposed

via contaminated food or water or through direct contact with animals ((Dho-Moulin et al., 1999). What about secondary transmission it can occur between humans, usually in day care centres or nursing homes. In other hand food can be caused by contamination through poor cooking practice, such as in contact with other food and uncooked meat could cause a contamination. But also contamination of food can expose symptomatic or asymptomatic food handlers especially when hand hygiene is inadequate for hygiene. It is well accepted that bacteria such as ***E.coli*** can move passively to humans through a variety of routes, including the food chain. Because of the similarity between some human and animal E.coli. Similarities in strains isolated from different host species should be expected, particularly with respect to specific virulence factors (VF). These VF are important to ExPEC survival in these extraintestinal environments. Extraintestinal pathogenic ***Escherichia coli*** (ExPEC) strains are the cause of most community and hospital-acquired extraintestinal E. coli infections, including infections of the urinary tract, bloodstream, and other anatomical sites (Russo et al., 2003).

In general, across the world, foodborne infection is classically known was held responsible for few Bacteria, Viruses and Parasites. In the last 30 years, Food production depends at every stage changes with a lot of microorganisms regain importance with new acquisitions or new gene was revealed to be associated with different diseases (Rodriguez-Siek et al., 2005). Again in no doubt that the most important microorganisms winning prefix with strong hardware such as transport systems can acquire new genes virulence from ***E. coli*** (Riley et al., 1983).

These microorganisms in food production laboratories sensitive techniques used in the western world was recognized as a major foodborne pathogen in the 1990s. These strains of food contamination and disease with the ability to create plasmids, bacteriophages and transpose those days like toxin encoded on mobile genetic elements, as they won the virulence genes adhesins and invade. These gains, irrational and implementations in human procure a various mutation and become responsible for the introduction of antibiotic resistance. Indeed, food

originated in infections of *E.coli* O157H7 not limited to this serotype O7: H10, O22: H16, O111: H (-), O119: H (-), O174: H21, O26: H11, O113: H21, O118: H16 O123: H11 and O177: H11 as Shiga toxicogenic E. coli winning strains with diarrheagenic *E. coli* strains in food derived responsibility in increasing the disease, which is indispensable for the human nutrition, especially undercooked beef, including meat, chicken, fruits, vegetables, they were isolated from acquired infections.

1.1. Escherichia coli

At the end of the 19th century, E.coli was discovered as "Bacterium Coli Commune" by Theodor Escherich German bacteriologist and paediatrician (1857 - 1911). *E.coli* has a rod-shaped and it is a gram-negative and facultative anaerobic bacterium which belongs to the Enterobacteriaceae family of gamma-proteobacteria. The family of Enterobacteriaceae consists of more than forty different genera (amongst others *Citrobacter, Salmonella, Klebsiella, Shigella, Erwinia, Proteus, Yersinia, Enterobacter and Escherichia*) containing commensal or a pathogenic as well as pathogenic strains. *Escherichia coli* are usually a commensal resident of the intestinal microflora as well as a pathogen causing various human and animal diseases by the acquisition of virulence factors (VFs), (Dobrindt et al., 2001).

Uropathogenic *Escherichia coli* (UPEC) encodes a variety of genetic determinants that are associated with virulence (Guyer et al, 2000). Most strains of *E. coli* are harmless and colonize as normal flora of the gut of warm-blooded humans and animals and these strains can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria (Reid et al., 2001; Bentley et al.,,1982). However, some *E. coli* strains have several factors and serotype virulence, which make *E. coli* as the cause of serious and various diseases.

1.1.1. *Escherichia coli* morphologic and genome structure

E. coli is a gram-negative, straight, non-spore forming rod (1.1- 1.5 µm x 2.0- 6.0µm) (Quim et al., 1994). *E. coli* is facultatively anaerobic and grows at 37°C. *E. coli* colonies usually grow rapidly 24 hours incubation and are relatively large on blood agar. On blood agar, the colonies are shiny, grey, round, smooth, rarely mucoid and some strains are hemolytic. *E. coli* colonies are bright pink on MacConkey agar, because of *E. coli* can ferment lactose in agar and produce acidic metabolic products which decrease the pH in agar to be bright pink. A characteristic 'coliform' (faecal) smell can be noted. The term 'coliform' refers to members of Enterobacteriaceae which can ferment lactose such as, *Klebsiella, E. coli and Enterobacter*. *E. coli* colonies are a metallic sheen on EMB (Eosin methylene blue) agar and this characteristic is unique for *E. coli* colonies, so EMB is usually used for identification of *E. coli* (Bettelheim, 1994). The biochemical and fermentation tests are used for identification of *E. coli* (Gyles et al., 2008). Characteristically, *E. coli* is positive in the catalase test, negative in cytochrome oxidase test and ferment glucose (carbohydrates) to produce gas and acid (Holt et al., 1994). The production of indole, fermentation of lactose, negative KCN, gelatin and malonate reactions are important tests which differentiate *E. coli* from other general (Martin, 2000). The IMVIC (indole, methyl red, Voges –Proskauer, citrate) is a quick and conventional method to the identification of *E. coli* because almost no other lactose positive member of the family Enterobacteriaceae produces this combination of results (Quim et al.,1994). The *E. coli* genome is around 4. 6 and 5.5 × 106 bp in size found in all *E. coli* strains and it can be well-known into a core genome. Clearly, the core genome embraces the so-called housekeeping genes, encoding for proteins of translation, transcription and replication, and the basic metabolism genes. Furthermore, some studies recommended that some genes of the core genome are important in the lifestyle (Dobrindt et al., 2002; Touchon et al., 2009).

If a GEI includes virulence genes and if this GEI is present in the genome of a pathogenic *E.coli*, but absent in non-pathogenic strains, this GEI is referred to as a pathogenicity island (PAI) (Dobrindt, 2005; Dobrindt et al., 2001).

E. coli has a highly versatile species because of substantial genome plasticity and this gives an ability to constantly alter its genome content by Horizontal Gene Transfer (HGT) and to provide adaptation, fitness and competitiveness by deletion events to different growth conditions and habitats.

Although, *Escherichia coli* can be an innocuous resident of the gastrointestinal tract, additionally, *E. coli* has the pathogenic capacity to be mainly responsible for diarrheal. Extraintestinal diseases can be the main cause of public health problems as they have low infectious doses and are transmissible via ubiquitous mediums, including food and water as shown in Figure 1. 1. Nowadays, Pathogenic variants of *E.coli* procure much morbidity and mortality across the world. Consequently, pathogenic *E.coli* is deeply studied in a different area such as humans, animals, food and the environment. While there are many common details that these pathotypes utilize to colonize the intestinal mucosa and cause disease and complications vary significantly. But the seriousness of pathogenic *E.coli* is demonstrated by national and international surveillance programs; unfortunately, this surveillance is often lacking in many countries.

1.1.2. Different catagorie of *E. coli*

The three major categories of *E. coli* strains are diarrheagenic *E.coli*, commensal *E.coli,* and extraintestinal pathogenic *E.coli* (ExPEC) (Kaper J. B et al., 2004; Schneider et al., 2004).

Uropathogenic *Escherichia coli* (UPEC) are associated with virulence because of encoding a variety of genetic determinants (Guyer et al., 2000). VFs often located on pathogenicity islands (PAIs) or smaller inserts defined specific pathotypes, a successful combination of VFs occur it can cause a broad spectrum of diseases in healthy individuals (Kaper et al., 2004). Most intestines commensal or

symbiotic approximately living in the more than 700 serotypes of *E. coli* strains are classified with clinicopathological features, as well as antigenic properties. Serotyping strains of *E.coli* as defined in the classification, Commensal, intestinal pathogens (InPEC) and non-intestinal tract pathogens (ExPEC) (Table 1.1) is divided into 3 groups depending on the site of infection intestinal pathogenic *E.coli* (InPEC), extraintestinal *E.coli* (ExPEC), and commensal *E.coli* (Kaper et al.,2004, Russo et al.,2003). Different types of diarrheal diseases can be caused by Diarrheagenic*E.coli* strains and those are among the most important enteric bacterial pathogens (Nataro et al., 1998). Members of the normal intestinal flora of humans, other mammals are called commensal *E.coli* strains (Dho-Moulin et al., 1999). Certain commensal intestinal isolates are capable of causing infections in extraintestinal tissues, and these pathogenic strains have been collectively termed ExPEC (Russo et al., 2000). Among ExPEC, human urinary tract infections (UTIs) is caused most common by uropathogenic *E. coli* (UPEC) (Marrs et al., 2005). Avian pathogenic *E. coli* (APEC) strains to share some virulence traits with ExPEC strains from human infections and are associated with extraintestinal infections of poultry (Rodriguez-Siek et al., 2005, Ron , 2006).

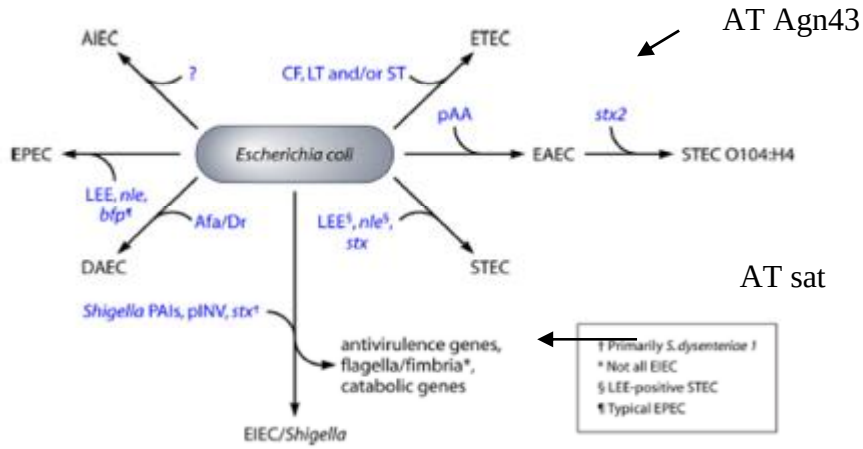

Figure 1.2. Pathogenic of *E.coli* (Croxen et al., 2013)

Acquisition of genes transfers often from mobile elements such as transposons, prophages, and plasmids. Based on clinical pathological features in this classification which is decisive for the *E. coli* strains, and that they have gained virulence factor is the type and number. Commensal, intestinal pathogens (InPEC) area within the pathotype and last year with the name of food-borne infections are the most commonly cited Shiga toxigenic *E. coli* strains, Shiga-like toxin 1 (stx1), Shiga-like toxin 2 (stx2) as defined and lead to intestinal tract pathology in humans potent carry a toxin gene. Moreover, some special conditions specific *E. coli* strain is caused by a large field of morbidity and mortality. These strains showed that it was responsible for diarrhoea as well as intestinal pathology hemorrhagic colitis and toxic megacolon as a severe intestinal system and hemolytic uremic syndrome.

In these systems, namely in pathogenicity islands, are mobile genetic elements, are transferred from other organisms and they can transfer their virulence genes (Al-Hasani et al., 2001). Therefore, secretion or transport systems in E.coli strains were included is a virulence determinant for microorganisms. *E.coli* O157: H7 reservoirs are large and small animals. But poultry is a source of contamination in deer and pigs (Delicato et al., 2002). Meat carcass during slaughter often become contaminated as a result of contact with infected faces of animals. Other EPEC, ETEC, and as EIEC diarrheagenic *E. coli* contamination of food in a similar manner that infected humans and/or animals are shaped by contact with the stool (Patel et al., 2004).

Besides the specific pathotypes (Table 1.1), the major categories are: depending on the site of infection intestinal pathogenic *E. coli* (IPEC), extraintestinal *E.coli* (ExPEC), and commensal *E.coli* (Dobrindt, 2005; Köhler et al., 2011). *E. coli* strains can be classified according to their phylogeny into clonal groups (A, B1, B2, C-I to C-V, D E and F) (Clermont et al., 2000; Walk et al., 2009).

Table.1.1. Intestinal and extraintestinal *Escherichia coli* pathotypes (Zude, 2014)

Intestinal pathogenic *E.coli* (IPEC)	**Extraintestinal pathogenic *E.coli* (ExPEC)**
Adherent invasive *E.coli* (AIEC)	Avian pathogenic *E.coli* (APEC)
Diffusely adherent *E.coli* (DAEC)	Meningitis-associated *E.coli* (NMEC)
Enterotoxigenic *E.coli* (ETEC)	Septicemia-associated *E.coli* (SEPEC)
Enteropathogenic *E.coli* (EPEC)	Uropathogenic *E.coli* (UPEC)
Enteroinvasive *E.coli* (EIEC)	Putative Mammary pathogenic *E.coli* (MPEC)
Enteroaggregative *E.coli* (EAEC)	
Enterohemorrhagic *E.coli* (EHEC)	

***E. coli* as a pathogen:** The pathogens are widely divided into two main categories: the extraintestinal pathogens and the enteric pathogens. Enteric pathogens are the cause of most severe acute diarrhoea in humans and animals. The extraintestinal pathogenic *E. coli* are the cause of the urinary tract infections in all age categories or meningitis and sepsis in small children and young animals (Nataro et al.,1998) and (Levine, 1987). The major subcategories of these pathogens are described below;

Enteric pathogenic *E. Coli:* Enteric pathogenic *E. coli* are well characterized and common pathogenic *E. coli* found in the gastrointestinal tract (GIT). The major intestinal pathotypes are enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Nataro et al.,1998).

Enterotoxigenic *E. coli* (ETEC): ETEC infects both human and animals and is the influential cause of diarrhoea in young children and adults human and in new-born animals, including main piglet lambs (Alexander, 1994; Hodgson, 1994) and calves (Butler et al., 1994). The contamination of drinking water and food by faecal is the major trend of infection for humans. After ingestion, ETEC colonises the small bowel and contact to its mucosa. They are the main cause of dysfunction

of electrolyte and water transport in enterocytes which lead to the typical signs of ETEC infections, but they usually do not cause any remarkable local inflammation or histological change in the intestinal mucosa. Some signs of ETEC infection include cramps, vomiting, low-grade fever, watery diarrhoea, and nausea and are closer to those of cholera but generally milder (Nataro et al., 1998).

