**CHAPTER ONE**

**INTRODUCTION**

Antibiotic resistance has spread so wild and has created panic around the world. As much as man is trying to solve the problems of antibiotic resistance, the organisms have also been trying harder against the effort of man. The problem of antibiotic resistance is fast spreading due to the ability of such organisms to mutate, transmit and acquire plasmids or other genetic material that encode for antibiotic resistance from other bacteria (Nazneen *et al.,* 2014). Multi drug resistance in organisms such as *Klebseilla* spp, *Pseudomonas* spp, *Escherichia* coli and *Enterobacter* spp have emerged due to extensive use of antibiotics (Shobha *et al.*, 2009). *K. pneumoniae* is an opportunistic pathogen and has been implicated in infections such as pneumonia, urinary tract infections, septicemia and other soft tissue infections in hospitalized and immunosuppressed patients. Extended spectrum beta-lactamases (ESBLs) and carbapenemases are the major causes of multidrug resistance in *K. Pneumoniae* (E. Ruh *et al*., 2016). Resistance to antibiotics is currently an issue of great scientific concern as it both nosocomial and community acquired (Sibhghatulla *et al*., 2015).

Furthermore, there are now the Extended Spectrum Beta Lactamases (ESBLs) mostly observed in gram negative bacteria which can hydrolyze extended spectrum cephalosporin. They are effective against beta-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime and oxyiminomonobactam (Ghafourian *et al,* 2000). These antibiotics are mostly used in the treatment of

Pseudomonas and other gram negative bacteria (Shobha *et al.*, 2009).

The quest for drug resistance in UTI (Urinary Tract Infection) causing pathogens is gaining more attention the resistance mechanism of ESBL producing bacteria differs between species (Aruna and Mobashshera, 2012).

In Urinary Tract Infections (UTIs) pathogens are able to invade of the urinary tract and this will cause an inflammatory response of the urothelium. Urinary tract infection is as a result of the presence of these bacteria in the urinary tract. The pathogen, part of urinary tract infected, extent of infection and competence of host’s immune system are the factors that determine the clinical symptoms seen in different UTI cases (Geoffrey *et al*., 2013).

The large number of species that belong to the family Enterobacteriaceae has contributed more diagnostic problems and clinical complications in UTIs. Genes that are responsible for producing ESBLs are usually located on plasmids, and these genes can also carry resistance determinants for fluoroquinolones, aminoglycosides, chloramphenicol tetracyclines and even cotrimoxizole. This has made the microorganisms to be able to resist a broad spectrum of antibiotics (Aruna and Mobashshera, 2012).

A great percentage of ESBLs are plasmid mediated Beta-lactamases that can efficiently hydrolyze cephalosporins and monobactams. Never the less, ESBLs can be inhibited by Beta-lactamase inhibitors like the clavulanic acid. Detection of ESBLs was first observed in *Enterobacteriaceae*. Today, various groups of ESBLs are produced by these microorganisms, but the most common ESBLs are the CTX-M and SHV enzyme types (Aggeliki *et al*., 2014).

The plasmids that code for these ESBLs are on their own resistant to some classes of antibiotics. This has posed a big problem in the therapy against these microorganisms (Reuland *et al*., 2012).

The potency of B-lactam antibiotics are tempered with when B- lactatmases enter a covalent bond with the carbonyl C=O functional group of the B- lactam ring as contained in the antibiotics. This will then cause hydrolysis in the amide bond of the B- lactam ring (Medeiros 1997).

Several outbreaks of infections with the ESBL producing bacteria have occurred across globe over the past few years (Shobha *et al.*, 2009).

This research was carried out to reveal the faecal carriage of ESBL-producing enterobacteria in Northern Cyprus, and also to uncover some possible risk factors associated with the carriage.

**CHAPTER TWO**

**2.1 ENTEROBACTERIACEAE**

Enterobacteriaceae consists of a group of gram negative bacteria (Okoche *et al*., 2015). This group of microorganisms have a worldwide distribution as they can be found in soil, fruits, water, plants animals and also in man (Ibrahim and Hameed, 2015). Some members of this group of microorganisms are normal flora of the human gut (Okoche *et al*., 2015). Enterobacteriaceae are a group of gram negative bacilli with average length of 1-3 micro meters. Enterobacteriaceae consist of 44 genera of bacteria and approximately 176 known species (Ibrahim and Hameed, 2015).

The Enterobacteriaceae is a large heterogeneous family of gram negative bacilli and some typical bacteria in this group include: *Escherichia coli*, *Klebsiella* spp*.,* *Citrobacter* spp., *Enterobacter aerogenes*, *Erwinia* spp., *Proteus* spp., *Salmonella*, *Serratia* spp., *Shigella*, *Vibrio* spp., *Edwardsiella* spp. Obligate pathogens of the Enterobacteriaceae include: *Yersinia enterocolkitica*, *Shigella,* *Salmonella* and *Vibrio Cholera* as well *Vibrio parahaemolyticus*. *Proteus* and *Klebsiella* which inhabit mainly the urinogenital and respiratory tracts are opportunistic pathogens. *Escherichia coli* and *Enterobacter aerogenes* are normal microbiota, becoming pathogenic under very exceptional circumstances (Dauda, 2010).

80% of clinically significant isolates of gram negative bacilli can be attributed to Enterobacteriaceae (Ibrahim and Hameed, 2015).

Enterobacteriaceae are facultative anaerobes which are Catalase-positive, Oxidase- negative and grow well on MacConkey agar.

A large number of the Enterobacteriaceae are pathogenic and cause gastrointestinal infections, meningitis, peritonitis, pneumonia, septicaemia and urinary tract infections in humans. Enterobacteriaceae are able to acquire and transfer resistance genes easily through plasmids and transposons. These genes will then cause these microorganisms to produce B-lactamases (Paterson, 2006).

On the basis of lactose fermentation, the Enterobacteriaceae family is divided into two groups as follows: lactose fermenters and non lactose fermenters.

The lactose fermenters can be further subdivided into two: early lactose fermenters such as *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp.; late lactose fermenters, such as *Citrobacter* spp. and *Serratia* spp.

