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College of Medicine

Multiple Antibiotic Resistance and Integrons among Commensal *Escherichia coli*

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بسم**الله** الرحمن الرحيم

((وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ
اللَّه عَلَيْكَ عَظِيم۞))

صَدَقَ اللّٰهُ العَلِيُّ العَظِيم

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Dedication ----------

I Introduce This Project As Expression Of My Great Love

For

My Dears, Father & Mother

Certification of Examining Committee

We, the members of examining committee, certify the candidate thesis (Multiple Antibiotic Resistance and Integrons among Commensal *Escherichia coli*) and after examining the student in its contents, we have found adequate for the degree of Doctor of philosophy in Medical Microbiology.

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Summary

Commensal *Escherichia coli* (*E. coli*) is one of the most common bacteria in the environment and in the intestinal tract of humans and animals. The intensive use of antibiotics in both human and veterinary medicine, as well as in the fields of agriculture, is associated with an emerging resistance against therapeutic drugs, followed by the selection of resistance gene cassettes (such as integrons associated gene cassettes) carrying *E. coli* isolates in humans, animals and the environment. Phylogenetic analysis and association between multiple drug resistance (MDR) and integrons among commensal *E. coli* in Iraq are not studied well therefore, the current research was carried out in order to determine the phylogenetic groups of commensal *E. coli* and their role as reservoir and source of MDR in human gut.

In present study, 350 fecal samples are collected from healthy individuals with age range from 1 to 80 years among which only 301 individuals harbor commensal *E. coli* in their gut that identified by cultures and biochemical tests, then their susceptibility to sixteen antibiotics was assessed by the disc diffusion method. After deoxyribonucleic acid (DNA) extraction, samples subjected to multiplex polymerase chain reactions (PCR) for determining the phylogenetic groups and conventional PCR for integrons detection.

In this study phylogenetic group B2 represent the majority of the collected isolates (63%) followed by group A (23%) and D (14%) but no isolates were found to belong to group B1and most abundant subgroup are $B2_3$ (40%) flowed by $B2_2$ (23%). Also results revealed that 70% of isolates have integrons from which 37% have only integron class 1, 10% have merely integron class 2 and 23% have both integron class 1and 2 while other 30% of isolates not carry any class of integron. Healthy individuals who have commensal *E. coli* are divided into seven

groups according to age and results show highest frequency of integron class 1 detected in isolates in individuals in group 1 (74%) while highest frequency of integron class 2 observed in isolates in group7(31%). Also results observed that highest distribution of integron class1 among phylogenetic groups recorded in group A (52%) flowed by group D (48%) and group B2 (29%) while integron class2 demonstrated only in group B2 (15%) and group A (3%). On other hand 29% of group B2 have both classes of integron flowed by 17% of group D and 9% of group A.

Results showed all *E. coli* isolates (phylogenetic groups) in different age are resist to Lincomycin, Amoxicillin, Cephalexcin and Ampicillin (100%). Also high resistance detected to Cefotaxime, Tetracyclines, Trimethoprim and Chloramphenicol. Most isolates are resisted to 11 antibiotics (19%) and among MDR isolates, over 20 resistance pattern were observed, the most frequent resistance patterns were AM-AX-L-CL (100%) flowed by AM-AX-L-CL-CTX (93%) and AM-AX-L-CL-CTX-TE (74%). Present study showed all integrons positive phylogenetic groups are resisted to six or more antibiotics and resistance patterns demonstrated more clear in integrons positive phylogenetic groups compared with integrons negative phylogenetic groups.

In conclusion, commensal *E. coli* isolates are abundant in this population and investigated in different age groups and the most common phylogenetic group among commensal *E. coli* isolates are group B2 (mainly subgroup $B2_3$) while group B1 are absent. Integron class 1 and class 2 are determent in part of tested isolates and integron class 1 is the most common. All isolates are MDR and have many resistance patterns that significantly reported in integrons positive commensal *E. coli*, this indicate that human fecal *E. coli* is a important reservoir and pool of antibiotic-resistant genes that transfer by horizontal ways in bacterial population

1. Introduction and Literature Review

1.1. Introduction

The emergence of antibiotic resistance among pathogenic and commensal bacteria has become one of the most significant global health challenges of this century. Resistance to antibiotics has a high prevalence in bacterial isolates from developing countries which are resulted from the overuse and abuse of antibiotics (Bailey *et al*., 2010; Chantziaras *et al*., 2014; Hiltunen *et al*, 2017; Schroeder *et al*., 2017).

Commensal *E. coli* inhabits the intestine of many mammals including human which are a potential reservoir for antimicrobial resistance genes and play an important role in the ecology of antimicrobial resistance of bacterial populations. The prevalence of resistance in commensal *E. coli* is a useful indicator of antibiotic resistance in bacteria in the community (Phongpaichit *et al*., 2008; Li *et al*., 2014; Al-Saedi *et al*., 2017).

Phylogenetic analysis have been shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) and that virulent extra-intestinal strains mainly belong to group B2 and to a lesser extent to group D whereas most commensal strains belong to group A and B1. (Derakhshandeh *et al.*, 2013; Logue *et al*.,2017).

Commensal *E. coli* strains efficiently exchange genetic material with other pathogens such as *Salmonella*, *Shigella*, *Yersinia* and *Vibrio*, as well as pathogenic *E. coli* (Betteridge *et al*.,2011; El-Shennawy, 2011; Oluyege *et al*., 2015; Schroeder *et al*., 2017). Recently this exchange of many different and diverse genes responsible for antibiotic resistance has been linked to genetic structures called integrons, that integrate and mobilize individual gene cassettes encoding antibiotic resistance determinants (Sepp *et al*., 2009; Cury *et al.*, 2016; Nourbakh *et al*., 2017).

Integrons are a central players in the worldwide problem of antibiotic resistance, because they can capture and express diverse resistance genes. In addition, they are often embedded in promiscuous plasmids and transposons, facilitating their lateral transfer into a wide range of bacterial strains. Understanding the origin of these elements is important for the practical control of antibiotic resistance and for exploring how lateral gene transfer can seriously impact on human activities (Gillings *et al.*, 2008). There are many types of integron have been identified and distinguished by their respective integrase (*IntI*) genes. Class 1 integrons are strongly associated with multi-resistance in *Enterobacteriaceae* in the hospital environment while class 2 integron were present among *Acinetobacter*, *Shigella*, *E. coli* and *Salmonella* isolates (Cocchi *et al*., 2007). The class 2 integron is related to the class 1 integron (46% amino acid identity) and both these integrons are also present in resistant intestinal *E. coli* isolated from subjects living in the community (Jelesić *et al*., 2011; Mazurek *et al*., 2015).

Unfortunately most studies on antimicrobial agents and resistance genes focused on pathogenic bacteria, and only few studies carried out on commensal bacteria (Dyar *et al.*, 2012; Dureja *et al.*, 2014). Although the commensal bacteria are acted as an important reservoir of antibiotic resistant genes and they are capable of facilitating the spread of resistant genes to pathogenic strains. Moreover it can cause serious extra-intestinal infection. Also most phylogenetic studies on *E. coli* in Iraq and other countries carried out on pathogenic *E. coli* isolates which are considered as a causative agent for recurrent urinary tract infection (UTI) and other diseases, and worldwide a little attention was paid on the importance of the multiple antibiotic resistance among commensal *E. coli*. So we believed that under taking such studies is not only beneficial for

providing new information, but also important for initiating the first step towards future studies.

Aim of the study

The present study aims to identify the role of commensal *E. coli* as reservoir and source of multiple antibiotic resistance genes that can be spread to surrounding bacterial populations by transposable elements, therefore the current study is conducted to:

1. Isolation of commensal *E. coli* from stool of healthy individuals
2. Determine phylogenetic groups and subgroups of commensal *E. coli*.
3. Determine the prevalence of antibiotics resistance among commensal *E. coli* isolates.
4. Determine the prevalence of integrons genes (class1 and class2) among commensal *E. coli*.
5. Determine the association between antibiotics resistance (single or multiple antibiotic resistance) and integrons among commensal *E. coli* isolates that can transmit to pathogenic or commonsal bacteria by horizontal ways.

1.2. Literature Review

1.2.1. *Escherichia coli*

Escherichia coli is a member of the family *Enterobacteriaceae,* a gram-negative, rod shaped and facultative anaerobic bacterium (Nicolas *et al*., 2012; Hertz, 2014). *E. coli* bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich. Escherich also showed that certain strains of the bacterium were responsible for infant diarrhea and gastroenteritis and that an important public health discovery. Although *E. coli* bacteria were initially called Bacterium coli, the name was later changed to *Escherichia coli* to honor its discoverer (Tamerat,, *et al*., 2016). It is a highly versatile bacterial species comprised of both harmless commensal strains and different pathogenic variants with the ability to cause either intestinal or extraintestinal diseases (Pitout, 2012). Consequently, *E. coli* strains are broadly classified into three major groups of commensal *E. coli*, intestinal pathogenic *E.coli* (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Carroll, 2013). The non pathogenic strains of *E. coli* referred to as commensal strains are harmless and are useful, not only in digesting and breaking down food, but also in protecting against harmful organisms which may be introduced into the gastrointestinal tract through food and water (Chinen and Rudensky, 2012; Hertz, 2014).

The pathogenic *E. coli*, IPEC and ExPEC, can be each one further subcategorized into specific pathotypes. This classification is based on clinical manifestations of disease and the pathogenic traits such, as presence of virulence factors (Clermont *et al*., 2013; Logue *et al*.,2017). The most prevalent ExPEC pathotypes are the uropathogenic *E. coli* (UPEC) and meningitis-associated *E. coli* (MNEC) (Keys *et al*., 2005; Coura *et al*., 2015). IPEC cause diseases of the intestinal tract that involve six different *E. coli* pathotypes including enteropathogenic *E. coli*

(EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). Often intestinal non-pathogenic *E. coli* and IPEC can be distinguished by genome content and phenotypic traits, but the discrimination between commensal *E. coli* and extraintestinal pathogens is not easy (Jørgensen *et al.*, 2010; Igbeneghu and Lamikanra, 2014). ExPEC strain are habitually found as part of the commensal flora of healthy individuals without causing enteric disease (Clermont *et al*., 2013; Horner *et al*., 2014). While IPEC cause diseases of the intestinal tract, ExPEC can cause a range of diseases in almost any anatomical niche such as UTI, bacteraemia, meningitis and intra abdominal infections (Clermont *et al*., 2013; Manges, 2016).

1.2.1.1. *Escherichia coli* Phylogenetic Groups

Several techniques can be performed to determine phylogenetic group of *E. coli*, such as multilocus enzyme electrophoresis, ribotyping, random amplified polymorphic DNA analysis, fluorescent amplified-fragment length polymorphism analysis, PCR phylotyping using the presence/ absence of three genomic DNA fragments, analysis of variation at mononucleotide repeats in intergenic sequences and multilocus sequence typing (MLST). MLST is now clearly the "gold standard" technique but it is costly and time-consuming (Chaudhuri and Henderson, 2012). PCR phylogenetic technique that described by Clermont *et al* was based on a triplex PCR using a combination of two genes (*chuA*, the outer-membrane hemin receptor gene, and *yjaA*, which encodes an uncharacterized protein) and a DNA fragment (TSPE4.C2) that has been recently identified as part of a putative lipase esterase gene (Clermont *et al.*, 2000; Tenaillon *et al.*, 2010). This method, whose results strongly correlate with those obtained by other standard methods,

is an excellent technique for rapid and inexpensive assigning of *E. coli* strains in different phylogenetic groups (Barzan *et al.*,2017).

By triplex PCR, commensal and pathogenic *E. coli* collectively classified into four different phylogenetic groups; A, B1, B2, and D. (Derakhshandeh *et al.,* (2014) as in Figure (1-1). Phylogenetic groups A and B1 are sister groups whereas groups D and B2 exhibits the highest diversity at both the nucleotide and the gene content level, supporting its early emergence in the species lineage and suggesting that it has subspecies status (Tenaillon *et al.,* 2010).

These phylogroups apparently differ in their ecological niches, life-history and some characteristics, such as their ability to exploit different sugar sources; their antibiotic resistance profiles and their growth rate (Jang *et al.,* 2014; Clermont *et al.,* 2015). The distribution (presence/ absence) of a variety of genes thought to enable a strain to cause extra-intestinal disease also varies among strains of the four phylogenetic - groups. Several studies have shown the relation between phylogeny and pathogenicity of *E. coli* strains and revealed that most commensal strains belong to A and B1 groups, and that the virulent extra-intestinal strain return mainly to group B2 and to a lesser extent to group D (Katouli , 2010; Derakhshandeh *et al.,* 2013; Iranpour *et al.,* 2015).

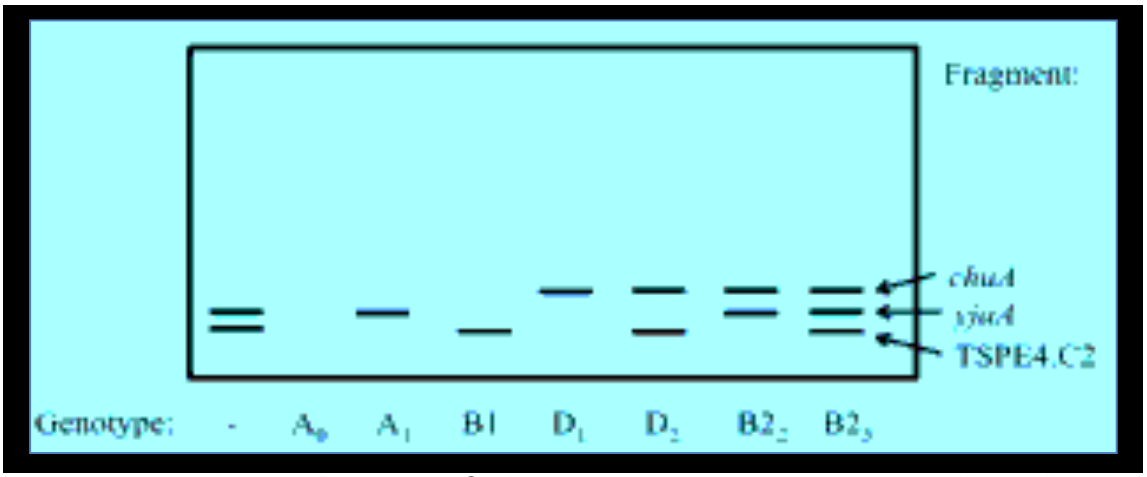


Figure (1-1): The Triplex PCR of Clermont That Represent a Hypothetical Gel Image of The Phylogenetic Group of *E. coli* (Anantham, 2014).

1.2.1.2. Normal Flora in Gastrointestinal Tract

Commensal bacteria asymptomatically colonize the skin, oral cavity, nasal cavity, gastrointestinal system and other surfaces in the human body that come into contact with the external environment soon after birth (Alekshun and Levy, 2006; Tenaillon *et al*., 2010). In humans the gut flora is established at one to two years after birth, and by that time the intestinal epithelium and the intestinal mucosal barrier that it secretes have co-developed in a way that is tolerant to, and even supportive of, the gut flora and that also provides a barrier to pathogenic organisms (*Jonkers, 2017).*

The relationship between gut flora and humans is not merely commensal (a non-harmful coexistence), but rather a mutualistic relationship. The normal microbiota in gut provides a first line of defense against pathogens, assist the host organism in biochemical process such as digestion, play role in toxin degradation and contribute to immune system maturation (Alteri and Mobley, 2012; *Linda et al.,* 2013; Kwong *et al*., 2017). Shift in normal microbiota in GIT may cause diseases as inflammatory bowel disease. Many aerobic and anaerobic commensal bacteria colonize GIT and occasionally individuals are found without normal flora (Bailey *et al*., 2010; Tenaillon *et al*., 2010).

The microbial composition of the gut flora varies across the digestive tract. Stomach have lower concentration of micro-organisms because acidic environment. Lower concentration in upper small intestine of micro-organisms also appeared due to peristaltic motility and the rapid transit of the intestinal content in this part of GIT while lower intestine and large intestine content highest concentration of micro-organisms as *Bacteriodes, E. coli, Clostridium, Enterococci* and many other species. (Peter *et al*., 2002; Anantham , 2014; Hertz, 2014; Sam *et al*., 2017).

Colon contains a densely-populated microbial ecosystem with up to 10^{12} cells per gram of intestinal content. These bacteria represent between 300 and 1000 different species. However, 99% of the bacteria come from about 30 or 40 species (*Saxena* and *Sharma*, 2016*).*

Commensal *E. coli* is used because it have the ability to transfer resistance between different strains and species of bacteria within the gastrointestinal tract and between *E. coli* strain itself, they are found in high numbers in warm mammals including humans and they reside primarily in mammalian hosts, thus being subjected to the pressures of antimicrobial use and other environmental factors (Bartoloni *et al*., 2006; Vittecoq *et al*., 2017). This makes them an ideal agent for surveillance and research into factors that may contribute to the select spread of resistant bacteria. Further, these bacteria are abundant in the environment making them a predominant vehicle for the transmission of resistance genes (Abdelhaleem, *et al*., 2014).

1.2.1.2.1. Role of Commensal *Escherichia coli* in Infectious Diseases

Once commensal *E. coli* strains enter a sterile anatomical site such brain, urinary tract and spinal cord can cause an infection and their ability to cause diseases are varies. The severity of infections by commensal *E. coli* depend on bacterial virulence factors and immune system of the body (Bien *et al*., 2012; Chaula *et al*., 2017).The most common infections cause by *E. coli* strains are urinary tract infections especially in women with recurrent UTI (Moreno *et al*., 2008; Moreno *et al*., 2009; Süzük *et al*., 2015).

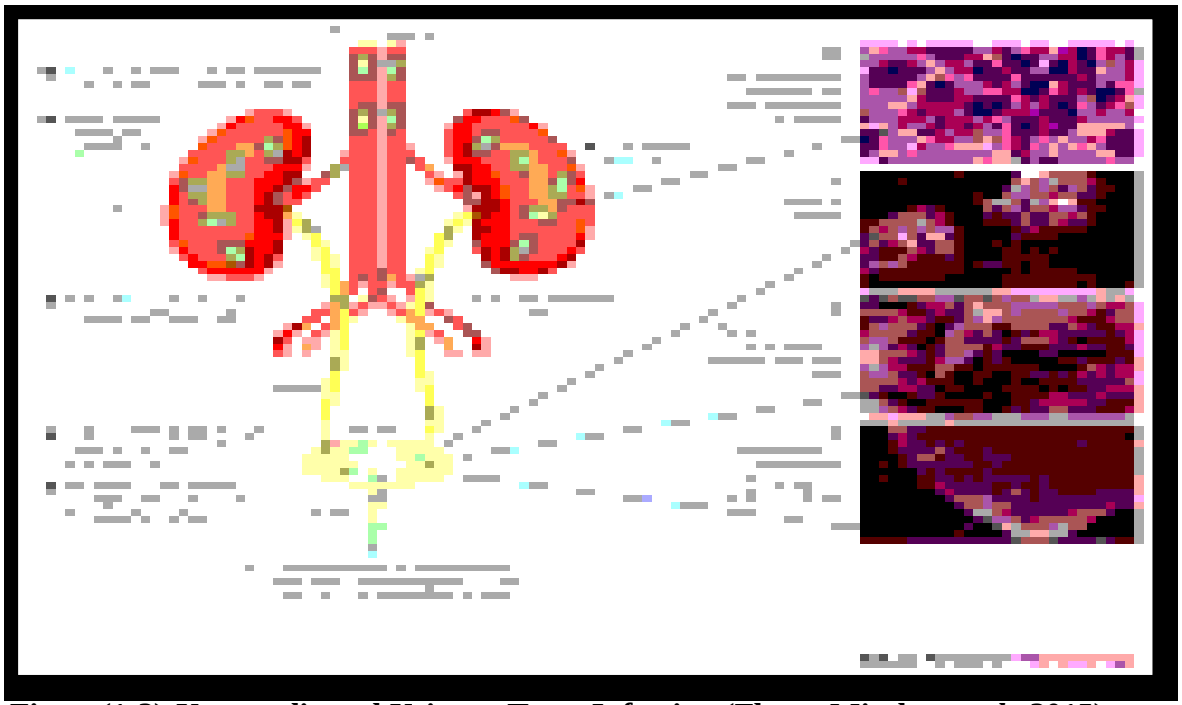
Urinary tract infection is the presence of significant numbers of pathogenic bacteria in the urinary tract (Wiles *et al*., 2008; Elizabeth 2010; Katouli, 2010; Hannan *et al*., 2012; Rosello *et al*., 2017). The primary route of UTI is ascending way that result from contamination by

feces that contain commensal and pathogenic bacteria. UTIs are among the most common infections that affect humans when fifty percent of all women will experience at least one UTI in their lifetime. In 90% of uncomplicated UTIs, the most common bacterium is *E. coli* (Giray *et al*., 2012; Jafri *et al*., 2014; Nielsen *et al*., 2014). It may be symptomatic in some individuals, particularly amongst the elderly population, who have asymptomatic bacteriurea. UTI can be divided into several categories including bacteriuria, cystitis, pyelonephritis and urosepsis, and the severity of infection increases as the infection ascends from the bladder to the bloodstream (figure 1-2). From a clinical perspective, the pathology of *E. coli* infections is quite well understood, particularly in the case of urinary tract infections, which have been studied since the 1960s (Wiles, *et al*., 2008;Hannan *et al*., 2012;Pitout 201Hannan and Hunstad, 2016).

Bacteriurea is the presence of *E. coli* in the urine but the symptoms usually associated with cystitis, such as pain. Cystitis and bacteriuria are defined as uncomplicated UTI of the lower urinary tract but in some cases, *E. coli* can ascend the urinary tract and infect the kidney, causing pyelonephritis, a more serious form of UTI. Severe kidney infections can result in urosepsis, which is defined as a bloodstream infection that is caused by bacteria originating from the urinary tract (Giray *et al*., 2012; Flores-Mireles *et al*., 2015). *E. coli* strains that cause infections usually express traits that are called virulence factors. Attempts to establish which commensal *E. coli* strain may be caused a UTI are difficult this is because fecal sampling in prior studies often begins post-infection, once symptoms have emerged, which is normally days after the onset of infection. At this point, the strain composition in the colon could have changed (Sabate *et al*., 2006; Anantham, 2014).

Two theories that attempt to describe which commensal strains can cause UTI have emerged. These are the special pathogenicity and the prevalence theories (Anantham, 2014) . The prevalence theory states that the numerically dominant strain in the feces at the time of infection was likely to be the cause as it had a greater opportunity to cause infection. The alternate special pathogenicity theory postulates that *E. coli* with specific virulence factors, regardless of their abundance in the feces, cause infections (Anantham, 2014).

Another major infection caused by *E. coli* strains is neonatal meningitis, where infection of the cerebrospinal fluid of newborns occurs and it has a greater risk of mortality than uncomplicated UTI (Pitout, 2012). *E. coli* also cause of nosocomial pneumonia in patients who have underlying disease such as diabetes mellitus, alcoholism, chronic obstructive pulmonary disease. *E. coli* pneumonia usually manifests as a bronchopneumonia of the lower lobes and may be complicated by empyema. *E. coli* bacteremia precedes pneumonia and is usually due to another focus of *E coli* infection in the urinary or GI tract (Madappa, 2017).



Figure(1-2):Uncomplicated Urinary Tract Infection (Flores-Mireles, *et al*., 2015).

1.2.1.2.2.Commensal *Escherichia coli* and Antibiotic Resistance

Antibiotic therapy can affect not only the pathogenic bacteria, but also commensal microorganisms in the gut flora of humans, which might serve as a reservoir of antimicrobial resistance genes. Commensal *E. coli* are considered to be a major reservoir for antibiotic resistant genes (Marshall *et al*., 2009; Adelowo *et al*., 2014; NARMS, 2012-2016). As antibiotic susceptible *E. coli* can acquire resistance genes via horizontal gene transfer from resistant isolates, thus transforming susceptible isolates into resistant organisms. The fitness cost associated with a naturally acquired antibiotic resistance gene is usually offset by compensatory mutations in the genome that allow the resistance gene to be retained even in the absence of selection. Hence, resistant strains could persist in the colon, and, the continuous acquisition of resistance genes could make these strains multiply antibiotic resistant (Meervenne *et al.*, 2012; Zurfuh *et al.*, 2016).

There are many mobile genetic elements (transposable elements) chief among them such as transposons, insertion sequences, gene cassettes and integrons. These mobile genetic elements are believed to be the most important means of horizontal dissemination of antibiotic resistance genes (Partridge, 2011; Rahube, 2013).

1.2.1.2.2.1 Multidrug Resistance

Multidrug resistance is defined as insensitivity or resistance of a microorganism to two or more of the administered antimicrobial agents (which are structurally unrelated and have different molecular targets) despite earlier sensitivity to it (Singh 2013; Popęda *et al.*, 2014). According to world health organization (WHO), these resistant microorganisms (like bacteria, fungi, viruses, and parasites) are able to combat attack by antimicrobial drugs, which leads to ineffective

treatment resulting in persistence and spreading of infections. Persistence of microbes after conventional or standard treatments points out different types of MDR which is an expanding problems in medical world as reduced treatment options, increased mortality rates, longer hospital stays and increased costs (Tanwar *et al*., 2014; WHO, 2014).

Although the development of MDR is a natural phenomenon, extensive rise in the number of immune-compromised conditions, like HIV-infection, diabetic patients, individuals who have undergone organ transplantation and severe burn patients makes the body an easy target for hospital acquired infectious diseases, thereby contributing to further spread of MDR (Nikaido, 2009; Yao *et al.*, 2016). Multidrug resistance in bacteria is often caused by the accumulation of genes, each coding for resistance to a single drug, on R plasmids. The assembly of resistance genes on a single R plasmid is achieved by mechanisms provided by transposons and integrons. Integrons, for example, are especially powerful in producing multidrug resistance because they assemble several resistance genes in a correct orientation and supply a strong promoter for their expression. Furthermore, the resistance gene once incorporated into an integron becomes tagged, so that it could easily become a part of another integron (Lin *et al.*, 2015; El-Sokkary and Abdelmegeed, 2015).

Multidrug resistance can be classified (Figure 1-3) as primary or secondary resistance.

- **Primary Resistance.** It occurs when the organism has never encountered the drug of interest in a particular host (Vranakis *et al*., 2013; Tanwar *et al*., 2014 ; Berendonk *et al*., 2015).
- **Secondary Resistance.** Also known as "acquired resistance," this term is used to describe the resistance that only arises in an organism after an exposure to the drugs (Loeffler and Stevens,2003;

Khalilzadeh *et al*., 2006). It may further be classified as intrinsic resistance and extensive resistance (Loeffler and Stevens, 2003; Lee *et al*., 2013; Marks *et al*., 2014).

- **Clinical Resistance**. In addition to the above-mentioned types, clinical resistance is defined by the situation in which the infecting organism is inhibited by a concentration of an antimicrobial agents that is associated with a high likelihood of therapeutic failure or reappearance of infections within an organism due to impaired host immune function. In other words, the pathogen is inhibited by an antimicrobial concentration that is higher than could be safely achieved with normal dosing (Tanwar *et al*., 2014).

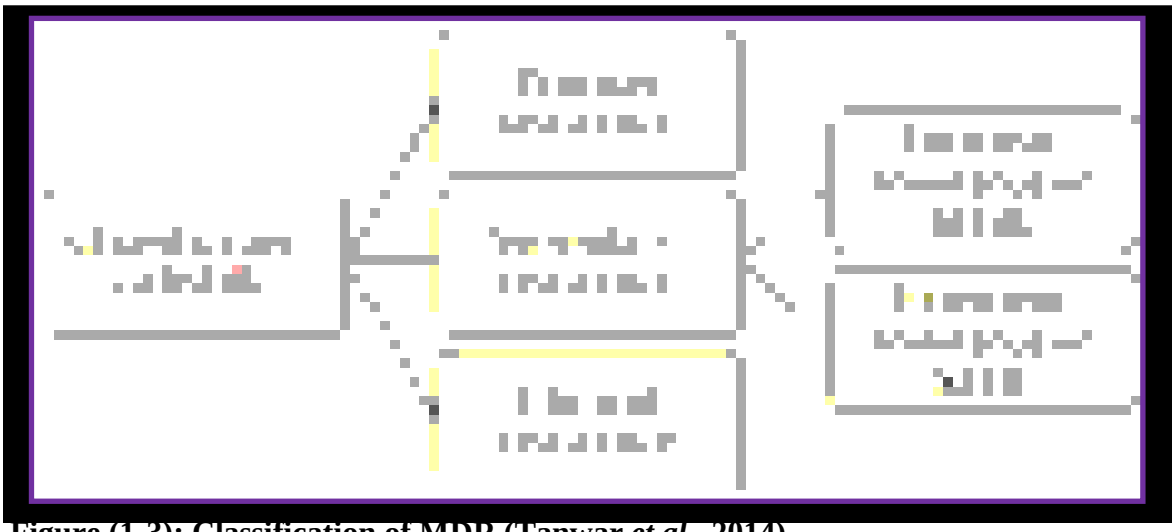


Figure (1-3): Classification of MDR (Tanwar *et al*., 2014).

1.2.1.2.2.2. Mechanism of Antibiotic Action and Resistance in *Escherichia coli*

An ideal antimicrobial drug exhibits selective toxicity. This term implies that the drug is harmful to a parasite without being harmful to the host. The most successful antimicrobial agents are those whose targets are anatomic structures or biosynthetic functions unique to microorganisms (Byarugaba, 2009; Buckland, 2017). There are five different modes of action for antimicrobial agents. These include interference with cell wall synthesis (β-lactams and Glycopeptides

agents), inhibition of protein synthesis (Macrolides and Tetracycline s), interference with nucleic acid synthesis (FluoroQuinolones and Rifampin), inhibition of a metabolic pathway (Trimethoprim-Sulfamethoxazole), and disruption of bacterial membrane structure (Polymyxins and Daptomycin) (Byarugaba, 2009) as in (figure 1-4).

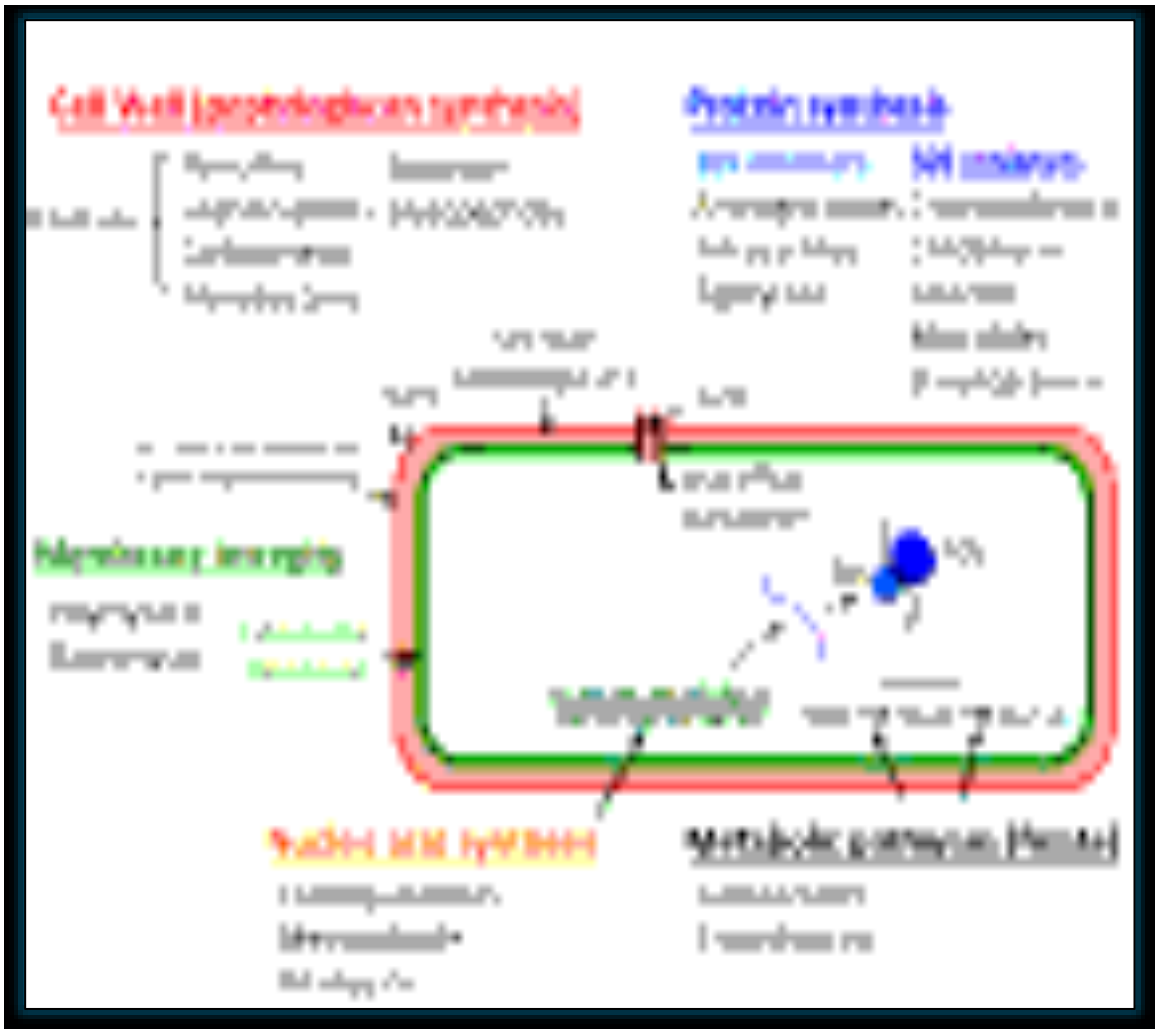


Figure (1-4): Action of antibiotics on *E. coli* (Penesyan *et al.*, 2015)

Bacteria may manifest resistance to antibacterial drugs through a variety of mechanisms which include (i) enzymatic modification, (ii) down-regulation or alteration of an outer membrane protein channel that the drug requires for cell entry, (iii) antibiotics target site modification, and (iv) presence of active efflux pumps (Figure 1-5). Enzymatic modification is an important mechanism of resistance to β-lactam family and Aminoglycoside family of antibiotics (Martins *et al.*, 2013; Lin *et*

al., 2015; Penesyan *et al*., 2015). The antimicrobial-resistant phenotypes is gained from extra-chromosomal genes that may confer resistance to an entire antimicrobial class. Most of these resistance genes are associated with transferable plasmids, on which may be other DNA mobile elements, such as transposons and integrons. These DNA mobile elements played an important role on dissemination of resistance genes among different bacteria (Deng *et al*., 2015; Dar *et al*., 2016). The mechanisms of action and resistance of seven major categories of antimicrobial agents are described below.