Enteropathogenic *E. coli* (EPEC): EPEC is another main cause of diarrhoea in third world countries, which have affected only the small children below age two and they do not represent a source of traveller's diarrhoea. EPEC has several symptoms which are relatively severe and children present with symptoms of fever, profuse watery diarrhoea and vomiting. EPEC have a limited number of serogroups (mainly O14, O55, O86, O111, O119, O125, O126, O127, O128, and O142) (Kaper, 1994). The contamination of food is the major route to transmit the EPEC, which have the ability to colonize the small intestine where they join firmly to the epithelial cells of the villus tips and are a cause of typical lesions called attaching and erase lesions (Kaper,1994). EPEC usually neither invade the intestinal mucosa nor produce a toxin similar to ETEC toxins and the exact mechanisms which are the cause of EPEC-associated diarrhoea are still under investigation (Donnenberg et al., 1997). There are strains similar to EPEC in rabbits, which are responsible for severe diarrhoea and histological lesions which are similar to those observed in humans (Peeters, 1994).

Enterohaeomorrhagic *E.coli* (EHEC): Shiga-toxin producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC), is a cause of a wide range of symptoms in humans (Griffin, 1999), which include uncomplicated diarrhoea, and many severe diseases as haemorrhagic colitis and the often deadly haemorrhagic uremic syndrome. STEC are responsible for severe forms of infection which are observed mainly in the elderly and young children. The main reservoir of STEC are cattle, STEC usually is transmitted to human by faecal contamination of food, and the infections can be also transmitted from human to human in outbreaks. STEC has also been ascertained to be a cause of diarrhoea in

calves (Butler et al., 1994), and some species serotypes have represented oedema disease in pigs (Gyles, 1994). STEC belong to a very large variety of serotypes, but the majority of clinical infections registered in humans and particularly in food-borne outbreaks are related with the serotypes and serogroups O157: H7, O157: H-, O26, O103, O111, O113, and O145. The severe diseases and outbreaks are caused by these serotypes, which also called enterohemorrhagic *E. coli* (EHEC) (Levine, 1987). The colon is usually colonized by STEC where they are responsible for necrosis of villus tips, in contrast, the intestinal mucosa is not invaded by them.

Enteroaggregative *E. coli* (EAEC): EAEC presents a typical adherence pattern on cell cultures with bacteria aligning in parallel clusters like bricks in a wall. EAEC infections occur as sporadic cases or outbreaks mainly in developing countries, but they have also been observed in industrialized countries. The clinical signs of EAEC infections are usually watery mucoid (sometimes bloody) diarrhoea with low-grade fever and little or no vomiting. Typically, it is the persistence of diarrhoea for often more than 14 days. The pathogenic potential of EAEC has been demonstrated during applying many experiments on volunteers and on animal models. EAEC are responsible for pediatric diarrhoea with lesions which are the same as those observed in animal models that have subsequently confirmed their virulence (Nataro et al., 1998).

Diffusely adherent *E. coli* (DAEC): In worldwide DAEC are mainly responsible for urinary tract infections, but they have a role as a causative agent of diarrhea which are controversial. Sources implicated in outbreaks of DAEC include contaminated food, especially undercooked ground beef, contaminated water and contact with livestock and other animals (Gióon et al.,1991).

Some commensal intestinal isolates are capable of causing infections in extraintestinal tissues, and these pathogenic strains have been collectively termed ExPEC (Russo et al, 2000). Among ExPEC, uropathogenic *E. coli* (UPEC) is the most common cause of human urinary tract infections (UTIs) (Marrs et al., 2005). Avian pathogenic *E. coli* (APEC) strains to share some virulence traits with ExPEC

strains from human infections and are associated with extraintestinal infections of poultry, such as airs vasculitis, pericarditis, cellulitis, and septicemia (Rodriguez-Siek et al., 2005; Ron. 2006).

1.2. Autotransporter proteins

The most common secretion pathway for the transport of molecules across the outer membrane of Gram-negative bacteria is the type V secretion pathway. New discoveries about structure, function, and assembly mechanisms have enlarged the classification regrouped such as Type Va ‘‘autotransporters’’, Type Vb ‘‘two-partner systems’’, Type Vc ‘‘trimetric auto-transporters’’, Type Vd ‘‘Patatin-like proteins’’ and Type Ve ‘‘intimins and invasins’’ (Tame, 2011). The Type Va group of proteins it’s called auto-transporter and are defined by three domains.

Within each broad category, a number of subgroups were identified in the previous study and most subgroups contained at least one characterized AT protein. Auto-transporter (AT) proteins are found in all *Escherichia coli* pathotypes and are often associated with virulence. Various auto-transporters have been identified among *Escherichia coli* associated with intestinal or extra-intestinal infections.

Sat, a 107 kDa protein, was first identified in the UPEC CFT073 strain, but later also found in clinical isolates of Shigella, EAEC, and DEAC species (Boisen et al., 2009). Tsh was the first SPATE discovered by virtue of its hemagglutinin activity nearly two decades ago, from the Avian pathogenic E. coli (APEC) strain x7122— a pathogen which causes respiratory tract lesions and septicemia in poultry (Provence et al.,1994).

The vat was found broadly distributed among human ExPEC including UPEC and invasive *E. coli* causing neonatal septicemia and meningitis. The vat gene in ExPEC strains have been arbitrarily annotated as HBP or Tsh, yet the

SPATE sequence in ExpEC has 96.5/ 97% identity/homology to Vat and 69.6/79% identity/homology to Tsh/Hbp. (Parreira et al.,2003)

Three major groups of distinct domain architecture: Secretion of proteins from gram-negative bacteria that direct their own secretion across the outer membrane result because of Auto-transporters Family and this secretion mechanism it also called type V secretion (Dutta et al., 2002; Henderson et al., 2004). Auto-transporters Family we can found generally among Gram-negative bacteria. They can have large different functions and many of them have a role in virulence. All AT proteins share a common characteristic structure consisting of three functional domains:

(i) large precursors with an N-terminal signal sequence, that mediates transport across the inner membrane into the periplasm, which initiates the SecA-dependent transport (ii) an α- or passenger translocator domain, which encodes for functional traits, that mediates the transport of the connected passenger domain across the outer membrane (OM) to the bacterial cell surface and (iii) an outer membrane-embedded C-terminal β- or translocation domain like integral outer membrane proteins, the translocator domain folds in a β-barrel structure and requires the Bam machinery for its insertion into the outer membrane as Figure.1.3 show bellow (Benz et al., 2011; Desvaux et al., 2004; Jose et al., 1995).

The passenger may stay connected via the translocator domain to the bacterial cell surface or it is proteolytically released into the extracellular milieu after transport across the outer membrane. This one is based on the size of the translocator domain and its position relative to the passenger in the precursor, and some research showed that the auto-transporters are regrouped into four sub-categories.

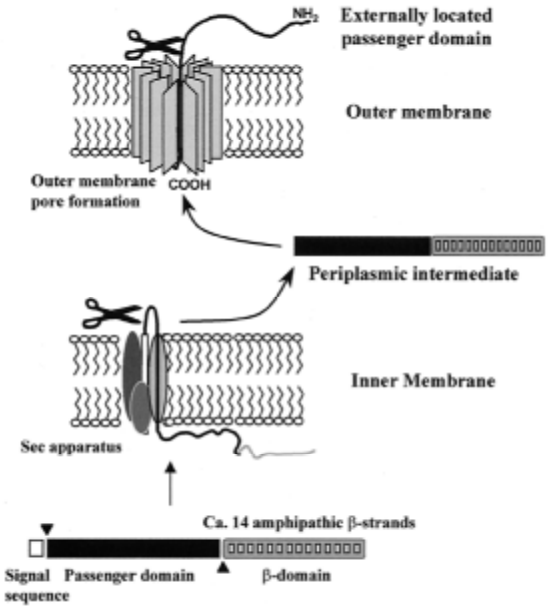

Figure 1.3. Model of autotransporter (type V) secretion mechanism (Henderson et al., 1998).

The translocation domain appears to be highly homologous for some AT subtype-specific, overall ATs show diversity in their passenger domain that determines their individual functional properties. The passenger domains secreted via the autotransporter mechanism mediate virulence and this diversity provide multiple functions such as mediating physical adhesion via protein-protein interactions (‘‘adhesins’’) (Benz et al., 1989) , autoaggregation (Diderichsen, 1980), biofilm formation (Danese et al., 2000), lipolytic attack of host cell membranes (‘‘esterases’’), -haemagglutination, serum resistance (Henderson et al., 2001), or proteolytic degradation of select host proteins (‘‘proteases’’) activity (Pohlner et al., 1987) or toxin activity and perhaps other as yet uncharacterized activities as well. (Cover, 1996).

The type V secretion pathway is the most widespread secretion pathway for the transport of molecules across the outer membrane of Gram-negative bacteria. This system comprises proteins secreted by the autotransporter (AT)

pathway (also known as AT-1 or type Va), the two-partner secretion pathway (also known as type Vb) and the Oca system [also known as AT-2, type Vc or the trimeric AT adhesin (TAA) pathway (Henderson et al., 2004). AT proteins represent the largest group of type V secreted proteins. Proteins are separated into three major groups based on protein domains: SPATE (red shading), TAA (yellow shading) and the remaining proteins in the broad group of AIDA-I-type AT proteins (Figure1.3) (blue shading). Subgroups are named after the best-characterized representative from that group.

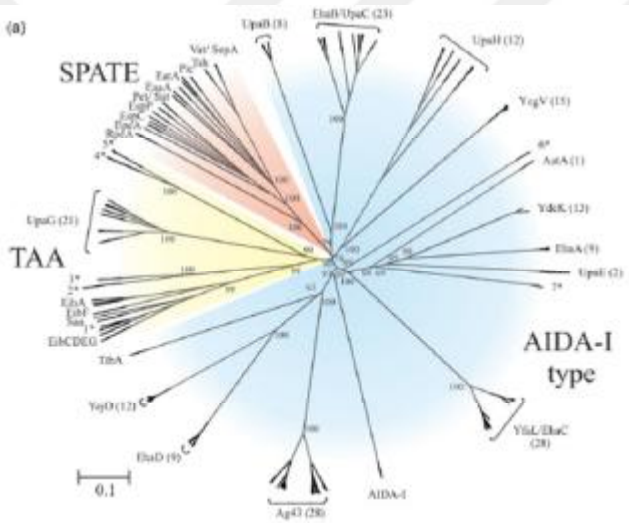

Figure 1.4. Classification of Autotransporter proteins (Wells et al., 2010)

This project was carried out at Cukurova University, Departement of Food Engineering and Medical Microbiology. In our study, a total of 100 isolates(35 food and 65 clinical) were used.

The aim of this study was:

-Determination of seven Autotransporter genes following as Antigen 43 (Agn43), temperature sensitive hemagglutinin (Tsh), Serine auto-transporter toxin

(Sat), Vacuolating autotransporter toxin (Vat), UpaB, UpaC and UpaJ genes in *E.coli* strains isolated from food and clinical samples to show virulence-related of those specific genes.

-Comparison between the distributions of autotransporter related genes in clinical strains and food strains

-And finally identification phylogenetic relationship of *E.coli* strains by PFGE method.

2. LITERATURE REVIEW

2.1. Isolation of *E.coli*from food

Riley et al. (1983) in their study showed the different species sero/ biotype of E.coli O157: H7 in humans caused foodborne.

Several studies demonstrated the prevalence of E. coli in food samples. Badri et al. (2009) performed bacteriological analysis of E.coli in different types of sample and they found that the prevalence of E. coli was 48%, 45%, 35.5%, 30%, 8.3% and 0% in good water, ground beef, seafood and domestic water, respectively.

Iyer et al. (2013) have studied the prevalence of both pathogens Salmonella spp. and E. coli in meat samples and PCR confirmed these findings. They found that the rate of incidence of E. coli in open butcher shops was a high (65%) when compared to groceries and hypermarkets which were (40%) and (20%) respectively. With respect to Salmonella, the rate of incidence was 45%, 25%, and 5% in butcher shops, groceries, and hypermarkets respectively.

Ahmed et al. (2014) showed that in the US approximately 76 million people are affected by foodborne infections each year and, therefore, 325,000 people died in 5,200 of these patients are to be monitored in the hospital. Foodborne infections also it has been reported in the same study to be around the US $ 7 billion of annual costs.

Abdaslam et al. (2014) have studied the prevalence of four types of bacteria including E.coli, Salmonella, Klebsiella, and S. aureus in 90 samples of fish, chicken, beef, and prawn. They have found that the E.coli was found in 45 (50%) of total samples while the prevalence of Salmonella, Klebsiella and S. aureus were 21 (23.3%), 2 (2.2%) and 27 (30%) respectively. 5 out of 45 of identified E.coli strains were E.coli O157. Sorbitol Mac Conkey Agar was used as a selective medium to identified non-sorbitol fermenters of E.coli after that rapid

latex agglutination test was performed on typical colorless colonies to identify the E.coli O157. H7

Adwan et al. (2015) have reported that *E. coli* was found in 95% of meat samples. The prevalence of E. coli was in beef 100%, chicken 93. 3% and turkey meat 90%. In this study, the results demonstrated that 89. 5% of meat samples which contaminated with *E.coli* belonged to *E. coli* pathotypes tested. The total prevalence of uni-infected samples with ETEC was 60% and with EAEC was 5%. The prevalence of more than one pathotype was found in 21% of the tested samples. Tanih et al. (2015) have studied the prevalence of bacterial pathogens in a various abattoir and animal types. They found that the *E. coli* was the most prevalent bacteria was found in 67. 5% (104/154) of samples, while S. aureus was found in 32.5% (50/154) of samples. No *Salmonella* was found in this study. Both S. aureus and *E. coli* were more found in pork with percentages of 40% (20/50) and 48. 1% (50/104), respectively, than in cattle across the different abattoirs.

Jabur et al. (2016) found that 34. 4% (21) of food samples was positive for *E. coli.* Macconkey agar with sorbitol (McA and SMCA) was used to isolate *E. coli* and the biochemical tests were used to confirm *E. coli* strains. Seven isolates of out 21 *E.coli* isolates were confirmed as *E. coli* O157: H7 on HiCrome *E coli* O157: H7 selective agar.

Mohammed Hamzah et al. (2013) have done other study which reported that, the E. coli O157: H7 was found in 5 of the 375 chicken samples and E. coli O157: H7 was detected in none of the samples. Al-Jobori et al. (2016) used the multiplex PCR method for detection of three food-borne pathogens after enrichment.

The amplification of the PCR products were visualized in agarose gel and invA gene (a 284 base pair fragment) was identified for the samples belonging to the S. aureus spp., uidR gene (153 base pair fragment) was used in the positive samples for the genus E. coli; and Listeria gene (a 217 base pair fragment) was identified for the samples which belong to the L. monocytogenes. One or more of

the bacterial pathogens were found in 58% (87 of 150) of samples. Uid R gene was found in 67 samples, the inv Agene was found in 26 samples and LISgene was present in eleven samples in this study.