The non lactose fermenters include *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Proteus* spp., *Edwardsiellatarda*, *Hafnia*, *Morganella morganii*, *Providencia* spp. The lactose fermenting Enterobacteriaceae are termed intestinal coliforms and are always used alongside other non intestinal coliforms as indicators of contamination in drinking water and pose a big risk to human health. Some of these indicators include coliforms such as *Escherichia coli*, enterococci, *Clostridium perfringens*, and *Pseudomonas aeruginosa.* Contamination of fruits and vegetable is often as a result of application of organic fertilizers and irrigation water (Ibrahim and Hameed, 2015).

**2.1.1 Antigenic Structure of Enterobacteriaceae**

The antigenic structure of the Enterobacteriaceae is a complex one. The antigenic structure is subdivided into three groups:

* 150 different heat-stable somatic O antigens (Lipopolysaccharides)
* More than 100 heat-stable K antigens (Capsular antigen)
* And more than 50 H antigens (Flagella antigen).

The most external parts of the cell wall are where the O antigens are located. The O antigen is somatic; it consists of layers of polysaccharides with distinctive sugars. O antigens have been linked with specific human disease in a number of cases. Example of such is the specific O type antigen of *E. coli* in diarrhoea and urinary tract infections (Dauda, 2010).

K antigens are capsular. K antigens have been linked with upper urinary tract infections in many cases, and antibody to the K antigen mediate some level of protection in experimental infections (Todar, 2004). Some K antigens are polysaccharides including the K antigens to *E. coli* and someothers are proteins (Dauda, 2010). These k antigens either polysaccharides or proteins, may be able to induce bacterial virulence by lowering the ability of antibodies and also decrease ability of complement to bind to the bacterial surface. K antigens also are able to cause inability of phagocytes to recognize and engulf the bacterial cells.  The best studied K antigen, K-1. This is made up of a polymer of N-acetyl neuraminic acid (sialic acid) (Todar, 2004). *E. coli* strains producing K-1 antigens are always identified in neonatal meningitis. K antigens of *E. coli* cause attachment of the bacteria to epithelial cells prior to gastro-intestinal or urinary tract invasion. H antigens are common in enterobacteria that are mobile as they are located on the flegalla. H antigens are heat labile and can be removed by alcohol; they contain flagella protein called flagellins. These flagellins are able to cause agglutinatination of IgG12 (Dauda, 2014).

**2.2 Some Enterobacteriaceae of Clinical Importance**

***Escherichia coli***

*Escherichia coli* are Gram negative, non spore forming bacilli. They are approximately 0.5 µm in diameter and 1.0–3.0 µm in length. Within the periplasm is a single layer of Peptidoglycan. Escherichia coli are commonly motile in liquid by means of peritrichous flagella. *Escherichia coli* is one of the well known colonizers of the human gastrointestinal tract, *E. coli* appears soon after birth and persists for a long time in the gastrointestinal tract. On a larger scale E. coli are considered as commensals in the intestinal tract of humans, but also some isolates have been identified to be pathogenic (Moriel *et al*., 2010).

*E. coli* is the most elaborate specie among the facultative anaerobic bacteria that make up the intestinal normal flora of humans, and plays an important role in maintaining a balance in the functions and activities of the intestinal organs. Theobald Escherich, a German paediatrician was the first to describe the organism. He classified it with the name “Bacterium coli commune” He further explained *E. coli* to be a short, plump rod that had initially been isolated from normal infant faeces and regarded them to be harmless saprophytes. And for more than fifty years *E. coli* was regarded as the predominant commensal in faeces which was non pathogenic. In subsequent times this idea changed as there have been substantial evidences acknowledging that *E. coli* has the ability to cause disease in man. To cause infections in man, *E. coli* may colonize the mucosal surface of intestinal organs or can spread throughout the body (Deborah and Frankel, 2005). Many strains of *E. coli* are considered harmless and have also shown to provide some advantages to their host by preventing the gut of their host from being colonized by other pathogenic microorganisms. But with time, some strains of *E. coli* have been found to be pathogenic and can cause a wide spectrum disease such as severe diarrhoea (Rivas *et al*., 2015).

*E. coli* has been identified in numerous cases urinary tract infection. *E. coli* have also been implicated in many pathological cases in farm animals, especially in young animals. The pathogenic *E. coli* tends to be easily ingested with food, and this reason has made the human gastro-intestinal tract is vulnerable to diarrhoeagenic *E. coli* infections. Different strains of *E. coli* pathogens have been associated with some diarrhoeal illness. Diarrhoea is a major public health problem globally, and statistics have it that there are over two million deaths occurring each year and as such there is an urgent need to carry out intensive studies and research to reveal patterns and mechanisms of Pathogenic *E. coli* (Deborah and Frankel, 2005).

The pathotypes of *E. coli* that cause intestinal diseases have been divided into

Six groups: Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), and Diffusely adherent *E. coli* (DAEC) (Hussain, 2015).

**Enteropathogenic *E. coli* (EPEC)**

In the 1940s and 1950s EPEC was the number one strain of *E. coli* implicated in infantile diarrhoea outbreaks (Deborah and Frankel, 2005), and also as a common cause of infantile diarrhoea in developing countries. EPEC is non-invasive and non-toxigenic, it causes diarrhoea by disrupting the absorption-secretion balance in the small intestine. Symptoms include malaise, vomiting and diarrhoea, other symptoms that may occur include. EPEC affects mostly children below the age of one and is not commonly associated with foods. EPEC infection will start by first colonizing the epithelial linings of the intestine and then cause effacement and lesions in the intestinal epithelium (Hussain, 2015).

Diarrhoea associated with EPEC was referred to as “summer diarrhoea” and developed countries suffered frequent outbreaks of this summer diarrhoea with a high mortality before the 1950s. For some reasons still not known EPEC strains are no longer important causative agents of infant diarrhoea in developed countries in terms of outbreaks. Never the less, EPEC is still the causative agent of Infant diarrhoea in paediatric wards and day care centres in developed countries. In developing countries, EPEC remains a major cause of infant diarrhoea, with recent outbreaks having mortality rate as high as 30%. Recent studies in countries like in Brazil, Mexico, South Africa and

Bangladesh all indicated that 30–40% of infant diarrhoea is due to EPEC infection and is estimated to cause the deaths of several hundred thousand children annually (Deborah and Frankel, 2005).