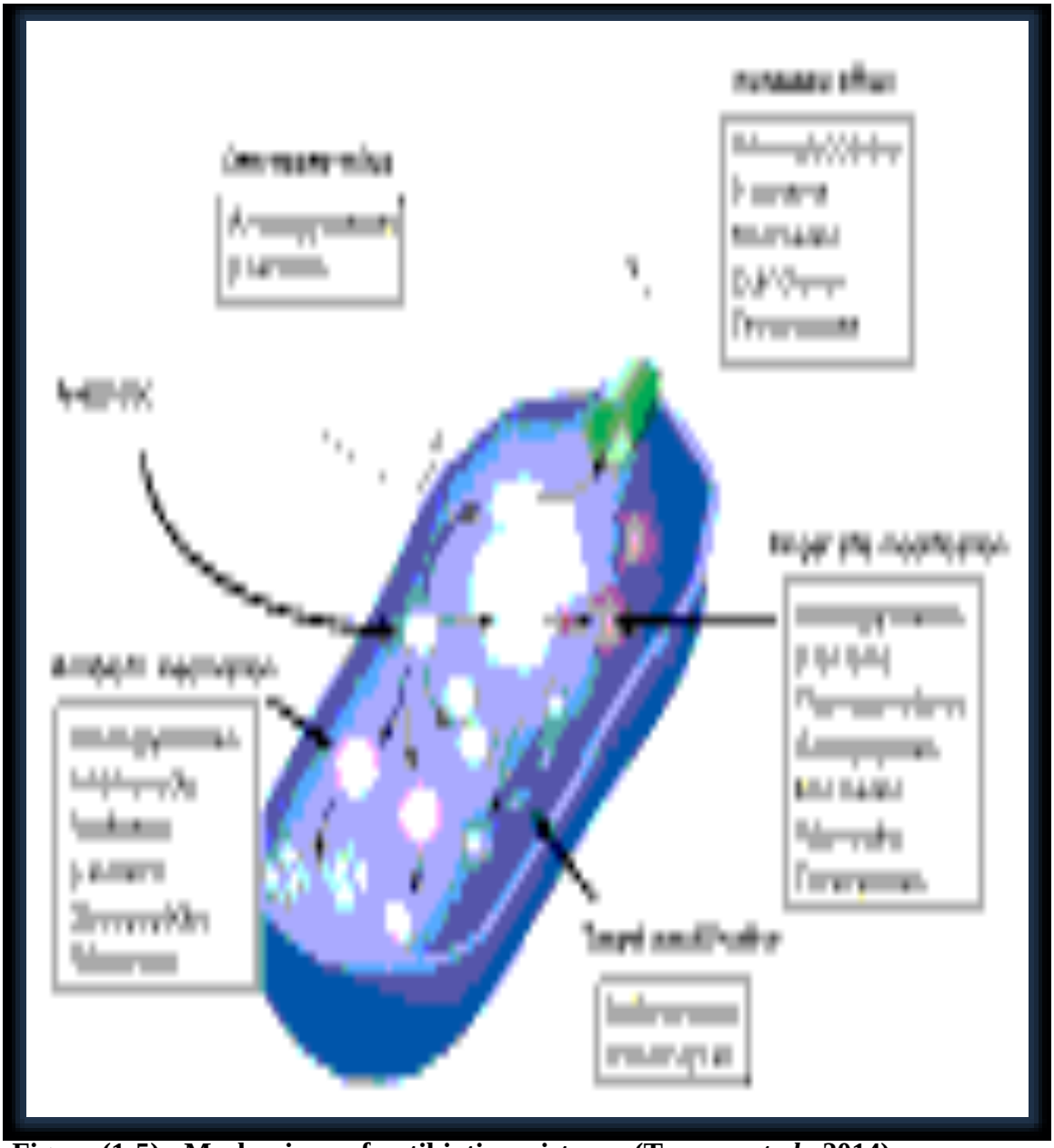


Figure (1-5) : Mechanisms of antibiotic resistance (Tanwar *et al*., 2014)

- **ß-Lactams**

ß- Lactams represent groups of antibiotics that include Penicillins, Cephalosporins, Monobactams and Carbapenems. These groups of drugs inhibit a number of bacterial enzymes, namely, Penicillin-binding proteins (PBPs), that are essential for peptidoglycan synthesis, thereby interfering with synthesis of petidoglycan of the cell wall (Chuma *et al*., 2013; Rahube, 2013). Of the various mechanisms of acquired resistance to ß- lactam antibiotics, resistance due to production of ß- lactamase by the cell is the most prevalent . ß- Lactamase included a family of enzymes with tremendous diversity. The *blaTEM-1* and *blaSHV-1*, which efficiently hydrolyze Penicillins and narrow-spectrum Cephalosporins but poorly hydrolyzes extended-spectrum Cephalosporins, are the most prevalent and most common in *E. coli* (Chuma *et al*., 2013; Al Mously *et al*., 2016). Recently, a plasmid-mediated AmpC ß-lactamase *blaCMY-2*, which causes resistance to extended spectrum ß-lactams (ESBLs) including ceftriaxone. The plasmid-mediated *blaCMY-2* was also detected in the recently emergent MDR-AmpC . In addition, alterations in the PBPs, acquisition of a novel PBP insensitive to ß-lactams, changes in the outer membrane proteins of Gram-negative organisms, and active efflux which prevent these compounds from reaching their targets can also confer resistance (El Salabi, 2011).

- **Aminoglycosides**

Aminoglycoside s are a class of drugs which inhibit bacterial protein synthesis by binding irreversibly to the bacterial 30S ribosome subunits. The Aminoglycoside -bound bacterial ribosome is unavailable for translation of mRNA during protein synthesis thereby causing bactericidal effects. The most common mechanism of Aminoglycoside resistance is antibiotic inactivation by plasmid-and transposon- encoded

modifying enzymes (Shi *et al*., 2013; Ashwlayan and Singh, 2016). There are three classes of Aminoglycoside -modifying enzymes: acetyltransferases (AAC), adenylytransferases (ANT) and phosphotransferases (APH). AAC enzymes acetylate amino groups, whereas ANT and APH enzymes adenlylate and phosphorylate hydroxyl groups, respectively. An ANT enzyme, AadA, which is encoded in integrons is commonly detected in Streptomycin -resistant gram negative bacteria (Ramirez *et al.*, 2013; Shi *et al*., 2013; Cairns *et al*., 2017) . In addition, the resistance also is caused by the decreased antibiotic uptake by outer membrane proteins and the mutation of ribosomal protein S12, which is the target of Aminoglycoside antibiotics (Lin *et al*., 2015).

- **Phenicols**

Phenicols include Chloramphenicol and Florfenicol. They inhibit protein synthesis by binding reversibly to the peptidyltransferase component of the 50S ribosomal subunit thus preventing the transpeptidation process of peptide chain elongation (Dale-Skinner and Bonev, 2009; Lysnyansky and Ayling, 2016). Chloramphenicol resistance in bacteria most commonly results from acquisition of plasmids encoding Chloramphenicol acetyltransferases (CAT), which enzymatically inactivate the drug. In gram negative bacteria, three types of CAT enzymes (types I, II, III) have been identified. In addition to enzyme resistance is also due to the decreased outer membrane permeability and acquisition of extra chromosomal efflux pumps (Nikaido, 2009; Kohanski *et al.*, 2010). cmlA is a major facility family transporter based on the amino acid sequence analysis. cmlA can also result in reduced expression of two outer membrane proteins (OmpA and OmpC) and decreased Chloramphenicol uptake. Recently, a new gene, *flo*, which shares 65% homology to cmlA, was found to confer resistance

to both Chloramphenicol and Florfenicol (El Salabi, 2011; Cattoir, 2016).

- **Sulfonamides and Trimethoprim**

Sulfonamide s were the first effective systemic antimicrobial agents used in the United States during the 1930s. They work by competitively inhibiting bacterial modification of para-aminobenzoic acid into dihydrofolate. Trimethoprim is a pyrimidine analog that inhibits the enzyme dihydrofolate reductase(DHFR), thus interfering with folic acid metabolism. This sequential inhibition of folate metabolism ultimately prevents the synthesis of bacterial DNA (Zhang, 2015). The widespread high-level resistance to Trimethoprim is likely due to the acquisition of exogenous DNA that specifies a supplementary DHFR which is less sensitive than the chromosomal enzyme to inhibition by Trimethoprim (Guillard *et al*., 2016). To date, at least 16 different DHFRs have been characterized in gram-negative bacteria. Type I DHFR is most commonly detected in gram negative bacterial and normally carried by integrons. Resistance to Sulfonamide is commonly due to acquisition of plasmids that encode a drug-resistant dihydropteroate synthase (DHPS). Two types (type I and II) of resistant DHPS, encoded by the *sulI* and *sulII* genes, respectively, have been identified in gram-negative organisms (Berglund, 2015).

The *sulI* gene is often linked to other resistance genes and is located in conserved segments of integrons in Tn21-like elements carried by large conjugative plasmids. The *sulII* gene is frequently linked genetically to a Streptomycin resistance gene on broad host- range plasmids and on small non-conjugative plasmids (Berglund, 2015).

- **Tetracycline**

The Tetracycline s act by inhibiting protein synthesis. They enter bacteria by an energy-dependent process and bind reversibly to the 30S ribosomal subunits of the bacteria. This process blocks the access of aminoacyl- tRNA to the RNA-ribosome complex, preventing bacterial polypeptide synthesis (Adesoji *et al.*, 2015; Huang *et al.*, 2016). Tetracycline resistance is the most common antibiotic resistance encountered in nature. Although it can result from chromosomal mutations affecting outer membrane permeability, more commonly it results from acquisition of exogenous DNA encoding proteins involved in active efflux of Tetracycline or in protection of the ribosome (Kobayashi *et al.*, 2007; Adesoji *et al.*, 2015).

In Gram-negative bacteria, six classes of *tet* efflux pumps including TetA, TetB, TteC, TetD, TetE, TetG, are of clinical importance. These efflux pumps use an antiport mechanism of transport involving the exchange of a proton for Tetracycline -cation complex. Another mechanism of Tetracycline resistance is ribosome protection (Kumar and Varela, 2013).

Modification of tRNA has been suggested to interfere with ribosomal protection, but its precise role remains to be determined. At least five classes of ribosomal proteins, which can interact with ribosomes such that the protein is unaffected by the presence of antibiotics, have been characterized . tetM is widely disseminated and is found in many gram-positive and gram-negative bacteria. Similar to some antimicrobial agents, Tetracycline resistance is also mediated by decreased membrane permeation and active efflux pumps (Sabouri Ghannad and Mohammadi, 2012; Kumar and Varela, 2013; Kester and Fortune, 2013; Von Wintersdorff *et al.*, 2016).

- **Quinolones / FluoroQuinolones**

Quinolones belong to a group of potent antibiotics biochemically related to nalidixic acid, which were developed initially as a urinary antiseptic. Newer Quinolones , also known as fluoroquinolones , have been synthesized by adding a fluorine atom attached to the nucleus at position 6. The primary target of Quinolones is DNA gyrase, an enzyme essential for DNA replication (Kohanski *et al*., 2010; El Salabi, 2011). Their therapeutic index stems from the fact that the clinically useful fluoroquinolones inhibit bacterial DNA gyrase at concentrations far below those required to inhibit mammalian topoisomerases. The mechanisms of Fluoroquinolone resistance include target gene mutation and removal Fluoroquinolones by efflux pumps (Bayramov and Neff, 2016; Ravn *et al*., 2016; Schroeder, *et al*., 2017)..

- **Lincomycin**

This class was first characterized in the 1960s and is now used for treatment of a broad spectrum of infections. It is mostly active against gram-positive organisms, including Methicillin-resistant *Staphylococcus aureus*. but also finds use against selected gram-negative anaerobes and protozoa (Leclercq, 2002; Rezanka *et al*., 2007). This antibiotics function by blocking microbial protein synthesis via binding to the 23S rRNA of the 50S subunit and mimicking the intermediate formed in the initial phase of the elongation cycle. Lincosamide-resistant microorganisms have emerged with antibiotic modification as one of their major resistance strategies. Inactivating enzymes LinB/A catalyze adenylylation of the drug. Other mechanisms of resistance are target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target and efflux of the antibiotic agent (Leclercq, 2002; Morar *et al*., 2009).

1.2.2. Integron

Gene cassettes carry a variety of genes, including antibiotic resistance genes and normally found integrated at a specific site in an integron. Integrons are potential mobile DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, by site-specific recombination (Cambray *et al*., 2010; Kargar *et al.*, 2014; Hajiahmadi *et al*., 2017). While integrons themselves are not able to transfer to other bacteria, they are frequently associated with transposons and plasmids. Plasmid-integrated integron carrying antibiotic resistance genes can be transferred to other bacteria through conjugation (Froehlich *et al.*, 2005; Avila *et al*., 2013). Integrons have an intergase gene *(IntI*), a nearby recombination site (attI), and a promoter (P). The amino acid sequences of integrase gene have been used as a basis for dividing integrons into 'classes'(Deng *et al*., 2015). The first integrons to be described, classes 1, 2, and 3, exhibit a number of features not typical of the more numerically dominant chromosomal integron classes (Tajbakhsh *et al*., 2015; Nourbakh *et al*., 2017)

There is experimental evidence that the antibiotic resistance genes found in class 1, 2, and 3 integrons were acquired by capturing gene cassettes from the vast pool of diverse cassettes that are prevalent in microbial communities. Class 1 and class 2 integrons seem to be more frequently associated with antibiotic resistance in enterobacterciae (Dureja *et al*., 2014; Tajbakhsh *et al*., 2015).

1.2.2.1. Integron Mobility

The mobility of integrons has been considered to be a major concern of clinically antibiotic resistance. Despite the defect of self-transposition, currently existent integrons (mostly class 1 integron) has been considered to be a potentially mobile genetic element and commonly found to be located on plasmids as facilitation of conjugative-

mediated transfer, as it contains gene cassettes that are mobile and capable of transferring to other integrons or to secondary sites in the bacterial genome (Boucher *et al*., 2007; Laroche *et al.,* 2009; Deng *et al.*, 2015; Cairns *et al*., 2016).

Mobile genetic elements, including conjugative plasmids, transposons, insertion sequences and genomic islands, may potentially be the vast reservoirs and massive genetic pool for integron, which will further be shared among bacteria (Laroche *et al.,* 2009). With mobility from gene cassettes, integrons play key role in the dissemination and spread of resistance genes, responsible for both spread and exchange of resistance genes to a wide range of distinct antibiotics among diverse bacteria (Su *et al*., 2006; Nemergut *et al*., 2008). Aside from clinical perspectives, a large number of reports on integrons from environmental microorganisms, as well as the high sequence diversity observed and various functional products other than resistance encoded by such cassettes, strongly indicates integrons are ancient genetic element within the genomes and may have played a critical role in evolution and adaptation for a considerable period (Deng *et al.,* 2015; Kheiri and Akhtari, 2016).

1.2.2.2. Integron Classification

Integrons are typically divided into mobile integrons and chromosomal integrons. Chromosomal integrons are usually stationary in the bacteria, while mobile integrons are readily disseminated between bacteria. Mobile integrons cannot mobilize and transfer themselves per se, they are often associated with genetic elements which can transmitted by conjugation, such as conjugative plasmids. Recent studies have also indicated that natural transformation may be important in the dissemination of integrons (Mazel, 2006; Cambray *et al*., 2010).

Moreover from the differences and divergence in the sequences of *intI*, integrons have been classified and divided into several classes (Tajbakhsh *et al*., 2015). There are at least 90 distinct integron classes, mostly located on chromosomes, and about 10% of sequenced bacterial genomes carry these elements, but there are 4 classes of integrons have been more identified and distinguished, termed classes 1–4 integrons. Multi-resistant integrons , classes 1–3 integrons are capable of acquiring same gene cassettes via similar recombination platform, which had been supported by the in vitro excision and integration occurred via recombination sites from such integrons (Gillings *et al.*, 2008). Most of the currently available studies on integrons had been conducted on class 1 integron, with focus on gram-negative microorganisms. As a distinct type of integron, class 4 integron was firstly identified on the small chromosome of *Vibrio cholerae* and found to be an integral component of many γ-proteobacterial genomes (Martin, *et al*.,2008), which had also been considered to be a leading concern on both antimicrobial resistance and bacterial genome evolution, despite the limitation of the associated reports within the species of *Vibrio*. The remaining classes of integrons may also contain antibiotic resistance gene cassettes, but their worldwide prevalence remains low (Urumova, 2016a). Current study focused on integron class 1 and 2, their structures and functions described below

- **Integron Class 1 and Antibiotic Resistance Genes**

Integrons class 1are central players in the worldwide problem of antibiotic resistance, because they can capture and express diverse resistance genes. In addition, they are often embedded in promiscuous plasmids and transposons, facilitating their lateral transfer into a wide range of pathogens. Class 1 integrons are the most prevalent in *Enterobacteriacae* clinical isolates (Venturini, 2011; Raherison *et al.*,

2017). Integrons class 1 have been found in commensal and pathogenic *E. coli* strains to confer resistance to all β- lactam antibiotics, Chloramphenicol , Aminoglycosides, Erythromycin, Trimethoprim, Streptothricin, Rifampin, Fosfomycin and Lincomycin. Class 1 integrons are the best characterized integrons which are frequently reported in clinical isolates, livestock, environmental isolates including rivers (Mukherjee and Chakraborty, 2006), fish farm , sewage treatment plant (Da Costa *et al.*, 2006; Lindberg *et al.*, 2007; Zhang *et al.*, 2009) and wastewater sources . Class 1 integrons, located on chromosome as well as plasmids and transposons, are the most prevalent integrons found in clinical isolates and are strongly associated with multi-antibiotic resistance in the hospital environment. They are now found in 40-70% of gram-negative pathogens isolated from clinical contexts (Van Essen-Zandbergen *et al.*, 2007) as well as in pathogens isolated from livestock. As shown in Figure(1-6) class 1 integrons contain a 5' and 3'conserved segments (CS) and a variable region. Like the other classes of integrons, their 5' conserved segment consists of an *intI1* gene (integrase), *attI1* site, and a promoter region (Pant) expressing the inserted gene cassettes (Bee, 2011; Marchiaro, *et al.* 2010). In fact, the Pant of class 1 integrons potentially contains two promoters, P1 and P2. On the other hand, the 3' conserved segment contains a truncated quaternary ammonium compounds gene (*qacΔ1*), a Sulfonamide resistance gene (*sul1*) and an open reading frame (*orf*5) of unknown function. These CS regions flank a variable region, in terms of length and sequence, which contain the gene cassettes of the particular integrons(Bee, 2011).

Gene cassettes are very simple genetic elements that typically consist of a single promoter, less resistant gene and a recombination site called a 59-base element (59-be) or *attC* (Urumova, 2016b). All integrons capture mobile gene cassettes using site-specific recombination

mechanism mediated by an integrase gene (*intI*). *IntI1* is a member of the tyrosine recombinase family. It catalyses two types of site specific recombination reaction which are recombination between two *attC* sites or recombination between *attI1* and a *attC* . However, studies have shown that the interaction between the *attC* and the *attI1* site is the preferred recombination reaction (Gillings *et al.*, 2008).

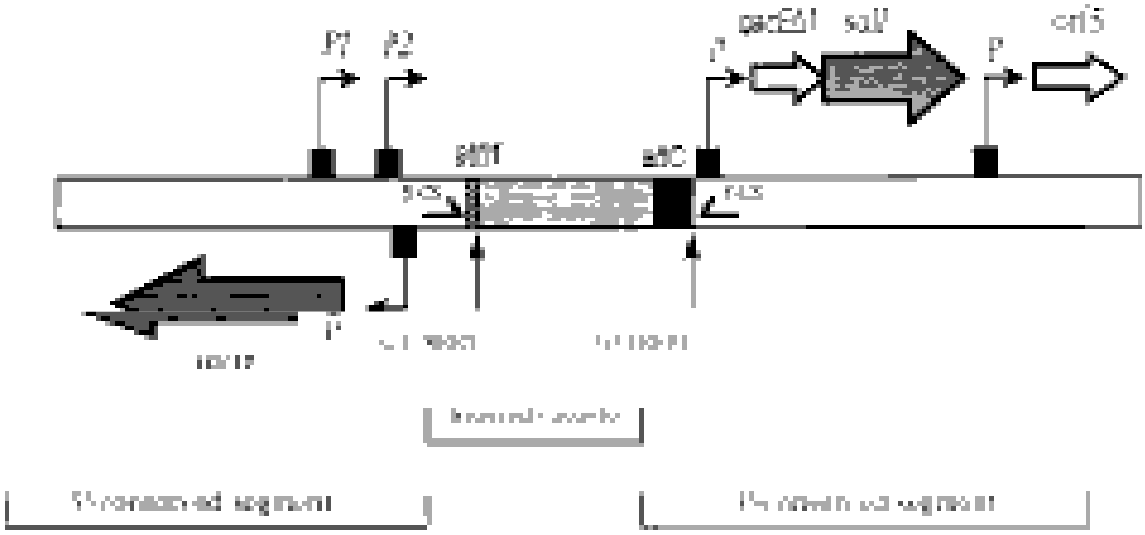


Figure (1-6): General Structure of Class 1 Integrons. The location and orientation of different promoters are shown with the arrow showing direction of transcription. The 5'CS consists of integrase gene, *intI1* and recombination site, *attI1*, while 3' CS contains antiseptic resistance gene (*qacEΔ1*), a Sulfonamide resistance gene (*sul1*) and an unknown function open reading frame (*orf5*). One inserted cassette is shown, with its associated *attC*. The sequence of GTTRRRY is the point of boundary for integration of gene cassettes (Bee, 2011).

The *attI1* site, located at the 3'end of the 5'CS, is less complex than the *attC* , whilst it is believed that *IntI1* binds stronger to *attI1* than to *attC* site. This recombination results in the assembly of new genes downstream of an integron-associated promoter Pant that directs transcription of the gene cassettes as illustrated in Figure (1-7). *IntI1* can also act on secondary sites containing a degenerate core site, but this type of recombination is infrequent. During recombination, gene cassettes can be inserted one after the other at the recombination site, *attI1*, to produce

tandem resistance gene arrays (Bennett, 2008). Therefore, the order of the gene cassettes from 5'CS indicates the order of addition, in which the nearest gene to 5' CS being the latest addition due to cassette insertion at the same point (*attI*). The number of gene cassettes can vary between zero for to five or more (Urumova, 2015b).

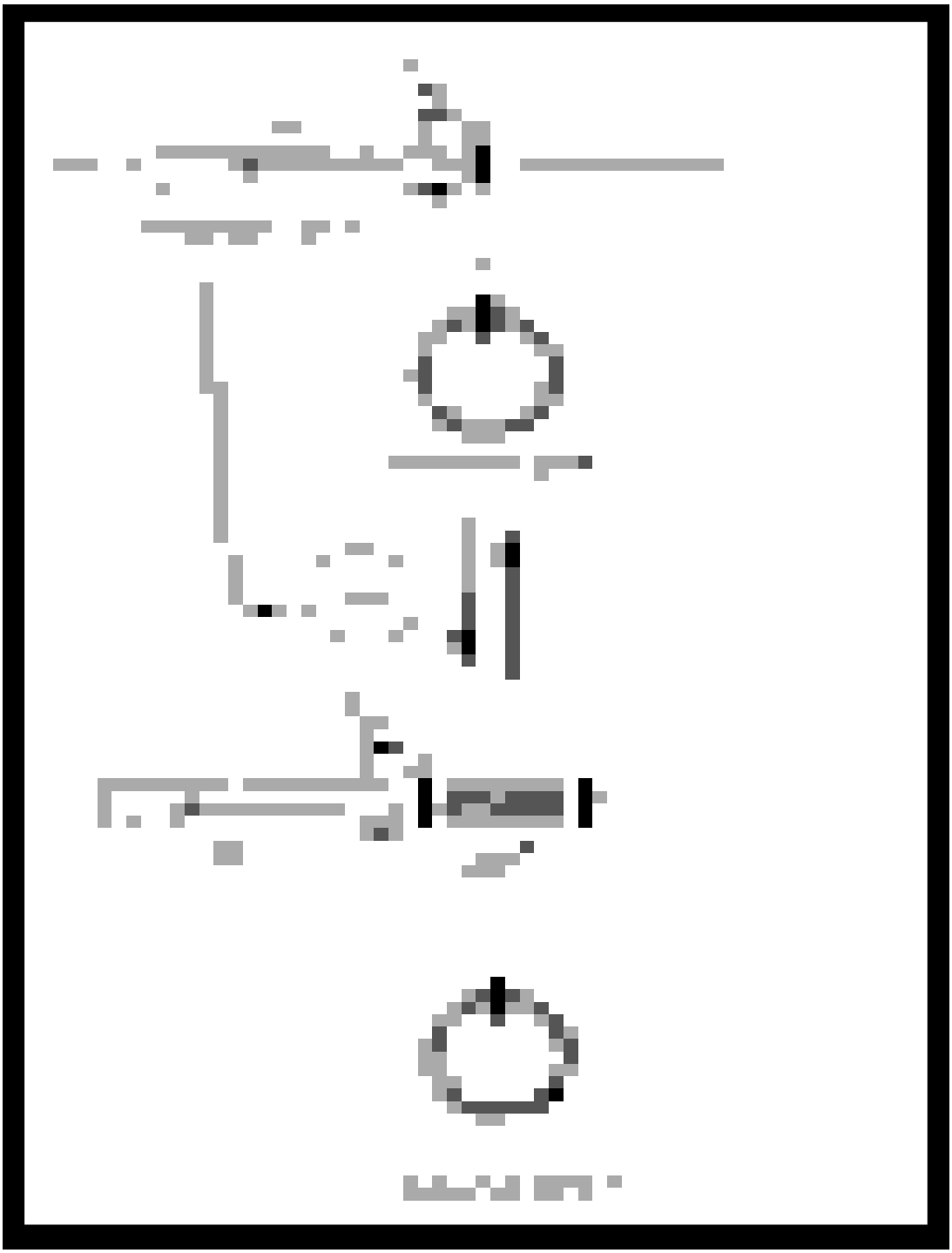


Figure (1-7): Schematic Representation of Site Specific Recombination For Class 1 Integron. Circular antibiotic resistance gene cassettes are inserted into a

specific attachment sequence (*attI*) and are placed downstream of a functional promoter element (Pant). The excision and integration of gene cassettes are mediated by integrase protein (IntI1) (Bee, 2011).

- **Integron Class 2 and Antibiotic Resistance Genes**

Class 2 integrons have been found to be embedded in the Tn7 transposon (Figure 1-8). Tn7 can be identified as a sophisticated mobile element containing a transposition module, formed by five *tns* genes of the transposition module that are *tnsA, tnsB, tnsC, tnsD,* and *tnsE* genes (Partridge *et al*., 2009; Ramírez *et al*., 2013; Singh *et al*., 2017).

The structure of class 2 integrons has not been fully characterized, but studies have shown that they are made up of a 5' conserved segment containing a pseudo-integrase gene (*intI2*) and gene cassettes encoding resistant to Trimethoprim (*dfrA1*), Streptothricin (*sat*) and Streptomycin (*aadA1*). The 3′- CS minimally consists of *qacEΔ* and *sulI* however, sometimes *orf5* and *orf6* make up part of it. Remnants of the Tn402 transposition module (*tni*) and inverted repeats can sometimes be found downstream of the 3′- CS (Avila, 2013). In Tn7 itself, the cassettes array appears to end with a truncated cassette known as *orfX*. Consequently, primers covering the *orfX* and the conserved *intI2* region of Tn7 have been used to amplify cassette arrays in class 2 integrons. The *intI2* gene is 40% identical to *intI1* gene. The *intI2* genes are so far described as non-functional because of the presence of an internal stop codon (Hansson *et al.*, 2002). However, studies by Hansson *et al.* (2002) claimed that replacement of the internal stop codon with a codon for glutamic acid yields a functional integrase and natural suppression of the stop codon in *IntI2* or the action of other *IntI* in trans may allow occasional acquisition of new cassettes (Partridge *et al*., 2009). Until now, class 2 integrons have been found in isolates of *Acinetobacter*, *Shigella*, and *Salmonella* and *E. coli* (Parks and Peter, 2009).

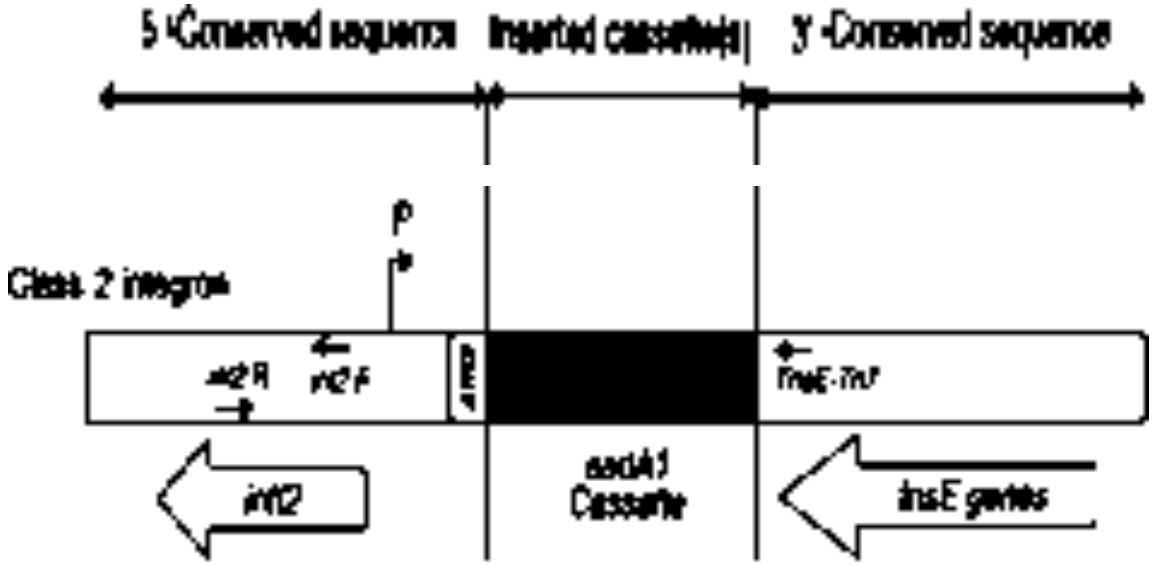


Figure (1-8): General Structure of Class 2 Integron. The arrows show the direction of transcription. The location and orientation of inserted cassette promoters are shown. For the class 2 integron, only the aadA1 cassette is symbolized (Bee, 2011).

2. Materials and Methods

2.1. Materials

2.1.1. Equipments and Instruments

The equipments and instruments that used in the current study are listed in Table (2-1).

Table (2-1) : Equipments and Instruments with Their Remarks

Equipments And Instruments	Remarks
Autoclave	Sturdy (Taiwan)
Beakers	Amsco (Germany)
Calibrated Loop 0.01	Himedia (India)
Cold Centrifuge	Hettich (Germany)
Cylinder (100 Ml)	Amsco (Germany)
Deep Freezer	Gfl (Germany)
Densichek Plus	Biomérieux / USA
Digital Camera	Sanyo (Japan)
Distilator	Gfl (Germany)
Eppendorf Tubes	Sterilin Ltd. / Uk
Flasks (Different Size)	Amsco (Germany)
Forceps	China
Fume Hood	Shinsaeng (South Korea)
Gel Electrophoresis System	Consort (Belgium)
Gloves	Broche (Malaysia)
Hot Plate With Magnetic Stirrer	Heidolph (Germany)
Hp Labtop	China
Incubator	Jarad (Syria)
Microcentrifuge	Lab Tech (Korea)
Microcentrifuge Tube	Sterilin Ltd. / Uk
Micropippetes (In Different Size)	Eppendorf (Germany)

Equipments And Instruments	Remarks
Petri Dish (Different Sizes)	China
Plain Tubes	Afco- Dispo (Jordan)
Polystyrene (Plastic) Tube 12 X 75mm	Biomérieux / USA
Printer Brother	China
Professional Tr/O Thermocycler	Biometra (Germany)
Rack	Sterellin Ltd. / Uk.
Sensitive Balance	Sartorius (Germany)
Sterile Swab	China
Tips	Sterellin Ltd. / Uk.
UV- transillumination	Consort (Belgium)
Vortex	Stuart (UK)
Water Bath	Kottermann (Germany)
Water Distillator	Lab Tech (Korea)
Wooden Sticks	Supreme (China)

2.1.2.Chemicals and Biological Materials

The chemicals and biological materials that are used in this study are listed in Table (2-2).