2.2. The prevalence of Autotransporter genes in *E. coli*

Provence et al. (1994) Tsh was first studied and showed as a temperature-sensitive hemagglutinating factor in avian pathogenic *E. coli* (APEC). Tsh was the first SPATE to be described and showed the presence of a conserved 7-amino-acid serine protease motif within Tsh classifies the protein in a subfamily of autotransporters, known.

Henderson et al. (1999) showed that Ag43 is the best characterized in E.coli K-12 and represent a member of the AIDA-I type which is phase-variable surface-located adhesin. Furthermore Schembri et al., 2003 showed that the early stages of biofilm formation are connected with Agn43 from E. coli.

Otto et al. (1998) Tsh have an identical homolog of Hbp and was described as a heme-binding protein with a serine protease activity targeted at hemoglobin. Researchers have found that Hbp expression enhances abscess formation in mice during mixed infections with Hbpexpressing E. coli and Bacteroides fragilis.

Roche et al. (2001) UPEC is commonly associated with the surface-exposed fimbrial adhesins and adhesins of the autotransporter (AT) family. And antigen Ag43 is the best studied of these proteins. Ag43 mediating is well-studied cell aggregation adhesion and biofilm development as the causes of chronic UTIs.

Henderson et al. (2001) Multiple copies of the Agn43 (flu) gene encode UPEC strains with the variations to determine the biological function of the Ag43 protein (agn43a/fluACFT073 and agn43b/fluBCFT073)

Anderson et al. (2003) Non-pathogenic and pathogenic E.coli, Ag43 were found in many including UPEC, and studies showed that it is associated with the uro virulence disease.

Heimer et al. (2004) reported that uropathogenic E. coli also secretes a Tsh autotransporter protein and they reported in their study Tsh gene isolated from the food lead UPEC.

Guyer et al. (2002) have identified secreted autotransporter toxin (Sat) using culture supernatants of strain CFT073that is responsible for vacuolating cultured bladder and kidney epithelial cells. And they showed that Sat genes share a high degree of homology with an expanding class of serine protease autotransporters in *E.coli* (SPATEs).

Restieri (2006) among 491 E. coli isolates from human urinary tract infections, diarrheagenic E. coli, and avian pathogenic E. coli (APEC) and E. coli reference strains belonging to the ECOR collection. They determined the presence of 13 autotransporter sequences and five allelic variants of antigen 43 (Agn43) and the results showed that Agn43 were significantly associated with clinical isolates (93%) compared to commensal isolates (56%).

Timothy et al., (2006) used a bioinformatic analysis to analyze twenty-eight E. coli genome of a total 215 AT-encoding sequences that represented three major groups of distinct domain architecture: (i) serine protease AT proteins, (ii) trimeric AT adhesins and (iii) AIDA-I-type. They were identified as Autotransporter proteins. And showed seven subgroups contained no previously described proteins and concluded that The AIDA-I-type AT proteins represented the largest and most diverse group, with up to 16 subgroups.

Wells et al.,(2007) reported that autotransporter proteins constitute the largest family of secretion proteins in Gram-negative bacteria and and exhibit many different functions. Still now it is decribed more than 800 members, autotransporter proteins constitute the largest family of secretion proteins in Gram-negative bacteria.

Cotter et al., (2005) worked on AT and showed that AT protein plays a major role for cause UPEC. They determined the incidence of ATespecially of 8 virulence gene from the isolation of food and clinical samples.

Those virulence genes are secreted AT Toxin (SAT), vacuolating autotransporter toxin 4 %(Vat), 12% (Tsh), 5% Ag43, surface located proteins UpaB 1, UpaC 3 and Trimeric AT 2 UpaJ genes among E.coli strains based on the origins of the isolated and determine a phylogenetic relationship.

Wells et al., (2010). The most widespread and diverse group of the Autotransporter family are the AIDA-I-type AT proteins Some studies focus on the second group of virulence factors associated with adhesion, aggregation and biofilm formation that large family Agn43 autotransporter (AT) proteins syntheses (Henderson & Nataro, 2001; Henderson et al., 2004; Kajava & Steven 2006).

Allsopp LP et al., (2010).For surface located proteins, they performed a detailed molecular characterization of two of this AIDA-I-type conventional AT proteins, closely related AT adhesins from CFT073: UpaB (c0426) and UpaC (c0478). The previous research revealed that the UpaB and UpaC AT-encoding genes are common in *E. coli*. In their study, UpaB and UpaC genes will be characterized in a recombinant E. coli K-12 strain back ground.

Zude, et al., (2014) indicated that Autotransporter protein UpaJ and its positional ortholog EhaC were detected in 93 % of the *E. coli* strains tested Among 111 E.coli strain. Moreover, UpaJ is the most prevalent Autotransporter in *E. coli* and were observed to mediate redundant functions in comparison to the ATs characterized in their study.

3. MATERIAL AND METHOD

3.1. Material

This study was carried out from October 2016 to December 2017. A total of 48 samples were collected from retail vegetable stores and markets in which included) chicken (16), fruits and vegetables (16) and meat (16). All samples were randomly collected in sterile plastic bags, labeled and transported to the Microbiology laboratory in the Department of Food Engineering of Cukurova University for analysis. On the other hand, a total of 65 clinical samples which collected from urinary tract infections, and surgical site infections by Onler between 2014-2016 in Cukurova University Balcalı Hospital used in this study. (Onler, 2016). In a total of 100 ***E.coli*** strains isolates; (n=35) from food and (n=65) from clinical sources were our material in this study

3.1.1. Food samples: chicken, fruits and vegetables and meat

Table.3.1. Food samples with collection place

Numbre of sample	Market	food Sample	Numbre of sample	Market	food Sample
1	G1	V1	**25**	M11	C9
2	B1	V2	**26**	M12	C10
3	M1	V3	**27**	B8	C11
4	M2	V4	**28**	M13	C12
5	G2	V5	**29**	G9	C13
6	G3	V6	**30**	G10	C14
7	B2	V7	**31**	M14	C15
8	B3	V8	**32**	B9	C16
9	B4	V9	**33**	M15	M1
10	G4	V10	**34**	G11	M2
11	M4	V11	**35**	G12	M3
12	M5	V12	**36**	B10	M4
13	M6	V13	**37**	B11	M5
14	M7	V14	**38**	G12	M6
15	G5	V15	**39**	G13	M7
16	M8	V16	**40**	G14	M8
17	B5	C1	**41**	G15	M9
18	B6	C2	**42**	G16	M10
19	M9	C3	**43**	G17	M11
20	G6	C4	**44**	B12	M12
21	B7	C5	**45**	B13	M13
22	G7	C6	**46**	M16	M14
23	G8	C7	**47**	G18	M15
24	M10	C8	**48**	G19	M16

G:Grossery **M:** Micros **B:** Bazaar **V:** Vegetable **C:**Chicken **M:**Meat

A total of 48 samples were collected from September to October 2016 from retail vegetable stores and markets in which included vegetables (16 samples), chicken (16) and meat (16) as the Table 3. 1 show. All samples were randomly collected in plastic bags, labeled and transported to the Microbiology laboratory/ Department of food Engineering of Cukurove University for analysis.

3.1.2. Clinical samples

Table.3.2. Clinical samples

Number	Sample	Gender	Age	Numbre	Sample	Gender	Age
2	U1	M	0	**34**	U28	M	54
3	U2	M	82	**35**	U29	F	47
4	U3	F	7	**36**	W2	F	71
5	U4	F	71	**37**	W3	F	71
6	W1	M	52	**38**	U30	M	80
7	U5	F	15	**39**	U31	M	1
8	U6	F	22	**40**	U32	M	50
9	U7	F	0	**41**	U32	F	38
10	U8	F	43	**42**	U34	F	0
11	U9	F	25	**43**	A2	F	86
12	U10	F	0	**44**	U35	F	8
13	U11	F	58	**45**	W4	M	79
14	U12	M	0	**46**	U36	F	0
15	U13	F	4	**47**	A3	F	79
16	U14	M	68	**48**	U37	F	9
17	A1	M	69	**49**	W5	F	67
18	U15	F	35	**50**	U38	F	28
19	U16	F	39	**51**	U39	M	33
20	U17	F	65	**52**	U40	F	4
21	B1	F	74	**53**	U41	F	58
22	U18	F	56	**54**	U42	F	32
23	U19	F	86	**55**	U43	F	36
24	U20	F	67	**56**	U44	F	8
25	U21	F	76	**57**	U45	F	2
26	U22	F	79	**58**	U46	F	36
27	U23	M	82	**59**	U47	M	23
28	U24	M	3	**60**	U48	M	70
29	B2	M	70	**61**	U49	F	3
30	B3	F	87	**62**	U50	F	71
31	U25	F	41	**63**	U51	M	3
32	U26	M	67	**64**	U52	F	1
33	U27	F	7	**65**	U53	F	8
				66	U54	F	24

U:Urine **B:** Blood **W:** Wound **A:**Aspiration **F:** Female **M:**Male

On the other hand, clinical samples were collected from urinary tract infections, surgical site infections, pneumonia, meningitis strains between 2014-2016 in Cukurova university Balcali Hospital by Onler. The isolates obtained (N=65) were our material in this study. A total of 65 ExPEC isolates were used in this study, including 54 (83%) isolates from urine, 5 (8%) isolates from the wound, 3 (4%) isolates from blood and 3(4%) isolates from aspiration as Table 3.2. show. The majority of ExPEC strains were isolated from urine samples. There were 49 (71%) women and 19 (29%) men (Figure 4.5), their age ranged between 0 and 87 years as follow : twenty of patients were less than 10 years of age (30.7%), seventeen of patients were between 11-50 years of age (26.1%) and the remaining patients were 28 (43.04%) were between 50-90 years of age.

3.1.3. Mediums used for incubation and stockage of *E coli* strains

-*Escherichia coli* FLSB Meduim

Fluorocult® Lauryl Sulfate Broth (Merck 1,12588)

Fluorocult® LST Broth (LST-MUG Medium)

ISO 11886, DIN Norm 10183 and EWG 76/1604 with correspond standard.

- Sheep blood agar medium

Blood agar (LAB M MC2): 45 g/l

Meats extract (MERCK 1.03979):10 g/l

Pepton (DIFCO 63928JB):10 g/l

NaCl MERCK 1.06400):5 g/l

Agar (LAB MC2):15g/l

1000ml of distilled water has been used and then 60 ml of sheep blood was added. Subsequently, all prepared culture media and their components should be sterilized for 15 minat 121°C.

- Stockage media

250 mL Brain heart infusion

30 ml Gliserol

20ml Blood

Two loops of pure colony were added to 300 ml stockage media for later analysis, and stored at -20.

3.2. Method

3.2.1. Isolation and identificationof *E.coli* strains from the food by classic method

48 food samples including meat (n: 16), Chicken (n: 16) and vegetable (n: 16) have been used. Followed by homogenization of each 10-g sample of meat, chicken or vegetable for 2 min in 90 ml of peptone water and then applied the Fluorocult lauryl sulfate broth (FLSB).

3.2.1.1. Most probable number (MPN) method

It was used most probable number (MPN) to isolate *E.coli.* In the MPN test, the sample to be tested was prepared in 3-fold dilution series and then 1mL of each dilution were inoculated into triplicate broth culture tubes for incubation as the Figure 3. 2 show. After the culture medium was incubated for 24 to 48 hours at 35 °C aerobically. Then after 24 hours of incubation, the tubes were checked and controlled if gas formation was negative (Table 3. 1). To be sure incubation continued another 24 hours and then checked again the gas formation. The presence of *E. coli* was indicated by gas formation (fermentation of lactose) and a positive indole reaction(Table.3. 3). We checked the tubes under UV light (366 nm) too. Light blue fluorescence(Figure.3. 3) indicated to the presence of *E. coli.*

Figure.3.1. Gas and indol test used to determine *E.coli*

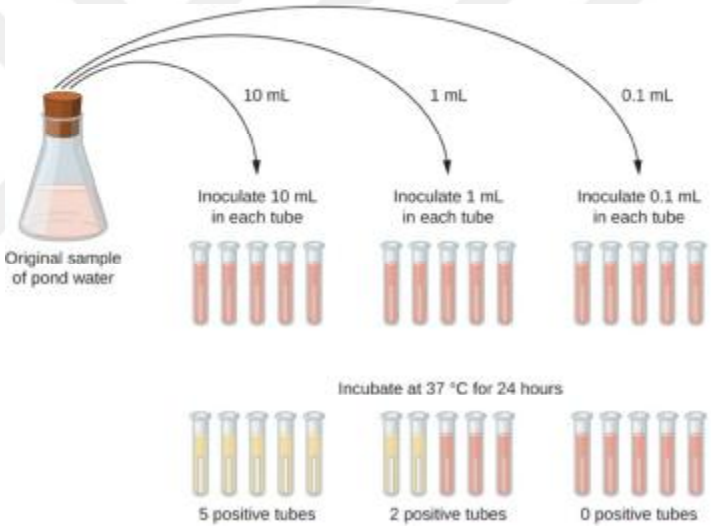

Figure.3.2. MPN Test (Nisha Rijal.,2017)

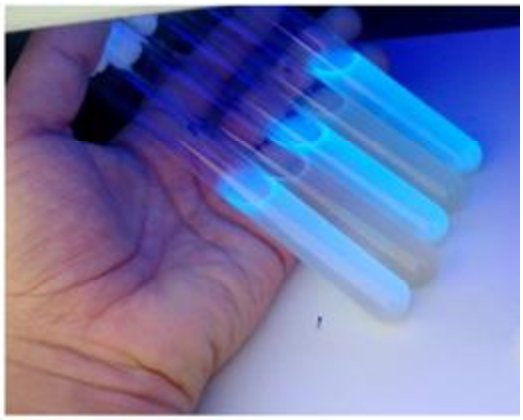

Figure.3.3. Fluorescence testused to determine *E.coli*

Table.3.3. Isolationand identificationof *E.coli* strains by fluorocult Laurly Sulfate Broth

Test strains	**Growth**	**Gas**	**Fluorescence**	**Indole**
Escherichia coli	Very good	+	+	+
Escherichia colio157:H7	Very good	+	-	+

Fluorocult *E. coli* O157: H7 Agar was used to culture the positive strains of *E. coli* (Table.3. 3.), the mode of action is as that Sodium deoxycholate inhibits the growth of the Gram-positive accompanying flora for the greater part. The role of Sorbitol is serving together with the pH indicator bromothymol blue and allow to determine the degradation of sorbitol. If Fluorocult *E. coli* O157: H7 Agar was used to culture the positive strains of *E. coli* (Table.3. 3.), the mode of action is as that Sodium deoxycholate inhibits the growth of the Gram-positive accompanying flora for the greater part. The role of Sorbitol is serving together with the pH indicator bromothymol blue and allow to determine the degradation of sorbitol. If the result the colonies turn yellow in color this show that sorbitol-positive microorganisms. On the other hand, Sorbitol-negative strains, do not lead to any change in the color of the culture medium (Table.3. 4.) and thus proliferate as greenish colonies. Conversation into 4-methylumbelliferone by β–D-

glucuronidase- to form pathogens is provided by 4-methylumbelliferyl-β-D-glucuronide (MUG); 4-methylumbelliferone fluoresces under UV light. A highly specific characteristic of *E. coli* occurs because of β-D-glucuronidase activity. In contrast to most of *E. coli* strains, *E.coli* 0157: H7 is not capable of forming β D glucuronidase. When irradiated with long-wave UV light (Schindler, 1991), fluorescence is formed.The results the colonies turn yellow in color this show that sorbitol-positive microorganisms. On the other hand, Sorbitol-negative strains, do not lead to any change in the color of the culture medium (Table.3. 4.) and thus proliferate as greenish colonies.