Persistent diarrhoea caused by EPEC is most times polymicrobial as it is commonly associated with parasitic infections such as *Cryptosporidium* and *Giardia* (Ochoa and Contreras, 2011).

EPEC causes watery diarrhoea, and in rare cases they produce bloody diarrhoea. Pathogenesis of EPEC is aided by EPEC adherence factor (EAF) which is a plasmid-encoded protein, and intimin (outer membrane protein). The EAF helps to localize adherence of bacteria to intestinal epithelium and the Intimin which is a non fimbrial adhesin is responsible for the final stages of adherence (Todar, 2004).

**Enterohaemorrhagic *E. coli* (EHEC)**

EHEC is a human pathogenic *E. coli* that is able to cause haemorrhagic colitis (bloody diarrhoea), which sometimes develops into haemolytic uraemic syndrome (HUS). HUS is a life threatening disease that causes kidney damage and is a severe complication of EHEC infection. EHEC belongs to the Shiga toxin producing *E. coli* (STEC). The STEC are also known as Verocyto toxin-producing *E. coli* (VTEC). Animals can carry other types of STEC/VTEC in their intestines that are not necessarily pathogenic for humans. These bacteria have accounted for various food borne infections over recent decades. The production of Shiga toxin (Shiga toxin 1 [Stx1] and/or Stx2) coupled with other virulence factors is what gives EHEC Pathogenicity. EHEC strains posse genes in which they use to develop characteristics used for attachment (Bohnlein *et al*., 2015).

Transmission of EHEC is through the faecal oral route. EHEC can spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. Birds and flies are potential vectors. In one experiment, EHEC O157:H7 was transmitted in aerosols when the distance between pigs was at least 10 feet. The organism was thought to have become aerosolized during high pressure washing of pens, but normal feeding and rooting behaviour may have also contributed. Humans become infected with EHEC O157:H7 mainly by ingesting contaminated food and water, or during contact with especially ruminant animals, faeces and contaminated soil. The infectious dose for humans is estimated to be from 10 to less than 100 organisms. EHEC O157:H7 has been implicated in food borne outbreaks involving undercooked meat, especially ground beef, but also other meats and sausages or unpasteurized milk and cheese. Some other outbreaks have attributed to ingestion of contaminated vegetables, unpasteurized cider, nuts and even pickled vegetables. Contaminated irrigation water is an important source of EHEC O157:H7 on vegetables (Kintz *et al*., 2017).

**Enteroinvasive *E. coli* (EIEC)**

EIEC are a group of *E. coli* are diarrhoeagenic. EIEC is related to *Shigella* spp. biochemically, genetically, and pathogenetically as it is known to cause shigellosis-like symptoms in both adults and children (Vieira, 2007). Both EIEC and *Shigella* have invasive plasmids which help to invade host tissues (Hussain, 2015). Researchers have given more attention to other pathogenic *E. coli* such as EPEC, EHEC, and ETEC, as well as *Shigella*. A major reason to the lack of attention to the epidemiology of EIEC is that it is often observed to be an inconsistent cause of diarrhoea as when compared to other diarrhoea causing *E. coli* (Vieira, 2007).

EIEC have the ability to cause dysentery (Bloody and mucoid diarrhoea) using the same method of invasion as Shigella does. EIEC invades the epithelial cells of the colon and then grows in these cells damaging the intestine by causing inflammation and ulceration (Van den Beld, 2012).

**Enterotoxigenic *E. coli* (ETEC)**

ETEC is the most common cause of travellers’ diarrhoea, the most frequent health problem among travellers visiting developing countries. With reference to the six diarrhoeagenic groups of *E. coli,* the most common is ETEC and most especially in the developing countries. The Pathogenicity of ETEC is determined by specific virulence factors such as Enterotoxins (which may be heat labile and/or heat stable) and other colonization factors. This differentiates ETEC from the rest of the diarrhoeagenic *E. coli*. The colonization factors allow ETEC to effectively adhere and colonize the small intestine and then cause diarrhoea. ETEC are also lactose fermenters (Qadri *et al*., 2005).

Transmission of ETEC is by the faecal oral route. Bacteria colonize the intestinal epithelia through the help of the Pili (fimbriae). Cytotonic enterotoxins secreted by ETEC are encoded on plasmids or bacteriophage DNA and these toxins induce watery diarrhoea, while cytotoxic enterotoxins which are also plasmid or Bacteriophage encoded induce tissue damage. The Plasmid encoded invasion factors permit invasion of the mucosa (Evans and Evans, 1996).

**Enteroaggregative *E. coli* (EAEC)**

Nataro *et al*. (1987) was the first describe EAEC in a child with acute diarrhoea in Lima, Peru. A number of previous studies in some developing countries have shown reasonable link between EAEC and diarrhoea, most especially persistent diarrhoea (Weintraub, 2007). This is observed mostly in children and adults, and has been identified as the cause of several outbreaks worldwide. EAEC is termed so because its characteristic “stacked brick” aggregative pattern of adherence to Human epithelial type 2 (HEp-2) cells. In addition, several possible EAEC virulence factors have been reported. However, the pathogenic mechanisms of EAEC infection are not fully understood (Abbasi *et al*., 2015)

The pathogenesis of EAEC is determined by the following factors which include adherence to intestinal cells, produce enterotoxins and cytotoxins, and induce inflammation. EAEC are able to aggregate intimately with each other, to human HEp-2 cells, and also attach to abiotic surfaces when grown in vitro (Okhuysen and DuPont, 2010).

**Diffusely adhering *E. coli* (DAEC)**

DAEC is regarded as a group of diarrhoeagenic *E. coli* strains that adhere to HEp-2 and HeLa cells in a distinctive manner that allow bacteria attach themselves evenly to the entire surface of the cell. DAEC is also regarded as a cause to diarrhoea in children. DAEC consist of a heterogeneous group of *E. coli*, with different enteropathogenicity, and these differences are factors that make strains of DAEC differ from one to another (Patzi-Vargas *et al*., 2013).