Table (2-2): Chemicals and Biological Materials with Their Remarks

Chemicals &Biological Materials	Manufacturers Name
Deionized water (Nuclease free)	Bioneer (korea)
Ethidium Bromide 10 mg/ml	Bioworld (USA)
Ethanol (96%)	England
Glycerol	Fluka (Switzerland)
Analytical profile of index (API) 20	Promega (USA)
Normal saline	china
Agarose	Promega (USA)
Tri-Borate EDTA Buffer (TBE buffer)10X	Promega (USA)

2.1.3. Antibiotic Discs

Antibiotic discs and their description that are used in the present study are listed in Table (2-3).

Table (2-3): Antibiotic Discs and Their Remarks

Antibiotics Class	Antibiotic subclass	Antibiotic Name	Symbol	Content (µg)	Origin
Penicillins	Aminopenicillin	Ampicillin	AM	25	Ankara/ Turkey
		Amoxicillin	AX	25	
Aminoglycosides		Amikacin	AK	10	
		Gentamicin	CN	10	
		Streptomycin	S	25	
Folate pathway inhibitors		Trimethoprim	TMP	10	
Lincosamides		Lincomycin	L	10	
Quinolones	Fluoroquinolone	Norfloxacin	NOR	10	
		Ciprofloxacin	CIP	10	
	Quinolone	Nalidixic Acid	NA	30	
Phenicols		Chloramphenicol	C	10	
Cephems	Cephalosporin	Cefotaxime	CTX	10	
		Ceftriaxone	CRO	10	
		Cephalexin	CL	30	
Tetracyclines		Tetracyclin	TE	10	
		Oxytetracyclin	T	30	

2.1.4. DNA Extraction Kit and PCR Master Mix

In Table (2-4) DNA extraction kit and PCR Master Mix that are used in PCR reaction with their companies and countries of origin are listed.

Table (2-4): DNA Extraction Kit and PCR Master Mix with Their Remarks

Type of kit	Manufacturer / State
DNA extraction and purification kit. This kit contain the followings**:** **1.** 2ml collection tubes **2.** Elution Buffer. **3.** GB Buffer. **4.** GD column **5.** Gram positive Buffer **6.** GT Buffer **7.** Lysozyme **8.** Proteinase K **9.** W1 Buffer. **10.** Wash Buffer.	Geneaid / USA
AccuPower™ PCR PreMix kit. This kit contains the followings**:** **1-** dNTPs (dATP,dCTP,dGTP and dTTP) 250µM **2-** KCl 30 µM **3-** $MgCI_2$ 1.5 µM **4-** Top DNA polymerase 1U **5-** Tris-HCl (pH 9.0) 10 µM **6-** Stabilizer and Tracking dye	Bioneer (south Korea)

2.1.5.Culture Media

Culture media with their origin of manufacture that are used in this study are listed in Table (2-5).

Table (2-5): Culture Media and Their Remarks

Media	Origin
Brain Heart Infusion broth	Himedia (India)
Cary-Blair transport media	Cypress (Belgium)
Eosin methylen Blue agar (Levine)	Oxoidltd, Hampshire/ England
MacConkey agar	Accumix(India)
Muller-Hinton agar	Cypress (Belgium)
Nutrient broth	Accumix(India)
Nutrient broth	Accumix(India)

2.1.6. Molecular Weight Marker

The molecular weight marker that used in this work, its description and source are depicted in Table (2-6)**:**

Table (2-6):Molecular Weight Marker with Their Description and Remark

DNA Ladder	Description	Source
100 bp DNA Ladder from Bioneer	100 bp DNA Ladder was designed to determine the size of double stranded DNA fragments that have molecular weight from 100 to 2,000 bp. The 100 bp DNA Ladder consists of 13 double stranded molecular weight markers ranging in sizes from 100 to 2,000 bp. The **500**, **1,000** and **2,000** bp bands are two times brighter for easy identification.	Bioneer/south Korea

2.1.7. PCR Primers

PCR primers and their references that are used in this study are listed in Table (2-7).

Table (2-7): **PCR Primers and Their References**

Gene	Oligoneucleotide primer sequence (5´-3´)		Reference
chuA	1	GACGAACCAACGGTCAGGAT	Clermont *et al*., 2000
	2	TGCCGCCAGTACCAAAGACA	
YjaA	1	TGAAGTGTCAGGAGACGCTG	Clermont *et al*., 2000
	2	ATGGAGAATGCGTTCCTCAAC	
TspE4C2	1	GAGTAATGTCGGGGCATTCA	Clermont *et al*., 2000
	2	CGCGCCAACAAAGTATTACG	
IntI1	F	GGTCAAGGATCTGGATTTCG	Kargar *et al*., 2014; Tajbakhsh *et al.*, 2015
	R	ACATGCGTGTAAATCATCGTC	
IntI 2	F	CACGGATATGCGACAAAAAGGT	Kargar *et al*., 2014; Tajbakhsh *et al.*, 2015
	R	GTAGCAAACGAGTGACGAAATG	

2.1.8. Standard Bacterial Strain

Two standard strains of *E. coli* are used in this study, one strain of Liofilchem (Italy) characterize by susceptible to Amoxicillin, Cephalexin and Ciprofloxacin and the other strain isolated in microbiology laboratory (Department of Microbiology/ College of Medicine/ University of Al-Qadisiyah) that have integrons and susceptible to Ampicillin,

Trimethoprim, Oxytetracyclin, Ceftriaxone Amoxicillin, Cephalexin and Lincomycin.

2.2. Methods

2.2.1. Study Design and Samples Size

The present research is a cross sectional study that involved data collected from a population at one specific point in that time. Prior to the initiation of the present study, sample size is determine based on frequency or percent (p) of integrons (class 1 and 2) that evaluated according to WHO and previous studies by use special equation**{sample size = $z^2 * p(1-p) / d^2$}** that estimated stander normal variation (Z) equal to 1.96 and precision (d) equal to 0.05. Therefore sample size of current study accordant to this equation involve 350 fecal samples.

2.2.2. Inclusion Criteria

This study was in agreement with ethics of Al-Diwaniya city/Iraq and verbal informed consent was obtained from all participants. Selection of samples depend on special criteria include:

- Samples collected from healthy individuals (males & females) represent different location of Al-Diwaniya city/Iraq.
- Individual age range from 1 to 80 years.
- Fecal samples collected from healthy individuals who not have any GIT infections or abdominal disorders.

2.2.3. Sample Collection

Fecal sample collected from health individuals their age from 1 to 80 year (students in College of Medicine of Al-Qadisiyah University with their families, Al-Diwaniya teaching hospital staff with their families and my relative families) in period from march to may in 2016. About 1g of stool is collected from each individuals at sterile containers and directly

transported to lab. Cary-Blair transported media are used to preserve samples that do not immediately bring to microbiology laboratory.

2.2.4. Isolation and Identification of *Escherichia coli*

All specimens are examined by general stool examination to identify the presence of bacterial cells, parasites and other substances. Selected specimens are seeded on MacConkey agar and incubated overnight at 37˚C in bacteriological incubator under aerobic conditions. The identification of *E. coli* was done depending on morphological features and the rose pink color of the colonies on MacConkey agar plats that confirmed by subculture on Eosin Methylene Blue agar and incubated for 24 hours at 37˚C, the typical greenish metallic sheen color indicated of *E. coli* then the result supported by biochemical tests (API 20) (MacFaddin, 2000; Kazemnia *et al*., 2014). After identification of *E. coli* bacterial cell cultured in Nutrient broth for DNA extraction for molecular study and cultured on Muller- Hinton agar for antibiotic susceptibility test.

2.2.5. Antibiotic Susceptibility Test

All significant *E. coli* isolates were subjected to susceptibility testing by modified disc-diffusion method (CLSI, 2013). Antibiotic resistance was determined by using 16 antibiotic discs listed in Table (2-4) according to the guidelines recommended by the CLSI (2013), corresponding to the drugs considered routine testing and reporting on *Enterobacteriaceae*.

By DensiCHEK Plus instrument, the 0.5ml suspension of bacterial cells are prepared from pure culture plate of Nutrient agar. The DensiCHEK Plus instrument provides values in McFarland units, proportional to microorganism concentrations. DensiCHEK Plus is indicated for use with polystyrene and glass test tubes and the reading

range is 0.0 – 4.0 McFarland. Steps for prepare 0.5 suspension from each sample including:

1. The DensiCHEK Plus instrument is first zeroed using a test tube filled with saline (blank)
2. Take one colony from plate by sterile swab and mix with normal saline in glass tubes or plastic tubes
3. A well-mixed organism suspension is placed into the instrument and the test tube is slowly rotated. The instrument display a series of dashes followed by a McFarland reading

Newly preformed 0.5 suspension seeded by the same swab that used in mixing on the plate of Muller-Hinton agar. The swab was streaked all over the surface of the medium several times. Antibiotic disks were applied to each plate by of sterile forceps. The disks were placed on a 15 cm plate, approximately 15 mm from the edge of the plate. Each disk gently pressed down to ensure contact with the medium. The plates were placed in an incubator at 37˚C for 18 hours. The diameter of each zone (including the diameter of the disc) were measured with a pair of electronic calipers, and recorded in mm. The results then interpreted according to CLSI (2013). The MDR phenotype was defined as resistant to at least 2 different classes or categories of drugs (Magiorakos *et al.*, 2012).

2.2.6.Preservation

2.2.6.1. Short Term Storage:

Fresh cultures of commensal *E. coli* were stored on nutrient agar at 4˚C until further test (Thomas, 2007)

2.2.6.2. Long Term Storage:

Fresh cultures of commensal *E. coli* isolates were frozen in brain heart infusion broth (15% glycerol) and stored at -20˚C until requisite (Thomas, 2007).

2.2.7. Molecular Techniques

2.2.7.1 Solution Preparation

A. TBE (1X) buffer: This solution was prepared by mixing 10 ml of stock TBE-10X buffer with 90 ml of distilled water, then stored at 4^0C until used in electrophoresis.

B. Ethidium bromide solution (0.5 %): 0.5 mg / ml concentration prepared from 10 mg / ml. The final concentration stored in sterilized flask. It was used in electrophoresis as specific DNA stain.

2.2.7.2. DNA Extraction

DNA was extracted from bacterial broth according to manufacturers' instructions of Genomic DNA Mini Kit (Geneaid).The extracted DNA was electrophoresed on agarose gel (0.5% agarose stained with 5**µ**L of ethidium bromide) to confirmation that DNA present in each sample then microcenterfuge tubes that contain DNA stored at -20ºC in deep freeze even used in PCR.

2.2.7.3. Primer Preparation

Specific primers (Table 2-7) were prepared according to manufacturer's instruction by dissolving the lyophilized primers with deionized distal water to form stock solution with concentration of 100 pmol / µl. Primer's stock solution diluted with deionized water (Neuclase free), using the equation to get final working solution (10 pmol / µl) for each primers.

2.2.7.4. PCR Reaction Mixtures

The PCR reaction mixtures were preformed according to manufactures procedures of the master mix and the genes that targeted in the methods.

- **PCR Reaction Mixtures for Phylogenetic Analysis**

Phylogenetic groups were determined by multiplex PCR. Amplification reaction for all primers were conducted in 0.2 ml tube of Accu Power PCR Premix tube according to the bioneer corporation's instruction as summarized in Table (2-8). The PCR tube vortexed until the lyophilized pellet dissolved and all mixtures are mixed, then PCR tube was entered into Professional TR/O Thermocycler for thermocycling condition.

Table (2-8): PCR Master Mix Reaction for Phylogenetic Analysis

PCR master mix reaction		**Volume / µl**
PCR premix (Lyophilized)		5
DNA template		5
chuA	Forward	1
	Reverse	1
yjaA	Forward	1
	Reverse	1
TspE4.C2	Forward	1
	Reverse	1
PCR water(nuclease)		9
Total volume		25

- **PCR Reaction Mixtures for Determine Integron Class 1 and 2**

Amplification reaction for each primer was conducted in 0.2 ml tube of Accu Power PCR Premix tube according to the bioneer

Corporation's instruction as summarized in Table (2-9). The PCR tube vortexed until the lyophilized pellet dissolved and all mixtures are mixed, then PCR tube was entered into Professional TR/O Thermocycler for thermocycling condition.

Table(2-9):PCR Master Mix Reaction for Determine Integron Class1 and 2

PCR master mix reaction		**Volume / µl**
PCR premix (Lyophilized)		5
DNA template		5
Primers	Forward	1.5
	Reverse	1.5
PCR water (nuclease free)		12
Total volume		25

2.2.7.5. Thermocycling Conditions

➢ **Thermocycling Conditions for Phylogenetic Determine**

The thermocycling condition for multiplex PCR reaction was performed with a Professional TR/O Thermocycler to detection the Phylogenetic groups and subgroups of *E. coli* under the following conditions (figure 2-1).

➢ **Thermocycling Condition for Integron Class1 and class 2**

According to study of Kargar *et al*., 2014, *IntI1* and *intI2* genes have the same PCR program although each one of them has special primers and molecular weight. The thermocycling condition for *IntI1* and *IntI2* genes PCR reaction was performed with a Professional TR/O Thermocycler under the following conditions (figure 2-2)

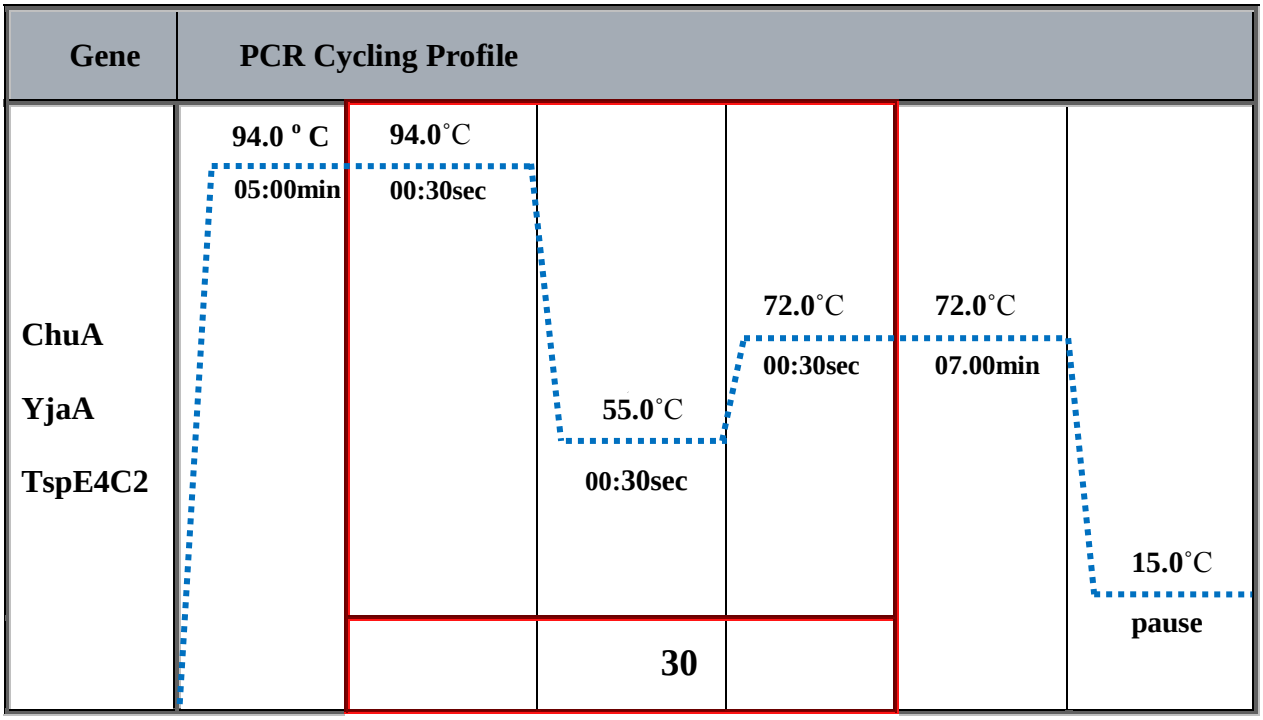


Figure (2-1): Thermocycling Conditions For Phylogenetic Determine (Clermont *et al.*, 2000)

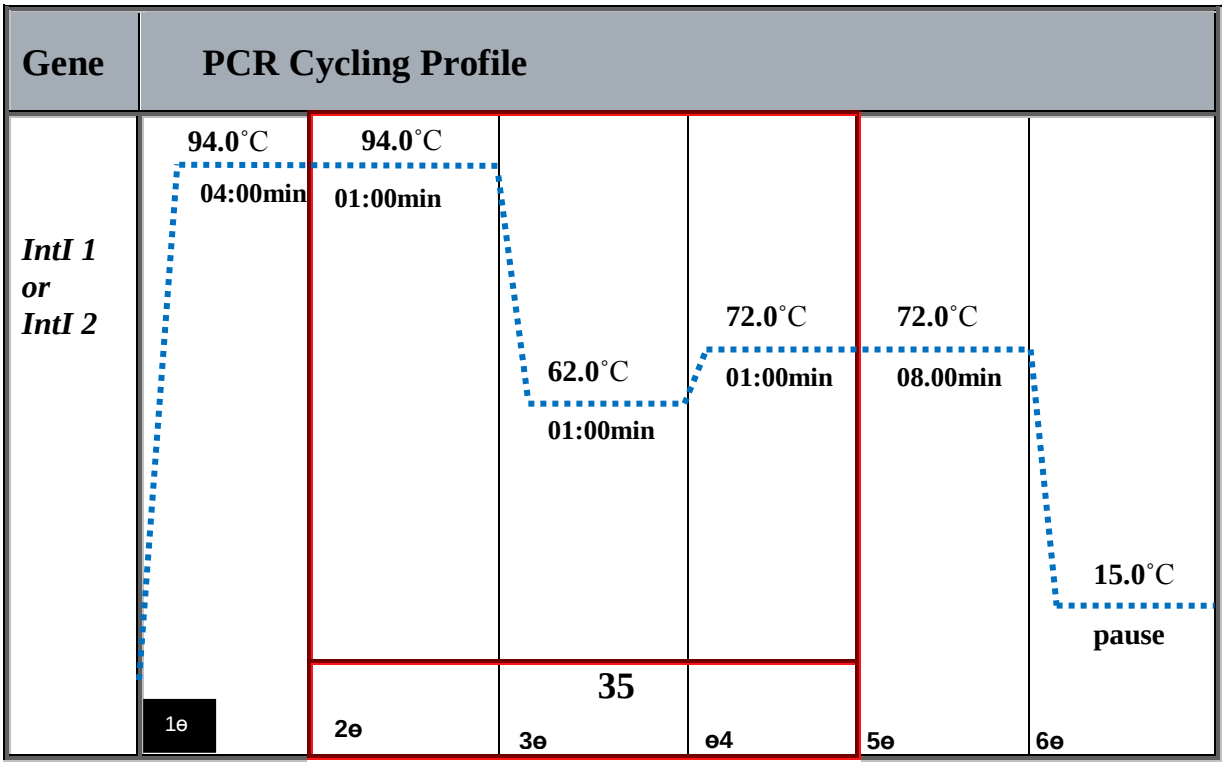


Figure (2-2):The Thermocycling Condition For *Inti1* and *Inti2* Genes PCR Reaction (Kargar *et al.*, 2014)

2.2.7.6. Gel Electrophoresis

❖ Gel Electrophoresis for Phylogenetic Analysis

Agarose gel was prepared in concentration of 2 % agarose for DNA profiling according to Clermont *et al*., 2000. The agarose gel was prepared as follow.

1. Agarose (1g) was dissolved in 50ml 1X TBE Buffer and heated on hot plate until all agarose particles were dissolved then left to cool until it reached 45-50 ^{0}C, then 5 µl (0.5 % concentration) of Ethidium Bromide stain was incorporated.
2. Agarose gel was poured into gel tray after fixing the two plastic walls and put the comb in a proper position and left to solidify for 25 minutes at room temperature, then the comb has removed gently from the tray and 5µl of PCR products were loaded in each well and 5µl of Ladder (100 bp DNA Ladder) added in one well as size standard.
3. The gel tray was fixed in the electrophoresis chamber in a correct position and filled by 1X TBE Buffer, the lid was closed, the electrodes were attached from the power supply and the DC power was turned on, voltage set for 100 volt, the run lasted for 30 min.
4. Band of PCR product was visualized by UV transillumination and photographed by a digital camera.
5. Positive bands have molecular weight 279 pb, 211 bp and 152 for *chuA, yjaA* and *TSPE4.C2* respectively.

Phylogenetic analysis was done on the basis of the presence or absence of the 3 DNA fragments (*chuA, yjaA* and *TSPE4.C2*) and phylogenetic groups detected as follows (Clermont *et al*., 2000; Derakhshandeh *et al*., 2013; Gudjónsdóttir, 2015; Staji *et al*., 2016):

Group A: *chuA*– and TspE4.C2–

- Subgroup A0:*chuA*–, *yjaA*– and TspE4.C2–

- Subgroup A1: *chuA*–, *yjaA*+ and TspE4.C2–

Group B1: *chuA*–, *yjaA*– and TspE4.C2+

Group B2: *chuA*+ and *yjaA*+

- Subgroup $B2_2$: *chuA*+, *yjaA*+ and TspE4.C2–
- Subgroup $B2_3$: *chuA*+, *yjaA*+ and TspE4.C2+

Group D: *chuA*+ and *yjaA*–

- Subgroup D1: *chuA*+, *yjaA*– and TspE4.C2–
- Subgroup D2: *chuA*+, *yjaA*– and TspE4.C2+

The results of triplex PCR product made it possible to establish a dichotomous decision tree for phylogenetic group as in Figure (2-3):

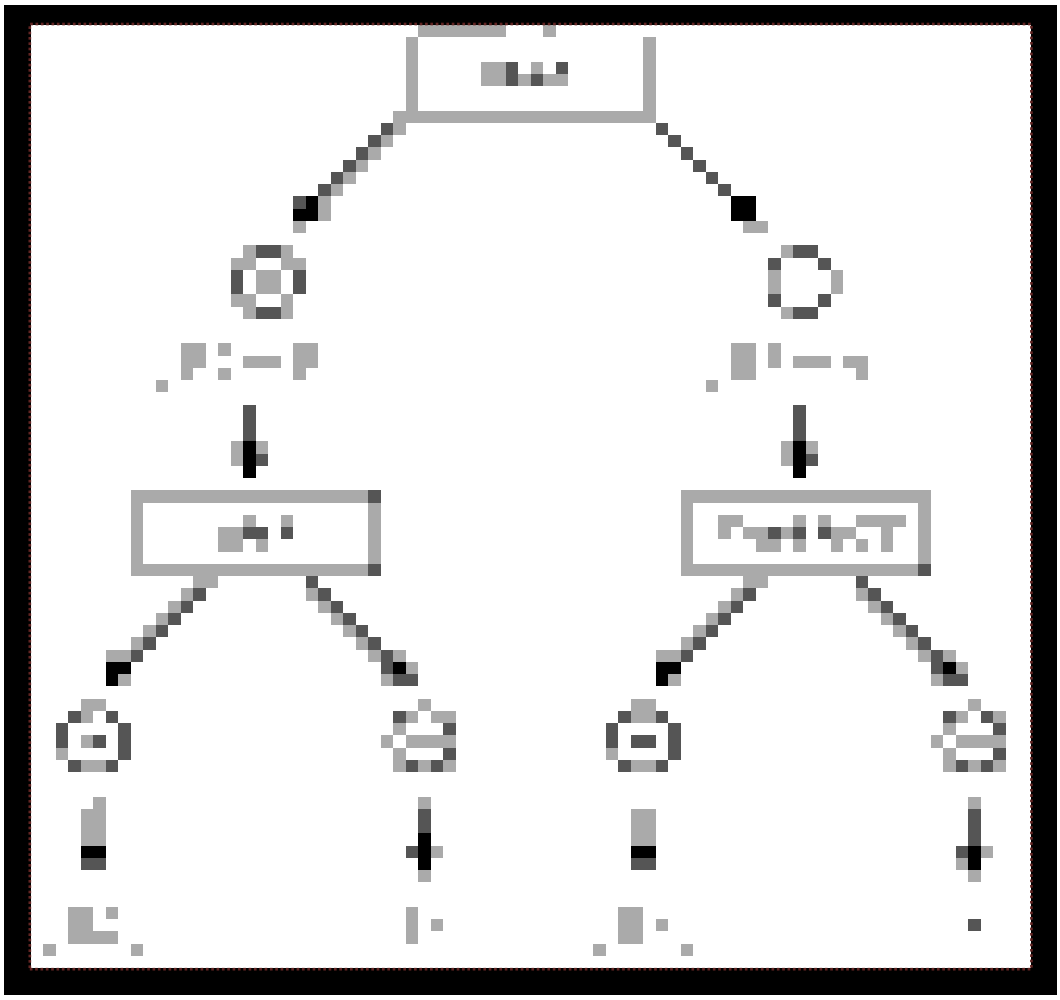


Figure (2-3): Dichotomous Decision Tree for phylogenetic Groups of *E. coli* (Gudjónsdóttir, 2015)

❖ Gel Electrophoresis for Determine Integron Class 1 and 2

Agarose gel was prepared in concentration of 1.5 % agarose for DNA profiling according to Kargar *et al.*, 2014 . The agarose gel was prepared as follow.

- Agarose (0.75 g) was dissolved in 50ml 1X TBE Buffer and heated on hot plate until all agarose particles were dissolved then left to cool until it reached 45-50 ^{0}C, then 5 µl (0.5 % concentration) of Ethidium Bromide stain was incorporated.
- Agarose gel was poured into gel tray after fixing the two plastic walls and put the comb in a proper position and left to solidify for 25 minutes at room temperature, then the comb has removed gently from the tray and 5µl of PCR products were loaded in each well and 5µl of Ladder (100 bp DNA Ladder) added in one well as size standard.
- The gel tray was fixed in the electrophoresis chamber in a correct position and filled by 1X TBE Buffer, the lid was closed, the electrodes were attached from the power supply and the DC power was turned on, voltage set for 100 volt, the run lasted for 40 min.
- Band of PCR product (*IntI 1* and *2*) was visualized by UV transillumination and photographed by a mobile or digital camera.
- Positive bands have molecular weight 439 pb for *IntI1* and 788 bp for *IntI2*

2.3. Statistical analysis

Statistical analysis was performed by Social Science Statistics and the Statistical Package For Social Sciences version 17 for Windows Software and Microsoft Excel 2010.

Descriptive analysis was done by calculating frequencies and approximate percentages for age groups, antibiotic susceptibility test, resistance patterns, integron classes and phylogenetic groups or

subgroups. The Pearson correlation (r) is used to measure the strength of a linear association between antibiotic resistance and integrons while the statistical significance of the relationship between resistance and the presence or absence of integrons was examined by the Fisher Exact Probability Test or Chi-Square test (χ^2) that also used to measure the strength of association between two categorical variables. All these statistical tests considered that *P*- value less than the 0.05 level was statistically significant (Viera, 2008; Parshall, 2013).

3. Results

3.1. Bacterial Isolates and Age Groups

The present cross sectional study including 350 stool sample collected from healthy individuals with age range from 1 to 80 years, from all these samples only 301 (86%)sample harbor commensal *E. coli* while remain 49 (14%) sample have negative culture for commensal *E. coli* (Figure 3-1).

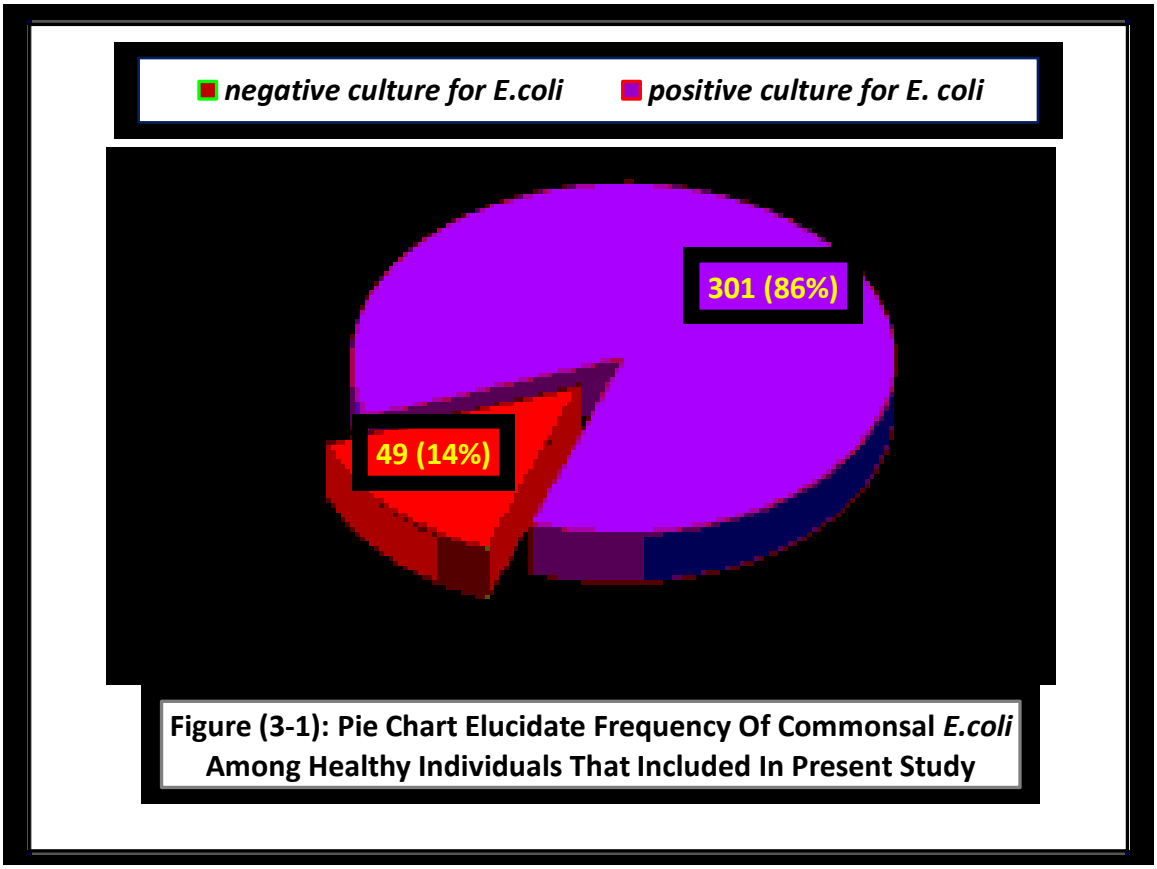


Figure (3-1): Pie Chart Elucidate Frequency Of Commonsal *E.coli* Among Healthy Individuals That Included In Present Study

In the current study, 301 healthy individuals who have commensal *E. coli* are constituted from 145 female and 156 male. Accordant to age, individuals are divided into seven age groups (Table 3-1) that including group1 (mean 6.7 year and SD± 5.1), group2 (mean 21.9 year and SD ± 1.7), group 3 (mean 28.1 year and SD ± 1.4), group4 (mean 37.6 year and SD ± 2.2), group5 (mean 48.8 year and SD± 3.6), group 6 (mean 60.2 year and SD± 3.8) and group 7 (mean 74 year and SD ± 7.2).

Table (3-1): Age Groups of Healthy Individuals Who Have Commensal *E. coli*

Age Range (year)	Age Groups	(Mean ± SD)	Females (N= 145)	Males (N=156)	Total N (%)
1-15	Group1	(6.7± 5.1)	22	20	42(14)
16-25	Group 2	(21.9 ± 1.7)	27	30	57(19)
26-35	Group 3	(28.1 ± 1.4)	22	26	48(16)
36-45	Group 4	(37.6 ± 2.2)	19	21	40(13)
46-55	Group 5	(48.8 ± 3.6)	18	18	36(12)
56-65	Group 6	(60.2 ± 3.8)	12	24	36(12)
66-80	Group 7	(74 ± 7.2)	25	17	42(14)

SD = Standard Deviation; N = Number

3.2.Phylogenetic Analysis of Commensal *Escherichia coli*

According to multiplex PCR-based phylogenetic analysis (Figure 3-2) a total of 301commensal *E. coli* isolated from fecal samples assigned into three phylogenetic groups (i.e. A, B2 and D) and six subgroups (i.e. A0, A1, $B2_2$, $B2_3$, D1 and D2). Group B2 represent the majority of the collected isolates (190 isolates, 63%) followed by group A (69 isolates, 23%) and D (42 isolates, 14%) but no strains were found to belong to group B1 (Figure 3-3 and Table 3-2). Most strains of group A (48 isolates, 16%) belonged to the subgroup A1 and the others (21 isolates, 7%) were related to subgroup A0. One hundred twenty one isolates (40%) of group B2 belonged to subgroup $B2_3$ and 69 isolates (23%) to subgroup $B2_2$. On other hand 21 isolates of group D (7%) fitted in subgroup D1 and others 21 isolates (7%) constituted to the subgroup D2.