Table.3.4. Isolation and identificationof *E.coli* strains by Fluorocult *E. coli* O157:H7 Agar

Test strains	**Growth**	**Colony color**	**MUG**	**Sorbitol**
Escherichia coli	Very good	yellow	+	+
Escherichia colio157:H7	Very good	colorless	-	-

After isolation and identification of *E. coli* and *E. coli* 0157: H7 strains, applied of biochemical tests to them.

3.2.1.2. Biochemical tests

E.coli and *E.coli* 0157: H7 strainswere grown on EMB agar. The culture incubated overnight for 37°C at 18-24 hours. *Escherichia coli* is gram-negative bacteria that ferment the lactose produce acid which turns the colonies dark purple as the acid acts upon the dyes. In addition, certain lactose-fermenting bacteria produce flat, dark colonies with a green metallic sheen (Figure.3. 4). *Escherichia coli* cultivated on blood agar. Cultivation 24 hours in an aerobic atmosphere, 37°C. Colonies are without hemolysis (Figure.3. 5). Additionally, It was used IMVIC (Indol, Methyl, Red-Voges Proskauer, citrate) test for biochemical identification of

E.coli strain (Figure.3.6.) and it was performed according to methods described by Barrow et al., 1993.

1. Indol test: *Escherichia coli* produce indole from amino acid tryptophan using the enzyme tryptophanase. Production of indole was detected using Kovac's reagent. Indole reacted with the aldehyde in the reagent to give a red color. An alcoholic layer concentrated the red color as a ring at the top.
2. Methyl red test(MR): the bacterium to be tested in inoculated into glucose phosphate broth, which contained glucose and a phosphate buffer and incubated at 37°C for 48 hours. Over the 48 hours, the mixed-acid producing organism must produce sufficient acid to overcome the phosphate buffer and remain acid. The pH of the medium was tested by the addition of 5 drops of MR reagent. Development of red color was taken as positive (*E. coli*). MR negative organism produced a yellow color.
3. Voges Proskauer(VP) test: Potassium treatment of culture media result in a production of red color reaction. The active product in the medium, which is formed by bacterial metabolism, is acetylmethylcarbinol that is described as a product of the butylenes glycol pathway.

   Bacterium to be tested was inoculated into glucose phosphate broth and incubated for at least 48 hours. 0.6 ml of alpha-naphthol was added to the test broth and shaken. 0.2 ml of 40% KOH was added to the broth and shaken. The tube was allowed to stand for 15 minutes. The appearance of red color was taken as a positive test This was indicating the presence of diacetyl, the oxidation product of acetoin. The negative tubes must be held for one hour since maximum color development occurs within one hour after addition of reagents, *Escherichia coli* was negative for this test.
4. Citrate utilization test: Bacterial colonies were picked up from a straight wire and inoculated into the slope of Simmon's citrate agar and incubated overnight at 37oC. If the organism had the ability to utilize citrate, the

medium changes its color from green to blue. *Escherichia coli*was negative for this test.

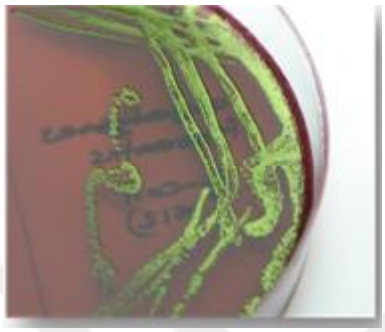

Figure.3.4. *E. coli* on EMB agar with metallic sheen

Figure.3.5. .*E. coli* on Blood agar green

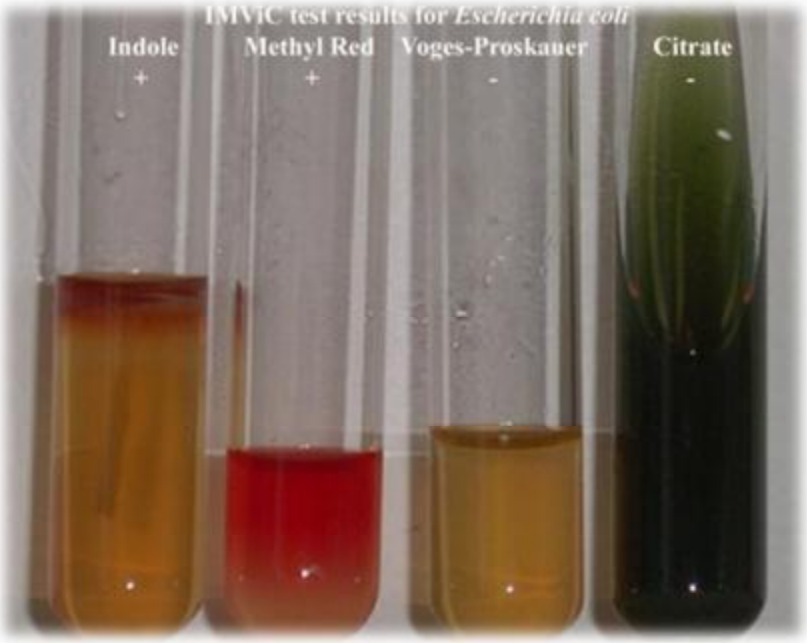

Figure.3.6. IMVIC (Indol, Methyl, Red-Voges Proskauer, citrate) test

3.2.2. Genotypic identification of *E.coli* strains

3.2.2.1. Chemical Solutions and Enzymes which used in DNA Extraction and PCR

TE Buffer

Tris HCl (SIGMA T-3253): 0,1576gr

EDTA (AMRESCO 0105-500): 0,0372gr

Distile Su: 100 ml

(pH 8.0)

dNTP Mixture

(25µmol each one) (Fermentas R0181)

(2,5 mM each one, total 10mM)

dATP (100 mM): 25 µl

dCTP (100 mM): 25 µl

dGTP (100 mM): 25 µl

dTTP (100 mM): 25 µl

Distilled water: 900µl.

Stored in -20°C.

Marker

Gene Ruler 200bp DNA Ladder Plus (Fermentas SM0323)

Gene Ruler 100bp DNA Ladder Plus (Fermentas SM0323)

Gene Ruler 50bp DNA Ladder (Fermentas SM0323)

Taq DNA polymerase enzyme

(Fermantas EP0402) 5U/ µl

10X PCR Buffer solution

25 mM $MgCl_2$

10xTBE Solution

Tris-base (SIGMA T-1503): 108gr

Boric Acid (MERCK 1.00165.0500): 55gr

EDTA (AMRESCO 0105-500): 7,44gr

(PH 8.4) and Completed to 1000ml with ultra-pure water..

Ethidium bromide

Ethidium bromide (Sigma E8751) (1000X, 5mg/ml): 0,5g

Distilled water: 100ml

To protect it from light we put it in aluminum foil then it was stored at + 4 ° C

3.2.2.2. Genomic DNA extraction and molecular identification of *E.coli* strains

We have used the uidA gene, encoded beta-D-glucuronidase, for molecular identification of *E.coli*. UidA gene has used as positive control in our study for amplification (Henrissat, 1991). Firstly for genomic DNA extraction we extracted it according to thaw and freeze protocol (Squirsr et al.1955) as follow:

1. To obtain pure colonies of *E.coli* strain it was cultured in Blood agar medium (Figure 3. 5).
2. The one pure colony of *E.coli* strain was taken and it was again cultured for one night at 37 °C.
3. The pure colony was taken and mixed with 2 ml of TE and the density was adjusted by MC Farland to be between 5-6.
4. One ml of the suspension was taken and put into a freezer at –20°C for 1.30 hours
5. Then the suspension was put in the dry heater at 95 °C for 15 min.
6. After that, the suspension was again put into a freezer at –20°C for 1.30 hours
7. Then it was put into a dry heater at 95 °C for 15 min.

8. Finally, the suspension was centrifuged at 13000 g x3 minutes then the supernatant was taken and put in a new tube, and it was used in the PCR.

After that polymerase chain reaction (PCR) to detect uidA gene 5 µl of DNA is used in a 25 µl reaction volume containing 12.5 µl master mix, 0.5µl uidA-F, 0.5µl uidA-R and 6.5 µl distilled water (AL-Zuwainy et al., 2012)). The product of PCR loaded in 2% gel of agarose in the electrophoresis device and the bands evaluated in imaging device Figure 3. 7.(Muller et al., 2007). It was used uidA gene for molecular identification of *E.coli* as Lane Maquer, The 100 bp DNA Ladder (100 – 1500 bp range).

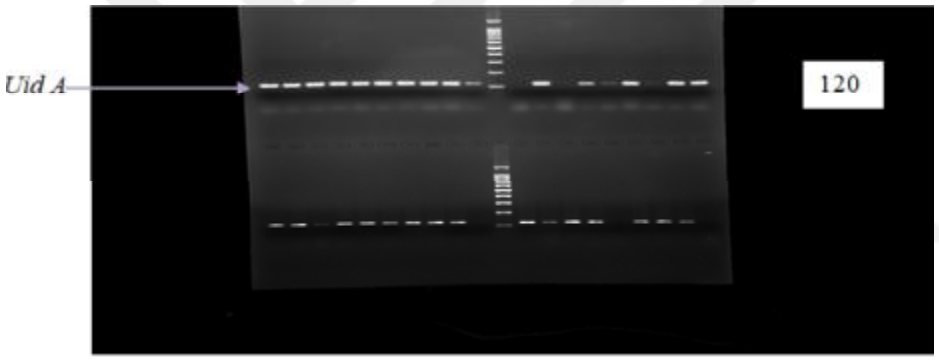

Figure.3.7. Using of *uid A* gene for molecular identification of *E.coli*

3.2.3. Identification of Autotransporter genes in *E.coli* strains by using polymerase chain reaction (PCR)

Saiki et al. (1988) was described first PCR method and showed that this method allows the exponential amplification of DNA regions in vitro by using a heat stable DNA polymerase from Thermophilus aquaticus (Taq). So this way will allow that even small amounts of template DNA can be amplified to high copy numbers and then easily visualized by using screening assays.

Standard PCR: This reaction was performed in a final volume of 25µl for a routine PCR. The thermal profile was designed according to the annealing temperature of the specific primers as shown in Table.3.5. and specific programmatic as described in Table 3.6.with time depending on the side of the expected amplification product.

Table.3.5. Primer Sequence for PCR

AT genes	Primer sequence Forward	Primer sequence Reverse	base pairs(bp)
Tsh	ACT ATT CTC TGC AGG AAG TC	CTTCCGATGTTCTGAACGT	824 Otto et al. (1998); Provence &Curtiss(1994)
Vat	TCCTGGGACATAATGGTCAG	GTGTCAGAACGGAATTGT	981 Shookohi et al. (2016)
Sat	ACT GGC GGA CTC ATG CTG T	AACCCTGTAAGAAGA CTG AGC	387 Guyer et al. (2000, 2002)
AG43-FLUA	TCA CGA TAA CAA CGG TAT	CACTGCCATGCCCGAACT TC	600 Mendez-Arancibiaet al. (2008)
UPA C	TGCTCTAGAAGGAATTGTTAT GCACTCCTGGAAAAAGAAA	CCGCTCGAGGCCCG CAAATCCTTGACGGGCA	435 Parham et al. (2004)
UpaB	TGCTCTAGAAGGAAT TGT TAT GGAGAATTTCTTCATGAAA	CCCAAGCTTAGTCGA CAGGGGAACCGACTGCT	345 Parham et al. (2004)
UpaJ	TGCTCTAGAAGGAATTGTTAT GAACAAAATTAAAGTT	CCCAAGCTTTGCTGA ATCACCCCGTAGGCCT	250 Parham et al. (2004)

Table.3.6. Program of PCR

Name of Gene	PCR program
Tsh	95°C for the 30s, followed by 35 cycles of denaturation, annealing at 55°C for 30s, and extension at 72°C for 1 min.A final extension at 72°C for 5 min.
Vat	Initial incubation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s. A final extension step of 72°C for 10 min was also included.
Sat	95°C for the 30s, followed by 35 cycles of denaturation, annealing at 60 °C for 30s, and extension at 72°C for 1 min.A final extension 1 cycle At 72°C for 5 min
Agn43	35 cycles of denaturation at 95°C for 45 s, annealing at 56°C for 45 s, and extension at 72 °C for 30 sec.
UpaB	At 94°C for 180s, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 60 s, and extension at 72°C for 60 s. A final extension step of 72°C for 600 s.
UpaC	
UpaJ	

Example for one sample Tsh gene

Initial denaturation 10 min, 95 °C
1. Denaturation 45 sec, 95 °C
2. Annealing 45 sec
3. Elongation 30 sec x min, 72 °C
Final elongation 10 min, 72 °C

35 cycles

As showed bellow in the Table 3.6

Mix for one sample:

reaction buffer

primer solution 1

primer solution 2

And 25 µl dH2O

3.2.4. Determination of phylogenetic relationship among *E.coli* strains between food and clinical samples by pulse field gel electrophoresis (PFGE) method

Pulse field gel electrophoresis is used in typing bacterial isolates and is considered the gold standard (Riley, 2004). It includes the extraction of DNA in a plug then restriction enzyme as XbaI and NotI used to cut the DNA and this process is called restriction digestion (Goering, 2010). The large DNA fragments 30-50 Kb which produced after restriction enzyme digestion are separated by alternating field direction (current) on an agarose gel. The large fragments move slower than the small fragments on the agarose gel which results in a DNA pattern specific to each clone (Goering, 2010). The PFGE is used in different laboratories around the world, because of the use of international databases byCenter for Disease Control (CDC), therefore the comparison of new outbreak strains and their clonal origin are very easy. Studies have demonstrated that discriminatory powers of PFGE are very high compared to other typing methodologies as RAPD and MLST. However, PFGE is labor intensive and needs specialized equipment; training and time compared to other genotyping methods (Goering, 2010).