Some strains of DAEC have been discovered to cause bloody diarrhoea and this has supported the fact that the DAEC is a heterogeneous group of *E. coli* (Ochoa *et al*., 2009).

***Klebsiella***

*Klebsiella* spp. are ubiquitous in nature. *Klebsiella* can be found in the environment, such as surface water, sewage, and soil and on plants. They can also be found on mucosal surfaces of mammals such as humans and animals which they colonize. The most clinically important specie is *Klebsiella pneumoniae.*

*Klebsiella pneumoniae* is one of the Enterobacteriaceae, it is gram negative, rod shape, lactose fermenting bacillus and has a capsule.

*K. pneumoniae* is an opportunistic pathogen that can be carried asymptomatically by healthy individuals in the intestinal tract, skin, nose, and throat of such individuals (Holt *et al*., 2015). Opportunistic *K. pneumoniae* mostly affects those that are immunocompromised or who are weakened by underlying infections (li *et al*., 2014). K. pneumoniae is also able to cause a number of infections in hospitalized patients, most commonly pneumonia, wound, soft tissue, or urinary tract infections. K. pneumoniae is among the leading causes of hospital-acquired (Nosocomial) infections and is also known to be a leading cause of neonatal sepsis worldwide. *K. Pneumoniae* also causes some serious community-acquired infections, including pneumonia, meningitis and pyogenic liver abscess (Holt *et al*., 2015). There is always the colonization of the gastrointestinal tract by opportunistic *K. pneumoniae* before the development of nosocomial infections, and *K. pneumoniae* can also colonize the urinary tract, respiratory tract and blood. When *K. pneumoniae* form biofilm on medical devices like catheters and endotracheal tubes, they provide a significant source of infection in patients that are catheterized with such catheters. Nosocomial infections caused by *K. pneumoniae* most of the time seems to be chronic because *K. pneumoniae* biofilms formed *in vivo* protect the pathogen from the host immune responses and antibiotics. Another reason for chronic infection with K. Peumoniae is that nosocomial isolates of *K. pneumoniae* usually display multidrug resistance phenotypes. This multidrug resistance is attributed to the presence of Extended Spectrum B-lactamases (ESBLs) or Carbapenemases and these enzymes will make it difficult to choose appropriate antibiotics for treatment. The capsule polysaccharide is recognized as the most important virulence factor of *K. Pneumoniae.* Hypervirulent *K. Pneumoniae* has increased production of capsule polysaccharide and this makes it much more invasive such that it can affect previously healthy persons, and cause life threatening community acquired infections, such as severe pneumonia pyogenic liver abscess, meningitis, necrotizing fasciitis and endophthalmitis (li *et al*., 2014).

***Salmonella***

The genus *Salmonella* was named so to honour Daniel E. Salmon, an American veterinarian, who was the first scientist to isolate the bacterium in 1885. Theobald Smith, a research assistant to Salmon was also part of the work. *Salmonella* spp. are rod shaped, gram negative, facultative anaerobes and posses peritrichous flagella. Productions of hydrogen sulfide, the use of tetrathionate as a terminal electron acceptor, are key factors that distinguish *Salmonella* from members of other genera of the Enterobacteriaceae family (Sterzenbach *et al*., 2013). Most of them produce hydrogen sulphide gas in Triple Sugar Iron agar (Dauda, 2014).

An infectious process can only begin after living *Salmonella* reach the gastrointestinal tract. Some might be killed in the stomach by the host defence systems, while the surviving *Salmonella* enter the small intestine and multiply in tissues forming localized infection. After the incubation period, endotoxins are released from the dead *Salmonella* and cause enteritis and gastrointestinal disorder. Some *Salmonella* species can proceed to cause systemic infections as they can pass through the lymphatic system of the intestine into the blood of the patients (typhoid form) and are carried to various organs like liver, spleen, kidneys *(*[*https://www.salmonella360.com/content/files/439/Tech*](https://www.salmonella360.com/content/files/439/Tech)*)*. Typhoid fever is caused by the strictly human pathogenic *Salmonella* *enterica* serotype Typhi (Raffatellu *et al*., 2008).

***Shigella***

*Shigella* is Gram-negative, non-motile bacilli belonging to the family

Enterobacteriacae. The genus Shigella includes four species: *S. dysenteriae*, *S. flexneri, S. boydii* and *S. sonnei*, also designated groups A, B, C and D, respectively. Infections caused by *Shigella* species have been implicated as one of the important causes of diarrhoeal diseases in developing and developed countries. Transmission of *Shigella species* is primarily through ingestion of contaminated food or contaminated water. Person to person contact is also a means of transmission, vectors such as flies especially *Musca domestica* also play an important role in the spread of these microorganisms. *S. dysenteriae* and *S. flexneri* are the predominant species in tropical countries, while *S. sonnei* is predominant in developed countries (Temu *et al*., 2007).

Diarrhoea infections caused by *Shigella* is termed Shigellosis and all species of *Shigella* cause acute bloody diarrhoea and this is a result of these organisms being able to invade colonic epithelium and cause patchy damages to the colon. This will then lead to the formation of ulcers and inflammatory exudates. Inflammatory cells (polymorphonuclear leucocytes, PMNs) and blood will then appear in stool. Shigella infection is a major public health problem in developing countries where sanitation is poor. Humans are the natural reservoir, although other primates may be infected. No natural food products harbour endogenous Shigella species, but a wide variety of foods may be contaminated. Shigellosis is characterized by passage of small liquid stools that contain visible blood, with or without mucus, abdominal cramps and tenesmus are common. Fever and anorexia are also common, but are not specific (WHO, 2005).

*Shigella dysenteriae* serotype 1 and Shiga toxin producing *Escherichia coli* (STEC) produce Shiga toxins which is cytotoxin. Although Shiga toxins have been associated with STEC and *Shigella dysenteriae* 1, recent studies have shown shiga toxins in *Sigella dysenteriae* serotype 4 and *Shigella flexneri.* These shiga toxins are encoded on large plasmids and for S dysenteriae 1 shiga toxin is not essential for virulence of but contributes to the severity of dysentery (Lamba *et al*., 2016).