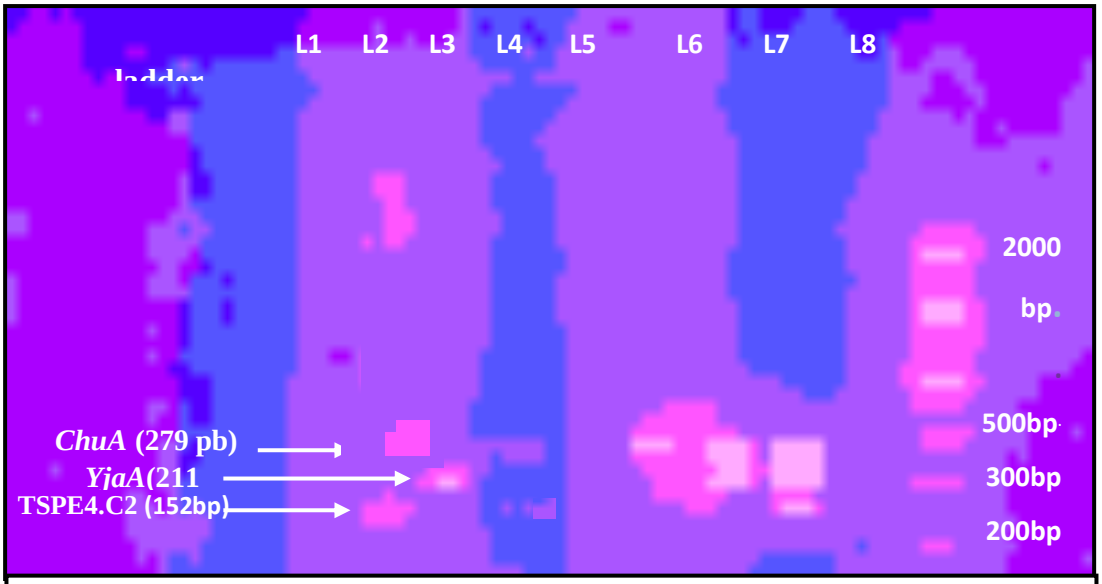


Figure (3-2): Triplex PCR profiles that specific for *E. coli* phylogenetic analysis. Combination of *chuA* gene, *yjaA* gene and DNA fragment, TSPE4.C2 are amplified to detection phylogenetic groups and their subgroups of *E. coli* isolates. lanes 1 and 2 represent group A (subgroup A0); lane 3 represent group D(subgroup D2); lane 4 include group A (subgroup A1); lane 6 represent group D (subgroup D1); lane 7 and 8 represent group B2 (subgroups $B2_2$ and $B2_3$ respectively). Standard strain in lane 5 (group D and subgroup D2. PCR products run on gel (2% agarose) at100 Volt for 30 min.

Group A: *chuA*– and TspE4.C2–
Subgroup A0:*chuA*–, *yjaA*– and TspE4.C2–
Subgroup A1: *chuA*–, *yjaA*+ and TspE4.C2–
Group B2: *chuA*+ and *yjaA*+
Subgroup $B2_2$: *chuA*+, *yjaA*+ and TspE4.C2–
Subgroup $B2_3$: *chuA*+, *yjaA*+ and TspE4.C2+
Group D: *chuA*+ and *yjaA*–
Subgroup D1: *chuA*+, *yjaA*– and TspE4.C2–
Subgroup D2: *chuA*+, *yjaA*– and TspE4.C2+

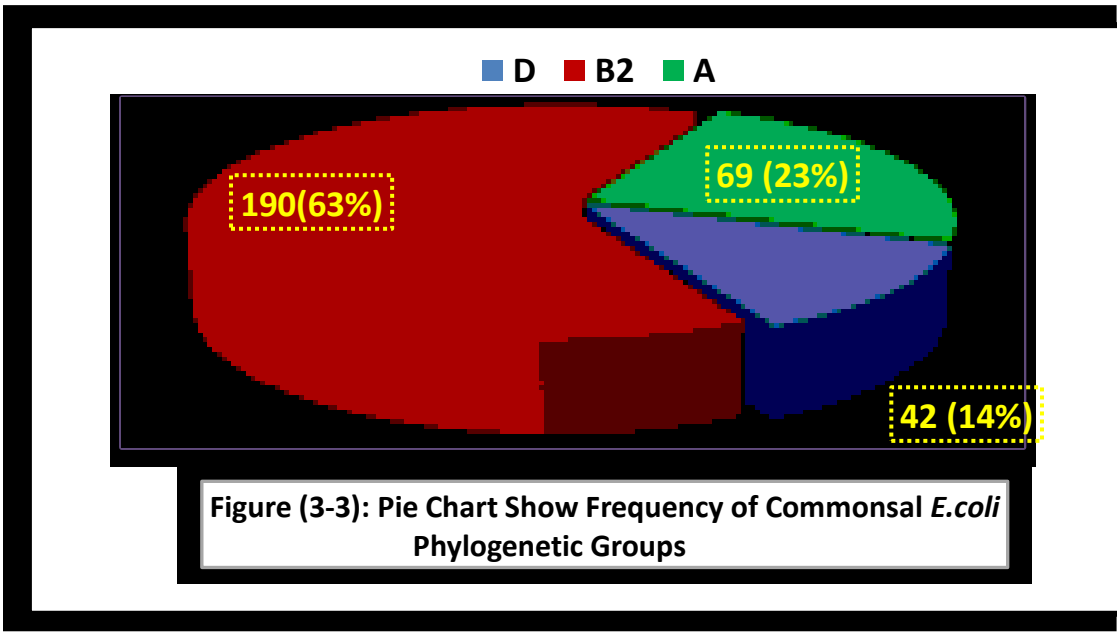


Figure (3-3): Pie Chart Show Frequency of Commonsal *E.coli* Phylogenetic Groups

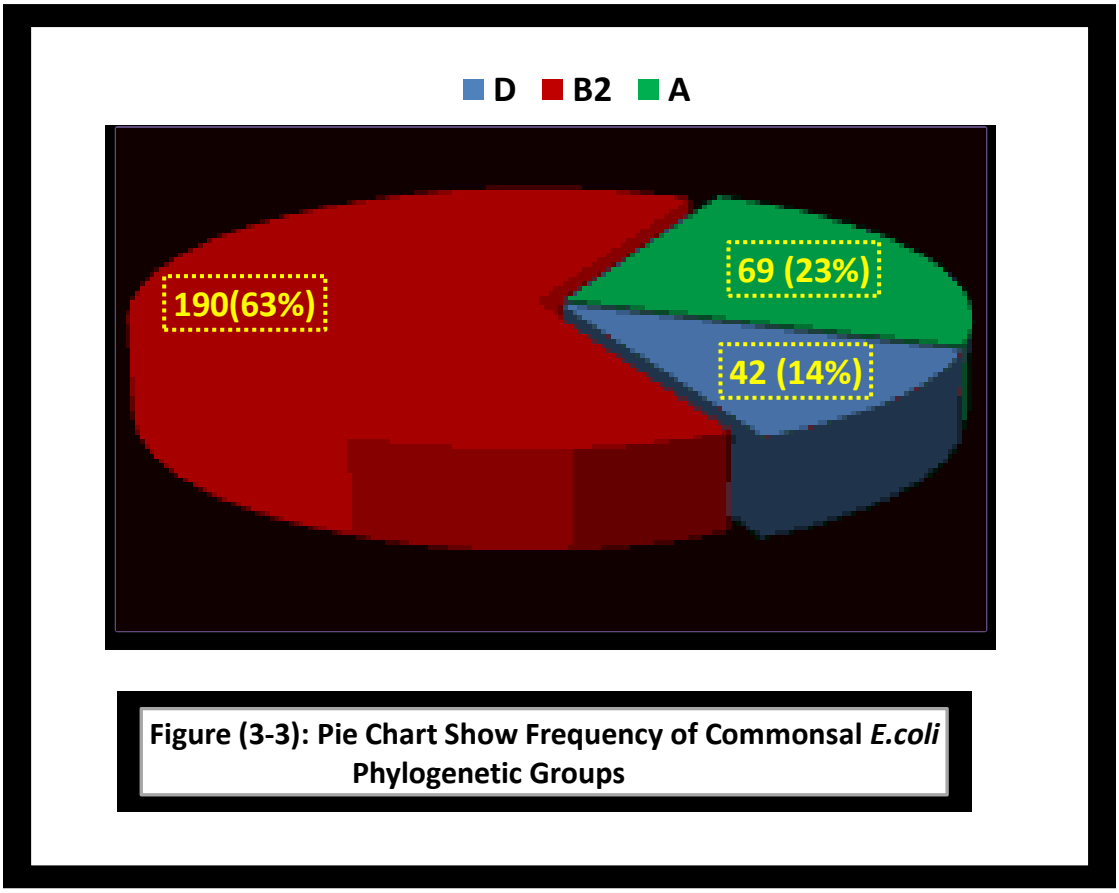


Figure (3-3): Pie Chart Show Frequency of Commonsal *E.coli* Phylogenetic Groups

Table (3-2): Distribution of Commensal *E. coli* Phylogenetic Groups and Subgroups Among Studied Population.

E. coli Groups	N (%)	Subgroups	N (%)
A	69 (23)	A_0	21 (7)
		A_1	48 (16)
B1	0 (0)		
B2	190 (63)	$B2_2$	69 (23)
		$B2_3$	121 (40)
D	42 (14)	D1	21 (7)
		D2	21 (7)

N = Number

3.3.Distribution of Commensal *Escherichia coli* Phylogenetic Groups among Age Groups

Present investigation clarified that whole commensal *E. coli* phylogenetic groups and subgroups together not indicated in most age

groups. Figure (3-4) and Table (3-3) illustrated that group A and their subgroups not seen in individuals in group 1, 5 and 7 but present mainly in individuals in group 4 [A (24 isolates, 60%), A0 (8 isolates, 20%) and A1(16 isolates, 40%)] followed by individuals in group 6 [A (14 isolates, 39%), A1(14 isolates, 39%) and A0 (0%)], group 3 [A (17 isolates, 35%), A0 (6 isolates,12%), A1 (11 isolates, 23%)] and group2 [A (14 isolates, 25%), A0(7 isolates, 12%) and A1 (7 isolates, 12)].

Group B2 predominant in all individuals and detected substantially in individuals in group7 (42 isolates,100%) and group 5 (36 isolates, 100%) followed by individuals in group 3 (31isolates, 65%), group 6 (22 isolates, 61%),group1 (23 isolates, 55%) and group2 (20 isolates, 35%). More ever subgroup $B2_3$also bring to light in all age groups and constituted 27(64%), 23 (55%), 20 (42%), 15 (42%), 16 (40%), 13 (23%) and 7 (19%) isolates of groups 7, 1, 3, 6, 4, 2 and 5 respectively whereas subgroup $B2_2$ not appeared in individuals in group 1and 4 but indicted in remaining age groups (29 isolates (81%), 15 isolates (36%), 11 isolates (23%), 7 isolates (19%) and 7 isolates (12%) of age groups 5, 7, 3, 6 and 2 respectively). Group D and their subgroups are prevailed only in individuals in group 1 [D (19 isolates, 45%), D1 (6 isolates, 14%)and D2(13 isolates, 31%)] and group2 [D (23 isolates, 40%), D1 (15 isolates, 26%) and D2 (8 isolates, 14%)].

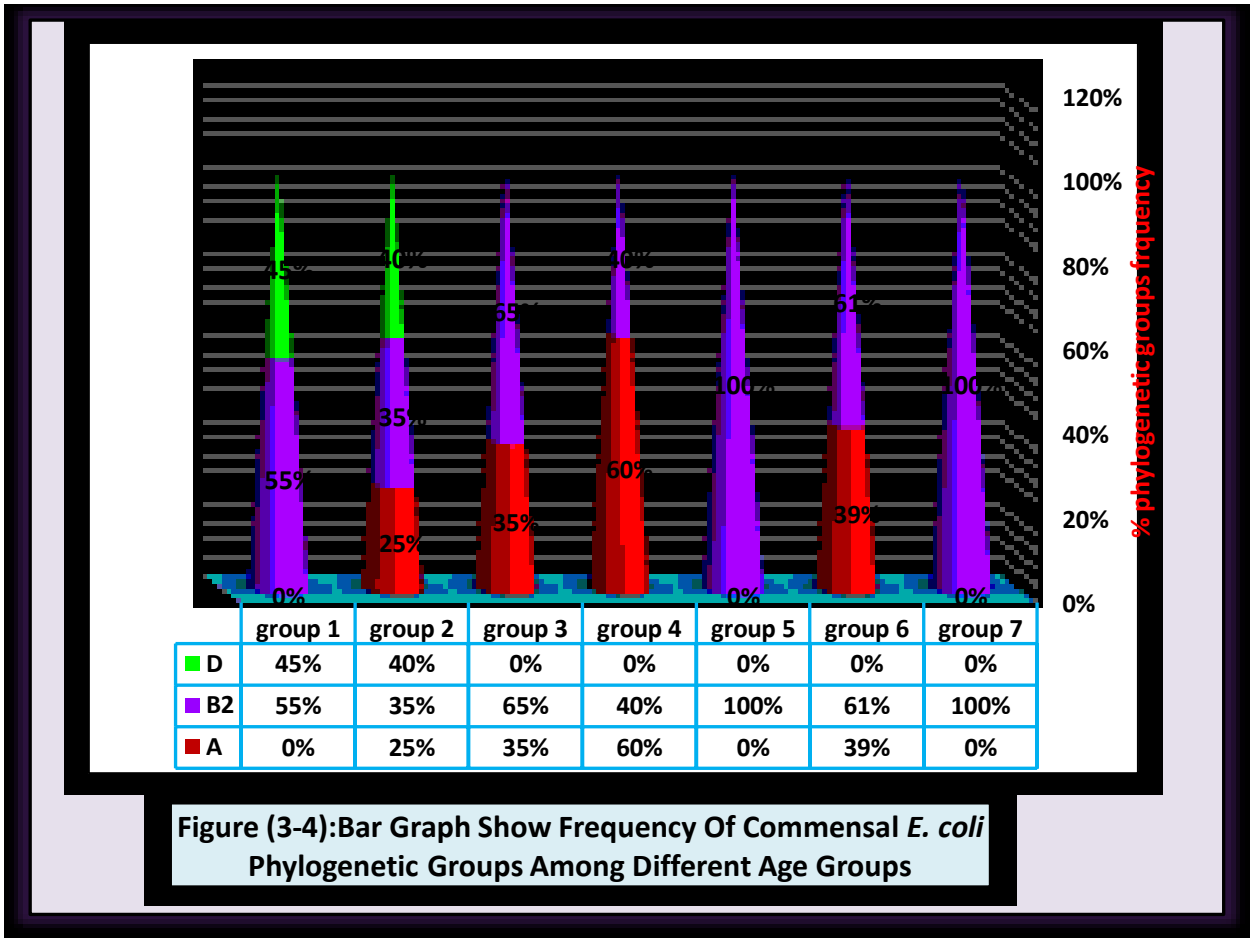


Figure (3-4):Bar Graph Show Frequency Of Commensal *E. coli* Phylogenetic Groups Among Different Age Groups

Table (3-3): Distribution of Commensal *E. coli* Phylogenetic Groups and Subgroups Among Different Age Groups

Groups or subgroups	Group 1 (N=42)	Group 2 (N=57)	Group 3 (N=48)	Group 4 (N=40)	Group 5 (N=36)	Group 6 (N=36)	Group 7 (N=42)	Total (N=301)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
A	0 (0)	14(25)	17(35)	24 (60)	0 (0)	14 (39)	0 (0)	69 (23)
A0	0 (0)	7 (12)	6 (12)	8 (20)	0(0)	0 (0)	0(0)	21 (7)
A1	0 (0)	7(12)	11(23)	16 (40)	0 (0)	14 (39)	0 (0)	48(16)
B2	23(55)	20 (35)	31 (65)	16 (40)	36 (100)	22 (61)	42 (100)	190 (63)
$B2_2$	0 (0)	7 (12)	11 (23)	0 (0)	29 (81)	7 (19)	15(36)	69 (23)
$B2_3$	23(55)	13 (23)	20 (42)	16 (40)	7 (19)	15 (42)	27 (64)	121 (40)
D	19(45)	23 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	42 (14)
D1	6 (14)	15 (26)	0(0)	0(0)	0(0)	0(0)	0(0)	21 (7)
D2	13 (31)	8 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	21 (7)

N = number

3.4. Prevalence of Integrons Among Commensal *Escherichia coli* Isolates

Integron genes detected in 211 (70%) out of 301 tested isolate by PCR amplification (Figure 3-5) among which 112 isolates (37%) have only integron class 1, 30 (10%) have only integron class 2 and 69 (23%) have both integron class 1and 2 while 90(30%) of isolates not have any one of these classes (Figures 3-6)

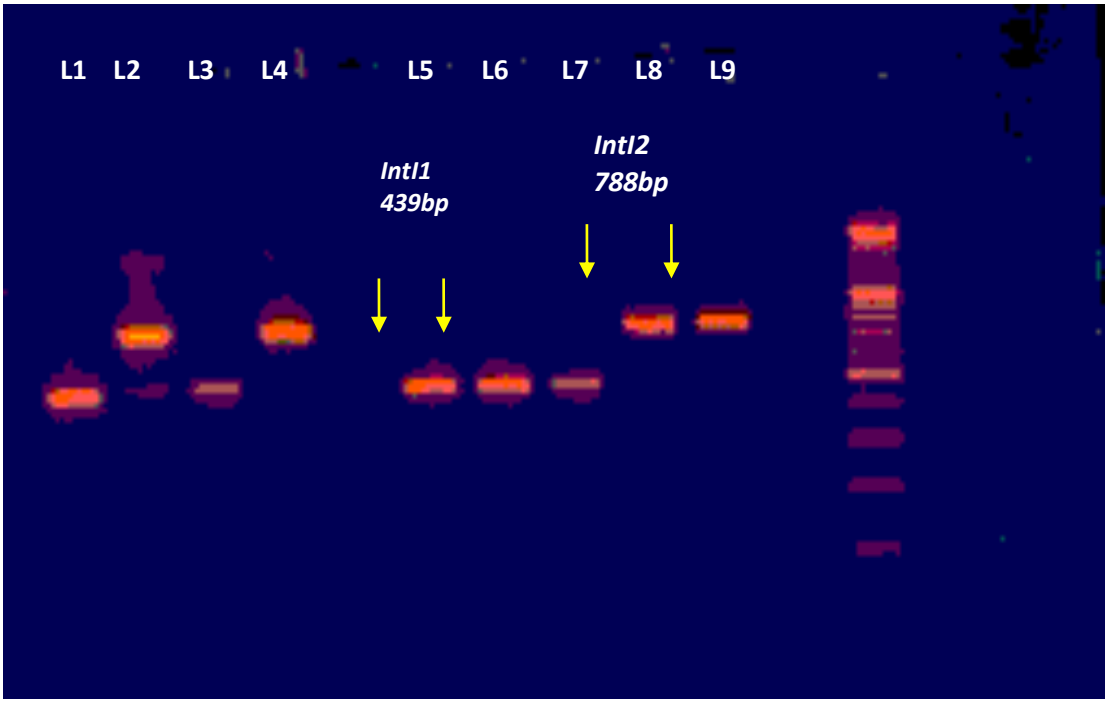


Figure (3-5): PCR Amplification of *IntI 1* and *IntI 2* Genes of Commensal *E. coli* Isolates. Standard strain in lines 1 and 2 (harbor both of *IntI 1 & 2* genes). lines 3 and 4 related to same isolated commensal *E. coli* isolate that contain both of *IntI1* and *IntI 2* genes. Lines 5, 6 and 7 below to isolated commensal *E. coli* isolates that have *IntI1* gene only while lines 8 and 9 represent isolated commensal *E. coli* isolates that have *IntI2* gene only. PCR products run on gel (1.5% agarose) at100 Volt for 40 min.

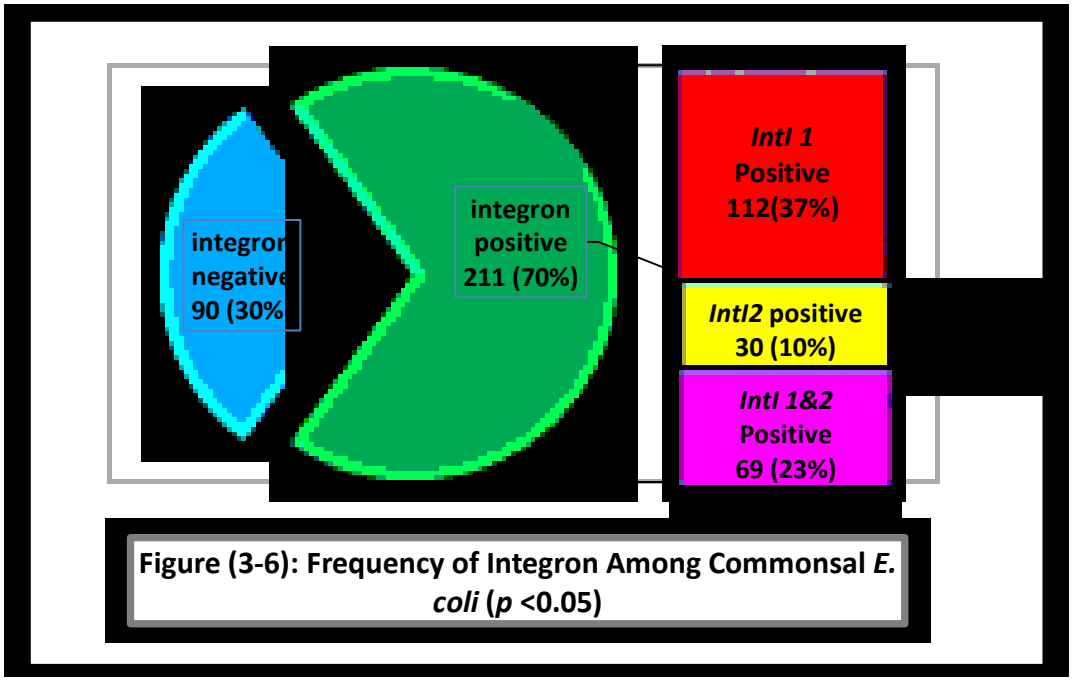


Figure (3-6): Frequency of Integron Among Commonsal *E. coli* (p <0.05)

3.5.Distribution of Integrons among Isolated Commensal *Escherichia coli* in Different Age Groups

Significant differences (P < 0.05) in integron distribution among *E. coli* isolates in different age groups, when all isolates (42isolates, 100%) in individuals in group 7 carried out integron also high predominant of integron (36 isolates, 86%) existed in isolates of individuals in group1. Isolates of individuals in groups 5, 2, 3 and 4 not revealed strong correlation with integron [18 isolates (50%),35 isolates (61%), 35 isolates(73%) and 32 isolates (80%) respectively] while isolates of individuals in group 6 show lower container of integrons among all age groups (13 isolates, 36%) (Figure 3-7).

Table (3-4) clarified significant differences (p < 0.05) in distribution of integron classes among *E. coli* isolates in different age groups and indicated high prevailing of integron class 1 mainly in isolates of individuals in group 1 (31 isolates, 74%) followed by groups 4,3,7 and 5 [25 isolates (62.5%), 27 isolates (56%), 14 isolates (33%) and 7 isolates (19%) respectively].Integron class1 have the lower prevalence in isolates

of individuals in group 2 (8 isolates, 14%) while not detected in those in group 6. In contrast to integron class 1, high frequency of integron class 2 located in isolates of individuals in group 7 (13 isolates, 31%) followed by groups 5 and 6 [11 isolates (30.5%), 6 isolates (17%) respectively] but not observed in isolates of individuals in groups 1, 2, 3 and 4. On other hand the highest prevalence of isolates that contain both classes of integron (27 isolates, 47%) detected in individuals in group 2 followed by groups 7, 6, 3, 4 and 1 [15 isolates (36%), 7 isolates (19%), 8 isolates (17%), 7 isolates (18%) and 5 isolates (12%) respectively] while isolates of individuals in group 5 not have any isolate has both classes of integron.

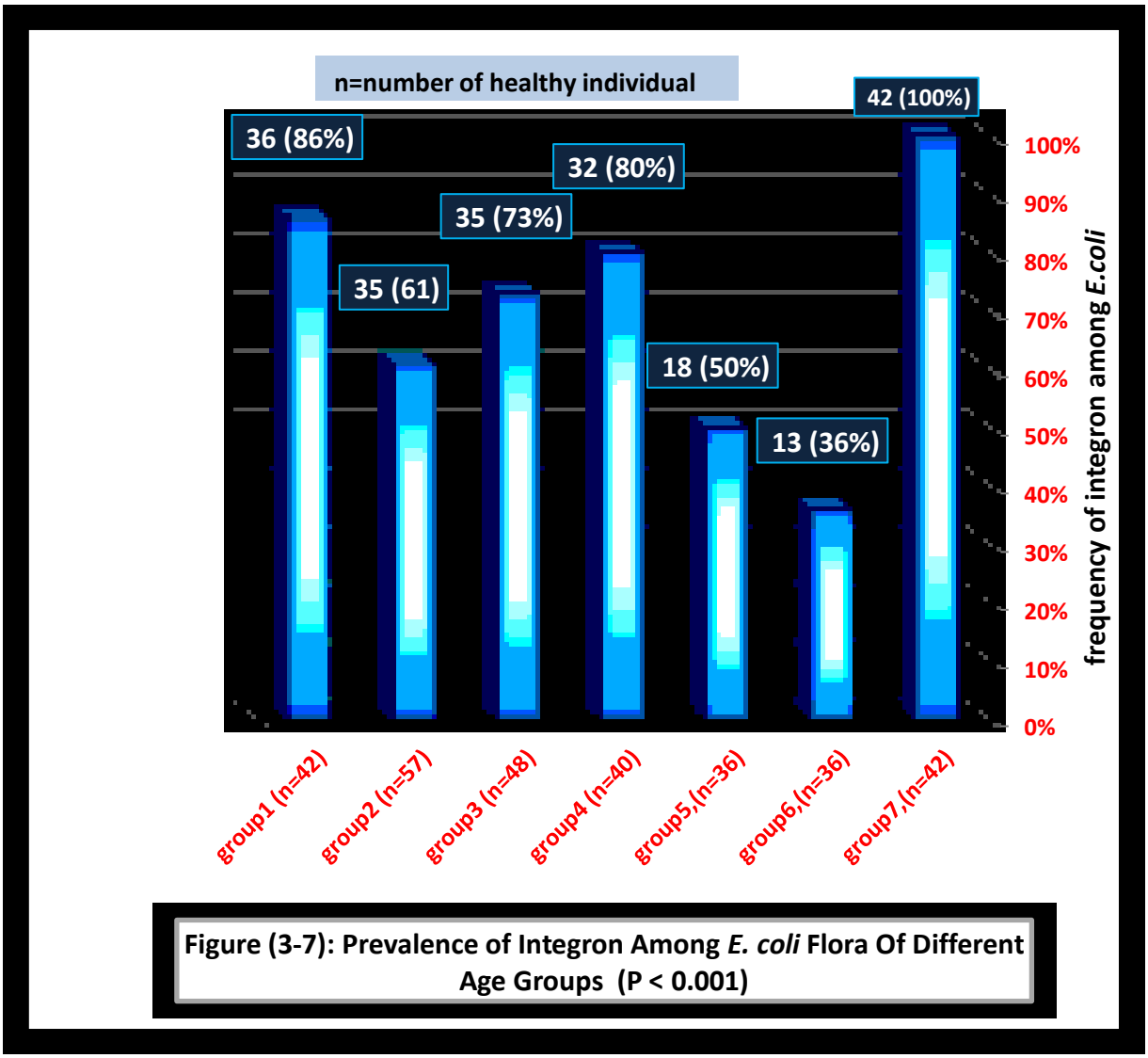


Figure (3-7): Prevalence of Integron Among *E. coli* Flora Of Different Age Groups (P < 0.001)

Table (3-4): Distribution of Integron Class 1 and 2 among *E. coli* in Different Age Groups

Age Groups	Positive *IntI 1* N (%)	Positive *IntI 2* N (%)	Positive *IntI 1& 2* N (%)	X^2	P value
Group 1 (N=42)	**31 (74)**	**0 (0)**	**5 (12)**	**60.45**	**< 0.0001***
Group 2 (N=57)	**8 (14)**	**0 (0)**	**27 (47)**	**38.67**	**<0.0001***
Group3 (N=48)	**27 (56)**	**0 (0)**	**8 (17)**	**41.80**	**< 0.0001***
Group 4 (N=40)	**25 (62.5)**	**0 (0)**	**7 (18)**	**43.65**	**< 0.0001***
Group 5 (N=36)	**7 (19)**	**11 (30.5)**	**0 (0)**	**11.036**	**0.0040***
Group 6 (N=36)	**0 (0)**	**6(17)**	**7 (19)**	**6.253**	**0.0439***
Group 7 (N=42)	**14 (33)**	**13 (31)**	**15 (36)**	**10.01**	**0.003***
Total	**112**	**30**	**69**		

N = Number; *Significant (P value < 0.05)

3.6. Distribution of Integrons among Commensal *Escherichia coli* Phylogenetic Groups and Subgroups

Commensal *E. coli* isolates of group B2 and subgroups D2 have highest container of integron among all tested phylogenetic groups and subgroups [140 isolates (74%) and 20 isolates(95%) respectively] as in Table (3-5).Current data showed significant differences (P < 0.05) in dissemination of integron class 1 and 2 among phylogenetic groups and subgroups (Table 3-5 and Figure 3-8) and observed that highest distribution of *IntI1*gene among phylogenetic groups recorded in group A (36 isolates, 52%) followed by group D (20 isolates, 48%) and group B2 (56 isolates, 29%) while *IntI2* gene mainly demonstrated in group B2 (28 isolates, 15%) followed by group A (2 isolates, 3%) but not determent in group D. In addition 29% (56 isolates) of group B2 have both classes of integron followed by 17% (7 isolates) and 9% (6 isolates) for groups D and A respectively. Results showed highest prevailing of *IntI1* gene seen in subgroup D2 (20 isolates, 95%) followed by A1(30 isolates, 62.5%), $B2_2$(28 isolates, 40.5%), A0 (6 isolates, 28.5%) and $B2_3$ (28 isolates, 23)but not detected in group D1. Whereas *IntI2* gene significantly detected in subgroups $B2_2$and $B2_3$[14 isolates (20%) and 14

isolates (12%) respectively] but not demonstrated in group D and subgroups A0, D1 and D2. Both classes of integron seen in clear picture in subgroup $B2_3$(42 isolates, 35%) and followed by subgroup D1 (7 isolates, 33%), $B2_2$ (14 isolates, 20%), A1 (5 isolates, 10%) and A0 (1 isolates, 5%) but not observed in subgroup D2 (0%).

Table (3-5): Prevalence of integrons class 1 and 2 among phylogenetic groups and subgroups

E. coli groups and subgroups	Integron N(%)	Integron class 1		Integron class 2		Integron class 1&2		X^2	*P* - value
		N	%	N	%	N	%		
A (N =69)	44 (64)	36	52	2	3	6	9	125.8	< 0.0001*
A0 (N = 21)	7 (33)	6	28.5	0	0	1	5		
A1 (N = 48)	37 (63)	30	62.5	2	4	5	10		
B2(N = 190)	140 (74)	56	29	28	15	56	29		
$B2_2$ (N =69)	56 (81)	28	40.5	14	20	14	20		
$B2_3$(N =121)	84 (69)	28	23	14	12	42	35		
D (N =42)	27 (64)	20	48	0	0	7	17		
D1 (N = 21)	7 (33)	0	0	0	0	7	33		
D2 (N = 21)	20 (95)	20	95	0	0	0	0		

N = number; * p- value & X^2 show association between *IntI* genes and subgroup

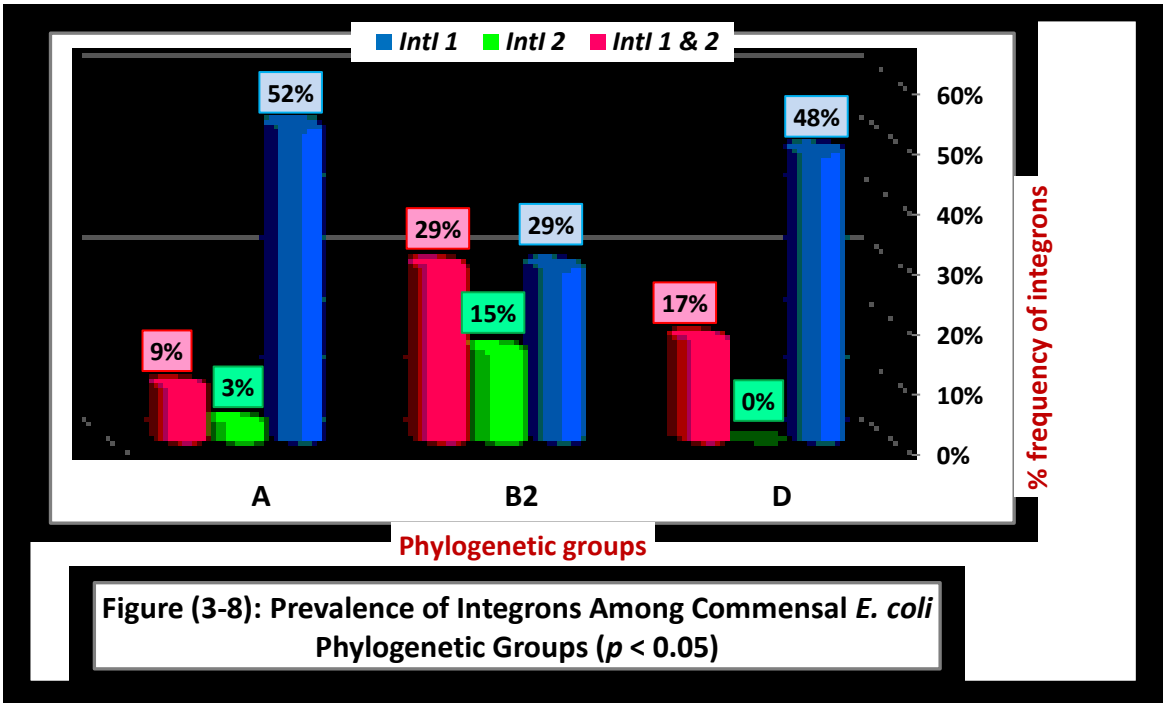


Figure (3-8): Prevalence of Integrons Among Commensal *E. coli* Phylogenetic Groups ($p < 0.05$)

3.7. Antimicrobial Resistance of *Escherichia coli* Isolates

Susceptibility to sixteen antibiotics (Ampicillin, Amoxicillin, Amikacin, Norfloxacin, Streptomycin, Trimethoprim, Lincomycin, Gentamicin, Ciprofloxacin, Nalidixicacid, Chloramphenicol, Cefotaxime, Ceftriaxone, Cephalexin , Tetracycline and Oxytetracyclin) was assessed by the disc diffusion method according to the CLSI (CLSI, 2013).