3.2.4.1. Preparation of isolates

1. One of *E.coli* colony was cultured at 37 °C overnight and the purity of culture was tested.
2. Then pure isolate was inoculated into an appropriate agar medium, and the plate was incubated overnight to ensure adequate growth.
3. A pure colony was suspended in 2 ml of Cell Suspension Buffer (pH8, 0.01M), then the density of suspension was adjusted to be between 5-6 by McFarland.
4. 1 ml of the bacterial suspension was taken, centrifuged (13000g x 3dk) then the supernatant part was dispensed and lastly, 1 ml of Cell Suspension Buffer was added to the pellet.

3.2.4.2. Preparation of Agarose Plugs

1. Agarose plugs were prepared in Cell Suspension buffer with 2% low melting agar. For this purpose, 9 ml of Cell Suspension buffer was added to 0. 2 gr low melting agarose in 10 ml screw-cap flask and shocked gently to disperse agarose. After that, it was placed in a microwave for 10 seconds, then mixed gently and placed again in the microwave until agarose was completely dissolved. After agarose was fully dissolved, placed in a water bath at 45-50°C. Then it was gently mixed with 1 ml of 10% sodium dodecyl sulfate solution. At least 200 µl of this mixture distributed in Eppendorf tubes and was placed in a water bath at 45-50 ° C until bacterial suspension added.
2. The same volume (200 µl) of bacterial suspension was mixed with 200 µl of low melting agarose at 50 °C.
3. Then 100 µl of the bacteria-agarose mixture was filled to each plug molds with an absence of air bubbles.
4. Then plug molds were stored for 20-30 min at +4°C.

In this stage, it is very important to keep the agarose plug molds in the cold to get quality DNA because of the homogenous solidification of agarose plug molds, reduced the endonuclease activity and early cell disruption.

3.2.4.3. Lysis of Cells in Agarose Plugs

1. For the lysis step, 0.5 ml of Lysis I solution, (1.5 mg/ml proteinase K and 2.5 mg/ml lysozyme) were added in 5 ml sterile short-lid tubes and agarose plugs were removed from molds and placed in each tube.
2. The agarose plugs in lysis solution were incubated at 37 °C for 1 hour in a slightly inclined position on the shaking water bath.
3. After that, it was placed into Lizis II buffer containing 400 µg / ml Proteinase K and incubated at 55 ° C for 2 hours in the shaking water bath.

3.2.4.4. Washing of Agarose plugs

1- After the lysis phase, the tubes were placed in ice for at least 15 minutes for solidification of agarose plate and the lysis solution aspirated carefully.
2- The agarose plugs were washed three times with 4 ml of ultrapure water at 50 °C for 15 minutes and then the agarose plugs were washed three times with 4 ml at 50 ° C1X TE for 15 minutes.
3- Finally, the agarose plugs were stored in 1X TE buffer at + 4 °C until the restriction enzyme phase.

3.2.4.5. Restriction Digestion of DNA in Agarose Plugs

1- The agarose plugs were placed on a slide and cut by a scalpel to 1/3 of their size, each of the fractions was placed in 100 µl of 1X enzyme buffer (Table.3.7.) and incubated at 37 °C for 10-15 minutes in a shaking water bath, after 30 minutes the liquid was dispensed (The other parts of the agarose plug are stored in TE buffer for + 4 °C for later use).

Table.3.7. The ratio of enzyme buffer and sterile water in agarose plugs.

	E.coli
Sterile water	**90ul**
Enzyme buffer	**10ul**
∑	**100ul**

1-The following mixture was prepared for each agarose plug

The agarose plugs were placed on a slide and cut by a scalpel to 1/3 of their size, each of the fractions was placed in 100 µl of (XbaI: 30U) 3ul enzyme and 100 enzyme buffer (Table.3. 8.) and then we added 87ul of Sterile water.

Table.3.8.The ratio of enzyme, enzyme buffer and sterile water in agarose plugs

	E.coli
Sterile water	87ul
Enzyme buffer	10ul
Enzyme	(XbaI: 30U) 3ul
∑	100ul

2- Agarose plugs which were washed by enzyme buffer were added to this mixture and then incubated in a shaking water bath for 4 hours at 37 ° C.
3- At the end of the incubation, the tubes were placed in the refrigerator for 15 minutes.
4- The agarose plugs were ready for electrophoresis

3.2.4.6. Gel Preparation and Casting

It was done as following steps :

1. The required components to cast a PFGE gel were assembled: casting stand and backplane, leveling table, bubble level, and comb. The casting stand and backplane were assembled and placed it on the leveling table. The bubble level was placed in the center of the backplane, and the leveling table was adjusted until the bubble was centered. The comb was positioned in the empty tray and ensured that it sat with the teeth completely level, in contact with the backplane. To prepare for sample loading, the comb was laid down across the casting stand, so that the teeth were horizontal and facing the user.
2. Totally 100mL of a 1% (w/v) agarose gel was prepared by weighing 1.1g of Agarose, and adding 100mL of 0.5X TBE (44.5 mM Trisma base, 44.5 mM Borik assist, 1 mM EDTA, pH 8.0) in a clean, dry 500mL Erlenmeyer flask and swirled gently to dislodge clumps.
3. The cap was placed loosely on the Erlenmeyer flask, and microwaved for 1 to 1.5 minutes, swirling occasionally until agarose was completely dissolved but not over boiled because agarose may superheat and be prone to bumping. Molten agarose was removed from the microwave, and transferred to a 45-50°C water bath until it became ready for use.
4. Restricted sample plug slices, carefully were applied to the teeth 2, 3, 4, 5, 6, 7,8, 9, 10, 11, 12, 13 and 14 of the comb by using a metal spatula and gently positioned against the edge. The molecular markers were inserted to the 1st and 15th teeth.
5. The comb was carefully rotated until it was upright, and set into position in the notches on either side of the casting stand. A metal spatula was used to adjust the plug slices so that they made contact with both the tooth of the comb and the backplane of the gel casting stand. We ensured that there were no air bubbles and that the plug slices made complete contact. `
6. Molten agarose was removed from a 55°C water bath, and allowed to cool until it could be comfortably held. The agarose was poured into the casting

stand, pouring slowly and steadily from a point near the center of the backplane. We took care to avoid introducing air bubbles or dislodging the plug slices from the comb. Air bubbles should be removed or moved to the margins using a metal spatula.

7. Allow the agarose to set for at least 15 minutes

3.2.4.7. Gel Loading and Electrophoresis

1. Using a bubble level, we ensured that the electrophoresis chamber was in level, that all hoses are properly attached, and that the system was in good working order.
2. The black gel frame was placed in the electrophoresis chamber, and we ensured that it was properly seated.
3. Two litters of 0.5X TBE by adding 100mL of 10X TBE to 1900mL in a 2000mL graduated cylinder was prepared and gently mixed.
4. All 1900 mL of 0.5X TBE were poured into the electrophoresis chamber.
5. We carefully removed the comb from the gel in a fluid motion, and unscrewed the side panels on the casting stand. Then we gently lifted the backplane from one side and removed the gel from the casting stand, taking care not to dislodge it or compromise its adherence to the backplane. a Kim Wipe was used, the excess agarose was removed from the bottom surface of the backplane, and the gel was carried over to the waiting PFGE system.
6. The gel carefully placed into the chamber, sliding the backplane securely into the frame in the center of the electrophoresis chamber. The gel slightly angled when submerging it to avoid trapping air bubbles underneath the gel.
7. The gel was allowed to equilibrate for several minutes and come down to the ambient temperature of the surrounding buffer.
8. We pressed “START” to begin the run.

9. We placed the completed PFGE worksheet on top of the electrophoresis(Table 3.7) chamber for reference and recorded the starting current. For a standard gel with 2L of 0.5X TBE buffer, the starting current should register between 110 and 160mA.

Table.3.9. Electrophoresis Conditions

Electrophoresis Conditions	*E.coli*
Initial time	5sn
Final time	30sn
Voltage	6v/cm
Time	20 saat
Current	150-160 mA

3.2.4.8. Post Staining and Imaging

1. We verified that the electrophoresis was completed successfully and documented any errors that had registered on the controller display.
2. We opened the electrophoresis chamber, and carefully removed the gel. We gently slid the gel off of the backplane and into a 25cm x 25cm gel staining tray.
3. Ethidium bromide post stain was poured over the gel to submerge it completely. Then it was placed on a rocker platform on low speed for 10 to 15 minutes.

Note: Ethidium bromide post stain consisted of 500µL of Ethidium bromide in 1L of water, and could be reused up to 10 times. It must be stored away from ambient light to prevent photobleaching.

4. The gel was resubmerged in reverse osmosis water and returned it to the rocker platform for 30 minutes to an hour to allow for complete destaining.
5. The gel was imaged according to the most current instructions for our gel documentation system software. We ensured that the gel was properly aligned and framed in the camera's field of view before activating the UV

transilluminator. When taking our picture, we took care not to over or underexpose the image, and avoided prolonged UV exposure of the gel. The image (Figure 3.7.)was saved in both the editable, native format for the gel documentation system, as well as a tagged image file format (TIFF). For Bio Numerics (Applied Maths, Austin TX), the TIFF should be saved as a black and white image, with a color depth of 8 bits per pixel (8bpp).

3.2.5.9. The solutions used in PFGE method

The solutions which used in the pulsed field gel electrophoresis method were prepared in our laboratory as described below.

a-Cell Suspension Buffer (1x/100ml) pH:8,0

100mM Tris-HCl :1.576gr

100mM EDTA : 3.722gr

Complete with 100 ml of ultra-pure water. Adjust pH :8with NaOH pellet.

b-Lysis I solution (1x/100ml) pH:8,0

50mM Tris-HCl 0 :788gr

50mM EDTA : 1.861gr

Complete with 100 ml of ultra-pure water. Adjust pH:8 with NaOH pellet.

c-Lysis II solution (1x/100ml) pH:8,5

500mM EDTA : 18.61gr

Complete with 100 ml of ultra-pure water.

However, NaOH pellets were added to dissolve EDTA. When the pH :8.5 was adjusted , 1gr N-lauryl sarcosine was added and then400 µg / ml proteinase K should be added to this solution.

d-TE solution (1x/1000ml) pH:7,6

10mMTris-HCl :1.576gr

0,1mM EDTA : 0.0372gr

Complete with 100 ml of ultra-pure water. Adjust pH:7.6 with NaOH pellet.

e-TBE solution (10x/1L)

Tris base : 107.8gr

Boric Acid : 55gr

EDTA : 7.44gr

Complete with 1 L of ultra-pure water.

f- and finally %10 of SDS solution was added.

4. RESULTS AND DISCUSSION

The aim of this current study was to identify seven autotransporter genes, including a Vacuolating autotransporter toxin (vat), Antigen43 (Agn43), temperature-sensitive hemagglutinin (Tsh), Serine auto-transporter toxin (Sat), UpaB, UpaC and UpaJ profiles of *E.coli* strains collected from food and clinical sources. A total of 100 isolates were used in this study.The uidA gene was used as a positive control to identify the *E.coli* strain. We selected oligonucleotides included known virulence genes and with so far unknown function genes.These genes are supposed to have impacts on human health. The presence of these genes was examined by PCR using oligonucleotides that are listed in Table 3.5. Before being amplified by PCR we checked specific sequence in the NCBI (The National Center for Biotechnological Information) database.

4.1. Presence of *E.coli* strain isolatedfrom food sources

A total of 48 food sources including chicken (n=16), raw fruit and vegetables (n=16), and meat (n=16) were used in this study. *E.coli* was obtained from 34 isolates (70.8%) where 11 isolates (22.9%) had *E. coli* O157:H7 as shown in Figure 4.1.

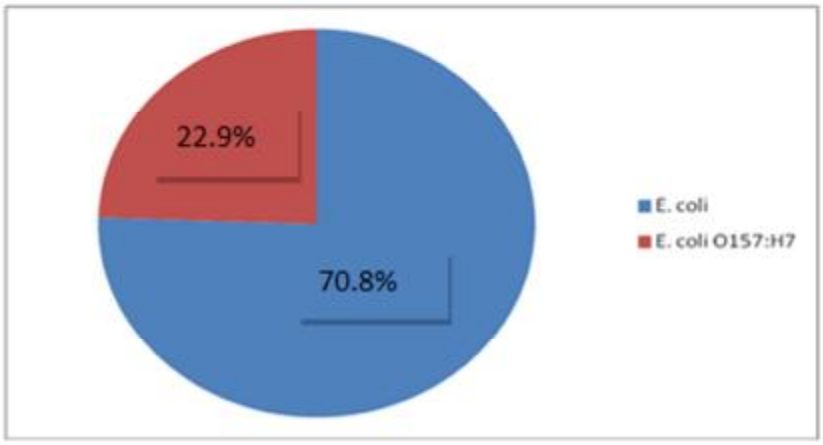

Figure 4.1. Distribution of *E. coli* and *E.coli* O157:H7 among total of food samples.

In this study, the presence of *E.coli were* in chicken, raw fruit and vegetables, and meat 93.75%, 62,5%, and 56,25% respectively (Table 4. 1.).

The highest percentage of *E.coli* (93.75%) was isolated from chicken, while the lowest percentage (56.25%) was obtained from meat. On the other hand, the occurence of total 11 isolates (22.9 %) of *E. coli* O157:H7 was 5 isolates, 3 isolates, and 3 isolates in raw fruit, vegetables, and meat, respectively.

Table 4.1. Number and percentages of *E.coli* isolated from raw vegetables, chicken and meat using MPN test.

	Sample	Number of sample	No. and % of isolated *E.coli* Type 1	No. of isolated *E.coli* O157:H7
1	Chicken	16	15(93.75)	5 (31.5%)
2	Fruit and Vegetables	16	10(62.5%)	3(18.75)
3	Meat	16	9(56.25%)	3(18.75%)
Total		48	34 (70.8)	11(22.9 %)

Autotransporter proteins are diverse regarding their genetic and clinical properties and are distinguished due to having some salient feactues , such as adhesions properties, protease activity and act as toxins (Hochhut et al., 2005; Johnson T. J. et al., 2006; Russo et al., 2003). However, Autotransporter proteins appeared to not posses a specific virulence factor or a set of factors, that are obligatory for the infection of a certain extraintestinal site (Johnson T. J.et al., 2006; Welch et al., 2002).