***Yersinia***

This is a genara of bacteria that are pleomorphic, gram negative rods, Catalase positive, oxidase negative and microaerophilic or facultative anaerobes. Animals are mostly the reservoir, but can induce severe human diseases. Yersinia consists of 17 known species (Savin *et al*., 2014)., of which Yersinia pestis which causes plague, Yersinia pseudotuberculosis and Yersinia enterocolitica which cause human diarrhoeal diseases have been identified as the species that are potential pathogens. *Yersinia* spp. are able to grow at low temperatures, and as such, even refrigerated foods are potential vehicles for growth and transmission of these organisms (Cocolin and Comi, 2005).

Yersiniosis is diarrhoeal disease caused by mostly *Y. enterocolitica* and rarely by *Y. pseudoturberculosis* which mostly cause gastroenteritis and pseudoappendisitis. The major source of yersiniosis is swine, but in recent times isolates of *Y. enterocolitica* were reported from contaminated chicken, milk, tofu, and water. Y. enterocolitica produces a heat labile enterotoxins but the role of this toxin in diarrhoeal related infections is not fully understood. Colonization of the intestinal tract will lead to ulceration and leukocytes appear in faeces, symptoms include fever, abdominal pain and diarrhoea (ranging from watery to bloody). In some few cases, the bacterium enters the lymphatic system causing bacteremia (Galindo *et al*., 2011).

Other members of the Enterobacteriaceae such as *Enterobacter, Citrobacter, Proteus* and *Serratia* are also of clinical importance as they can cause opportunistic infections in humans.

**2.2 Antibiotics**

The word antibiotic was formed from the word “antibiosis” which simply means “against life”. Antibiotic is usually defined as a chemical produced by one microorganism that is capable of killing or inhibiting the growth of other microorganisms. Antibiotics are drugs which are used in treating bacterial infections (Etebu and Arikekpar, 2016).

Antibiotics are the most commonly used antimicrobial agents in the treatment of bacterial infections, as well as the most abused antimicrobial agents globally. Antibiotics have been in used for more than half a century and have been saving human lives ever since the antibiotic golden age and post-antibiotic golden age. The emergence of the antibiotics brought about a positive change in the treatment of infections that used to claim the lives of millions of people before the antibiotic golden age. *Streptomyces*, *Penicilliums*, *Actinomycetes* and *Bacilli* are the major sources of antibiotics (Bbosa *et al*., 2014).

The antibiotics work in two ways. The first is by inhibition of microbial multiplication known as bacteriostatic effect. The second is by killing the microbial population known as bactericidal effect. The characteristic feature of an antibiotic for prescription is taken into the account in such a way that it should be selective target, bactericidal, narrow spectrum so that it does not kill the normal flora, minimum adverse effects, various route of administration, good absorption and emergence of resistance is low (Bernatova *et al*., 2013).

**2.2.1 Emergence of Antibiotics**

Antibiotics are one of the most successful forms of chemotherapy in the history of medicine. Antibiotics have saved so many lives around the world and have significantly added to the control of infectious diseases (Aminov, 2010). There is a common belief that has confined the exposure to antibiotics only to this modern day “antibiotic era,” However, several researches have been conducted and traces of antimicrobials have been discovered in skeletal remains of people who died a very long time ago. One of such is traces of tetracycline found in human skeletal remains from ancient Sudanese Nubia which dated as far back 350–550 CE (Bassett *et al*., 1980). Indeed these traces of tetracycline in the skeletal remains of these ancient people can be attributed to presence of tetracycline-containing materials included in the diet of these people (Aminov, 2010).

In 1910 Paul Ehrlich developed the first antimicrobial salvarsan for the treatment of syphilis, a disease that was almost incurable back then

A very remarkable event in the history of antibiotics is that of September 3, 1928 that led to the penicillin discovery by Fleming (1929) while he was conducting involving *staphylococcus* variants. He left some *Staphylococcus* culture plates on the bench which he observed frequently, and in the process the plates became contaminated. A large colony of a contaminating was found around *Staphylococcus* colonies as the *Staphylococcus* colonies became transparent and were obviously undergoing lysis. Further research was conducted on the mould subcultures and was identified as *Penicillium*, and that it had bactericidal and bacteriolytic properties (Aminov, 2010).

Between the 1950s and 1970s was described as the golden era (Golden Age) of discovery of novel antibiotics classes, and no new classes of antibiotics have been discovered since then (Chopra *et al*., 2002). Ever since the “Golden Age” many newer antibiotics and antibacterial agents have been semi synthetically or synthetically produced based on chemical modifications of pre-existing antibiotics (Bbosa *et al*., 2014). To solve the present day problems of antibiotic resistance has based on modification of existing antibiotics to produce different generations with improved efficacy and broad spectrum of activity (Aminov, 2010; Bbosa *et al*., 2014).

Ever since the antibiotics era in the early 1900s, antibiotics were grouped into three with reference to how they are being produced on a large scale. These groups include; Natural antibiotics which are compounds manufactured directly by large-scale fermentation of bacteria or fungi, examples include benzylepenicillin and gentamycine. Semi synthetic antibiotics are chemically-altered natural compounds and the natural compounds are used as the starting material, some examples include ampicillin and amilkacin. Synthetic antibiotics are compounds that are chemically designed in the laboratories. This is achieved by adopting a full synthetic route in the cause manufacturing. Examples of synthetic antibiotics include moxifloxacin and norfloxacin (Wright *et al*., 2014).

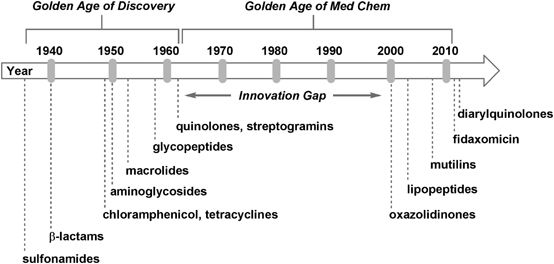


Figure : Emergence of antibiotics (Walsh, C. T., & Wencewicz, T. A. (2014). Prospects for new antibiotics: a molecule-centered perspective. Journal of Antibiotics, 67(1), 7).