Among the drugs under the study, Ampicillin, Amoxicillin, Cephalexin and Lincomycin not have any antimicrobial effect (100% of the total isolates were resistant). In addition, the high resistance rate was recorded to Cefotaxime (280 isolates, 93%), Tetracycline (223 isolates, 74%), Trimethoprim (202 isolates, 67%), Oxytetracyclin (201 isolates, 67%), Ceftriaxone(188 isolates, 63%), Nalidixic acid(168 isolates, 56%) and Streptomycin (161 isolates, 53%). On the other hand low resistance indicated to Gentamicin (64 isolates, 21%), Ciprofloxacin (77 isolates, 26%), Norfloxacin(92 isolates, 31%) and Chloramphenicol (105 isolates, 35%) while resistance to Amikacin was less common and seen in only 42 isolates (14%) as in Figure (3-9).

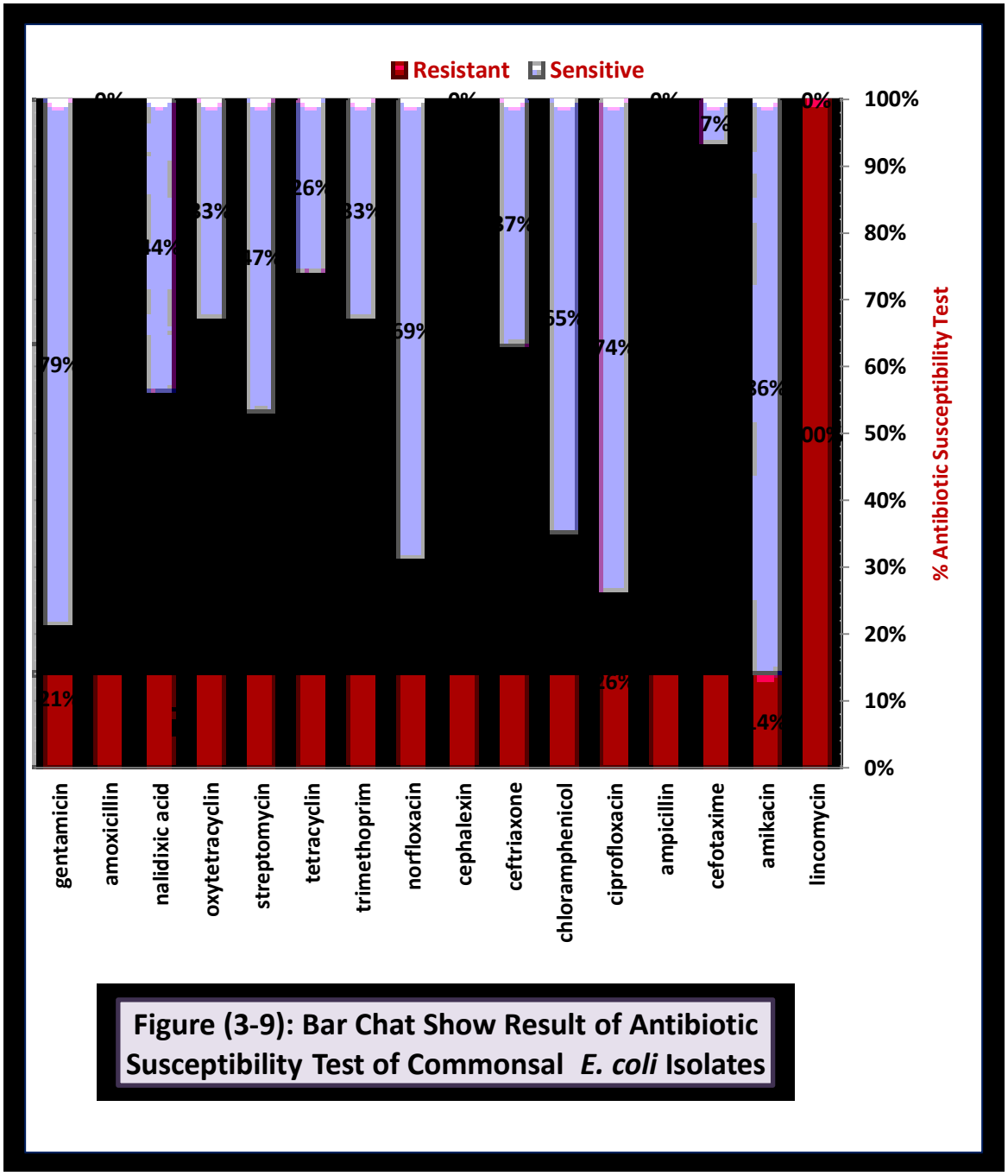


Figure (3-9): Bar Chat Show Result of Antibiotic Susceptibility Test of Commonsal *E. coli* Isolates

3.8.Distribution of Antibiotic Resistance among Commensal *Escherichia coli* in Different Age Groups

Table (3-6) was indicated significant association between commensal *E. coli* in different age groups and resistance ($p< 0.05$) for all antibiotics except Lincomycin, Amoxicillin, Cephalexcin and Ampicillin that have highest resistance among all *E. coli* isolates in different age

groups (301 isolates, 100%). All isolates in age groups 1, 4, 6 and 7 have resistance to Cefotaxime (301 isolates, 100%) also high resistance to Cefotaxime observed in isolates in group 3 (42 isolates, 88%), group 2 (49 isolates, 86%) and group 5 (29 isolates, 81%). In additional all isolates (301 isolates, 100%) in group 7 resist to Tetracycline s and Ceftriaxone. Resistance for Nalidixic acid mainly existed in isolates of group 2 (44 isolates, 77%) followed by group 4 (25 isolates, 63%) and group 6 (22 isolates, 61%) while resistance for Norfloxacin mainly appeared in group 7 (27 isolates, 64%) followed by group 2 (27 isolates, 47%). Low resistance to Ciprofloxacin mainly reported in group 6 (0%) and group 4 (7 isolates, 18%) while highest resistance to Ciprofloxacin reported in group 2 (21 isolates, 38%).

High rate of resistance among Aminoglycosides are seen for Streptomycin [mainly isolates in group 6 (28 isolates, 78%) followed by group 3 (31 isolates, 65%)] in comparison with Gentamicin and Amikacin. In general all isolates show low resistance to Gentamicin particularly isolates in group 3 (0%) followed by group 6 (4 isolates, 10%) while isolates in group1show highest resistance to Gentamicin (16 isolates, 38%). Amikacin has the lower resistance (42 isolates, 14%) among tested antimicrobial agents for which all isolates in groups 1, 5 and 7 are sensitive and only isolates in group 4 showed high resistance (16 isolates, 40%). More ever isolates in group 6 (21 isolates, 60%) followed by group 1 (18 isolates, 43%) showed highest resistance to Chloramphenicol among age groups. Trimethoprim have high resistance in all isolates in different age groups particularly group 4 (31 isolates, 78%) followed by group 5 (27 isolates, 75%).

Table (3-6): Distribution of Antibiotic Resistance Among Commensal *E. coli* in Different Age Groups

Antibiotic agents	Total (N = 301)	Group1 (N= 42)	Group2 (N=57)	Group3 (N=48)	Group4 (N=40)	Group5 (N=36)	Group6 (N=36)	Group7 (N=42)	*P* value
	R N(%)	R N (%)	R N (%)	R N (%)	R N (%)	R N (%)	R N (%)	R N (%)	
Penicillins									
Ampicillin	301 (100)	42 (100)	57 (100)	48 (100)	40 (100)	36 (100)	36 (100)	42 (100)	0.9997
Amoxicillin	301 (100)	42 (100)	57 (100)	48 (100)	40 (100)	36 (100)	36 (100)	42 (100)	0.9997
Cephalosporins									
Cefotaxime	280 (93)	42 (100)	49 (86)	42 (88)	40 (100)	29 (81)	36 (100)	42 (100)	0.0222*
Ceftriaxone	188 (63)	20 (48)	28 (49)	32 (67)	24 (60)	28 (78)	14 (39)	42 (100)	0.0069*
Cephalexin	301 (100)	42 (100)	57 (100)	48 (100)	40 (100)	36 (100)	36 (100)	42 (100)	0.9997
Quinolones									
Nalidixic acid	168 (56)	13 (31)	44 (77)	28 (58)	25 (63)	19 (53)	22 (61)	17 (40)	0.0005*
Norfloxacin	92 (31)	11 (26)	27 (47)	9 (19)	7 (18)	5 (14)	6 (17)	27 (64)	0.0008*
Ciprofloxacin	77 (26)	12 (28)	21 (38)	11 (23)	7 (18)	13 (36)	0 (0)	13 (31)	0.0024*
Aminoglycosides									
Amikacin	42 (14)	0 (0)	14 (25)	5 (10)	16 (40)	0 (0)	7 (19)	0 (0)	<0.001*
Gentamicin	64 (21)	16 (38)	20(35)	0 (0)	7 (18)	5 (14)	4 (10)	13 (31)	0.0001*
Streptomycin	161 (53)	23 (55)	35 (61)	31 (65)	15 (38)	16 (44)	28 (78)	13 (31)	0.0055*
Tetracyclines									
Oxytetracyclin	201 (67)	34 (81)	34 (60)	25 (52)	30 (75)	16 (44)	20 (56)	42 (100)	<0.0001*
Tetracyclin	223 (74)	34 (81)	41 (72)	30(63)	30 (75)	26 (72)	20 (56)	42 (100)	< 0.0001*
Phenicol									
Chloramphenicol	105 (35)	18 (43)	14 (25)	16 (33)	16 (40)	6 (17)	21 (60)	14 (33)	0.0105*
Anti-Folate									
Trimethoprim	202(67)	29 (69)	42 (74)	32 (67)	31 (78)	27 (75)	13 (36)	28 (67)	0.0013*
Lincosamide									
Lincomycin	301 (100)	42 (100)	57 (100)	48 (100)	40 (100)	36 (100)	36 (100)	42 (100)	0.9997

N = Number; R = Resistance; *Significant (P value < 0.05)

3.9. Distribution of Antibiotic Resistance among Commensal *Escherichia coli* Phylogenetic Groups and Subgroups

All phylogenetic groups and subgroups (100%) in current results are resisted to penicillins, Cephalexin and Lincomycin (Figure 3-10 and Table 3-7). Also all group A(100%) (including their subgroups A1and A0) and subgroups $B2_2$ (100%) and D2 (100%) resistance to Cefotaxime that also have high resistance in remaining groups (B2 (93%) and D(83%)) and subgroups ($B2_3$(88%) and D1(67%)). Resistance for Ceftriaxone mainly investigated in group B2 (70%) followed by group A (60) also 70% of subgroup $B2_2$ and $B2_3$ have resistance to Ceftriaxone followed by subgroups A0 (67%) and A1(56%) while 33% of subgroups D1 and D2 resist to Ceftriaxone (Table 3-7). Resistance to Nalidixic acid determent in high frequency in group A (70%) followed by group B2 (56%) and highest resistance among subgroups appeared in subgroup A1 (81%) followed by subgroups $B2_2$(61%) and $B2_3$ (53%).

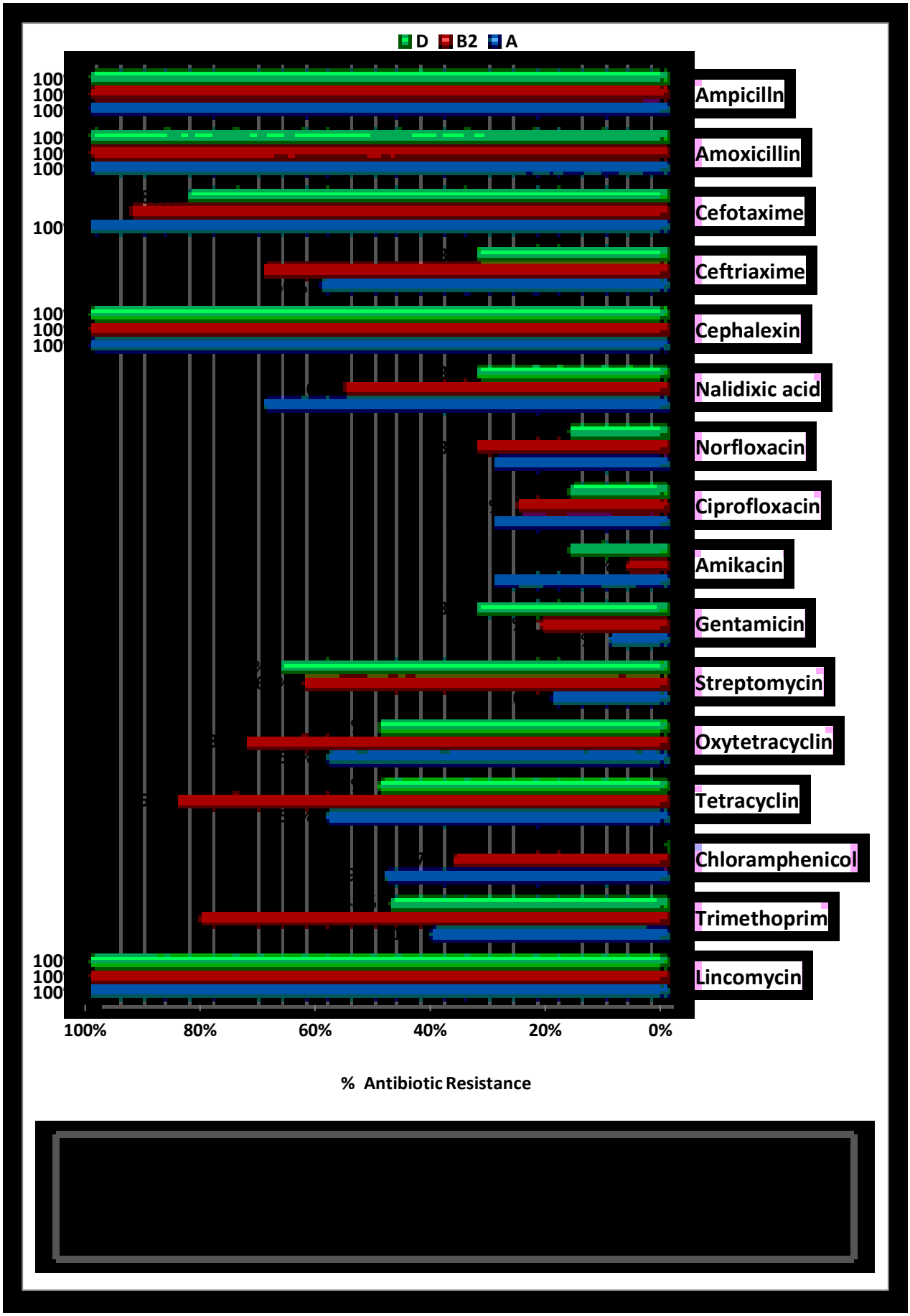
Resistance to Norfloxacin mainly detected in group B2 (33%) followed by group A (30%) and D (17%) whereas in subgroups, 51% of subgroup $B2_2$ resist to Norfloxacin followed by 44% of subgroup A1, 33% of subgroup D1 and 24% of subgroup $B2_3$while all subgroups A0 and D2 are sensitive. In addition, resistance to Ciprofloxacin mostly investigated in group A (30%) and subgroup A0 (33%) while all isolates in subgroup D2 are sensitive. Group A have the highest resistance to Amikacin (30%) among phylogenetic groups followed by D (17%) and only 7% of B2 are resist while resistance in subgroups mainly seen in A0 (38%) and D2 (38%) followed by A1 (27%). On other hand 33% of group D resist to Gentamicin followed by groups B2 (22%) and A (10%). Resistance to Gentamicin in subgroups mainly determent in D1(42%) followed by (29%) of both D2 and $B2_3$ while all isolates in subgroup A0 are sensitive. Resistance to Streptomycin investigated in all

groups (D(67%), B2 (63%) and A (20%)) and subgroups (mainly in $B2_2$ (70%) and 67% in both D1 and D2).

High resistance to Oxytetracyclin and Tetracycline recorded in group B2 (73% and 85% respectively) followed by group A (59% for both antibiotics) and D (50% also for both antibiotics). All isolates in subgroup $B2_2$ (100%) resist to Tetracycline s while other subgroups also have high resistance for both Tetracycline agents. Resistance to Chloramphenicol mainly reported in groups A and B2(49% and 37% respectively) and subgroups A1, $B2_2$ and $B2_3$ (71%, 30% and 41% respectively) while all isolates in group D and their subgroups are sensitive to Chloramphenicol.

Resistance to Trimethoprim appeared in all groups and subgroups and the resistance mainly detected in group B2 (81%) followed by group D (48%)and group A (41%). Among subgroups, $B2_3$ involve the highest resistance to Trimethoprim (82%) followed by $B2_2$(80%) whereas isolates in subgroup D2 have lower resistance to Trimethoprim (29%).

Table (3-9) showed significant differences ($p < 0.05$) in distribution of antibiotic resistance among phylogenetic groups and subgroups for all antibiotics except Lincomycin, Amoxicillin, cephalexcin and Ampicillin.


D
B2
A
Ampicilln
Amoxicillin
Cefotaxime
Ceftriaxime
Cephalexin
Nalidixic acid
Norfloxacin
Ciprofloxacin
Amikacin
Gentamicin
Streptomycin
Oxytetracyclin
Tetracyclin
Chloramphenicol
Trimethoprim
Lincomycin
100%
80%
60%
40%
20%
0%
% Antibiotic Resistance

3.10. Association Between Antibiotic Resistance and Integrons of Isolated Commensal *Escherichia coli*

In order to assess the effect of integron carriage on antibiotic susceptibility profile, the pattern of resistance by disc diffusion test of integrons positive *E. coli* isolates was compared with resistance pattern of integrons- negative *E. coli* isolates and results revealed strong positive linear correlation between antibiotic resistance and *IntI* genes (r =0.982, 0.975 and 0.980 for *IntI1*, *IntI2* and both classes respectively) as in Table (3-8). Although high resistance of integrons positive isolates to penicillines, cephalosporins, Tetracyclines, trimthoprim, chloramephnicol and Lincomycin significant correlation not determent for gthese antibiotic agents (P> 0.05). Resistance to Cefotaxime, Ciprofloxacin, Oxytetracyclin , Tetracycline and Trimethoprim significantly associated with *IntI1* gene positive isolates (p <0.0001).

Resistance to Cefotaxime, Ceftriaxone, Streptomycin, Tetracycline s and chloramephenicol significantly associated with *IntI2* gene positive isolates (p = 0.0008, 0.0342, 0.021, 0.0001and 0.0005 respectively) while isolates that contain both class 1 and class 2 significantly correlated with resist to Cefotaxime (p = 0.0001), Streptomycin (p = 0.0241), Chloramphenicol (p = 0.0003), Tetracycline (p < 0.0001), Oxytetracycline (p < 0.0001) and Trimethoprim (p = 0.00013).

3.11.Association Between Integrons Positive phylogenetic Groups and Antibiotic Resistance

Significant correlated between integrons positive phylogenetic groups and antibiotic resistance are determent by compared antibiotic resistance of integrons positive with resistance of integrons- negative *E. coli* phylogenetic groups. *IntI1* positive group A significant association

with resistance to Tetracycline ($p < 0.0001$), Oxytetracyclin e ($p < 0.0001$) Norfloxacin ($p = 0.00014$), Amikacin ($p = 0.00014$), Streptomycin ($p = 0.004$), Chloramphenicol ($p = 0.00014$) and Trimethoprim ($p< 0.0001$) while *IntI2* positive group A significant association with resistance to Norfloxacin ($p = 0.0001$), Ciprofloxacin ($p <0.0001$), Ceftriaxone ($p = 0.022$), Amikacin ($p < 0.0001$), Gentamicin ($p < 0.0001$), Chloramphenicol ($p = 0.019$) and Trimethoprim(0.004) (Tables 3-9 and 3-10). Group A that contain both *IntI1* and *IntI2* genes are significantly associated with resistance to Norfloxacin ($p < 0.0001$), Amikacin ($p < 0.0001$), Gentamicin ($p < 0.0001$), Tetracycline ($p = 0.0017$), Oxytetracyclin e ($p < 0.002$) and trimthoprim ($p < 0.0001$) (Table 3-13). On other hand *IntI1* positive group B2 are significantly association with resistance to Tetracycline ($p < 0.0001$), Oxytetracycline ($p < 0.0001$), Nalidixic acid ($p = 0.010$), Chloramphenicol ($p = 0.008$), Cefotaxime ($p< 0.0001$), Ciprofloxacin (0.007) and Trimethoprim ($p = 0.0061$) while *IntI2* positive group B2 are significantly association with resistance to Cefotaxime ($p = 0.001$), Streptomycin ($p = 0.002$), Tetracycline ($p < 0.0001$) Chloramphenicol ($p = 0.0007$) and Ceftriaxone ($p = 0.033$`). *IntI1* and *IntI2* genes positive group B2 are significantly associated ($p < 0.05$) with resistance to Oxytetracyclin e ($p = 0.0045$), Tetracycline s ($p < 0.0001$) and Chloramphenicol (0.025).

Integron class 1 positive group D mainly associated with antibiotic resistance to Ceftriaxone ($p < 0.0001$). *IntI1* and *IntI2* positive group D also significantly associated with resistance to Amikacin ($p = 0.00031$) while, alone *IntI2* gene not indicted in any isolates of group D

3.12.Multiple Drug Resistance Among Commensal *Escherichia coli* Isolates

All isolates in present study are multiple antibiotic resistance isolates and have significant differences in distribution of number of

antibiotic resistance (P <0.05). Figure (3-11) showed resist to 5 and 15 antibiotics reported in 5% of isolates. On other hand resistance to 8, 9, 10 and 12 antimicrobial agents determent in 9% of isolates while resistance to 14 antimicrobial agents detected in 7% of isolates. Resistance to 4,6,11and 13appeared in 2% , 14%, 19% and 12% of isolates respectively.

Among MDR isolates 20 resistance pattern were observed. The most frequent pattern was AM-AX-L-CL (100%) followed by AM-AX-L-CL-CTX (93%) and AM-AX-L-CL-CTX-TE (74%)while resistance patterns; AK-CN-S (Aminoglycosides) and AX-AM-CTX-CL-TE-T-CIP-NOR-AN-TMP-C-L-AK-CN-CRO are appeared in only 2% and 5% of tested isolates respectively (Table 3-12).

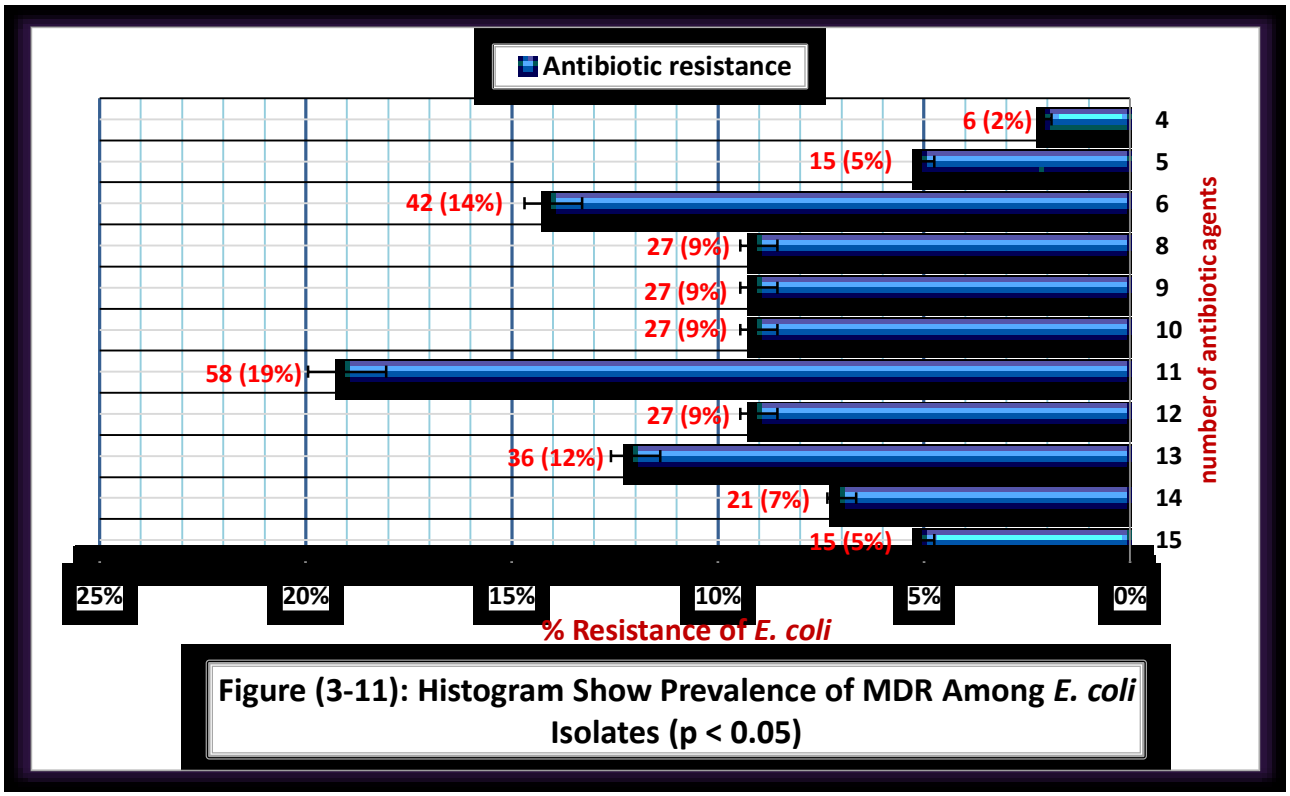


Figure (3-11): Histogram Show Prevalence of MDR Among *E. coli* Isolates (p < 0.05)

3.13.Multiple Drug Resistance profile of Commensal *Escherichia coli* Isolates in Different Age Groups

Current results (Figure 3-12) showed most *E. coli* that isolated from individuals in group 1 (29% and 28%) resist to 11 and 13 antimicrobial agents respectively and also 14% of this isolates have resistance to 6 and 14 antimicrobial agents. In additional 15% of isolates in group 1 resist to 8 antibiotics while resistance to 4, 5, 9, 10, 12, and 15 antibiotics not recorded (0%) in any isolate. In group two, 12% of isolates resist to 4,11,14 and 15 antibiotics and 13% of this isolates resist to 6 and 13 antibiotics. Farther more 11% and 14% of isolates in group 2 resist to 10 and 12 antibiotics whereas resistance to 5, 8 and 9 are absent (0%). Resistance to 5, 6 and 9 antibiotics demonstrated in 11% of isolates in group 3 and 23% of it resist to 11 and 13 antibiotics also 12% of isolates resist to 8 antibiotics but resistance to 4, 12, 14 and 15 not detected any in isolates of this age group. In isolates ofgroup 4 resist only to 12 (21%), 14 (20%), 5 (20%), 11 (20%) and 10 (19) antibiotics. On other hand 20% of isolates in group 5 resist to 6, 9, 10, 11 and 12antibiotics while resistance to other tested antimicrobial agents not observed (0%). Most MDR isolates (42%) in isolates of group 6 resist to 6 antibiotics followed by 28% of isolates resist to 9 antibiotics and 14% of those isolates resist to 11 and 13 antibiotics whereas resistance to 4, 5, 8, 10, 12, 14 and 15 not appeared (0%).

Resistance of MDR commensal *E. coli* in group 7 mainly recorded for 8 antibiotics (32%), 15 antibiotics (32%) and 11 antibiotics (30%) and only 1%, 2% and 3% of MDR isolates resist to 14, 6 and 12 antibiotics respectively while resistance to 4, 5,9, 10 and 13 antibiotics not observed(0%).

Patterns of antibiotic resistance are demonstrated in the seven age groups with different frequency and *P*- values (Table 3-13). In general resistance pattern of AM-AX-L-CL the most frequent among all isolates in different age groups (100%). Resistance pattern of AM-AX-L-CL-CTX also predominant among isolates in all age groups [group 1(100%), group 2 (88%), group 3 (90%), group 4(100%), group 5 (100%), group 6 (86%) and group 7 (100)]. Some of remaining resistance pattern also appeared clear in age groups such as in group 1, resistance patterns of AM-AX-L-CL-CTX-TE, AM-AX-TE-T, CTX-CRO-CL and T-TE-TMP are clear observed (86%, 86%, 74% respectively and 71%) while in group 2 other more common resistance patterns are M-AX-L-CL-CTX-TE (75%), AM-AX-TE-T (63%) and AX-NA-CTX-T-CL-TMP-AM-L (63%). Other significantly appeared resistance patterns in group 3 are AM-AX-L-CL-CTX-TE (67%), AM-AX-L-CL-CTX-TE-NA (56%), AM-AX-TE-T(56) and CTX-CRO-CL (56%), group 4 are AM-AX-L-CL-CTX-TE (83%), AM-AX-L-CL-CTX-TE-NA (63%), AM-AX-TE-T (80%) and T-TE-TMP (80%), group 5 are AM-AX-L-CL-CTX-TE (81%) and CTX-CRO-CL (50%) and group 6 are AM-AX-L-CL-CTX-TE (72%) and AM-AX-L-CL-CTX-TE-NA (58%).

Most resistance patterns indicated more clearly in group 7 in compared with other age groups such as AM-AX-L-CL-CTX-TE (100%), AM-AX-TE-T (100%), CTX-CRO-CL (100%), AX-CTX-T-CRO-TE-CL-AM-L (100%) and AX-CTX-T-TE-S-CL-TMP-AM-L (69%).

Remaining resistance patterns are less common in age groups but really existed in group 7 such as AX-AM-CTX-CL-TE-T-CIP-NOR-AN-TMP-C-L-AK-CN-CRO (31%), AX-CTX-T-CRO-S-TE-CL-TMP-AM-L(67%), AX-NA-CTX-CIP-S-TE-CL-TMP-AM-CN-L (33%), NOR-CIP-NA-CTX-CRO-CL (33%), AX-CTX-T-TE-S-CL-TMP-AM-L(67%) and CTX-T-CRO-TE-TMP (67%)

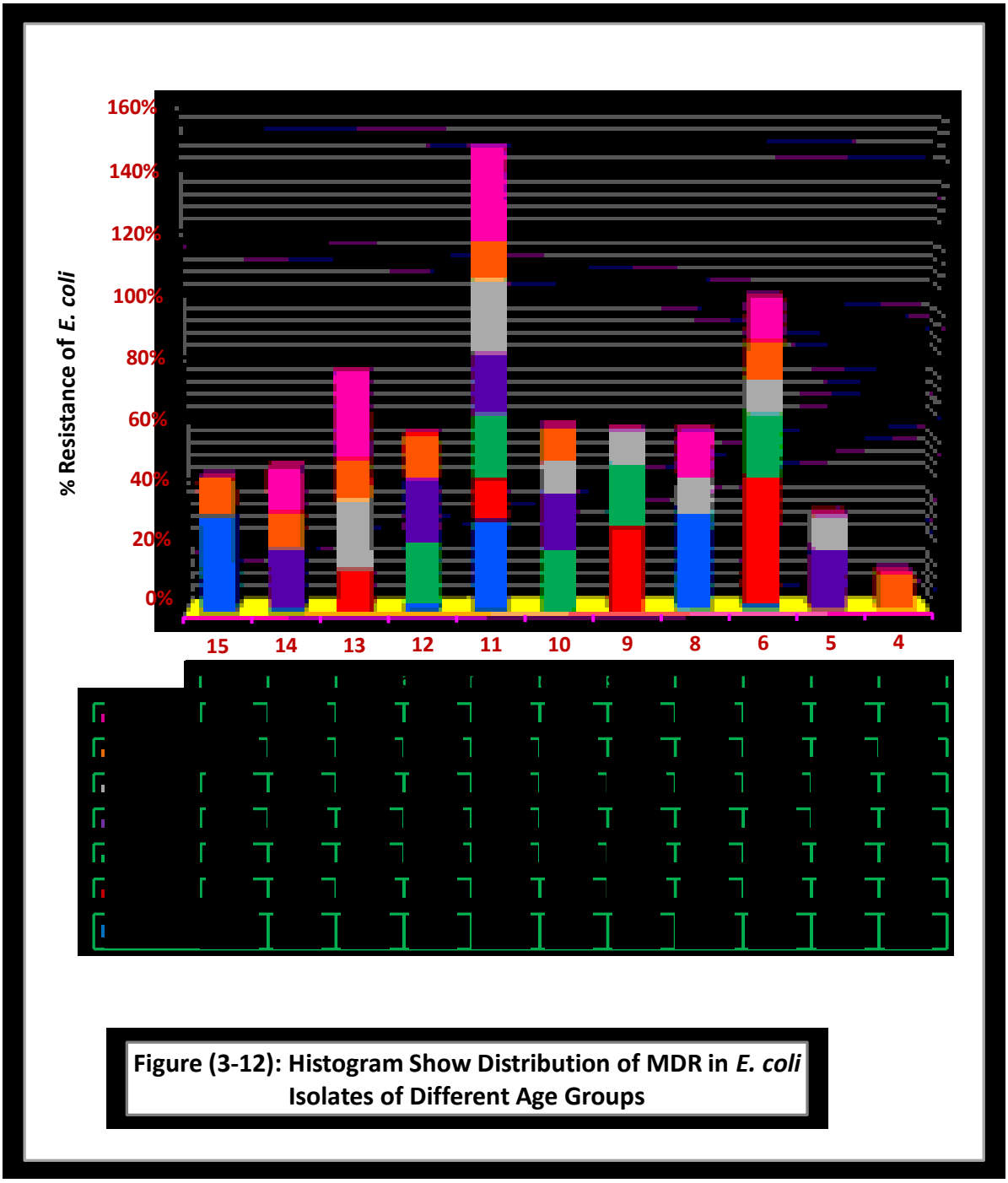


Figure (3-12): Histogram Show Distribution of MDR in *E. coli* Isolates of Different Age Groups

3.14. Correlation Between Multiple Drug Resistance and Integrons of Commensal *Escherichia coli*

All integrons positive isolates are resist to more than five antibiotics (Table 3-14). Among *IntI1* gene positive isolates, resist to 6 (9%), 8 (7%), 9 (8%), 10 (16%), 11 (24%), 12 (12%)13 (12%), 14(9%) and 15 (4%) antibiotics are observed but significantly association ($p < 0.05$) demonstrated to resistance of 10, 11 and 12 antibiotics only.