E.coli strains isolated from food and clinical sources were used to determine the incidence of autotransporter genes such us Antigen 43 (Agn43), temperature sensitive hemagglutinin (Tsh), serine auto-transporter toxin (Sat), vacuolating autotransporter toxin (Vat) UpaB, UpaC, and UpaJ to show virulence-related of those specific genes. In our study, we aimed to determine the occurrence of those specific genes in 34 isolates (70.8%) of *E. coli* Type 1 isolated from chicken, fruit, vegetables and meat. While the rest of 11 isolates (22.9%) of *E.coli* O157: H7 isolates were reserved to be used for another project in the futher study.

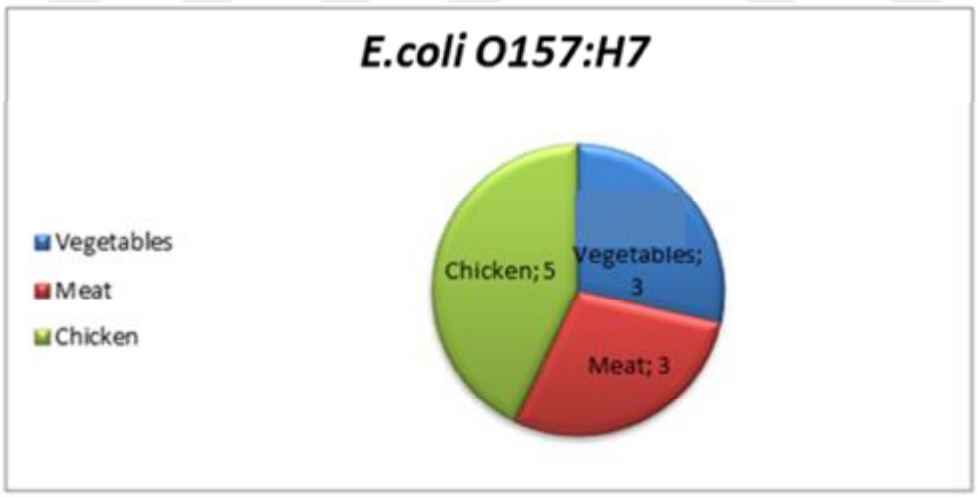

Figure 4.2. Distribution of *E. coli* O157:H7 among chicken, fruits and vegetables and meat samples

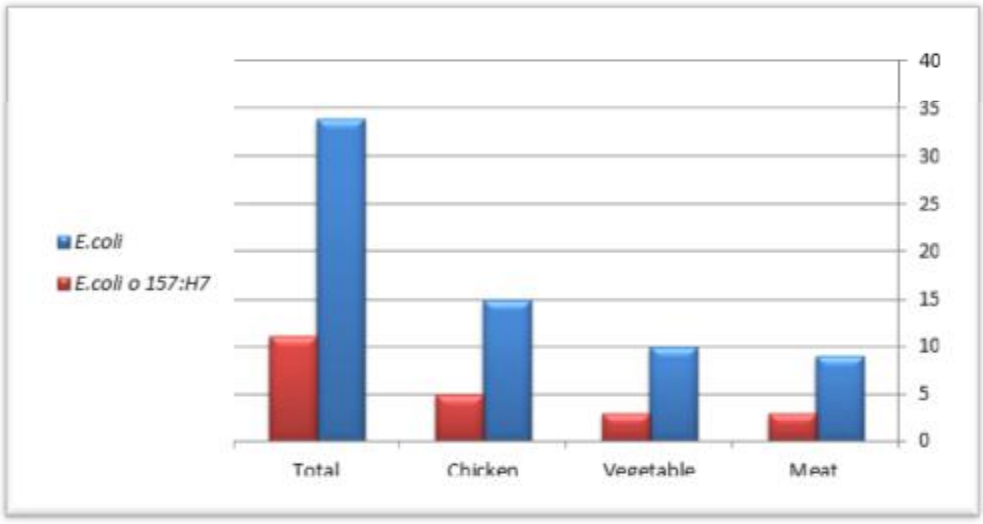

Figure 4.3. Analyze of *E.coli* and *E.coli* O 157:H7 isolated from raw vegetables, chicken and meat.

Foodborne pathogens are considered as the major threat to food safety and security, particularly in developing countries where hygiene and sanitation facilities are poorly defined. *E.coli* have a different pathogenic strains includes Enterohemorrhagic *E. coli* (EHEC) and Enteropathogenic *E. coli* (EPEC).. *Escherichia coli* O157: H7, which belongs to a family of pathogenic *E. coli* called enterohemorrhagic *E. coli* (EHEC), is one of the main causes of outbreaks of foodborne diseases and thus the most studied strains. *E.coli* is the main causes of human foodborne infections throughout the globe with complex disease such as hemolytic uremic syndrome and eventually renal failure. The food, including meat, chicken, water, raw milk, fruit juice and vegetables, can be contaminated by *E. coli* that can also be transmitted to humans through consumption of contaminated food

Nkanga et al. (1981)performed in Benin City, Nigeria using *Staphylococci* in both traditional markets, supermarket and abattoir meat samples and reported that meat was frequently contaminated due to the poor sanitary environment through the slaughter of animals and during transportation, usage, and handling.

In our study it was shown that *E.coli* Type 1was detected in 70,8 % of samples which is higher than those conducted in Iraq by Saeed et al. (2013)who found that *E.coli* was present in 19,3% of unwashed raw vegetables. The authors used only unwashed raw vegetables samples difference whereas in our study chicken, fruit, vegetable, and meat samples were used.

Enabulele et al. (2009) found that *E. coli* were present in 85.65 %, while *E.coli* O157: H7 was detected in 4.63% of fresh meat samples which is lower than our findings. In comparison, our study showed that the presence of *E.coli* and *E.coli* O157: H7 was 70,8% and 22,9 %, respectively.

In Thailand, Suthienkul et al. (1990) was found that *E.coli* was isolated from 9% of market beef specimens, 8 to 28% of fresh beef specimens at slaughterhouses, and from 11 to 84% of fecal specimens from cattle. Furthermore, Suthienkul et al. (1990) found 9 (1%) out of 107 chicken carcasses contaminated with *E. coli*.

In the UK, Adams et al. (1995)found that *E. coli* O157: H7 detected in 1-2% of lamb, chicken, and pork meat samples.

In Egypt, ,Abdul-Raouf et al. (1996)assayed 50 chicken meat samples and detected *E.coli* O157: H7 in 2 samples.

In France, , Vernozy-Rozand (1997) examined 320 samples in which 160 chicken drumstick samples and 160 chicken breast samples for *E.coli* O157: H7. The researchers documented that *E.coli* O157: H7 was detected in 2,5% (4) of drumstick samples.

Ahmed et al. (2014) worked on a total of 1600 food samples (800 meat products and 800 dairy products) and analyzed by using classic and PCR- based methods. *E.coli* O157: H7 was detected in 54 (3,4%) samples. In our study E.*coli* O157: H7 were detected in 11 (22,9%). They reported that *E.coli* O157:H7 was higher in dairy products (29; 3,6%) than in meat products (25; 3,1%).In our study *E.coli* O157: H7 was higher in chicken (5; 31,5%) than in meat products, fruits, and vegetables (3;18.75%).

Iyer et al. (2013), showed that a high incidence of *E.coli* was in open butcher shops (65%) and the results obtained are close to our findings. In our study, we found *E.coli* in 70,8% of isolates. On the other hand, *E.coli* O157:H7 was found in 11 isolates (22,9 %) as follow; fruit/vegetables 3 isolates (18.75%), meat 3 isolates (18.75), and chicken5 isolates (31,5%).This findings are close to that of Sharif et al. (2014) who also found the prevalence of *E.coli* O157: H7 ranged from 0% to 33% in onions and cabbage respectively. However, our study in contrary to other studies which did not find E*.coli* O157: H7 in their study of unwashed raw vegetables (Saeed et al., 2013).

Tanih et al. (2015) reported that *E.coli* was the most prevalent bacteria in meat sample 67,5% (104/154). Whereas, in our study, the presence of E.*coli* was the highest percentage (93.75%) in the chicken sample, and the lowest percentage (56.25%) was in themeat samples. Several studies have also been documented that the presence of *E. coli* O157:H7 in chicken samples. Karadal et al. (2013)found that *E. coli* O157: H7 was present in 1% (1/100) in processed chicken samples. Momtaz et al. (2013) who also detected E.*coli* O157: H7 in 7,3% (31/422) of chicken samples

The presence of many factors such as geographical area, samples, size and agricultural methods for vegetable production and different conditions of sanitary environments during slaughter and transportation of animals could contribute the difference of the results. In conclusion, the presence of *E.coli* Type I and *E.coli* O157: H7 in chicken, vegetable and meat determined in the present study, suggesting that they may be the main cause of human infections because of this organism. In order to protect public health, a series of control strategies should be developed and implemented at all stages of food production, system “from farm to table”, to eliminate contamination with this important human pathogen. More epidemiological studies are needed in order to determine the possible role of poultry and cattle as a source and/or reservoir of *E. coli* O157: H7. In addition, the

exact mode of entry of this bacterium into the food chain should be determined in order to develop and implement control measures.

4.2. Prevalence of Autotransporter genes in *E.coli* strain isolates from food and clinical sources

The isolates obtained from urine, blood, wound, and aspiration (N=65) collected by Onler, 2016. .A total of 65 ExPEC isolates including 54 (83%) isolates from urine, 5 (8%) isolates from the wound, 3 (4%) isolates from blood, and 3(4%) isolates from aspiration. The majority of ExPEC strains were isolated from urine samples shown in Figure.4. 4.

ExPEC strains were isolated from urine samples because *Escherichia coli* are a very diverse species of bacteria found naturally in the intestinal tract of all humans and many other animal species. A subset of ***E.coli*** is capable of causing enteric/diarrhoeal disease, and a different subset causes extraintestinal diseases and a different subset cause extraintestinal disease, including urinary tract infection (UTI) (Marrs et al., 2005).

Clinical samples collected from urinary tract infections, surgical site infections, pneumonia, meningitis strains between 2014-2016 in Cukurova University Balcali Hospital. The isolates were distributed as followed; 54 (83%) isolates from urine, 5 (8%) isolates from wound, 3 (4%) isolates from blood, and 3(4%) isolates from aspirtation Table 4. 4.

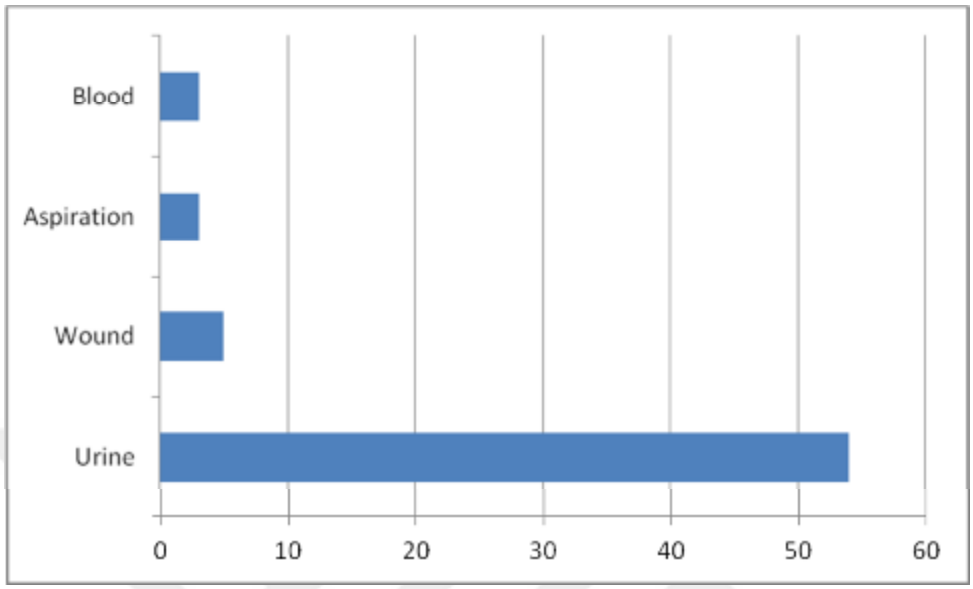

Figure 4.4. Distribution of clinical samples

The distribution of selected virulence genes in *E.coli* strains collected from food and clinical is shown in Figure 4.5. We found that 99% of the isolates were positive for at least one gene. Table 4.2 shows that one isolate harboured two genes simultaneously, ten isolates harboured three genes, and in twenty isolates four genes were detected. Ten isolates possessed five virulence genes. There was no strain that contained all 10 of the virulence-associated genes. However, nine isolates possessed six genes, while ten isolates had seven of the genes. Patterns and combinations of virulence-associated genes for 100 isolates collected in the present study are summarized in Table 2. Also, the prevalence of: Antigen 43 (Agn43), UpaC, UpaJ and temperature-sensitive hemagglutinin (Tsh) were 15%,6 %,8%, and 1% respectively.

The prevalence of autotransporter genes ranged from 1%for (tsh) to 15% for Antigene 43 (Agn43). . Overall, Serine auto-transporter toxin (Sat),Vacuolating autotransporter toxin (Vat), and UpaJ have not been detected in any isolate.

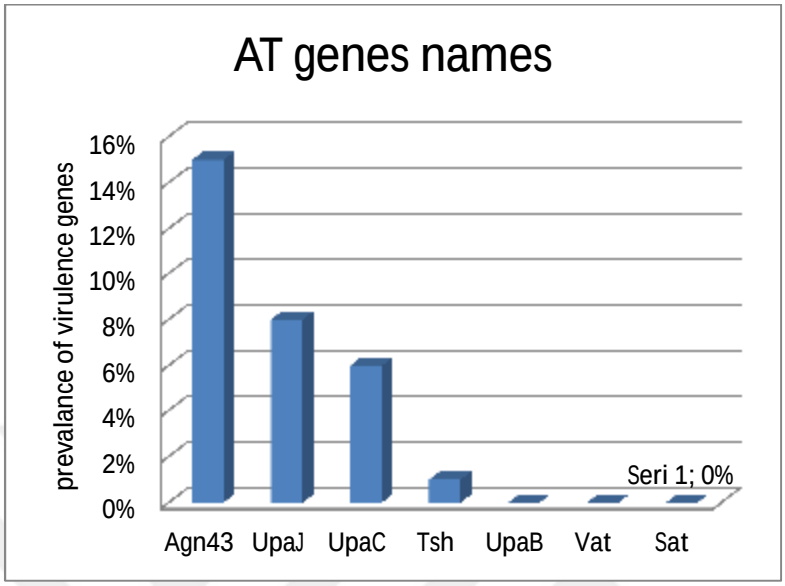

Figure 4.5. Analyze of autotransporter-related genes among *E.coli* strains

Our study investigated that the prevalence of Agn43 gene was present in 27% of ExPEC strains while Palma et al. (2016)) in Peru found that Agn43 was presented in 52.3% of 65 *Escherichia coli* isolates from blood cultures in children <5 years in Peru. Their result was higher than our result .