**2.3 Classification of Antibiotics based on Mechanism of Action**

There are numerous ways to classify antibiotics but the most common classifications depend on their mechanism of action, molecular structures and spectrum of activity (Calderon and Sabundayo, 2007). Antibiotics that are in the same structural class tend to express similar pattern in terms of their effectiveness, toxicity and even side effects (Etebu and Arikekpar, 2016).

Based on mechanism of action we can group antibiotics into four groups:

1. **Inhibitors of cell wall synthesis:** The various classes of antibiotics which come under this category are the B-lactam antibiotics (penicillins, cephalosporins, carbapenems, monobactams). Others are vacomycin and bacitracin.

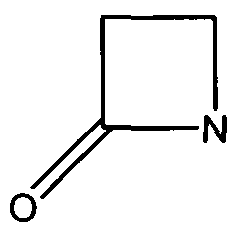
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Figure 2 B-lactam ring (Hasan, T., Sallum, U. W., & Verma, S. (2009). U.S. Patent Application No. 12/990,997).

**Penicillins**

Just like other B-lactams, penicillins inhibit synthesis of bacterial cell wall. Generally, penicillins are bactericidal and they do this by binding to the penicillin-binding proteins (PBP) which are the enzymes responsible for connecting different layers of peptidoglycan located within the cytoplasmic membrane of the cell wall. Once bound, penicillins can have various effects leading to cell death, but first they inhibit the cross-linking of the peptide chains and halt the development of normal peptidoglycan structure (Calderon and Sabundayo, 2007). Penicillins are a large family of antibiotics, different types of penicillins include Penicillin G, Penicillin V, Oxacillin (dicloxacillin), Methicillin, Nafcillin, Ampicillin, Amoxicillin, Carbenicilin, Piperacillin, Mezlocillin and Ticarcillin. Alexander Flemming was the first to discover Penicillin G from the fungus *Penicilliun notatum.* However, only gram positive bacteria (*streptococci*) and some gram negative bacteria such as *Treponema pallidum* and *meningococci* are susceptible to Penicillin G which show that Penicillin G has a narrow spectrum of activity (Etebu and Arikekpar, 2016).

**Cephalosporins**

These are similar to penicillin in their structure and mode of action (Etebu and Arikekpar, 2016). Cephalosporins are also bactericidal (Calderon and Sabundayo, 2007). First generation cepahlosoprins have a narrow spectrum against gram positive bacteria e.g. cefazolin; second generation cephalosporins have a better gram negative bacteria coverage e.g. cefuroxime; third generation cephalosporins are much active against Enterobacteriaceae and *Psuedomonas aeruginosa* e.g. ceftriaxone and the fourth generation cephalosporins have a broad spectrum against Enterobacteriaceae and *Psuedomonas* *aeruginosa* e.g. cefepime. Cephalosporins are known to have a range of side chains that give them the enablement to attach to the different penicillin-binding proteins (PBPs), this helps cephalosporins to resist breakdown by penicillinase producing bacterial strains and these side chains also help to facilitate entry into gram negative bacterial cells (Etebu and Arikekpar, 2016).

**Carbapenems**

The chemical structure of carbapenem is similar to that of penicillin. They are B-lactam antibiotics with wide spectrum against gram negative and gram positive bacteria, except, MRSA. They have excellent activity against both aerobic and anaerobic gram-positive and gram-negative bacteria. The carbapenems include imipenem, meropenem, and ertapenem and all show a wide spectrum and very good activity to most gram negative bacteria, including nosocomial pathogens such as *Psuedomonas aeruginosa*, except Ertapenem which do not have successful activity against *Psuedomonas aeruginosa.* Carbapenems are often used for treatment of mixed bacterial infections (Calderon and Sabundayo, 2007). A disturbing issue globally is that resistance to carbapenems have emerged and has been recorded in many cases (Etebu and Arikekpar, 2016).

**Monobactams**

They are monocyclic beta-lactam antibiotics. Their chemical structure is made up of the four beta-lactam ring and a side chain unlike Penicillin and cephalosporins which have five and six respectively. The spectrum activity of monobactams is limited to only gram negative bacteria and thus used in combination with other antimicrobials for empiric therapy (Calderon and Sabundayo, 2007).

Other non B-lactam antibiotics that also inhibit cell wall synthesis include vancomycin and bacitracin.

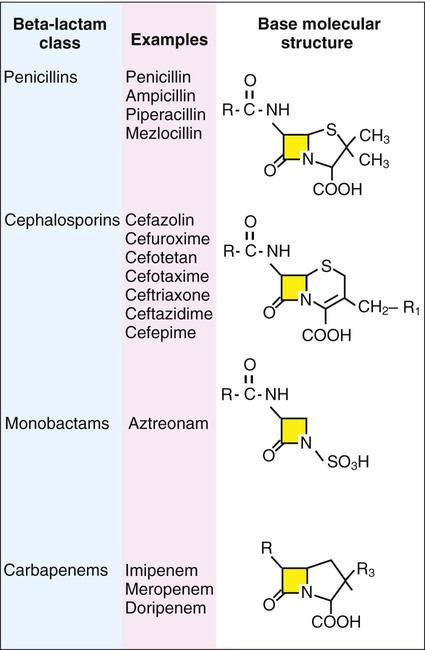
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Figure base structures of B-lactam antibiotic with B-lactam ring highlighted in each structure (Wilson, B. A., Salyers, A. A., Whitt, D. D., and Winkler, M. E. (2011). Bacterial pathogenesis: a molecular approach (No. Ed. 3). American Society for Microbiology (A

**Vancomycin**

Vancomycin adopts a different pattern in inhibiting the cell wall synthesis. It primarily stops the formation of peptidoglycan layers. It prevents addition of subsequent cross-linking by transpeptidation to the existing peptidoglycan layer by binding to D-alanyl-D-alanine residues of the bacterial cell wall (Courvalin, 2006).