Although *IntI2* gene positive isolates have resistance to 6 (10%), 8 (7%), 9 (21%),10 (7%), 11 (7%), 12 (10%), 13 (13%), 14 (10) and 15 (17%) antibiotics, they significantly correlated ($p < 0.05$) with resistance of 9 and 15 antibiotics. In addition, 10 % of *IntI1&IntI2* positive isolates resist to 6, 10,14 antibiotics and 11% resist to 15 antibiotics while resistance to 8, 9, 12 and 13 antibiotics demonstrated in 8%, 8%, 9% and 21% respectively and these isolates only significantly association ($p < 0.05$) with resistance to 10 and 15 antibiotics.

Many resistance patterns reported among integrons positive isolates and most common resistance patterns are AM-AX-L-CL and AM-AX-L-CL-CTX and by compared resistance patterns between integron positive and integron negative isolates P value are determent. *IntI1* positive isolates are significantly associated ($P < 0.05$) with all detected resistance patterns except AM-AX-L-CL ($P = 0.655$), AX-AM-CTX-CL-TE-T-CIP-NOR-AN-TMP-C-L-AK-CN-CRO($P = 0.103$), AX-CTX-T-C-CRO-S-TE-CL-TMP-NA-L ($p= 0.076$), NA-CTX-CIP-T-C-CRO-S-TE-NOR-TMP ($p = 0.077$) and AX-CTX-T-C-CRO-S-TE-CL-TMP-AM-L ($p= 0.076$) (Table 3-15) while *IntI2* gene positive isolates have significant correlation ($p <0.05$) with most resistance patterns in Table (3-16) except resistance patterns; AM-AX-L-CL ($P = 0.571$), AM-AX-L-CL-CTX-TE-NA ($P = 0.633$), AM-AX-TE-T($p=0.535$), NOR-CIP-NA ($P = 0.779$), AX-CTX-T-TE-S-CL-TMP-AM-L ($P = 0.401$), CTX-T-CRO-TE-TMP ($P = 0.263$) and AX-CTX-T-CRO-TE-CL-AM-L ($P = 0.088$).

Isolates that have both classes of integron significantly associated ($p < 0.05$) with most resistance patterns except resistance patterns; AM-AX-L-CL ($P = 0.255$), NOR-CIP-NA ($P = 0.646$), AM-AX-AK-CN-S ($P = 0.318$), AX-NA-CTX-CIP-S-TE-CL-TMP-AM-CN-L ($P = 0.412$), AX-

CTX-T-TE-S-CL-TMP-AM-L (P = 0.418) and AX-CTX-T-CL-TMP-AM-L (P = 0.140) as in Table (3-17).

3.15. Association between Multiple Drug Resistance and Integron Positive phylogenetic Groups

Present study showed all integrons positive phylogenetic groups are resist to six or more antibiotics and resistance patterns appeared more clear inintegrons positive phylogenetic groups compared with integrons negative phylogenetic groups. Resistance of integron class 1 positive group A are significantly reported ($p < 0.05$) for 10, 11, 12, 13 and 15 antibiotics and *IntI1* gene positive group B2 are resist to many numbers of antibiotics including 6 (7%), 8 (7%), 9 (12.5%), 10 (9%), 11 (30%), 12 (14%), 13 (12.5%) and 14 (14%) antibiotics but significant associated ($P < 0.05$) with resistance for 11 antibiotics only while *IntI1* positive group D significantly associated ($p < 0.05$) with MDR of 6 (26%), 8 (20%) and 10 (44%) (Table 3-18). On other hand, 50% of *IntI2* gene positive groupA resist to 15 and 13 antibiotics ($P < 0.05$). *IntI2* gene positive group B2 significantly associated ($P < 0.05$) with resist to 6 (14%), 9(21%), 13 (25%) and 15 (14%) antibiotics (Table 3-19).

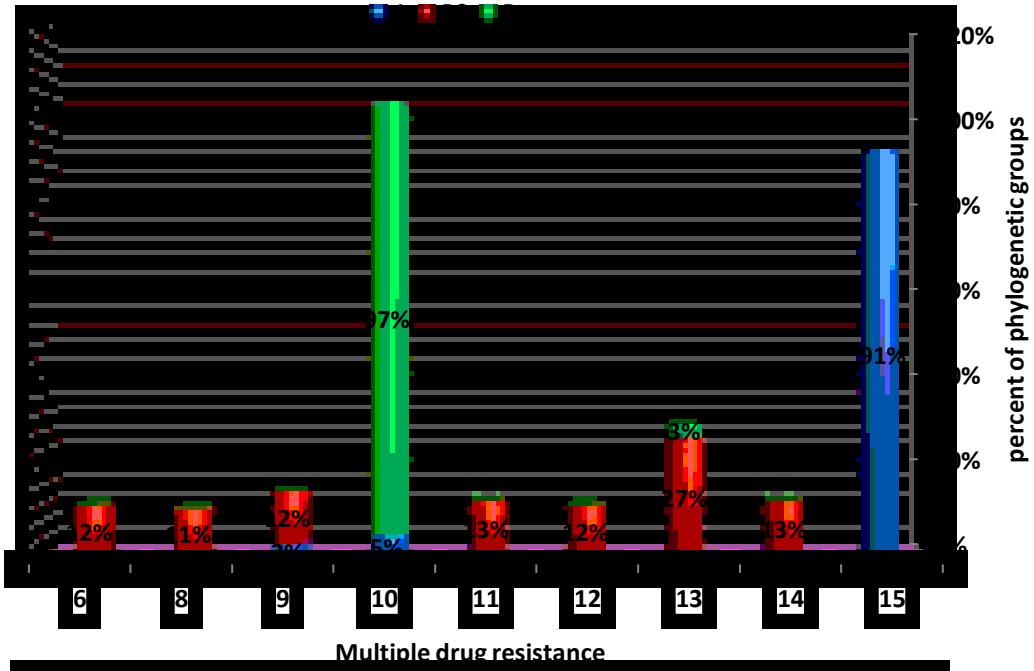
Most *IntI1&2* positive group A (91%) are significantly resist to 15 antibiotics ($p < 0.05$) while only 3% and 5% of these groups resist to 10 and 9 antibiotics respectively. *IntI1&2* positive group B2 are resist to 6 (12%), 8 (11%), 9 (12%), 11(13%), 12(12%),13(27%) and 14 (13%) antibiotics but significant associated ($P < 0.05$) with resistance to 13 and 6 antibiotics only whereas most *IntI1&2* positive group D (97%) are significantly resist to10 antibiotics ($P < 0.05$) and only 3% of this group resist to 13 antibiotics(Figure 3-13).

Resistance patterns that significantly correlated with *IntI1* positive isolates of group A are AM-AX-L-CL-CTX –TE ($P< 0.0001$), AM-AX-L-CL-CTX –TE- NA ($P< 0.0001$), AM-AX-TE-T ($P< 0.0001$), NOR-CIP-NA ($P= 0.0003$), C-TMP-L ($P = 0.0003$), AX-NA-CTX-T-CL-TMP-

AM-L (P=0.0002), AX-CTX-T-TE-S-CL-TMP-AM-L (P= 0.0141), CRO-TE-CL-TMP (P<0.0001), AX-AM-CTX-CL-TE-T-CIP-NOR-AN-TMP-C-L-AK-CN-CRO (P=0.0141), AX-CTX-T-TE-S-CL-TMP-AM-L (P=0.0141), AX-CTX-T-CRO-TE-CL-TMP-L (P=0.0141), NA-CTX-T-CRO-S-TE-TMP (P=0.0141), CTX-T-CRO-TE-TMP (P = 0.0003), T-TE-TMP (P < 0.0001) and AX-CTX-T-CRO-S-TE-CL-TMP-AM-L(P=0.0141)(Table 3-20), also *IntI1*gene positive B2 groups significantly associated with most detected resistance pattern (p < 0.05) as AM-AX-L-CL-CTX(P = 0.002), AM-AX-L-CL-CTX –TE (P< 0.0001), AM-AX-L-CL-CTX-TE-NA (P< 0.0005), AM-AX-TE-T (P< 0.0001), NOR-CIP-NA (P= 0.022), C-TMP-L(P = 0.016), AX-NA-CTX-T-CL-TMP-AM-L (P = 0.0003), AX-CTX-T-TE-S-CL-TMP-AM-L (P = 0.044), AX-CTX-T-CRO-TE-CL-TMP-L(P< 0.0001), CTX-T-CRO-TE-TMP(P< 0.0001), T-TE-TMP(P< 0.0001), AX-CTX-T-CRO-TE-CL-AM-L (P< 0.0001)and AX-CTX-T-CRO-S-TE-CL-TMP-AM-L (P = 0.0034) while *IntI1*gene positive D groups significantly associated (p < 0.05) with three resistance pattern only that included; AM-AX-L-CL-CTX(P < 0.0001), AM-AX-TE-T (P= 0.0012) and CTX-CRO-CL (P= 0.005)(Table 3-20).

Correlation between resistance patterns and *IntI2*gene positive phylogenetic groups are appeared mainly in group A and B2(Table 3-21). Integron class 2 positive group A significantly associated (p <0.05) with most resistance patterns except AM-AX-L-CL-CTX-TE (P=0.463),AM-AX-L-CL-CTX-TE-NA (P=0.0907), CTX-CRO-CL (P= 0.078), AX-CTX-T-TE-S-CL-TMP-AM-L (P= 630), AX-CTX-T-C-CRO-S-TE-CL-TMP-NA-L (P = 0.864), NA-CTX-CIP-T-C-CRO-S-TE-NOR-TMP (P = 0.864), NA-CTX-T-CRO-S-TE-TMP (P = 0.630), AX-CTX-T-C-CRO-S-TE-CL-TMP-AM-L(P = 0.914) and AX-CTX-T-CRO-S-TE-CL-TMP-AM-L (P= 0.681). Integron class 2 positive group B2 significantly associated (p < 0.05) with resistance patterns; AM-AX-L-

CL-CTX(P=0.001), AM-AX-L-CL-CTX –TE (P< 0.0001), C-TMP-L (P = 0.0044), CTX-CRO-CL (P = 0.001), CRO- TE-CL-TMP (0.002), AX-CTX-T-C-CRO-S-TE-CL-TMP-NA-L (P = 0.0001), NA-CTX-CIP-T-C-CRO-S-TE-NOR-TMP (0.004), NA-CTX-T-CRO-S-TE-TMP (P = 0.0311) and T-TE-TMP (P = 0.0355) and AX-CTX-T-C-CRO-S-TE-CL-TMP-AM-L (P = 0.0001). Most resistance patterns in Table (3-20) significantly associated ($p < 0.05$) with *IntI1* and *IntI2* positive isolates of group A except resistance patterns of AM-AX-L-CL (P= 0.275), AM-AX-L-CL-CTX (P= 0.275),CTX-CRO-CL (P = 0.117), AX-CTX-T-TE-S-CL-TMP-AM-L (P= 0.316), CRO-TE-CL-TMP (P0.275), AX-CTX-T-C-CRO-S-TE-CL-TMP-NA-L (P = 0.316), NA-CTX-T-CRO-S-TE-TMP (P = 3.91), NA-CTX-CIP-T-C-CRO-S-TE-NOR-TMP (P = 3.91), AX-CTX-T-C-CRO-S-TE-CL-TMP-AM-L (P = 0.316)and AX-CTX-T-CRO-S-TE-CL-TMP-AM-L (P = 0.316).Also Most resistance patterns in Table (3-22) significantly associated ($p < 0.05$) with *IntI1* and *IntI2* positive isolates of group B2 except resistance patterns of AM-AX-L-CL (P = 0.925), NOR-CIP-NA,AX-CTX-T-TE-S-CL-TMP-AM-L (P= 0.464), AX-AM-CTX-CL-TE-T-CIP-NOR-AN-TMP-C-L-AK-CN-CRO (P = 0.927) and AX-CTX-T-CRO-TE-CL-TMP-NA-L(P = 0.273) and NA-CTX-CIP-T-C-CRO-S-TE-NOR-TMP (P = 0.927) that have high p value ($p> 0.05$) . on other hand *IntI1* and *IntI2* positive isolates of group D not have significant association with any resistance patterns in Table (3-22).



Figure(3-13): Frequency of MDR among *IntI&IntI2* Positive Phylogenetic Groups. MDR appeared significantly associated ($p < 0.05$)between integron positive groups; D and 10 antibiotics; A and 15 antibiotics; B2 and 13 and 6

4. Discussion

4.1.Distribution of Commensal *Escherichia coli* and Phylogenetic Analysis

Results in Figure (3-1) and Table (3-1) showed commensal *E. coli* present in 86% of samples and present in different age groups these agreement with most studies that found high frequency of commensal *E. coli* in their populations (Sepp *et al*., 2009; Tenaillon *et al*., 2010; Bailey *et al.*, 2010).Large distribution of commensal *E. coli* could be explained by easiness of dissemination of commensal *E. coli* in different ecosystems through food chain and contamination that reach it to different new hosts at any age. Many researchers regarded that gut of human and animals represent good niche for the growth of *E. coli* that latter an important indicator of fecal contamination in aquatic environments and food (Roe *et al*., 2003; Duijkeren *et al*., 2005; Ozgumus *et al*., 2007; Ghaderpour *et al*., 2015; Loong *et al*., 2016). In current study some individual (14%) not harbor commensal *E. coli* in their gut this may be related to the present many of factors that may be effected on the frequency of gut flora as diet, pregnancy, immunity, treatment and hygiene (*Reinoso, 2016).*

Phylogenetic analysis in Figure (3-3) and Table (3-2) reported that most commensal *E. coli* strains related to group B2 flowed by A and to lesser extent to group D and not revealed any isolate belong to group B1,whereas numerous previous studies recorded that virulent extra-intestinal strains of *E. coli* mostly belong to group B2 , and to a lesser extent to group D, while most commensal strains belong to group A and B1, this result illustrated by studies of other researchers that found strains which involved in commensal *E. coli* phylogenetic groups not or less virulent when compared with strains that belong to pathogenic *E. coli*

phylogenetic groups which may be due to presence of many virulence factor in group B2 or A that cause UTI or diarrhea and not existed in strains of group B2 or A that included in commensal *E. coli*, this mean that commensal *E. coli* constituted from commensal strains that associated with special properties regardless on phylogenetic analysis (Anantham, 2014; Süzük *et al*., 2015; Losada *et al*., 2016).Commensal *E. coli* isolates in the current study mainly related to group B2 (especially subgroup $B2_3$) followed by A group (especially subgroup A1) and this similar to results of many studies in various populations (Zhang *et al*., 2002; Nowrouzian *et al.*, 2005; Bailey *et al*., 2010; Massot *et al*., 2016). Massot, (2016) and his coworkers revealed that the frequency of isolation of phylogenetic group B2 strains has increased with a parallel decrease of that of phylogenetic A strains that may be a consequence of modifications in the lifestyle of the Parisian population that included changing food processing and hygiene procedures whereas, Nowrouzian, (2005) and his colleagues showed that group B2 strains appear to possess yet unidentified traits that enhance their survival in the human intestinal microflora independent of carriage of all investigated virulence-factor genes, including genes for P fimbriae.

Present study showed that commensal *E. coli* mainly related to group B2 (subgroup $B2_3$) and not have any isolate belong to group B1.Absent of strains of group BI in gut flora may be because the gut conditions that not optical for their growth or may be those strains very sensitive for antimicrobial agents that used in vicious and extensive manner in this population and these results agreement with study in Tokyo in 2002 by Obata-Yasuoka *et al.*, who found B2 conformed 44% of commensal *E. coli* isolates, while B1 not detected in any isolate (0%). Also study of Derakhshandeh *et al*.,(2013) in Iran included pathogenic and commensal *E. coli* collected from different samples not found B1

group but this results unlike other researches that found A and B1 groups which conformed high frequencies among commensal *E. coli* isolates (Nowrouzian *et al*., 2006; Moreno *et al*., 2008).

The differences in distribution of the phylogenetic groups among the strains of geographically distinct populations in different studies as in Table (4-1) may be due to the health status of the host, geographic climatic conditions, dietary factors, use of antibiotics, host genetic factors, also some *E. coli* strains may be primarily adapted to the gut conditions of certain populations (Duriez *et al*., 2001;Tenaillon *et al*., 2010). All these causes may be lead to dramatic shifts in the proportions of some phylogenetic groups (such as B2 &A) in many populations as France, Japan, Sweden and others (Gordon *et al*., 2005). Therefore this study suggested that phylogenetic analysis used for molecular identification *E. coli* but not distinguishing between commensal and pathogenic *E. coli* .

In Figure (3-4) and Table (3-3) distribution of phylogenetic groups among age groups brings attention due to registration isolates that belonging to group D or their subgroups in group1and 2 but not detected in other ages also recorded isolates that related to group A (mainly subgroup A1) in group 2, 3 , 4 and 6 but not recorded in group 1,7 and 5 while group B2 (subgroup $B2_3$) existed in all ages. Sensitivity of group D or their subgroups to changes in hormones, immune system, nutrition, hygiene, treatment and other life style that occur with age may be interpret their found in group1 and 2 only at same time, this may reflect acclimated and resistant of group B2 to those changes. Unfortunately, there is no previous study noted or explained the distribution of commensal *E. coli* phylogenetic groups at different ages and stated the reasons for those.

4.2. Distribution of Integrons

4.2.1.Distribution of Integrons among Commensal *Escherichia coli* Isolates

Figure (3-5) shows wide dissemination of integrons (70%) among commensal *E. coli* isolates that revealed high prevalence of isolates that harbor integron class1, class 2 and both classes compared with many studies in different populations (Table 4-2), this may be because of the widespread of horizontal transfer of mobile elements such as plasmids and transposones that associated with integons between commensal *E. coli* isolates and *Enterobacteriaceae*, also increased antibiotic consumption in animals and human and decreased healthy status that contribute in appearance and distribution of integrons in this population and this agree with the explanation of Sunde (2005) that showed integrons conformed a part of successful plasmids or transposons with a wide, perhaps global dissemination.

In line with other studies (Reyes *et al*, 2003; Mathai *et al*, 2004), this study noted that class 1 integrons were common in *E. coli* isolates. Skurnik*et al* (2005) found that integrons persist in commensal *E. coli* isolates in subjects who have not taken antibiotics for at least one month.

The prevalence of class I integron more than class 2 noted in current results and this like the previous reports where frequency of class 1 integron is much more than class 2 (van Essen-Zandbergen *et al*., 2007; Dureja *et al*., 2014), this may be due to ability of integron class 1 to integrate to different mobile genetic elements as plasmids and transposones and this explained by Lee *et al*., (2006)who suggest that class 1 integrons harbored by commensal *E. coli* isolates may be acquired from other pathogenic or commensal bacteria by horizontal transfer of R-plasmids carrying class 1 integrons. Barlow and Gobius (2006) come back lower frequency of class 2 integron to the replacement of the

internal termination codon with glutamic acid and production of a shorter and inactive polypeptide,which was unable to catalyse the recombination reaction.

Integrons play a role in the spread and maintenance of antimicrobial resistance among bacterial population, and increase frequency of *IntI1*and *IntI2* genes among commensal *E. coli* phylogenetic groups which confirmed the role of *E. coli* flora as reservoir and pool of multiple antibiotic resistance genes for many other bacteria in environment and gut of human and animal (Rahube, 2013; Tajbakhsh *et al*., 2015), also results showed high frequency of integron class 1 in group A in human gut in agreement with Cocchi and his coworkers (2007) who studied distribution and characterization of integrons in *Escherichia coli* strains of animal and human origin. In general present results (Figure 3-8 and Table 3-5) showed high integrons prevalence appeared in group B2 compared with other phylogenetic groups and this results unlike results of Skurnik *et al.,* (2005) who reported that *Escherichia coli* B2 phylogenetic group strains tend to carry less integrons (*IntI1*gene (4%) and *IntI2* gene (0%)) than other phylogenetic groups in commensal environments.

4.2.2. Distribution of Integrons among *Escherichia coli* Isolates from Different Age Groups

Integron-containing bacteria (pathogenic or normal flora) that enter human gut through infection, contamination or food chain may be transmitted their integron to gut colonized flora that lead to accumulation of many movable genetic element including integrons in gut flora of individuals at any age (*Mikaelyan et al*, 2017). Increase antibiotic consuming also may be other cause for those as detected in this study that showed highest frequency of integron containing bacteria existed in most

age groups and mainly in group7 (100%) and group1 (86%) as in Figure (3-7) . High frequency of integrons in isolates that collected from elderly may be resulted from repeat exposure to integron containing bacteria and different antibiotics for long period, Nahid (2007) also detected the selective pressure of antibiotics consumed during a lifespan associated with emergence of resistance genes as integrons in commensal *E. coli* of elderly individuals. Table (3-4) showed that isolates in group 1 have wide domain of integrons (86%) especially class 1 (74%) compared with other age groups. This results unlike research of Sepp *et al* (2009) who found significantly higher frequency of the *intI1*gene in the dominating intestinal *E. coli* populations in healthy antibiotic-naive children (53%) compared to healthy elderly persons (17%), also El-Shennawy (2011) detected a significantly high frequency of the *intI1* gene in the intestinal *E. coli* population among healthy antibiotic-naive children (64%) compared to healthy elderly persons (18%). El-Shennawy and Sepp *et al.*, indicated that the possible explanation of high prevalence of class 1 integrons in children related to the gastrointestinal micro-ecosystem is not yet fully completed and colonization resistance (supported by anaerobes, lactobacilli, and other endogenous commensal bacteria) against exogenous bacteria is weaker; hence, they may be colonized by high numbers of *E. coli* strains from the environment, containing different virulence or resistance genes, e.g. integrons. Isolates in group 2 and groups 3 and 4 also show high prevalence of integrons especially class 1 and this similar to investigate of many researchers who study integrons in adult (Saenz *et al.*, 2001; Yang *et al*., 2009).

Finally , poor information a viable about distribution of integrons in isolates of different age groups because of previous studies focused on detection integron class1 in adults only.

4.3. Antibiotics Susceptibility

4.3.1.Evaluation of Antibiotic Resistance of Commensal *Escherichia coli* Isolates

Present study (in Figure 3-9) and many other studies demonstrated high resistance of commensal *E. coli* (or phylogenetic groups) to antimicrobials that commonly used as amoxicillin, ampicillin, cephalexin, cefotaxime and tetracyclines that may be due to the miss, extensive and long-term use of these antibiotics in treatment of humans and livestock (Hsu *et al*, 2006; Phongpaichit *et al*., 2008; Hiltunen *et al*, 2017). Current study similar to study in Mexico that showed resistance to Ampicillin is100% of commensal *E. coli* also agree with studies of Marshall *et al*., (2011) and Li *et al.*, (2014) who showed high resistances of gut flora to streptomycin, nalidixic acid, tetracycline and amoxicillin that may be due to widely use of these antimicrobial agents especially streptomycin and tetracycline as growth promoters or prophylactic agents in animal husbandry (Urumova, 2016a,b; Sohrabi and Zeighami, 2017). Some of other studies indicated that animals might be responsible for the transfer of ESBL-producing bacteria and/or ESBL-encoding genes to humans, either through contact or *via* food chain (Jensen *et al*., 2006; Ewers *et al*., 2012;Jones-Dias *et al.*, 2015).

Most recent researches suggest increase and spread antibiotics resistance related to horizontal transfer of resistance genes such as integrons among bacterial populations. Transport resistance genes from commensal *E. coli* to pathogenic bacteria represents a potential risk to public health (Marshall *et al*., 2009; Mendonça*et al*., 2016; Schroeder *et al*., 2017). Marshall *et al*., (2009) elucidating that transfer of antibiotic resistance genes from commensals to pathogens depends on the density of donor and recipient cells, the availability of a transfer mechanism,

nutrition and selective pressures when the intestinal environment is considered optimal. Low resistance for aminoglycosides (amikacin and gentamicin) among commensal *E. coli* isolates recorded in this study which agreement with results of Phongpaichit and his coworker (2008) but this results unlike results of Yang *et al.*, (2009) in Taiwan that showed high resistance rates in fecal strains of *E. coli* were observed for streptomycin (52%), ampicillin (50.2%), trimethoprim (47.6%) and chloramphenicol (33.8%). Data of Shin and Cho (2013) indicated that fecal *E. coli* isolates from fishery workers showed higher resistance to cephalothin and cefoxitin than to other cephem antibiotics and to gentamicin than to other aminoglycosides, whereas current study articulate highest resistance to cefotaxime among cephem and to streptomycin among aminoglycosides. Low resistance of ciprofloxacin reported in current study, and this agreement with previous data available for studies carried out about 12 years earlier which indicated a significant increase in the resistance rates for most of the antibiotics as well as the appearance of resistance to ciprofloxacin (Igbeneghu and Lamikanra, 2014). However the various percentages of antibiotic resistance in different parts of the world are due to differences in the prevalence of antibiotic consumption in each country (WHO, 2012; WHO, 2017).

Phylogenetic group B2 and their subgroups ($B2_2$ and $B2_3$) have highest resistance to most antimicrobial classes as penicillins, tetracylines, trimethoprim and cephalosporins in compared with other groups and subgroups and also have clear resistance to remaining tested antibiotics and this property may be from causes that made group B2 more common and persist in gut (Figure3-10 and Table 3-7). In additional group A have highest resistance to nalidixic acid, ciprofloxacin and amikacin among phylogenetic groups. Increase resistance in phylogenetic groups of commensal *E. coli* particularly B2 and A may be related to their

high carriage of resistance genes, in addition, the number of isolates (samples) which related two groups A and B2 perhaps reflected on the clarity of resistance to antibiotics in these groups compared with the group D, this viewpoint also remembered by Woerther *et al.*, (2013).

Present result showed lower resistance to quinolones and most tested antibiotic existed in group D among phylogenetic groups and this contrastive with results of Skurnik *et al.*, (2009), who reported that *Escherichia coli* B2 phylogenetic group strains are more susceptible to antibiotics, especially to quinolones, than other phylogenetic groups in commensal environments.

In additional to the role of antibiotic resistant commensal *E .coli* as source of MDR to other bacteria in gut, it may be reach to other area of the body (extra-intestines) and causes infections that not response to therapies as UTI and meningitis that appear more clear in immune-compromised patients.

4.3.2.Prevalence of Antibiotic Resistance in Commensal *Escherichia coli* Isolates from Different Age Groups

Resistance to penicillines, cephalosporins, tetracyclines, trime-thoprim and lincomycin higher than to other antibiotics in commensal *E. coli* in all age groups in present study and highest values of resistance of these drugs indicated mainly in isolates of individuals in group 7 flowed by group 1, as in Table (3-9). High resistance may be due to extensive and long- term use of these antibiotics by those individuals, in additional to weakness immune system in elderly and incomplete immunity in children that associated with high GIT colonization by antibiotic resistant commensal and pathogenic bacteria, this opinion like to what mention in the studies of Bee (2011) and Bajaj *et al* (2016). Farra and his coworkers (2002) suggested that age can act as a risk factor for carriage of resistant

bacteria, independent of increased antibiotics that were used. Farther more current study similar to study in Mexico that showed resistance to Ampicillin is100% in children (Süzük *et al*.2015), but these values are higher compared to similar study reported by Dyar *et al.,* (2012) on antibiotic resistance of commensal *Escherichia coli* among children in rural Vietnam that observed high prevalence of resistance to tetracycline (74%), co-trimoxazole (68%), ampicillin (65%), chloramphenicol (40%), nalidixic acid (27%) and only two isolates were resistant to ciprofloxacin. Highest resistance of quinolones among normal flora seen to nalidixic acid and the lower resistance reported for ciprofloxacin so resistance for both these drugs stated mainly in isolates of individuals of group 2 while resistance to norfloxacin mainly determent in isolates of individuals in group 7 this may be results from extensive use of these drugs for treatment. On other hand highest resistance for chloramephenicol and streptomycin investigated in isolates of individuals in group6. Amikacin and gentamicin have the least resistance among tested antibiotics this may be due to restrictive use of these antibiotics in this population (Venturini,2011). Highest resistance to amikacin seen in isolates in group 4 (40%) and group 2 (25%) while all isolates in age groups 1, 5 and 7 sensitive to amikacin. High resistance to gentamicin recorded in isolates in groups1 (38%), 2 (35%) and 7 (31%) compared with high sensitivity in isolates in individuals in other age groups. However these results agreement with most previous studies carried out on gut flora of infants, children, adults or older that showed high resistance to older antibiotics such as penicillines and cephalosporines (Phongpaichit *et al.,* 2008; Dureja*et al*., 2014;Blair *et al*., 2015) but disagreement with Li and his coworker (2014) in china who showed low resistance of cefotaxime in adults (15%).

Finally present results showed high antibiotic resistance when compared with previous studies therefore, restrictive use of all antibiotics is needed to prevent the spread and development of antibiotic resistance.

4.4. Antibiotic Resistance and Integrons

Integron is one of the important causes for increased antibiotic resistance in different populations. Higher percentage of resistance to some antimicrobial agents (aminoglycoside, cephalo-sporines, quinolones, and beta-lactam agents) were observed among integron-positive strains with respect to integron-negative strains (Table 3-10). The fact could be explained by the presence of resistance genes of these antibiotics in the conserved or variable region of integrons, or by the inclusion of resistance genes in the same mobile elements that carry integrons (Vinue´ *et al*., 2008; Li *et al*., 2014; Baloch *et al*., 2017).

Significant correlated between *IntI1* gene positive isolates and resistance to cefotaxime, tetracycline and trimethoprim ($P < 0.05$) also determented in results of Li and his coworkers in china that also detected clear association between *IntI1* gene and resistance to nalidixic acid and ampicillin/sulbactam. Other study conducted in Taiwan has shown that fecal *E. coli* isolates have high levels antibiotic resistance gene cassette containing class 1 integron (Yang *et al*., 2009; Navidinia *et al.*, 2014).

Integron class 2 positive isolates significantly associated with antibiotic resistance to cefotaxime, ceftriaxone, streptomycin, tetracycline and chloramephenicol ($p < 0.05$), while study of Kargar and his colleagues (2014) indicated substantial correlation between integron class 2 and antibiotic resistance to aminoglycosides, co-trimoxazole, cefalexin, ampicillin, and chloramphenicol.

Previously, studies reported distribution of antibiotic resistance mainly among integron positive pathogenic *E. coli* phylogenetic groups

and integron positive commensal *E. coli* phylogenetic groups that isolated from animal sources but not recorded any clear information about antibiotic resistance of integron positive commensal *E. coli* phylogenetic groups or subgroups that isolated from human fecal samples. However, current results (in Tables 3-9, 3-10 and 3-11) not showed significant association (P> 0.05) between integrons positive phylogenetic groups and antibiotic resistance to penicillines, cephalexine and licomycin because all isolates with or without integrons resist to these antibiotics while significant association between integrons positive phylogenetic groups and resistance of some antibiotic may be due to integrated resistance genes of these antibiotic in integrons (khachatran *et al.*, 2008).