Agn43 gene was higher in clinical samples 8 (12%) while that Tsh gene was higher in food samples 2 (3%). Ag43 is a common phase-variable surface protein on many E. coli strains, and it is encoded by the flu (agn43) gene in E.coliK-12 (Diderichsen, 1980). Tsh was the first SPATE discovered by virtue of its hemagglutinin activity nearly two decades ago from the Avian pathogenic E. coli (APEC) strain ((Provence et al., 1994).

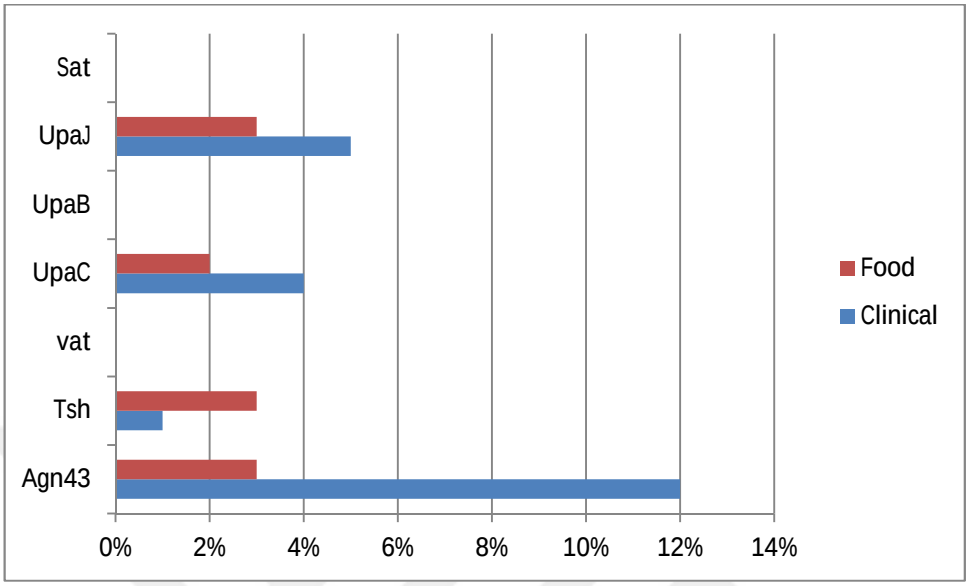

Figure 4.6. Comparison between the distributions of autotransporter related genes in clinical strains and food strains.

Shookohi et al. (2016) in Iran found that tsh was present in 1% among 100 UPEC isolates, and this was similar of our study Tsh (1%).However, they found vat (is a member of the SPATE subfamily of the autotransporters) was present in 18% while in our study we could not detected.

Guyer et al. (2000) identified a 107 kDa protein that was expressed significantly more often by *E. coli* strains associated with the clinical syndrome of acute pyelonephritis than by faecal strains (P = 0.029). Protein isolated from *E. coli* CFT073, a strain cultured from the blood and urine samples collected from a patient with acute pyelonephritis. Sat shows serine protease activity that is cytopathic for relevant targets, including cultured kidney, bladder and epithelial cells, that elicits an immune response in mice by wildtype *E. coli* CFT073. Genomic sequences homologous of sat are found in the majority of pyelonephritogenic strains but occasionally in faecal isolates. Comparing with other studies, Sat gene was not detected in our studydue to our materiel was not a patient with acute pyelonephritis.

Agn43 gene was higher in clinical samples 8(12%) while that Tsh gene was higher in food samples 2(3%). Ag43 is a common phase-variable surface protein on many *E. coli* strains, and it is encoded by the flu (agn43) gene in E.coliK-12 (Diderichsen, 1980). Tsh was the first SPATE discovered by virtue of its hemagglutinin activity nearly two decades ago from the Avian pathogenic *E. coli* (APEC) strain (Provence et al., 1994). Bacterial adhesins mediate attachment to host tissues and allow the first critical step in colonization to happen. UPEC have a range of different adhesins, many of which belong to the autotransporter proteins. We reported here the identification and characterization of the UpaJ (8%)in which 5 from clinical (3 unine and 2 wounds) and 3 from foods (2 fruits and 2 vegetables). And UpaC (6 %) in which 3 clinical (2 unine and 1 aspiration) and 3 foods (3 chicken).AT protein from *E.coli* Strains collected the clinical sources. However, half of all EXPEC isolates possess none, or only one AT, of the virulence factors characterized thus far, and hence other as yet uncharacterized bacterial factors may be important.

Table 4.2. Comparison between the distributions of autotransporter-related genes in clincal strains and food strains.

Genes name	**ExPec strains** N=65(%)	**Isolated place**	**Food strains** N=35(%)	**Isolated place**
Tsh	0(0%)	0	2(3%)	2 Chicken
Agn43	8(12%)	5 urine 2 wound 1 aspi**r**ation	1(3%)	1 meat
UpaC	3(4%)	2 urine 1aspiration	3 (2%)	3 chicken
UpaJ	5 (7%)	3 urine 2 wound	3 (8%)	2 chicken 1 vegetable
UpaB	0	0	0	0
Sat	0	0	0	0
Vat	0	0	0	0

The validation of theprimer sets and optimization of the specific primer sequence were used according previous studies. Following the optimization conditions, all primers used of seven different autotransporter genes were amplified using specific primers ofthe expected sizes (Figure 3.5).

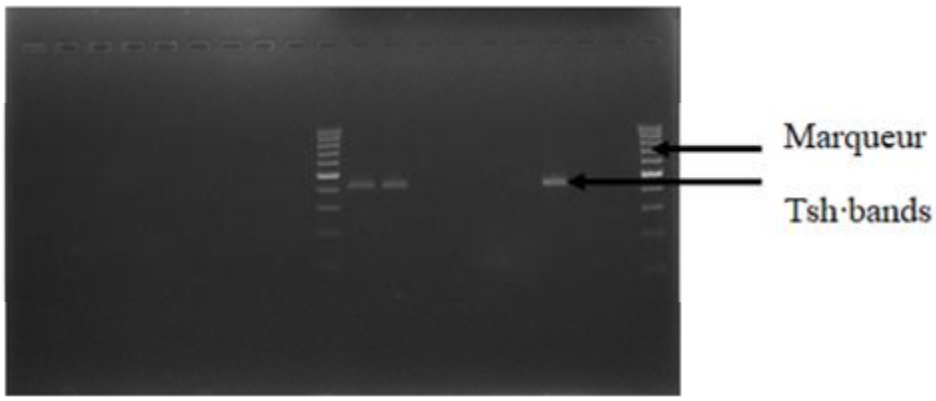

Figure 4.7. PCR amplification of Tsh (824bp) gene from *E.coli*. PCR product was visualized on 2% agarose

Agarose gel showing PCR products amplified TSH from PCR using specific primer sequence described in Materials and Methods (Table 3.5).

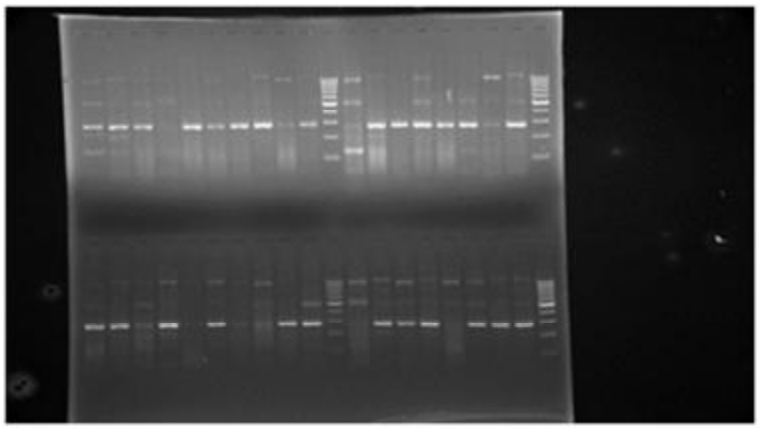

Figure 4.8. PCR amplification of UpaJ (250bp) gene from *E.coli*. PCR product was visualized on 2% agarose

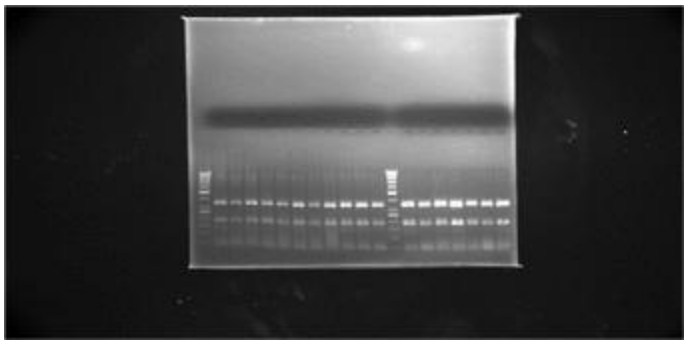

Figure 4.9. PCR amplification of UpaC (300bp) gene from *E.coli*. PCR product was visualized on 2% agarose

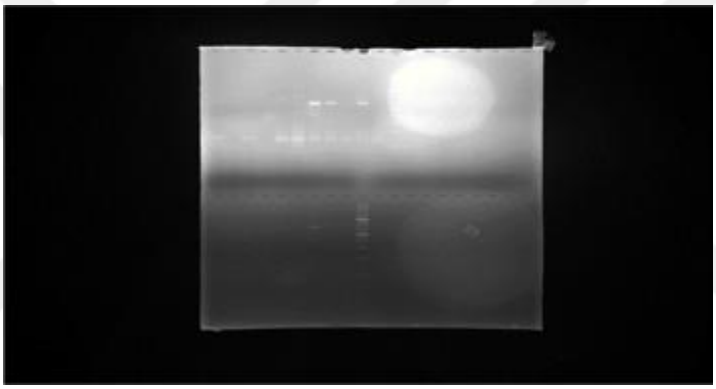

Figure 4.10. PCR amplification of Ag43 (600bp) gene from *E. coli*. PCR product was visualized on 2% agarose

Extraintestinal *Escherichia coli* (ExIPEC) are a diverse group of pathogenic *E. coli* strains, which have high ability to colonize several extraintestinal sites, as the blood stream, the meninges and the urinary tract, and this ability is dependent upon specific virulence factor genes. The great differences have been showed through comparison of whole-genome of *E.coli* strains which cause different diseases (i.e. intestinal and extraintestinal infections) (Welch et al., 2002). These differences were also present within ExIPEC strains which were a cause of the same disease and infect the same host tissues (Dobrindt et al., 2002). ExIPEC have a high ability to effectively infect the gastrointestinal tract (Johnson et al., 2000) and this ability depends up on their virulence factors (VFs) which

enable the strains to avoid or subvert local and systemic host defense mechanisms, colonize host mucosal surfaces, injure or invade the host, scavenge essential nutrients such as autotransporter (Bower al., 2005). Autotransporters are secreted proteins that plays role in virulence in avian pathogenic *E. coli* and some other types of pathogenic *E. coli*. The autotransporter proteins secreted from gram-negative bacteria that represent bacterial virulence factors, such as adhesins, proteases, and toxins. Various autotransporters have been identified among *Escherichia coli* associated with intestinal or extraintestinal infections.

Recent studies described the existence of several autotransporters in the UPEC strain CFT073, including the SPATE proteins, sat, and TSH (Parham et al., 2004) and theyalso demonstred the presence of those autotransporter protein. Such conservation within a subset of pathogenic strains suggests that TSH might be important in allowing *E.coli* to cause UTI (Parreira et al., 2003). In our study, we demonstated presence of Tsh 1%.Furthermore, Heimer et al.(2004) demonstrated by reverse transcription-PCR that tsh was expressed in mice during experimental urinary tract infection. In summary, we have demonstrated that tsh is specifically associated with extraintestinal phylogenetic groups of *E.coli* but have not been able to demonstrate a correlation with a specific clinical manifestation(Oelschlaeger et al.;2002).We report here the characterization of tsh, a highmolecular- height secreted protein, and its corresponding gene from highly virulenturopathogenic E. coli CFT073, a strain cultured from the blood and urine of a woman (Bien et al.,2012). The prevalence of the detected genes among the studied isolates and the frequency of concurrent detection of various virulence factors are summarized in Figure 4. 2. Antigen 43 (Agn43) 15%, temperature-sensitive hemagglutinin (Tsh) 1%, UpaC 6 % and UpaJ 8% were detected. On the other hand no copy of vat, sat and UpaB genes was not detected in any of the isolates. Our results showed a lower prevalence of temperature-sensitive hemagglutinin (Tsh) and hight prevanlce ofAntigen 43 (Agn43) 15% in *E. coli* strains collected from food and clinical sources isolates.A study by Palmaet al., (2016), in contrary to our study reported

presence of Agn43 (72.3%) in their study.Among of 65 *Escherichia coli*isolates from blood cultures in children, older than 5 years in Peru and Brazil. The Agn43 gene is an autotransporter involved in biofilm formation (Danese et al., 2000) and is widely distributed in pathogenic *E. coli* (Restieri et al., 2007).This resultat show higher prevalence of Ag43 than our resultat. We can explicate that Agn43 is present more in children and these difference may be due the difference of country and geographical location. In our study it is indicated that the prevalence of genes coding for autotransporter such as UpaJ, UpaC and tsh were present in 15%,8% ,6% and 1% of all ExInP isolates. Presence of antigen 43 (Agn43) was high while the presence of temperature-sensitive hem agglutinin (Tsh) was low. We could not find sat and vat genes because it was not easy to detect toxin genes and demonstate by just using specif primer. “Sat is a serine auto-transporter toxin, which displays cytopathic activities on the kidney and bladder epithelium in vitro and in vivo “ (Guyer et al., 2000; Guyer et al., 2002; Anderson et al., 2004). In our study, we used clinical samples collected from blood, urine, wound and aspiration. This can be reason we could not be able to detect sat gene in our study. UpaB and vat were not detected too because a lack of enough information.