**Bacitracin**

Bacitracin is a widely used antibiotic produced by *Bacillus subtilis* and *Bacillus licheniformis* with a potent bactericidal activity directed primarily against Gram positive organisms. Bacitracin prevents cell wall synthesis by preventing the transport of cell wall precursors to the developing cell wall. It does this by binding to a lipid pyrophosphate which transports these precursors, and binding to this molecule denies it the ability to accept cell wall precursors. Bacitracin has spectrum of activity against gram positive cocci, few gram negative organisms and also the anaerobe *Clostridium defficile* (Husain, 2004).

1. **Inhibitors of Protein synthesis**

Protein synthesis is a complex process which deals with building amino acids to form specific protein and this takes place in the ribosome. The ribosome is divided into two sub units based on their coefficients of sedimentations. The prokaryotic ribosome is designated 30s and 50s sub units. Antibiotics in this class are bacteriostatic while some bactericidal (Etebu and Arikekpar, 2016). Antibiotics in this class include:

**Tetracyclins**

They include tetracycline, doxycyclineand minocycline and are effective against *Chlamydia, Mycoplasma,* and *Rickettsia* species. They are employed in treatment against malaria, elephantiasis, amoebic parasites and rickettisia, but cause teeth discolouration if administered to children below the age of eight. They bind to the 30S ribosomal subunit thereby preventing the binding of tRNA to the mRNA ribosomal complex (Calderon and Sabundayo, 2007).

**Aminoglycosides**

They have a broad spectrum of activityagainst aerobic gram negative rods and some gram positive bacteria. Streptomycin happens to be the oldest aminoglycoside, it is gotten from soil *Streptomyces* and is effective in treating bubonic plague, tularemia and tuberculosis. Other aminoglycosides areGentamicin, Neomycin, Tobramycin and Amikacin. Gentamicin being less toxic has gotten much more attention in treating infections caused by gram negative rods (*Escherichia, Pseudomonas, Shigella and Salmonella*). Tobramycin, specifically, is employed in cases of Pseudomonas infections in cystic fibrosis patients. Aminoglycosides also carry out their action against bacteria by binding to th 30S subunit of the ribosome to halt protein synthesis (Etebu and Arikekpar, 2016; Talaro and Chess, 2008).

Other drugs that inhibit bacterial protein synthesis by acting on the 30S ribosomal subunit include Chloramphenicol, Macrolides, Ketolide, Clindamycin, Linezolid, Streptogramins, Retapamulin (Calderon and Sabundayo, 2007; Talaro and Chess, 2008).

1. **Inhibitors of Nucleic acid Synthesis**

Some group of antibiotics will inhibit synthesis of DNA (quinolones: spectrum-nalidixic acid, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin), while others will inhibit synthesis of RNA (rifamicins). Quinolones block DNA synthesis by inhibiting two bacterial enzymes Topoisomerase II (DNA gyrase) and Topoisomerase IV. Inhibition of the DNA gyrase will prevent unwinding of double stranded DNA into a single stranded structure, which is needed for replication and also mRNA synthesis. Inhibition of topoisomerase IV interferes with separation of replicated chromosomal DNA into daughter cell during cell division. Rifampin inhibits synthesis of mRNA by binding to DNA-dependent RNA polymerase (Adegboye *et al*., 2012; Calderon and Sabundayo, 2007).

1. **Inhibitors of membrane function**

Some Peptide antibiotics ( Polymyxin B or E) are able to bind to lipid A, the anchor for lipopolysaccharide in gram negative bacteria. Polymyxins accumulates in the outer membrane, then penetrates into the inner membrane and finally into the cytoplasm. This process distorts the cell membrane and cause leakage of proteins and nitrogen bases, leading to cell death. These drugs have narrow spectrum of activity against gram negative bacteria (Epand *et al*., 2016).

**2.3.1 Antibiotic Resistance**

Previous studies and research has have shown that antibiotic resistance for different antibiotics tend to develop in a relatively short space of time after the production of the antibiotics. In short while after Alexander Flemming discovered penicillin, he was able to discover some *staphylococci* that were resistant to it. Flemming foresaw and predicted that indiscrete use of antibiotics would subsequently result to clinical failures in the future. Not until this discovery, all *staphylococci* were considered to be sensitive to penicillin and many gram negative organisms were known to be naturally resistant (Iyalomhe *et al*., 2011). Usually, antibiotic resistance occurs within a few years after the introduction of that particular antibiotic. This is not amazing because most of the modern day antibiotics are products of microorganisms either directly or indirectly (Iredell *et al*., 2016).

Bacteria use the use antibiotic resistance features as a fitness trait which are developed to survive in whatever environment they find themselves, and this has added to the account of microbial existence in any part of the earth regardless of the unfavourable conditions. It is possible for bacteria to have intrinsic resistance that protect them from a particular antibiotic, bacteria can also acquire resistance through chromosomal mutation or acquisition of genetic materials from other bacteria either through vertical or horizontal transfer of genes (Adegboye *et al*., 2012). Globally, there is a general increase in the number of resistant microorganisms, although the patterns of resistance vary across countries. In a previous study, it showed that there has been a substantial increase across Europe in the percentages of *Klebsiella pneumoniae* resistant to fluoroquinolones, third-generation cephalosporins, and aminoglycosides, and also a significant combined resistance to all three antibiotic groups. Significant increase of *Escherichia coli* resistance to third-generation cephalosporins has also being recorded from 9.6 % to 12.0 % between 2011 and 2014 (population-weighted European Union/European Economic Area (EU/EEA) mean percentage of resistance). Another case of global concern is the high level prevalence of Carbapenem-resistant Enterobacteriaceae (CRE), particularly in *K. pneumoniae* which has presented infections almost impossible to treat. Greece, Italy, and Republic of Malta in Europe, the USA, South America, and Asia have significantly been affected by these bacteria. Such is the grade of scourge that the US Centres for Disease Control and Prevention has identified CRE as one of the top three in the cases of most urgent antimicrobial resistant challenges (Karam *et al*., 2016).