4.5. Multiple Drug Resistance

4.5.1.Multiple Drug Resistance Profile of *Escherichia coli* Isolates and Phylogenetic Groups

In present study all isolates (or phylogenetic groups) are MDR isolates (Figure 3-11) this may be output from the co-selection of several resistance genes cassettes present in the same genetic element, from cross-resistances to more than one class of antibiotic and\or from exposure to several antibiotics, according to this can be expectation that resistance to less than 5 antibiotics may occur from cross-resistances, such as the resistance to amoxicillin or to tetracycline may conferring resistance to several members of the same class of antibiotics, but the multiple resistance of the *E. coli* involving as many as 10, 11 or 15 antibiotics that corresponding to eight classes of antibiotics, cannot arise from only one resistance mechanism such as cross -resistance and that may be mainly related to existed resistance genes-containing plasmids (Bartoloni *et al*., 2006; Laroche*et al.*, 2009; Jitwasinkul *et al*., 2016; Adefisoye and Okoh, 2016), for example, Szczepanowski *et al*. (2005)

have isolated two plasmids from activated sludge, one of which confers resistance to 10 antibiotics, and the other to 12, these plasmids consisted of a mosaic of transposons and integrons. The repertoire of antibiotic-resistant genes found in the commensal *E. coli* might serve as a pointer to the possible presence of other antidrug-resistant genes conferring resistance to other classes of antibiotics that were not targeted in this study, and our finding is in line with other reports on the detection of multiple antibiotic-resistance gene in some commensal and pathogenic strains of *E. coli* (Bailey *et al*., 2010; Karczmarczyk *et al.*, 2011; Munita and Arias, 2016; Singh *et al*., 2017).This results also indicated there are other factors that responsible of MDR rather than the presence of the resistance gene, of these factors could be poor medical scholarship, hygiene and the random use of antibiotics without a doctor request in this population (Abdelhaleem *et al.*, 2014;Ternent *et al.*, 2015). In this results, *E. coli* flora resist to at least four antibiotics because of all these isolates resist to ampicillin, amoxicillin, cephalexine and licomycin. These four antibiotics perform resistance pattern (AM-AX-L-CL) that present in all isolates (100%). Also other common antibiotics such as cefotaxime and tetracycline in collected with ampicillin, amoxicillin, cefalexine and lincomycin lead to investigation other common resistance pattern as AM-AX-L-CL-CTX(93%) and AM-AX-L-CL-CTX-TE (74%) as in Table (3-12). On other hand some antibiotics such as kanamycin, gentamicin and streptomycin present in low frequency that make them less appearance in resistance patterns (AK-CN-S (2%), AX-AM-CTX-CL-TE-T-CIP-NOR-AN-TMP-C-L-AK-CN-CRO (5%)), while researches of Fricke *et al*., (2008), khachatran *et al*., (2008) found multiple drug resistance profile of *E. coli* mainly include ampicillin, streptomycin, celphonamide, trimethoprime, tetracycline and kanamycin this results from carriage and expression of several different resistance genes that often plasmid borne.

Also present study reported high value of resistance compared with study of Phongpaichit and his coworkers (2008), that showed MDR phenotypes were common (89%) and only seven isolates (4%) were resistant to a single drug and ten isolates (6%) were susceptible to all antimicrobial agents and the most frequent pattern was SMX (sulphamethoxazole)-TMP-AM-TE-S (21%), followed by SMX-TMP-AM-TE-KAN (kanamycin)-S (14%) and SMX-TMP-NA-NOR-CIP-AM-AX-TE-S (7%).

In present results, resistance patterns and MDR appeared more clear in phylogenetic groups than subgroups that may be effected by number of cases and frequency of groups and subgroups. However high resistance to amoxicillin, ampicillin, cephalexine, lincomycin, ceftraixone and tetracyclines that lead to demonstration common resistance patterns as AM-AX-L-CL, AM-AX-L-CL-CTX and AM-AX-L-CL-CTX-TE that mainly investigated in group B2 (mainly subgroup $B2_3$) and group A (mainly subgroup A1) and this results unlike results of Anantham (2014) that found high level of resistance gene cassettes of chlormphenicol and tetracycline among group D and less observed in group B2, also this results contradictory with previous research of Skurnik *et al*., (2005) that showed pathogenic and commensal B2 phylogenetic group appear to be the least resistant to antibiotics compared with other groups that associated with MDR.

4.5.2.Multiple Drug Resistance Profile among Commensal *Escherichia coli* Isolates of Different Age Groups

In this study resistance of commensal *E. coli* investigated to large number of antibiotics in different age groups in compared with other previous studies and main causes of these results are remain misuse of antibiotics and transferability of resistance traits by mobile elements in

bacterial population. Common resistance to pencillin, amoxicillin, cephalexine, lincomycin, cefotaxime and tetracycline among *E. coli* in all age groups result in indicated common resistance patterns consisted from these antimicrobial agents as AM-AX-L-CL, AM-AX-L-CL-CTX and AM-AX-L-CL-CTX-TE (Table 3-13).

In present results (Figure 3-10) all *E. coli* flora in children (group1) resist to at least six antibiotics and most of them resist to 11 antibiotics, while study of Bartoloni *et al*., (2006) on multidrug-resistant commensal *Escherichia coli* in children of Peru and Bolivia showed most isolates resist to three antibiotic and most common resistance pattern is AMP-TET-SXT whereas study of Igbeneghu and Lamikanra (2014) on pre-school children under the age of 2 years in Nigeria showed collected *E. coli* isolates are MDR isolates and most them resist to 8 antibiotics also Igbeneghu and Lamikanra found this property could be transferred by conjugation to other organisms, pathogenic or flora, especially where the multiply resistant strain have the ability to persist in the guts by virtue of the ability of such strains to produce any of colicin, capsule, and haemolysin or all concurrently as observed among some of the recovered strains. Also research of Oluyege *et al*., (2015) showed 78% of commensal *E. coli* in children are multiple resistant to 3 or more of the 8 tested antibiotics that result from most commonly prescribed of those drugs for children, especially for acute respiratory illness and diarrhea. Findings of Shin and Cho (2013) showed the multiple resistance patterns in the *E. coli* isolates of young and adults fishery workers are similar to those of young and adults restaurant workers when 55% of fishery workers and 49% of restaurant workers showed resistance to two or more antibiotics and approximately 12% of the isolates showed resistance to four or more antibiotics. The most frequently observed resistance patterns in the fishery workers were to tetracycline/ sulfamethoxazole and to

tetracycline/sulfamethoxazole/ cephalothin. In the restaurant workers, the most frequently observed resistance pattern was to cefoxitin/tetracycline.

4.6. Multiple Drug Resistance Phenotypes and Integrons

All isolates in present study (Table 3-14) are multiple drug resistance isolates and are resist to at least four antibiotics but integron positive isolates are resist to at least six antibiotics this may be related to present many resistance genes associated with integrons or may be due to presence of more than one integron in the same isolate and each one carry out different resistance genes for different antibiotics. Current results in line with study of Phongpaichit *et al*., (2008), who found multiple drug resistance was more frequent in integron-positive isolates (89%) than those in integron-negative *E. coli* (57%). Moreover study of Dureja and his coworker (2014) showed the class 1 integron positive isolates contain eight different gene cassettes in five different combinations, namely dfrA12-orfF-aadA2, dfrA1- aadA1, dfrA17-aadA5, dfrA5 and dfrA7, this similar to those observed earlier by Karczmarczyk *et al* (2011),which revealed that class 1 and class 2 integrons in the collection were found to contain trimethoprim (*dfr*) and streptomycin (*aad*) resistance-encoding genes, which are frequently reported in *E. coli* isolates recovered from various sources, including human, animal, and environmental samples. The presence of more than one gene cassettes in most integrons positive samples support the literature suggesting that since 1990 there is a prevalence of class 1 integrons carrying multiple gene cassettes (Kang *et al.*, 2005; Dureja *et al.*, 2014; Hajiahmadi *et al.*, 2017).These data indicate that human fecal *E. coli* is a reservoir of antibiotic-resistant genes that poses a significant risk of the spread of microbial resistance in the community (Cury *et al*., 2016).

In this study *IntI1* gene positive isolates significantly associated with most detected resistance patterns while these patterns less

prevalence among *IntI2* or both *IntI1* and *IntI2* positive isolates (Tables 3-15, 3-16 and 3-17) this may be due to high frequency of integron class 1 that integrated with resistance gene cassettes for multiple antibiotics resistance also in class 1 integrons, the 5´ conserved region encodes a site-specific recombinase (integrase, *IntI1*) and a strong promoter or promoters that ensure expression of the integrated resistance gene cassettes for multiple antibiotics in the same *IntI1* gene positive isolates (Phongpaichit *et al.*, 2008).This high frequency of multidrug resistance among *Inti1*-positive isolates supports the hypothesis of an association between the presence of class 1 integrons and emerging multidrug resistance in *E. coli* (Yu *et al.*, 2016)

Previously, numerous studies have shown that integrons play a considerable role in the dissemination of MDR in clinical isolates and environmental samples. Fewer studies have aimed at investigating the role of integrons in the propagation and maintenance of MDR phenotypes in non-clinical commensal isolates from humans. There are probably at least two reasons for the lack of studies investigating integrons in commensal isolates from healthy people. One reason may be due to the lack of availability of samples. While bacteria are routinely obtained from hospitalized patients in order to identify and treat infections, healthy people may be far less likely to voluntarily submit to a rectal swab or fecal samples. Perhaps a second reason is that studying bacteria isolated from clinical samples and from individuals with infections can easily seem like a more pressing matter, and seem more vital to understanding the problem of the rapid spread of multidrug-resistance.(Avila, 2013)

4.7.Association Between Multiple Drug Resistance and Integrons of Phylogenetic Groups

Current results showed integrons positive phylogenetic groups are resisted to more antibiotic agents compared with integrons negative phylogenetic groups and also integrons positive phylogenetic groups significantly associated with most resistance patterns particularly groups B2 and A (Figure 3- 13 and Tables 3-18, 3-19, 3-20, 3-21 and 3-22) that indicated role of integrons which found in plasmid or chromosome as major means for transporting and incorporating antibiotic resistance genes (Marshall *et al*., 2009; Von Wintersdorff *et al*., 2016) and this results like previous study of Cocchi *et al*., (2007) that observed nonpathogenic commensal *E. coli* strains (mainly phylogenetic group A) represent an important reservoir of integrons, and consequently of multiple antibiotic resistance gene cassettes and this is in agreement with the hypothesis that virulent strains may acquire these factors from commensal strains and survive in an environment where a high antibiotic pressure is present (Johnson *et al*., 2003).

The present study detected isolates in phylogenetic groups particularly group D possessing integrase gene and failed to express antibiotic resistance and this findings agreement with some investigators that found antibiotic susceptible *E. coli* and *salmonella* can harbor specific resistance gene cassettes within integron but the expression of these gene cassettes is weak (Zhao *et al*, 2001; 2003; Sunde, 2005; Phongpaichit *et al*., 2008) and possible that these susceptible isolates may not have resistant cassette or the expression of their gene cassette is weak. Also, it may be a result of the distance between the cassette and the promoter that transcribes the cassette genes. Another reason could be a weak promoter version or mutation in the region between two specific sequences of the promoter (Zhao *et al*, 2001; 2003; Sunde, 2005).

Gene cassettes in integrons can have a variable expression and this may caused by several factors, for example, the integron is located on a

high-copy-number plasmid or not, several versions of the integron promoter located in the 50-conserved segment of the integron causing differences in the strength of the promoter, the expression of a cassette in an integron containing more than one inserted cassette is also influenced by the position of the cassette and the expression weakens as the cassette is situated nearer the 30-conserved segment. All these factors may lead to considerable variations of MDR values when gene cassettes in integrons are responsible for antimicrobial resistance (Sunde, 2005).

Conclusions:

1. The most common phylogenetic group among commensal *E. coli* are group B2 (mainly subgroup$B2_3$) flowed by groups A (mainly subgroup A1) and D while group B1 not present.

2. Phylogenetic group A and B2 and their subgroups existed in most age groups while group D and their subgroups found in age group1 and 2 only

3. High frequency of integrons in commensal *E. coli* especially class 1 that primarily notable in isolates of individuals in children and elderly.

4. All tested commensal *E. coli* are multiple drug resistance and resistances for lincomycin, amoxicillin, cephalexine and ampicillin are appeared in all isolates, this investigation reflect misuse of antibiotics in this population

5. Antibiotic resistance significantly observed in integrons positive commensal *E. coli* (mainly B2 and A phylogenetic groups) that indicate that human fecal *E. coli* is a important reservoir of antibiotic-resistant genes that poses a significant risk of the spread of antimicrobial resistance in the community.

6. Multiple drug resistance and resistance patterns seen in clear picture in individuals in children and elderly that also appeared widely among integrons positive phylogenetic groups especially group A and B2.

Recommendations:

1. Restrictive use of all antibiotics especially amoxicillin, ampecillin, cephalexine and lincomycin.
2. Further investigations are necessary on integron genetic elements by sequencing the variable region to identify the carried antibiotics resistant genes.
3. Try to identify new techniques to control activity or spread of integrons in bacterial population.
4. The data of this study provides useful information regarding the dissemination of antibiotic resistance among healthy humans in the community and continued surveillance of other normal flora should be carried out to predicting the antimicrobial resistance trends of clinical *Enterobacteriaceae* isolates in this population.
5. Probing commensals and understanding the role they play in antibiotic resistance should help toward developing effective interventions to control resistance and preserve the efficacy of antibiotics.
6. Current study suggesting can depend on phylogenetic or genotyping analysis for molecular identification of *E. coli* but not distinguishing between commensal and pathogenic *E. coli* because of the not consensus among the current study and previous studies, which in turn are also often inconsistent about any groups represent commensal or pathogenic *E. coli* and that need to be development new methods for differentiation between commensal and pathogenic bacteria.
7. Other studies about effects of hormonal changes, immune system and nutrition on frequency and types of gut flora.

- **Abdelhaleem, A. A.; Homeda, H. E.; Hershan, A. A.; Makeen, A. M. and Alsanosy, R.M., (2014).** Class 1 integrons gene in drug resistant *E. coli* and isolated from different clinical specimens in Jazan area K.S.A. International journal of current research, 6 (10): 9283-9286.
- **Adefisoye, M. A. and Okoh, A. I., (2016).** Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa. Microbiology open, 5(1): 143–151.
- **Adelowo, O. O.; Fagade, O. E. and Agersø, Y., (2014).** Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, Southwest Nigeria. J Infect Dev Ctries., 8(9):1103-1112.
- **Adisoji, A. T.; Ojungubi, A. A.; Olatoye, I. O. and Douclas, D. R., (2015).** Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in Southwestern Nigeria. Ann Clin Microbiol Antimicrob., 14 (35): 1-8.
- **Al Mously, N.; Al Arfaj, O.; Al Fadhil, L. and Mukaddam, S., (2016).** Antimicrobial susceptibility patterns of ESBL *Escherichia coli* isolated from community and hospital-acquired urinary tract infections. Original article, 4 (2): 133-139.
- **Alekshun, M. N. and Levy, S. B., (2006).** Commensals upon us. Biochem Pharmacol., 71(7**):**893-900.
- **Algburi, A.; Comito, N.; Kashtanov, D.; Dicks, L. M. and Chikindas, M. L., (2016).** Control of biofilm formation: antibiotics and beyond. Appl Environ Microbiol., 02508-16.
- **Al-Saedi, F.; Vaz, D. P.; Stones, D. H. and Krachler, A. M., (2017).** Commensal adhesin enhances *E. coli* retention by mucin, while mucin

desulfation by mucin-foraging bacteria enhances its transmigration through the mucus barrier. International. J., 11:1-18.

- **Alteri, C. J. and Mobley, H. L., (2012).** *Escherichia coli* physiology and metabolism dictates adaptation to diverse host micro-environments. Curr Opin Mircobiol., 15:3-9.
- **Anantham, S., (2014).** Analysis of persistent and antibiotic resistant commensal *Escherichia coli* from healthy adults. PHD thesis. School of Molecular Bioscience, University of Sydney, Australia. 1-279.
- **Ashwlayan, V. D. and Singh, G., (2016).** Analysis of Aminoglycosides. Int. J. Pharm. Sci. Rev. Res., 39(1): 282-293.
- **Avila, A. L., (2013).** Prevalence and characterization of integrons in multidrug-resistant non-clinical enteric bacterial isolates. PHD thesis, California State University, Sacramento. 1-96.
- **Bailey, J. K.; Pinyon, J. L.; Anantham, S. and Hall, R.M., (2010).** Commensal *Escherichia coli* of healthy humans: a reservoir for antibiotic-resistance determinants. Journal of Medical Microbiology. 48 (9).
- **Bajaj. P.; Singh, N. S. and Virdi, J. S., (2016).** *Escherichia coli* β-Lactamases: what really matters. Front Microbiol., 7:417-423.
- **Baloch, A. B.; Yang, H.; Feng, Y.; Xi, M.; Wu, Q.; Yang, Q.; Tang, J.; He, X.; Xiao, Y. and Xia, X., (2017)**. Presence and antimicrobial resistance of *Escherichia coli* in ready-to-eat foods in Shaanxi, China. Journal of Food Protection, 80(3):420-424.
- **Barlow, R. S. and Gobius, K. S., (2006).** Diverse class 2 integrons in bacteria from beef cattle sources. J Antimicrob Chemother., 58:1133–1138.
- **Bartoloni, A.; Pallecchi, L.; Benedetti, M.; Fernandez, C.; Vallejos, Y.; Guzman, E.; Villagran, A. L.; Mantella, A.; Lucchetti, C.; Bartalesi, F.; Strohmeyer, M.; Bechini, A.;**

Gamboa, H.; Rodríguez, H.; Falkenberg, T.; Kronvall, G.; Gotuzzo, E.; Paradisi, F. and Rossolini, G. M., (2006). Multidrug-resistant commensal *Escherichia coli* in children. Peru and Bolivia emerging infectious diseases, 12 (6): 907-913.

- **Barzan, M.; Rad, M.; Tabar, G. R. and Azizzadeh, M.,(2017).** phylogenetic analysis of *Escherichia coli* isolates from healthy and diarrhoeic calves in mashhad, iran. Bulgarian journal of veterinary medicine, 20(1):11–18.
- **Bayramov, D. F. and Neff, J. A., (2016).** Beyond conventional antibiotics—new directions for combination products to combat biofilm. Adv Drug Deliv Rev., 1-11.
- **Bee, K. S., (2011).** Prevalence and characterization of integrons in clinical isolates of *Enterobacteriaceae* and *Pseudomonas* from hospitals in Malaysia. MSc thesis. Faculty of Science, University Tunku Abdul Rahman. 1-179.
- **Bennett, P. M., (2008).** Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. British Journal of Pharmacology. 153: 347-357.
- **Berendonk, T.U.; Manaia, C.M.; Merlin, C.; Fatta-Kassinos, D.; Cytryn, E.; Walsh, F.; Burgmann, H.; Sørum, H.; Norström, M. and Pons, M. N., (2015).** Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol., (13): 310–317.
- **Berglund, B., (2015).** Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. Infection ecology and epidemiology, 5: 1-10.
- **Betteridge, T.; Partridge, S. R.; Iredell, J. R. and Stokes, H. W., (2011).** Genetic context and structural diversity of class 1 integrons from human commensal bacteria in a hospital intensive care unit. Agents and chemotherapy, 55 (8): 3939–3943.

- **Bien, J.; Sokolova, O. and Bozko, P., (2012).** Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. International journal of nephrology, 1-16.
- **Blair, J. M.; Webber, M. A.; Baylay, A. J.; Ogbolu, D. O. and Piddock, L. J., (2015).** Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol., (13):42–51.
- **Boucher, Y.; Labbate, M.; Koenig, J. E. and Stokes, H. W., (2007).** Integrons:mobilizable platforms that promote genetic diversity in bacteria. Trends microbiol., 15:301–309.
- **Brooks, B. D. and Brooks, A. E., (2014).** Therapeutic strategies to combat antibiotic resistance. Adv Drug Deliv Rev., 78: 14–27.
- **Buckland, D., (2017).** Antimicrobial resistance and the race to find new antibiotics. New antibiotics/Analysis, 1-4.
- **Byarugaba, D. K., (2009).** Mechanisms of antimicrobial resistance. department of veterinary microbiology and parasitology, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda.15- 26.
- **Cairns, J.; Becks, L.; Jalasvuori, M. and Hiltunen, T., (2017).** Sublethal streptomycin concentrations and lytic bacteriophage together promote resistance evolution. Phil. Trans R Soc., 372: 20160040. (doi:10.1098/rstb.2016.0040)
- **Cairns, J.; Jalasvuori, M.; Ojala, V.; Brockhurst, M. and Hiltunen, T., (2016).** Conjugation is necessary for a bacterial plasmid to survive under protozoan predation. Biol Lett. 12:1-10.
- **Cambray, G.; Guerout, A. M. and Mazel, D., (2010).** Integrons. Annu Rev Genet., 44:141-66.
- **Carroll, K. C., (2013).** Bacteriology, in Brooks, G. F., Carroll, K.C., Butel, J. S., Morse, S. A., Mietzner, T. A. "Jawetz, Melnick&

Adelberg's Medical Microbiology", 26th Edition, McGraw-Hill Companies, Inc, USA, PP (229-240).

- **Cattoir, V., (2016).** *Streptococcus pyogenes* : Basic Biology to Clinical Manifestations. Mechanisms of Antibiotic Resistance, 1-45.
- **Chantziaras, I.; Dewulf, J.; Boyen, F.; Callens, B. and Butaye, P., (2014).** Antimicrobial resistance prevalence of pathogenic and commensal *Escherichia coli* in food-producing animals in Belgium. Vlaams Diergeneeskundig Tijdschrift., (83): 225-233.
- **Chaudhuri, R. R. and Henderson**, **I. R., (2012).** The evolution of the *Escherichia coli* phylogeny Infection. Genetics and Evolution, 12 (2). 214–226**.**
- **Chaula, T; Seni, J; Ng'walida, N; Kajura, A.; Mirambo, M; DeVinney, R. and Mshana, S. E., (2017).** Urinary tract infections among HIV-positive pregnant women in Mwanza city, Tanzania, are high and predicted by low CD4+ Count. International journal of microbiology, 1-7.
- **Chinen, T. and Rudensky, A. Y., (2012).** The effects of commensal microbiota on immune cell subsets and inflammatory responses. Immunology Review., 245:45-55.
- **Chuma, T.; Miyasako, D.; Dahshan, H.; Takayama, T.; Nakamoto, Y.; Shahada, F.; Akiba, M. and Okamoto, K., (2013).** Chronological change of resistance to beta-Lactams in *Salmonella enterica* serovar infantis isolated from broilers in Japan. Front Microbiology., 4:113.
- **Clermont, O.; Bonacorsi, S., and Bingen, E., (2000).** Rapid and simple determination of the *Escherichia coli* phylogenetic group. Applied and environmental microbiology, 66(10):4555–4558.

- **Clermont, O.; Christenson, J. K.; Denamur, E. and Gordon, D. M., (2013).**The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylogroups. Environ Microbiol Rep., 5(1):58–65.
- **Clermont,O.; Gordon, D. and Denamur, E., (2015).** Guide to the various phylogenetic classification schemes for *Escherichia coli* and the correspondence among schemes. Microbiology, 161: 980–988.
- **Clinical and Laboratory Standards Institute (CLSI), (2013).** Performance standards for antimicrobial susceptibility testing, 21th, informational supplement Approved standard M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute.
- **Cocchi, S.; Grasselli, E.; Gutacker, M.; Benagli, C.; Convert, M. and Piffaretti, J., (2007).** Distribution and characterization of integrons in *Escherichia coli* strains of animal and human origin. FEMS Immunol Med Microbiol., 50: 126–132.
- **Coura, F. M.; Diniz, S. D.; Silva, M. X.; Mussi, J. M.; Barbosa, S. M.; Lage, A. P. and Heinemann, M. B., (2015).** Phylogenetic group determination of *Escherichia coli* isolated from animals samples. The scientific world journal,1-5.
- **Cury, J.; Jov, T.; Touchon, M.; N´eron, B. and Rocha, E., (2016).** Identification and analysis of integrons and cassette arrays in bacterial genomes. Nucleic Acids Research, 1-12.
- **DaCosta, P. M.; Vaz-Pires, P. and Bernardo, F., (2006).** Antimicrobial resistance in *Enterococcus* spp. isolates in inflow, effluent and sludge from municipal sewage waste treatment plants. Water Research, 40:1735-1740.
- **Dale-Skinner, J. W. and Bonev, B. B., (2009).** Molecular mechanisms of antibiotic resistance: The need for novel antimicrobial therapies. Microbiol. Mol. Biol. Rev., 1-46.

- **Dar, D.; Shamir, M.; Mellin, J. R.; Koutero, M.; Stern-Ginossar, N.; Cossart, P. and Sorek, R., (2016).** Term-seq reveals abundant ribo-regulation of antibiotics resistance in bacteria. Science, 352-9822.
- **Davies, J. and Davies, D., (2010).** Origins and evolution of antibiotic resistance. Microbiol. Mol Biol Rev., **(**74):417–433.
- **Deng, Y.; Bao, X.; Ji, L.; Chen, L.; Liu, J.; Miao, J.; Chen, D.; Bian, H.; Li, Y. and Yu, G., (2015).** Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob., 14(45): 1-11.
- **Derakhshandeh, A.; Firouzi, R. and Naziri, Z., (2014).** Phylogenetic group determination of faecal *Escherichia coli* and comparative analysis among different hosts. Iranian Journal of Veterinary Research, Shiraz University, 15(1);13-17.
- **Derakhshandeh, A.; Firouzi, R.; Moatamedifar, M.; Motamedi, A.; Bahadori, M. and Naziri, Z., (2013).** Phylogenetic analysis of *Escherichia coli* strains isolated from human samples. Molecular biology research communications, 2(4):143-149.
- **Duijkeren, E.; Box, A.T.; Schellen, P.; Houwers, D. J. and Fluit, A. D., (2005).** Class 1 integrons in *Enterobacteriaceae* isolated from clinical infections of horses and dogs in The Netherlands. Microb Drug Resist., 11:383-386.
- **Dureja, C.; Mahajan, S. and Raychaudhuri, S., (2014).** Phylo-genetic distribution and prevalence of genes encoding class i integrons and CTX-M-15 extended-spectrum b-lactamases in *Escherichia coli* isolates from healthy humans in Chandigarh. India. Plos One, (9): 1-6.
- **Duriez, P.; Clermont, O.; Bonacori, S.; Bingen. E.; Chaventre, A.; Elion, J.; Picard, B. and Denamur, E., (2001).** Commonsal *Escherichia coli* isolates are phylogenetically distinct human populations. Microbiology, 147: 1671-1676.

- **Dyar, O. J.; Hoa, N. Q.; Trung, N. V.; Phuc, H. D.; Larsson, M.; Chuc, N. T. and Lundborg, C. S., (2012).** High prevalence of antibiotic resistance in commensal *Escherichia coli* among children in rural Vietnam. BMC Infectious Diseases, 12 (92): 1-8.
- **El salabi A. A., (2011).** Characterization of antibiotic resistance mechanisms in gram-negative bacteria from Tripoli and Benghazi, libya. Phd thesis. School of Medicine, Cardiff University,1-37.
- **Elizabeth, K. S., (2010).** Correlates of protection in response to mucosal vaccination with uropathogenic *Escherichia coli* antigens and the role of IL-17a during urinary tract infection. Phd thesis. University of Michigan, 1-227.
- **El-Shennawy, G. A., (2011).** Detection of class 1 integrons mediated antibiotic resistance among commensal *Escherichia coli*. Egyptian journal of medical microbiology, 20 (1): 1-10.
- **El-Sokkary, M. M. and Abdelmegeed, E. S., (2015).** Characterisation of class 1 Integron among *Escherichia coli* isolated from Mansoura University Hospitals in Egypt. Advances in Microbiology, 5: 269-277.
- **Ewers, C.; Bethe, A.; Semmler, T.; Guenther, S., and Wieler, L. H., (2012).** Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clinical Microbiology Infection., 18: 646–655.
- **Farra, A.; Skoog, G.; Wallén, L.; Kahlmeter, G.; Kronvall, G. and Sorberg, M., (2002).** Antibiotic use and *Escherichia coli* resistance trends for quinolones and cotrimoxazole in Sweden. Scand J Infect Dis., 34:449-455.
- **Flores-Mireles, A. L.; Walker, J. N.; Caparon, M. and Hultgren, S. J., (2015).** Urinary tract infections: epidemiology, mechanisms of

infection and treatment options. Article In Nature Reviews Microbiology, 3: 2-17.

- **Fricke, W. F.; Wright, M. S.; Lindell, A. H.; Harkins, D. M.; Baker-Austin, C.; Ravel, J. and Stepanauskas, R., (2008).** Insights into the environmental resistance gene pool from the genome sequence of the multiple–resistance environmental isolate *Escherichia coli*. Journal of the bacteriology, 190 (20): 6779-6794.
- **Froehlich, B.; Parkhill, J.; Sanders, M.; Quail, M.A., and Scott, J. R., (2005).** The pCoo plasmid of enterotoxigenic *Escherichia coli* is a mosaic cointegrate . Journal of Bacteriology., 187(18):6509-6516.
- **Ghaderpour, A.; Ho, W.; Chew, L.; Bong, C.; Chong, V.; Thong, K. and Chai, L., (2015).** Diverse and abundant multi-drug resistant *Escherichia coli* in Matang mangrove estuaries, Malaysia Frontiers in Microbiology, 6 (977): 1-13.
- **Gillings, M.; Boucher, Y.; Labbate, M.; Holmes, A.; Krishnan, S.; Holley, M. and Stokes, H. W., (2008).** The evolution of class 1 integrons and the rise of antibiotic resistance. Journal of bacteriology, 190: 5095-5100.
- **Giray, B.; Ucar, F. B. and Aydemir, S. S., (2012).** Characterization of uropathogenic *Escherichia coli* strains obtained from urology outpatient clinic of Ege Medical Faculty in İzmir. Medical Microbiology., 42 (1): 1328-1337.
- **Gordon, D. M.; Stern, S. E. and Collignon, P. J., (2005).** Influence of the age and sex of human hosts on the distribution of *Escherichia coli* ECOR groups and virulence traits. Microbiology, 151**:**15–23
- **Gudjónsdóttir, H. B., (2015).** ESBL-producing *Escherichia coli: a* molecular study on bacterial relatedness and resistance genes in isolates from the period 2006-2014. Master thesis, Faculty of Medicine, University of Iceland, 1-143.

- **Guillard, T.; Pons, S.; Roux, D.; Pier, G.B. and Skurnik, D. (2016).** Antibiotic resistance and virulence: Understanding the link and its consequences for prophylaxis and therapy. BioEssays, 38:682–693.
- **Hajiahmadi, F.; Ghale, E.; Alikhani, M. Y.; Mordadi, A. and Arabestani, M. R., (2017).** Detection of Integrons and Staphylococcal Cassette Chromosome *mec* Types in Clinical Methicillin resistant Coagulase Negative *Staphylococci* Strains. Osong Public Health Res Perspect, 8(1):47–53.
- **Hannan, T. J. and Hunstad, D. A., (2016).** A murine model for *Escherichia coli* urinary tract infection. Methods Molecular Biology., (1333): 159-75.
- **Hannan, T. J.; Totsika, M.; Mansfield, K. J.; Moore, K. H.; Schembri, M. A. and Hultgren, S. J., (2012).** Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection. FEMS Microbiology Review., 36:616-48.
- **Hansson, K.; Sundstrom, L.; Pelletier, A. and Roy, P. H., (2002).** *IntI2* integron integrase in Tn7. Journal of bacteriology, 184: 1712-1721.
- **Hertz, F. B., (2014).** ESBL-producing *Escherichia coli:* antibiotic selection, risk factors and population structure. PHD thesis. Department of Biology, Faculty of Science,University of Copenhagen, 1-211.
- **Hiltunen, T.; Virta, M. and Laine, A., (2017).** Antibiotic resistance in the wild: an eco-evolutionary perspective. Phil Trans R. Soc B., 372: 1-7.
- **Horner, C.; Fawley, W.; Morris, K.; Parnell, P.; Denton, M. and Wilcox, M., (2014).** *Escherichia coli* bacteraemia: 2 years of

prospective regional surveillance (2010-12). Journal Antimicrobal chemotherby., 69(1):91–100.

- **Hsu, S. C.; Chiu, T. H.; Pang, J. C.; Hsuan-Yuan, C. H.; Chang, G. N. and Tsen, H. Y., (2006).** Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmo*nella enterica serovar choleraesuis strains isolated from humans and swine in Taiwan. Journal Antimicrobial Agents, 27(5):383-91.
- **Huang, H.; Chen, Y.; Zheng, X.; Su, Y.; Wan, R. and Yang, S. (2016).** Distribution of tetracycline resistance genes in anaerobic treatment of waste sludge: The role of pH in regulating tetracycline resistant bacteria and horizontal gene transfer. Bioresour Technol., 218:1284–1289.
- **Igbeneghu, O. A. and Lamikanra, A. O., (2014)**.Multiple-resistant commensal *Escherichia coli* from Nigerian children: potential opportunistic pathogens. Tropical Journal of Pharmaceutical Research, 13 (3): 423-428
- **Iranpour, D., Hassanpour, M., Ansari, H., Tajbakhsh, S., Khamisipour, G. and Najafi, A., (2015).** Phylogenetic groups of *Escherichia coli* strains from patients with urinary tract infection in iran based on the new clermont phylotyping method. Biological Medical Research International, 1-7.
- **Jafri, S. A.; Qasim, M.; Masoud, M. S.; Rahman, M.; Izhar, M. and Kazmi, S., (2014).** Antibiotic resistance of *E. coli* isolates from urine samples of urinary tract infection (UTI) patients in Pakistan. Bioinformation,10 (7):419-422.
- **Jang, J.; Di, D. Y.; Lee, A.; Unno, T.; Sadowsky, M. J. and Hur, H., (2014).** Seasonal and genotypic changes in *Escherichia coli*

phylogenetic groups in the Yeongsan River Basin of south Korea. Plos One, 9(7):1-8.