4.3. Determination of the phylogenetic relationship among *E.coli* strains, which were isolated from food and human

In this study, the phylogenetic relationships between *E. coli* strains isolated from food and human were studied by PFGE method (PFGE) using restriction enzyme XbaI, to constitute evidence of transmission of pathogenic E. *coli* strains to human from food, and the distribution of siderophore genes have also studied using PCR method. Gel-compar II program was used to evaluate and interpret the DNA fragment profiles obtained from PFGE method. The relationship between strains was determined by similarity coefficient of DNA profiles and cluster analysis was performed on the basis of ≥ 80% of similarity ratio and the

similarity ratios were shown in dendograms (Figure 4.12). The genotype analysis of *E. coli* strains by XbaI-PFGE method show that:

- Only 13 (20.96%) out of the 62 clinical strains were clonally related to each other and these strains were distributed in a total of 5 clusters (Ah, Ch, Eh, Fh and Gh). The cluster F included two of subgroup with 4 members (hu34-hu35-hu43-hu42), the remaining strains were distributed in unrelated clusters with one member.
- Only 4 (14.81%) out of 27 the food strains, were clonally related to each other, and they were in two clusters (A and D), the remaining strains form single member clusters.
- Three strains of clinical samples and 3 strains of food borne samples were collected in 2 clusters, 2 members in B cluster and 4 members in A cluster and there was no clonal relationship between other clinical and food isolates (Table 4. 4).

As a result, the evaluation of PFGE analysis showed that 6 (6. 8%) out of 89 human (Figure 4.11) and foodborne (Figure 4.12.) isolates had a clonal relationship. And we deducted there was no significant relationship between the other isolates. this number of evaluated isolates was not sufficient to produce the statistical significance, and there was no strong evidence of transmission of ExPEC to human from food.

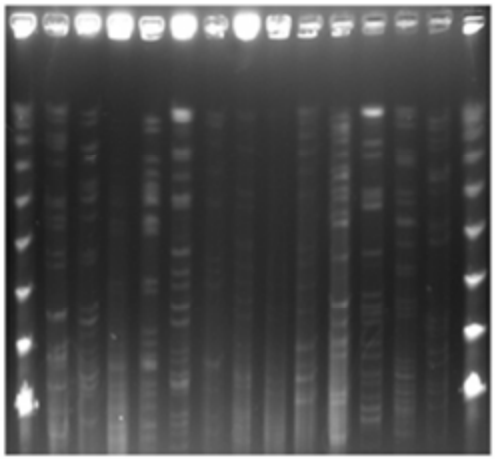

Figure 4.11. Pulsed-field gel electrophoresis (PFGE) profiles of selected *Escherichia coli* isolates from humans.

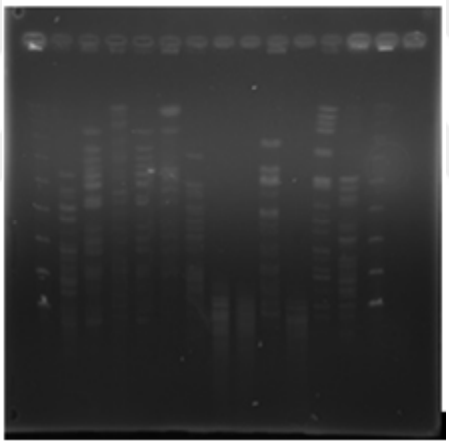

Figure 4.12. Pulsed-field gel electrophoresis (PFGE) profiles of selected *Escherichia coli* isolates from food.

Furthermore, the existence of multiple SPATEs suggests an element of functional redundancy such that the absence of Vat may be compensated for by the presence of other SPATEs (Parham et al., 2005). Investigation of the ECOR collection revealed that SPATE proteins were clustered in the B2 and D groups (Table 4. 3) and a subgroup of the a phylogenetic branches.

Table 4.3. Phylogenetic relationship among *E. coli* strains.

Sample	ClusterSubset	İsolate		Philogenetik relation (%)
Human origin	**A**	**ai1**	**in52**	**85**
		ai2	**in22**	
	C	**c_i1**	**in17**	**87**
		c_i2	**in18**	
		c_i3	**in26**	
	E		**in33/in41**	**80.6**
	Ff_i 1in34/in35			**88.9**
		f_i 2	**in43/in42**	
	Gin37/in45			**88.7**
Food origin	**A**	**a_g1**	**g9**	**85**
		a_g2	**g11**	**80.3**
	D		**g10/g12**	
Human–food origin	**A**	**a1**	**in52/g9**	**85**
		a2	**in22/g011**	**80.1**
	B		**in25/g2**	

The phylogenetic analysis between the human *E. coli* and food-borne *E. coli* strains were studied by PFGE method and the prevalence of autotransporter genes in PFGE clusters of these strains were examined, and the result was as that:

- UpaB, Sat and Vat gene were not found in any of the isolates.
- Agn43 gene was found in large.
- The presence of the tsh and the absence of vat genes were the common features between one isolate of human and one isolate of foodborne which were in subgroups of A1.

- The simlarity of autotransporter genes of two strains of foodborne in D group was 90% and the simlarity of autotransporter genes of two strains of human in f1 and one strain in f 2 was also 90%.
- PFGE analysis of isolates belonging to human and food samples, which had 93,2% of similarity and located in subgroup 2, they had same 75% of autotransporter genes.

The prevalence of the autotransporter genes in food-borne isolates was less than human-borne isolates, and it was concluded that the ability to transport protein to surface is easy in human *E. coli* isolates than food. As a result, the analysis of autotransporter genes distribution alone and with PFGE analysis showed that there were not clonal correlations between food-borne strains and clinical strains. It has been concluded that clinical strains may have been the result of mobile genetic elements or chromosomal mutations in human and animal intestinal systems and this confirmed the hypothesis that individual virulence gene acquisitions can occur concurrently against a back ground of horizontal gene transfers of pathogenicity islands. Over time, this could enable specific clonotypes to respond to host selection pressure and to evolve into new strains with increased virulence.

Table 4.4. The prevalence of autotransporter genes in PFGE clusters of *E. coli* strains.

Cluster	Subset	Strain No	Tsh	Agn43	UpaC	Vat	UpaB	UpaJ	Sat
A	a1	hu52	-	+	-	-	-	-	-
		f9	-	-	-	-	-	+	-
	a2	hu22	-	-	+	-	-	-	-
		f11	+	-	-		-	-	-
B	b1	hu25	+	+	-	-	-	+	-
		f2	-	-	-	-	-	-	-
C	c1	hu17	-	+	-	-	-	+	-
		hu18	-	+	-	-	-	-	-
	c2	hu26	-	-	-	-	-	-	-
D	d1	f10	-	+	+	-	-	+	-
		f12	-	-	-	-	-	-	-
E	e1	hu33	-	-	-	-	-	-	-
		hu41	-	+	-	-	-	+	-
F	f1	hu34	-	+	+	-	-	-	-
		hu35	-	-	-	-	-	+	-
	f2	hu43	-	+	-	-	-	-	-
		hu42	-	+	-	-	-	+	-
G	g1	hu37	-	+	-	-	-	+	-
		hu45	-	-	+	-	-	+	-

Only one instance of a close match between chicken and a human isolate by PFGE analysis. Our study found that three strains of human and three strains of food were collected in 2 clusters, 2 members in B cluster and 4 members in A cluster (figure 4.12).

The analysis of food and clinical isolates classified by phylogenetic group further illustrated the phylogenetic associations of some AT in *E. coli*. Tsh sequences were dispersed among different phylogenetic groups of food strains.

And Agn43 allele in general was associated with strains belonging to phylogenetic group B2.

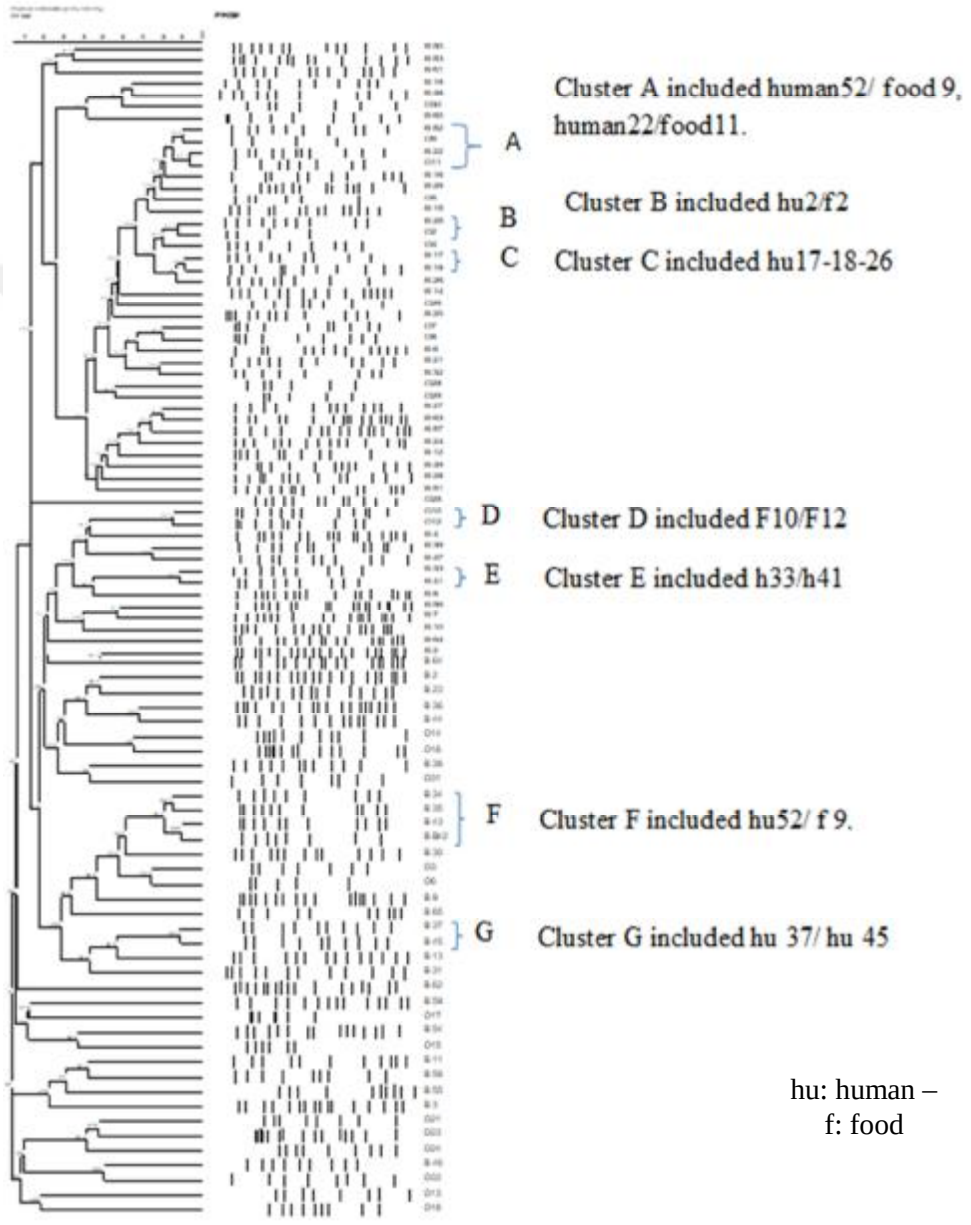

Figure 4.13. Dendrogram depicting inferred phylogeny of 69 isolates using PFGE method.

5. CONCLUSION AND RECOMMANDATION

5.1. Conclusion

Autotransporters are one category of secreted proteins implicated in virulence of *E. coli.* The autotransporter proteins constitute a distinct family of proteins secreted from gram-negative bacteria that represent bacterial virulence factors, such as adhesins, proteases, and toxins. Members of this family include virulence factors of important human pathogens, such as the immunoglobulin A1, TSH and vat from avian pathogenic *E. coli,* Sat from uropathogenic E. coli. The autotransporters have an overall unifying structure comprising (i) an N-terminal signal sequence, (ii) the secreted mature protein (or passenger domain), and (iii) a C-terminal domain, which forms a pore in the outer membrane through which the passenger domain passes to the cell surface.

Properties associated with virulence of *E. coli* include temperature-sensitive hemagglutinin (TSH), Antigen 43 (Agn43), Serine auto-transporter toxin (Sat), Vacuolating autotransporter toxin (Vat), UpaJ, UpaC, and UpaB in ExPEC isolates. Agn43 gene was higher in clinical samples 8 (12%) in which 5 urine, 2 wounds and 1 aspiration while that TSH gene was higher in food samples 2 (3%). The UpaJ (8%) in which 5 from clinical (3 unine and 2 wounds) and 3 from foods (2 fruits and 2 vegetables). And UpaC (6 %) in which 3 clinical (2 unine and 1 aspiration) and 3 foods (3 chicken).E. coli strains isolated from clinical specimens were found to have higher prevalence autotransporter genes than E. coli strains isolated from food samples.

These virulence factors are protein structures located at the cell surface, proteins that are secreted to the bacterial cell surface, or proteins that are released into the external environment.

The autotransporter, or type V, secretion system is a dedicated protein translocation mechanism which allows the organism to secrete proteins to and beyond the bacterial surface. Seven autotransporter subgroups represented by

Antigen 43 (Agn43) 15%, temperature-sensitive hemagglutinin (Tsh) 1%, and Upac 6%, Serine auto-transporter toxin (Sat), Vacuolating autotransporter toxin (Vat), UpaJ, and UpaB were not detected in this study and thus may have more specific functions. Being able to identify particular pathotypes of a bacterial species has proved very helpful in understanding the particular disease processes involved and in identifying particular virulence factors.

As a result, the evaluation of PFGE analysis showed that 6 (6.8%) out of 89 human and foodborne isolates have a clonal relationship, while there was no significant relationship between the other isolates, this number of evaluated isolates was not sufficient to produce the statistical significance, and there was no strong evidence of transmission of ExPEC to human from food.

5.2. Recommandation

In this current work, the analysis of strains focused on mostly extraintestinal isolates from human and food samples. It would be of further interest to examine a set of characterized E. coli isolates belonging to different pathotypes such as diarrheagenic E. coli of UPEC. Clearly, we need to determine the distributions of Autotransporter, both those studied in this study and others as they are discovered, in large collections of epidemiologically because it has not been described and not yet characterized yet. This process will be facilitated by the existence of a complete genome and understand if there relate a relationship between food and human isolates.A great deal of research is required to understand both the roles of these proteins and the fundamental features of autotransporter secretion and processing. Among different geographical regions, because of differences in social, economic, health, hygiene and environmental conditions more studies can be occurred to identify the virulence factors of ExPEc. Furthermore, studies are needed to determine the relationship between the expression of virulence factors and antibiotic resistance.

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