- **Jelesić, Z.; Gusman, V.; Ukropina, M.; Kulauzov, M. and Medić, D., (2011).** Resistance of *Escherichia coli* from healthy donors and from food–an indicator of antimicrobial resistance level in the population. Med Preg., 64(7-8): 397-402.
- **Jensen, L. B.; Hasman, H.; Agersø, Y.; Emborg, H. D. and Aarestrup, F. M., (2006).** First description of an oxyimino-cephalosporin-resistant, ESBL-carrying *Escherichia coli* isolated from meat sold in Denmark. Journal of Antimicrobial Chemotherby., 57: 793–794.
- **Jitwasinkul, T.; Suriyaphol, P.; Tangphatsornruang, S.; Hansen, M.A.; Hansen, L. H.; Sørensen, S. J.; Permpikul, C.; Rongrungruang, Y. and Tribuddharat, C., (2016).** Plasmid metagenomics reveals multiple antibiotic resistance gene classes among the gut microbiomes of hospitalized patients. Journal of Global Antimicrobial Resist., 6:57–66.
- **Johnson, J. R.; Kuskowski, M. A.; Owens, K.; Gajewski, A. and Winokur, P. L., (2003).** Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. Journal of Infectious Disease., 188: 759–768.
- **Jones-Dias, D.; Manageiro, V.; Ferreira, E.; Barreiro, P.; Vieira, Luís.; Moura, I. and Caniça, M., (2015).** Architecture of class1,2 and 3 integrons from gram negative bacteria recovered among fruits and vegetables. Frontiers in Microbiology, 7: 1-13.
- *Jonkers, D. M., (2017). Microbial perturbations and modulation in conditions associated with malnutrition and malabsorption. Best Practice & Research Clinical Gastroenterology, 30 (2): 161–172.*

- **Jørgensen, R. L.; Nielsen, J. B.; Friis-Møller, A.; Fjeldsøe-Nielsen, H. and Schønning, K**., **(2010).** Prevalence and molecular characterization of clinical isolates of *Escherichia coli* expressing an AmpC phenotype. Journal of Antimicrobial Chemotherby, 65(3):460–4.
- **Kang, H.Y.; Jeong, Y.S.; Oh, J. Y; Tae, S. H.; Choi, C. H; Moon, D. C.; Lee, W.K.; Lee, Y. C.; Seo, S. Y.; Cho, D. T. and Lee, J.C. (2005).** Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. Journal of Antimicrobial Chemotherby, 55: 639–644.
- **Karczmarczyk, M.; Walsh, C.; Slowey, R.; Nola Leonard, N. and Fanning, S., (2011).** Molecular characterization of multidrug-resistant *Escherichia coli* isolates from Irish cattle farms. Application in Environmental Microbiology., 77(20): 7121–7127.
- **Kargar, M.; Mohammadalipour, Z.; Doosti, A.; Lorzadeh, S. and Japoni-Nejad, A., (2014).** High prevalence of class 1 to 3 integrons among multidrug-resistant diarrheagenic *Escherichia coli* in South-west of Iran. Osong Public Health Res Perspect., 5(4): 193-198.
- Katouli, M., (2010). Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. Katouli. Iran Journal of Microbiology., 2 (2): 59-72.
- **Kazemnia, A.; Ahmadi, M. and Dilmaghani, M., (2014).** Antibiotic resistance pattern of different *Escherichia coli* phylogenetic groups isolated from human urinary tract infection and *Avian coli bacillosis*. Iranian biomedical journal., 18 (4): 219-224.
- **Kester, J. C. and Fortune, S. M., (2013).** Persisters and beyond: mechanisms of phenotypic drug resistance and drug tolerance in bacteria. Crit Rev Biochemical Molecular Biology., 1-13.

- **Keys, C.; Kemper, S. and Keim, P., (2005).** Highly diverse variable number tandem repeat loci in the *E. coli* O157:H7 and O55:H7 genomes for high-resolution molecular typing. J Appl Microbiol., 98(4):928–40.
- **Khachatran, A. R.; Besser, T. E. and Call, D. R., (2008).** The streptomycin- sulfadiazine- tetracycline antimicrobial resistance element of calf-adapted *Escherichia coli* is widely distributed among isolated from Washington state cattle. Applied and environmental microbiology, 74 (2): 391-395.
- **Khalilzadeh, S.; Boloorsaz, M. R.; Safavi, A.; Farnia, P. and Velayati, A. A., (2006).** Primary and acquired drug resistance in childhood tuberculosis. Eastern mediterranean health journal, 12 (6):909–914.
- **Kheiri, R. and Akhtari, L., (2016).** Antimicrobial resistance and integron gene cassette arrays in commensal *Escherichia coli* from human and animal sources in IRI. Gut Pathog., 8(40):1-10.
- **Kobayashi, T.; Suehiro, F.; Tuyen, B. C. and Suzuki, S., (2007).** Distribution and diversity of tetracycline resistance genes encoding ribosomal protection proteins in Mekong river sediments in Vietnam. FEMS Microbiol Ecol., 59:729–737.
- **Kohanski, M. A.; Dwyer, D. J. and Collins, J. J., (2010).** How antibiotics kill bacteria: from targets to networks. Nat Rev Microbio., 8(6): 423-435.
- **Kumar, S. and Varela, M., (2013).** Molecular mechanisms of bacterial resistance to antimicrobial agents. Microbial pathogens and strategies for combating them: science, technology and education, 522-534.

- **Kwong, W. K .; Mancenido, A. L. and Moran, N. A., (2017).** Immune system stimulation by the native gut microbiota of honey bees. R Soc Open Sci., 4:1-9.
- **Laroche, L. E.; Pawlak, B.; Berthe, T.; Skurnik, D. and Petit, F., (2009).** Occurrence of antibiotic resistance and class1, 2 and 3 integrons in *Escherichia coli* isolated from a densely populated estuary (Seine, France). FEMS Microbiol Ecol., 68: 118–130.
- **Leclercq**, **R., (2002).** Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Antimicrobial Resistance, 34:1-11.
- **Lee, C. R.; Cho, I. H.; Jeong, B. C. and Lee, S. H., (2013).** Strategies to minimize antibiotic resistance. International Journal of Environmental Research and Public Health, 10 (9): 4274–4304.
- **Lee, J. C.; Kang, H. Y.; Oh, J. Y.; Jeong, J. H.; Kim, J.; Seol, S. Y.; Cho, D. T. and Lee, Y. C., (2006).** Antimicrobial resistance and integrons found in commensal *Escherichia coli* isolates from healthy humans. Journal of bacteriology and virology, 36 (3): 133 **–** 139.
- **Li , B.; Zhao, Z.; Wang, M.; Huang, X.; Pan, Y. and Cao, Y., (2014).** Antimicrobial resistance and integrons of commensal *Escherichia coli* strains from healthy humans in China. Journal of chemotherapy, 26(3): 190-192.
- **Lin, J.; Nishino, K.; Roberts, M.; Tolmasky, M.; Aminov, R. I. and Zhang, L., (2015).** Mechanisms of antibiotic resistance. Frontiers in microbiology | antimicrobials, resistance and chemo-therapy, 6: 1-3.
- *Linda, S.; Joanne, W.* **and** *Christopher, W., (2013).* Prescott's Microbiology *(9th^{ed}.). New York: McGraw Hill. pp. 713–721..*
- **Lindberg, R. H.; Bjorklund, K.; Rendahl, P.; Johansson, M. I.; Tysklind, M. and Andersson, B. A., (2007).** Environmental risk

assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Research, 41(3): 613-619.

- **Loeffler, J. and Stevens, D. A., (2003).** Antifungal drug resistance. Clinical infectious diseases, 36(1):S31–S41.
- **Logue, C. M.; Wannemuehler, Y.; Nicholson, B. A.; Doetkott, C.; Barbieri, N. and Nolan, L. K., (2017).** Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. Front. microbiol., 8:283-290.
- **Loong, S. K.; Mahfodz, N. H.; Seri, A.; Wali, H. A.; Abd Gani, S. A.; Wong, P. and AbuBakar, S., (2016).** Genetic characterization of commensal *Escherichia coli* isolated from laboratory rodents. Springer Plus, 5:10351-9.
- **Losada, L.; DebRoy, C.; Radune, D.; Kim, M.; Sanka, R.; Brinkac, L.; Kariyawasam, S.; Shelton, D.; Fratamico, P.M. and Kapur, V., (2016).** Whole genome sequencing of diverse Shiga toxin-producing and non-producing Escherichia coli strains reveals a variety of virulence and novel antibiotic resistance plasmids. Plasmid, 83: 8–11.
- **Lysnyansky, I. and Ayling, R. D., (2016).** *Mycoplasma bovis*: Mechanisms of Resistance and Trends in Antimicrobial Susceptibility. Front Microbiol., 7(595): 1-7.
- **MacFaddin, J. F., (2000).** Biochemical tests for identification of medical bacteria, 3rd edition. lippincott williams and williams.1-122
- **Maes, P. W.; Rodrigues, P. A.; Oliver, R.; Mott, B. M. and Anderson K. E., (2016).** Diet related gut bacterial dysbiosis correlates

with impaired development, increased mortality and Nosema disease in the honey bee (Apis mellifera). Mol Ecol., 5439–5450.

- **Manges, A. R., (2016)**. *Escherichia coli* and urinary tract infections: the role of poultry-meat. Clin Microbiol Infect., 22(2):122-9.
- **Marchiaro, P.; Viale, A. M.; Ballerini, V.; Rossignol, G.; Vila, A. J. and Limansky, A., (2010).** First report of a Tn*402*-like class 1 integron carrying blaVIM-2 in *Pseudomonas putida* from Argentina. J Infect Devel Count., 4 **(**6**):**412–416.
- **Marks, S.M.; Flood, J.; Seaworth, B.; Hirsch-Moverman, Y.; Armstrong, L.; Mase, S.; Salcedo, K.; Oh, P.; Graviss, E. A.; Colson, P. W.; Armitige, L.; Revuelta, M.; Sheeran, K. and the TB Epidemiologic Studies Consortium, (2014).** Treatment practices, outcomes, and costs of multidrug-resistant and extensively drug-resistant tuberculosis, United States, 2005–2007. Emerging infectious diseases, 20 (5):812–821.
- **Marshall, B. M. and Levy, S. B., (2011).** Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev., 24:718–33.
- **Marshall, B. M., Ochieng, D. J. and Levy, S. B., (2009).** Commensals: Underappreciated reservoir of antibiotic resistance Microbe, 4:231-38.
- **Martin, B. S.; Lapierre, L.; Cornejo, J. and Bucarey, S., (2008).** Characterization of antibiotic resistance genes linked to class 1 and 2 integrons in strains of *Salmonella* spp. isolated from swine. Can J Microbiol., 54:569–576.
- **Martins, A.; Hunyadi, A. and Amara, L., (2013):** Mechanisms of resistance in bacteria: an evolutionary approach. The open microbiology journal, 7: 53-58.

- **Massot, M.; Daubie, A. S.; Clermont, O.; Jaureguy, F.; Couffignal, C.; Dahbi, G.; Mora, A.; Branger, C.; Mentre, F.; Eddi, A.; Picard, B. and Dennamur, E., (2016).** Phylogenetic, virulence and antibiotic resistance characteristics of *Escherichia coli* from community subjects in the paris area in 2010 and evolution over 30 years. Microbiology, 162(4):642-650.
- **Mathai, E.; Grape, M. and Kronvall, G., (2004).** Integrons and multidrug resistance among *Escherichia coli* causing community-acquired urinary tract infection in Southern India. APMIS., 112:159-64.
- **Mazel, D., (2006).** Integrons: agents of bacterial evolution. Nat Rev Microbiol., 4: 608-20.
- **Mazurek, J.; Bok, E.; Stosik, M. and Baldy-Chudzik, K., (2015).** Antimicrobial resistance in commensal *Escherichia coli* from Pigs during metaphylactic trimethoprim and sulfamethoxazole treatment and in the post-exposure period. J. Environ. Res. Public Health., 12: 2150-2163.
- **Meervenne, V. E.; Coillie, E. V.; Kerckhof, F. M.; Devlieghere, F.; Herman, L.; Leen De Gelder, S. P.; Top, E. and Boon, N., (2012).** Strain-specific transfer of antibiotic resistance from an environmental plasmid to food borne pathogens. Journal of biomedicine and biotechnology, 1-9.
- **Mendonça, N.; Figueiredo, R.; Mendes, C.; Card, R. M.; Anjum, M. F. and Silva, G. J., (2016).** Microarray evaluation of antimicrobial resistance and virulence of *Escherichia coli* isolates from Portuguese poultry. Antibiotics, 5 (4):1-9.
- *Mikaelyan, A.; Thompson, C.; Hofer, M* **and** *Brune, A., (2017). The deterministic assembly of complex bacterial communities in germ-*

freecoc kroach guts. Applied and Environmental Microbiology, 82 (4): 1256–1263.

- **Morar, M.; Bhullar, K.; Hughes, D. W.; Junop, M. and Wright, G. D., (2009).** Structure and mechanism of the lincosamide antibiotic adenylyltransferase LinB. Structure, 17: 1649–1659.
- **Moreno, E.; Andreu, A.; Pigrau, C.; Kuskowski, M. A.; Johnson, J. R. and Prats, G., (2008).** Relationship between *Escherichia coli* strains causing acute cystitis in women and the fecal *Escherichia coli* population of the host. J Clin Microbiol., 46:2529-2534.
- **Moreno, E.; Johnson, J.R.; Perez, T.; Prats, G.; Kuskowski,M. A., and Andreu. A., (2009).** Structure and urovirulence characteristics of the fecal *Escherichia coli* population among healthy women. Microbes Infect., 11:274–280.
- **Mukherjee, S., and Chakraborty, R., (2006).** Incidence of class 1 integrons in multiple antibiotic-resistant gram-negative copiotrophic bacteria from the River Torsa in India. Research in Microbiology, 157: 220-226.
- **Munita, J. and Arias, C., (2016).** Mechanisms of antibiotic resistance. in virulence mechanisms of bacterial pathogens, fifth edition; American Society of Microbiology, 481–511.
- **Nahid, A., (2007).** Antibiotic resistance and fitness of *Escherichia coli* in the infantile commensal microbiota. PHD thesis. Goteburg University, Department of Clinical Bacteriology.1-214.
- **National Antimicrobial Resistance Monitoring System (NARMS) Strategic Plan, 2012-2016**. 1-11.
- **Navidinia, M.; Peerayeh, S. N.; Fallah, F.; Bakhshi, B. and Sajadinia, R. S., (2014).** Phylogenetic grouping and pathotypic comparison of urine and fecal *Escherichia coli* isolates from children with urinary tract infection. Braz J Microbiol., 45(2): 509-14.

- **Nemergut, D. R.; Robeson, M. S.; Kysela, R. F.; Martin, A. P.; Schmidt, S. K. and Knight, R., (2008).** Insights and inferences about integron evolution from genomic data. BMC Genom., 9:1–12.
- **Nicolas-Chanoine, M. H.; Jarlier, V.; Robert, J.; Arlet, G.; Drieux L.; Leflon-Guibout, V.; Laouénan, C.; Larroque, B.; Caro V. and Mentré, F., (2012).** Patient's origin and lifestyle associated with CTX-M-producing *Escherichia coli*: a case-control study. Plos One., 7(1):e30498.
- **Nielsen, K. L.; Dynesen, P.; Larsen, P. and Faecal, F. N., (2014).** *Escherichia coli* from patients with *E. coli* urinary tract infection and healthy controls who have never had a urinary tract infection. J Med Microbiol., 63(4):582–589.
- **Nikaido, H., (2009).** Multidrug resistance in bacteria. Annual Review of Biochemistry. 78:119–146.
- **Nourbakh, F.; Rajai, M. and Momtaz, H., (2017).** Antibiotic Resistance and Carriage Integron Classes in Clinical Isolates of *Acinetobacter Baumannii* from Isfahan Hospitals, Iran. Zahedan J Res Med Sci, 1-7.
- **Nowrouzian, F. L.; Adlerberth, I. and Wold, A. E., (2006).** Enhanced persistence in the colonic microbiota of *Escherichia coli* strains belonging to phylogenetic group B2: role of virulence factors and adherence to colonic cells. Microbes Infect., 8:834–840.
- **Nowrouzian, F. L.; Wold, A. E. and Adlerberth, I., (2005).** *Escherichia coli* strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. J Infect Dis., 191**:**1078–1083.
- **Obata-Yasuoka, M.; Ba-Thein, W.;Tsukamoto, T.; Yoshikawa, H. and Hayashi. H., (2002).** Vaginal *Escherichia coli* share common

virulence factor profiles, serotypes and phylogeny with other extraintestinal *E. coli*. Microbiology, 148:2745–2752.

- **Oluyege, A. O.; Ojo-Bola, O. and Oludada, O. E., (2015).** Carriage of antibiotic resistant commensal *E. coli* in infants below 5 months in Ado-Ekiti. J Curr Microbiol App Sci., 4(5): 1096-1102.
- **Ozgumus, O. B.; Celik-Sevim, E.; Alpay-Karaoglu, S.; Sandalli, C. and Sevim, A., (2007).** Molecular characterization of antibiotic resistant *Escherichia coli* strains isolated from tap and spring waters in a coastal region in Turkey. J Microbiol., 45:379-387.
- **Parks, A. R. and Peters, J. E., (2009).** Tn7 elements: Engendering diversity from chromosomes to episomes. Plasmid. 61: 1-14.
- **Parshall, M .B., (2013).** Unpacking the 2 x 2 table. Heart &lung. 42: 221-226.
- **Partridge, S. R., (2011).** Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol Rev., 35:820-55.
- **Partridge, S. R.; Tsafnat, G.; Coiera, E. and Iredell, J. R., (2009).** Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiology Review, 33: 757-784.
- **Penesyan, A.; Gillings, M. and Paulsen, I. T., (2015).** Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. Molecules, 20(4):5286–5298.
- **Peter, F.; Weagant, S. D.; Grant, M. A. and Burkhardt, W., (2002).** Enumeration Of *Escherichia coli* and the coliform bacteria, in bacteriological analytical manual (8^{th}), chapter 4, 1-94.
- **Phongpaichit, S.; Wuttananupan, K. and Samasanti, W., (2008).** Class 1 integrons and multidrug resistance among *Escherichia coli* isolates from human stools. Southeast Asian J Trop Med Public Health., 39 (2):279-287.

- **Pitout, J. D. D., (2012).** Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. Front Microbiol., 3:1-9.
- **Popęda, M.; Płuciennik, E. and Bednarek, A.K., (2014).** Proteins in cancer resistance, Postępy Higienyi Medycyny Do´swiadczalnej, 68:616–632.
- **Raherison, S.; Jove, T.; Gaschet, M.; Pinault, E.; Tabesse, A.; Torres, C. and Ploy, M., (2017).** Expression of the *aac(6')-Ib*-cr gene in class 1 integrons. Antimicrob. Agents chemother., 61 (3):1-10.
- **Rahube, T. O., (2013).** Dissemination of antibiotic resistant bacteria and plasmids encoding antibiotic resistance genes in the environment. PhD thesis. University of Regina.1-224.
- **Ramirez, M. S.; Nikolaidis, N. and Tolmasky, M. E.,(2013).** Rise and dissemination of aminoglycoside resistance :the *aac (6')-Ib* paradigm. Front Microbiol., 4:121.
- **Ravn, C.; Tafin, U.F.; Bétrisey, B.; Overgaard, S. and Trampuz, A., (2016).** Reduced ability to detect surface-related biofilm bacteria after antibiotic exposure under in vitro conditions. Acta Orthop., 87:644–650.
- *Reinoso, W. C., (2016). Protective and pro-inflammatory roles of intestinal bacteria. Pathophysiology (Review), 23 (2): 67–80.*
- **Reyes, A.; Bello, H.; Dominguez, M.; Mella, S.; Zemelman, R. and Gonzalez, G., (2003).** Prevalence and types of class 1 integrons in aminoglycoside-resistant *Enterobacteriaceae* from several Chilean hospitals. J Antimicrob Chemother., 51: 317-21.
- **Rezanka, T.; Spizek, J. and Sigler, K. (2007).** Medicinal use of lincosamides and microbial resistance to them. Anti-Infect. Agents Med Chem., 6:133–144.

- **Roe, M. T.; Vega, E. and Pillai, S. D., (2003).** Antimicrobial resistance markers of class 1 and class 2 integron-bearing *Escherichia coli* from irrigation water and sediments. Emerg Infect Dis., 9:822-826.
- **Rosello, A.; Hayward, A. C.; Hopkins, S. ; Horner, C.; Ironmonger, D.; Hawkey, P. M. and Deeny, S. R., (2017).** Impact of long-term care facility residence on the antibiotic resistance of urinary tract *Escherichia coli* and *Klebsiella*. J Antimicrob Chemother., 1-8.
- **Sabate, M.; Moreno, E.; Perez, T.; Andreu, A. and Prats, G. (2006).** Pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. Clin Microbiol Infect., 12**:**880–886.
- **Sabouri Ghannad, M. and Mohammadi, A., (2012).** Bacteriophage: time to re-evaluate the potential of phage therapy as a promising agent to control multidrug-resistant bacteria . Iran J Basic Med Sci., (15) : 693–701.
- **Sáenz, Y.; Zarazaga, M.; Briñas, L.; Lantero, M.; Ruiz-Larrea, F. and Torres, C., (2001).** Antibiotic resistance in *E. coli* isolated from animals, foods and human in spain. Int J Antimicrob Agents, 18(4):353-8.
- **Sam, Q. H.; Chang, M. W. and Chai, L. Y., (2017).** The Fungal Mycobiome and Its Interaction with Gut Bacteria in the Host. Int. J. Mol. Sci., 18(330):1-11.
- *Saxena, R.* **and** *Sharma, V. K., (2016). A Metagenomic Insight Into the Human Microbiome: Its Implications in Health and Disease. In D. Kumar; S. Antonarakis.* Medical and Health Genomics, *Elsevier Science, pp.117.*
- **Schroeder, M.; Brooks, B. and Brooks, A. E., (2017).** The Complex relationship between virulence and antibiotic resistance. Genes, 8(39) :1-23.

- **Sepp, E.; Stsepetova, J.; Lõivukene, K.; Truusalu, K.; Kõljalg, S.; Naaber, P. and Mikelsaar, M., (2009).** The occurrence of antimicrobial resistance and class 1 integrons among commensal *Escherichia coli* isolates from infants and elderly persons. Annals of clinical microbiology and antimicrobials, 8 (34): 1-6.
- **Shi, K.; Caldwell, S. J.; Fong, D. H. and Berghuis, A. M., (2013).** Prospects for circumventing aminoglycoside kinase mediated antibiotic resistance. Front Cell Infect Microbiol., 3:1-22.
- **Shin, H. and Cho, S., (2013).** Prevalence of antimicrobial resistance in *Escherichia coli* strains isolated from fishery workers. Osong Public Health Res Perspect., 4(2): 72-75.
- **Singh, P. Chand, D. D.; Maury, A. P.; Paul, D.; Chakravarty, A. and Bhattacharjee, A., (2017).** Distribution of Class II integrons and their contribution to antibiotic resistance within *Entero-bacteriaceae* family in India. Original Article**,** 34; 303-307……..
- **Singh, T.; Das, S.; Ramachandran, V. G. ; Wani, S.; Shah, D.; Maroof, K. A. and Sharma, A. (2017).** Distribution of Integrons and Phylogenetic Groups among Enteropathogenic *Escherichia coli* Isolates from Children <5 Years of Age in Delhi, India. Frontiers in Microbiology, 8:1-13
- **Singh, V., (2013).** Antimicrobial resistance, in microbial pathogens and strategies for combating them. Science, technology and education, formatex research center, 1: 291–296.
- **Skurnik, D., Menac'h, A. L., Zurakowski, D., Mazel, D., Courvalin, P., Denamur, E., Andremont, A. and Ruimy, R., (2005).** Integron-associated antibiotic resistance and phylogenetic grouping of *Escherichia coli* isolates from healthy subjects free of recent antibiotic exposure. Antimicrobial agents and chemotherapy, 49(7): 3062–3065.

- **Sohrabi, R. and Zeighami, H., (2017).** Determination of phylo-genetic groups and antibiotic resistance in uropathogenic and commensal *Escherichia coli* isolated from patients in Zanjan city. Zums.ac.ir., 1-12.
- **Staji, H.; Khoshgoftar, J.; Vayeghan, A. J. and Bejestani, M. R. (2016).** Phylogenetic grouping and assessment of virulence genotypes, with antibiotic resistance patterns, of *Escherichia coli* strains implicated in female urinary tract infections. J Enteric Pathog., 4(1): e31609.
- **Su, J.; Shi, L.; Yang, L.; Xiao, Z.; Li, X. and Yamasaki, S., (2006).** Analysis of integrons in clinical isolates of *Escherichia coli* in China during the last six years. FEMS Microbiol Lett. 254(1): 75–80.
- **Sunde, M., (2005).** Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. Journal of antimicrobial chemotherapy, 56: 1019-1024.
- **Süzük, S.; Avciküçük, H.; Kaşkatepe, B. and Aksaray, S., (2015).** Antibiotic susceptibility of microbiota members *Escherichia coli* strains isolated from stool samples of patients attended Kırıkkale Yüksek İhtisas Hospital in ten months. Turk Hij Den Biyol Derg., 72(4): 289 - 296.
- **Szczepanowski, R.; Braun, S.; Riede, V.; Schneiker, S.; Krahn, I.; Pühler, A. and Schlüter, A., (2005).**The 120 592 bp IncF plasmid pRSB107 isolated from a sewage-treatment plant encodes nine different antibiotic-resistance determinants, two iron-acquisition systems and other putative virulence-associated functions. Microbiology,151(4):1095-10111.

- **Szmolka, A. and Nagy, B., (2013).** Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Frontiers in Microbiology, 4:1-13.
- **Tajbakhsh, E.; Khamesipour, F.; Ranjbar, R. and Ugwu, I. C., (2015).** Prevalence of class 1 and 2 integrons in multi-drug resistant *Escherichia coli* isolated from aquaculture water in Chaharmahal Va Bakhtiari province, Iran. Ann Clin Microbiol Antimicrob., 14(37):1-5.
- **Tamerat, N.; Muktar, Y. and Shiferaw, D., (2016).** Application of molecular diagnostic techniques for the detection of *E. coli* O157:H7: A Review. J Veterinar Sci Technol., 7(5): 1-10.
- **Tanwar, J.; Das, S.; Fatima, Z. and Hameed, S., (2014).** Multidrug resistance: an emerging crisis. Interdisciplinary perspectives on infectious diseases, 1-9.
- **Tement, L.; Dyson, R.J.; Krachler, A.-M. and Jabbari, S., (2015).** Bacterial fitness shapes the population dynamics of antibiotic-resistant and -susceptible bacteria in a model of combined antibiotic and anti-virulence treatment. J Theor Biol., 372:1–11.
- **Tenaillon, O.; Skurnik, D.; Picard, B. and Denamur, E., (2010).** The population genetics of commensal *Escherichia coli*. Nat Rev Micro., 8: 207-17.
- **Thallinger, B.; Prasetyo, E. N.; Nyanhongo, G. S. and Guebitz, G. M. (2013).** Antimicrobial enzymes: An emerging strategy to fight microbes and microbial biofilms. Biotechnol J., (8): 97-109.
- **Thomas, L. C., (2007).** Genetic methods for rapid detection of medically important nosocomial bacteria. Master Thesis of Science in Medicine, Faculty of Medicine, Department of Medicine, University of Sydney.1-244.

- **Urumova, V., (2016a).** Investigations on tetracycline resistance in commensal *Escherichia coli* isolates from swine. Bulgarian journal of veterinary medicine,1-10.
- **Urumova, V., (2016b).** Prevalence of resistance to sulfonamides and streptomycin among commensal porcine *Escherichia coli* isolates. Revue Méd Vét., 167 (1-2): 38-44.
- **Van Essen-Zandbergen, A.; Smith, H.; Veldman, K. and Mevius, D., (2007).** Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli, Salmonella* and *Campylobacter spp*. in the Netherlands. J Antimicrob Chemother., 59: 746–750.
- **Venturini, C., (2011).** Molecular characterization of multiple antibiotic resistance in *Escherishia coli* isolated from animals and humans. University of Wollongong thesis collection, 1-222.
- **Viera, A. J., (2008).** Odds ratios and risk ratios: what's the difference and why does it matter? . South Med J., 101 (7): 730-734.
- **Vinue, L.; Sa´enz, Y.; Somalo, S.; Escudero, E.; Moreno, M. A.; Ruiz-Larrea, F. and Torres, C., (2008).** Prevalence and diversity of integrons and associated resistance genes in faecal *Escherichia coli* isolates of healthy humans in Spain. J Antimicrob Chemother., 62:934-7.
- **Vittecoq, M.; Laurens, C.; Brazier, L.; Durand,P.; Elguero,E; Arnal, A.; Thomas, F.; Aberkane, S.; Renud, N.; Prugnoe, F.; Solassol, J.; Plerre, H.; Godreui, S. and Renud, F., (2017).** VIM-1 cabapenemase-producing *Escherichia coli* in gulls from southern France. Ecology and Evolution, 7: 1224-1232.
- **Von Wintersdorff, C. J.; Penders, J.; van Niekerk, J. M.; Mills, N. D.; Majumder, S.; van Alphen, L.B.; Savelkoul, P.H. and Wolffs, P. F., (2016).** Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol., 7:1-8.

- **Vranakis, I.; Goniotakis, I.; Psaroulaki, A.; Sandalakis, V.; Tselentis, Y.; Gevaert, K. and Tsiotis, G., (2013).** Proteome studies of bacterial antibiotic resistance mechanisms. J Proteomics, 1-5.
- **Wiles, T. J.; Kulesus, R. R. and Mulvey, M. R., (2008).** Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Exp Mol Pathol., 85:11-19.
- **Woerther, P. L.; Angebault, C.; Jacquier, H.; Clermont, O.; El Mniai, A.; Moreau, B.; Djossou, F.; Peroz, G. and Catzeflis, F., (2013).** Characterization of fecal extended-spectrum-b-lactamase producing *Escherichia coli* in a remote community during a long time period. Antimicrob agents chemother., 57: 5060–5066.
- **World Health Organization, (2012).** The evolving threat of antimicrobial resistance: options for action. Geneva: Available from: http://whqlibdoc. who.int/ publications/ 2012/ 9789241503181_eng.
- **World Health Organization, (2017).** Antibiotic-resistant priority pathogens list. Geneva: Available from: http://whqlibdoc. who.int/ publications/ 2017/ 9789241503181_eng, 1-11.
- **World Health Organization,(2014).** Antimicrobial Resistance Global Report on Surveillance, Geneva, Switzerland.
- **Yang, C. M.; Lin, M. F.; Lin, C. H.; Huang, Y. T.; Hsu, C. T. and Liou, M. L., (2009).** Characterization of antimicrobial resistance patterns and integrons in human fecal *Escherichia coli* in Taiwan. Jpn J Infect Dis., 62(3):177-81.
- **Yao, X.; Doi, Y.; Zeng, L.; Lv, L. and Liu, J. H., (2016).** Carbapenem-resistant and colistin-resistant *Escherichiacoli* co-producing NDM-9 and MCR-1. Lancet Infect Dis., 16: 288–289.
- **Yu, T.; Zhang, J.; Jiang, X.; Wu, J.; Dai, Z.,; Wu, Z.; Liang, Y. and Wang, X., (2016).** Characterization and horizontal transfer of

class 1 integrons in *Escherichia coli* isolates from cooked meat products. Infect Dev Ctries., 10(1):068-073.

- **Zhang, L.; Foxman, B. and Marrs, C., (2002).** Both urinary and rectal *Escherichia coli* isolates are dominated by strains of phylogenetic group B2. J Clin Microbiol., 40:3951–3955.
- **Zhang, T., (2015).** Daptomycin: mechanism of action and bacterial resistance. PHD thesis. University of Waterloo, Canda.1-152.
- **Zhang, X.; Zhang, T.; Zhang, M.; Fang, H. H. and Cheng, S. P. (2009).** Characterization and quantification of class 1 integrons and associated gene cassettes in sewage treatment plants. Applied microbiology and biotechnology, 82: 1169-1177.
- **Zhao, S.; Qaiyumi, S.; Friedman, S.; Foley, S. L.; White, D. G.; McDermott, P. F.; Donkar, T.; Bolin, C.; Munro, S.; Baron, E. J. and Walker, R. D., (2003).** Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J Clin Microbiol., 41: 5366-71.
- **Zhao, S.; White, D. G.; Ge, B.; Ayers, S.; Friedman, S.; English, L.; Wagner, D.; Gaines, S. and Meng, J., (2001).** Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Appl Environ Microbiol., 67: 1558-1564.
- **Zurfuh, K.; Poire, L.; Nordmann, P.; Nüesch-Inderbinen, M.; Hächler, H. and Stephan, R., (2016).**Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in ESBL-producing *Enterobacteriacae* in river water and imported vegetable samples in Switzerland. Antimicrob. Agents Chemother., 60:2594–1295.