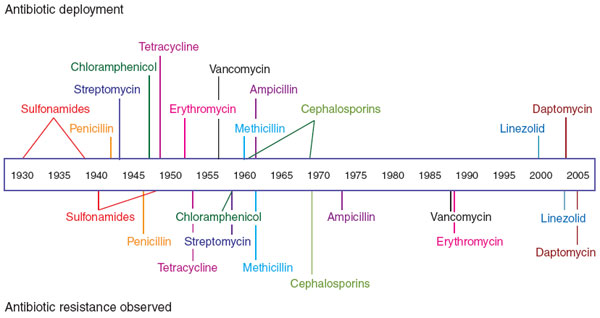


Figure 4 development of antibiotics and antibiotic resistance (Nestle, M. Currently browsing posts about: FDA. Looking ahead, 2011).

**2.3.2 Antibiotic resistance in Enterobacteriaceae**

Gram negative bacteria belonging to the Enterobacteriaceae family are important causes of urinary tract infections (UTIs), hospital and healthcare associated pneumonia, bloodstream infections and various intra abdominal infections. Some very important members of this family include *Escherichia coli* whichare a frequent cause of UTIs, *Klebsiella* sppand *Enterobacter* spp are important causes of pneumonia. So many members of the Enterobacteriaceae have been involved in bloodstream infections and in peritonitis, cholangitis, and other intra abdominal infections. *Salmonella* is also known to produce gastroenteritis, and in some patients it progresses to invasive infection subsequently (Paterson, 2006).

In recent times, there has been the problem of multidrug-resistant Enterobacteriaceae and this has staged a challenge to disease control. Bacteria belonging to the Enterobacteriaceae are known to cause serious infections and sadly enough, most important members of this family are becoming more and more resistant to currently available antimicrobials. The whole idea of having a wide spread of infections caused by Enterobacteriaceae and fewer treatment options has urged great public health concern globally. For example, extended-spectrum B-lactamases (ESBLs) related resistance in Enterobacteriaceae is a major highlight (Azevedo *et al*., 2015), but there are also other mechanisms or resistance in which some of these Enterobacteria also use (Paterson, 2006).

According to a 2014 report on global resistance by World Health Organization, Africa, the Americas, the eastern Mediterranean, Europe, South East Asia, and the western Pacific are points of interest as regards resistant Enterobacteriaceae. The ability to produce extended spectrum B-lactamases (ESBLs) provides resistance to many penicillins and cephalosporins and this ability is usually accompanied with mechanisms that confer resistance to some other types of antibiotics. The prevalence of *E. coli* and *K. pneumoniae* ESBL related resistance shows a wide variation across different nations and there are speculations that this is related to factors such as waste and water management, antibiotic availability and restriction, and the general standard of living and healthcare. In some regions around the globe, report has it that 60 percent or more of *E. coli* and *K. pneumoniae* show resistance to B-lactam antibiotics, such as third generation cephalosporins (for example, cefotaxime), and these resistant strains are regularly imported to countries with lower prevalence of such type of resistance (Iredell *et al*., 2016).

Since Enterobacteriaceae are gram negative bacteria, they are more resistant to antimicrobial agents than gram positive bacteria because they possess an outer membrane structure and some defence features such as periplasmic beta-lactamases. This has contributed to the factors that have denied novelty in the development of antibiotics against gram negative bacteria (E. Ruh *et al*., 2016).

Antibiotic resistance in Enterobacteriaceae is not just confined to hospital and healthcare environments, as they can also be found in the community and domestic food related environment. In a study where 125 Enterobacteriaceae isolates were examined, it showed that 8 of the isolates showed resistance to more than one antibiotic. Among these 8 resistant isolates, 4 showed (Multi-drug resistance) resistance to more than three antibiotics and these multidrug resistant Enterobacteriaceae were kitchen isolates. The up rise in multi-drug resistance among Enterobacteriaceae in recent time is alarming and causing a major threat to treatment of infections caused by these bacteria (Azevedo *et al*., 2015).

**2.3.3 General Mechanisms of Antibiotic Resistance**

Resistance to antibiotics can be in any of the two forms; intrinsic resistance or acquired resistance.

**Intrinsic or passive Resistance:** In this form of resistance the bacteria are naturally without sites for the antibiotics to act on or they show low level permeability to such antibiotics, principally for antibiotics that need to gain access into the cytoplasm in order to be effective. Examples of bacteria with intrinsic resistance include *Pseudomonas aeruginosa* which has intrinsic resistance to some B-lactam antibiotics, *Citrobacter* against ampicillin and *Mycoplasma* against penicillin.

**Acquired or active Resistance:** This is the type of resistance that is a consequence of genomic changes of the bacteria. This may be as a result of mutation and subsequently passed down to daughter bacteria cells. There can also be a horizontal transfer of these resistance genes and it happens more between bacteria of same specie but can also happen between bacteria of different species (Toma and Deyno, 2015).

The mechanisms of resistance to antibiotics are often referred to in term so of acquired resistance and they include:

* **Inactivation of drug by enzymes**

This is usually plasmid mediated. Example of such enzymes is the B-lactamases (penicillinases) which inactivates to penicillins and cephalosporins, and erythromycin esterase which inactivates macrolides (Byarugaba, 2010). The B-lactamase is the most prominent amidase that hydrolysis the B-lactam rings of penicillins and cephalosporins (Dzidic *et al*., 2008).

* **Alteration of membrane permeability**

Most antibiotics will want to get to the cytoplasmic membrane or into the cytoplasm to be effective. Most gram negative bacteria will tend to have modifications in their porins or transport proteins to decrease permeability of antibiotics.

* **Active efflux pumps**

The efflux system is a mechanism in which bacteria use to eject antimicrobials out of the cell. A number of bacteria use this system actively against tetracyclines, quinolones and macrolides (Toma and Deyno, 2015).

* **Mutation of target of the antibiotics**

Some bacteria species can modify target sites of antibiotics and this will make the antibiotic not to bind properly with its target (Dzidic *et al*., 2008). Such is seen in some gram negative rods as they can alter the 30s subunit of their ribosome which is the target site for aminoglycosides (Toma and Deyno, 2015).

The quinolones interact with the DNA gyrase (topoisomerase II) and topoisomerase IV enzymes to interfere with DNA replication and transcription. When this target sites are altered, the bacteria become resistant to the quinolones (Dzidic *et al*., 2008), the fluoroquinolone resistant *Pseudomonas aeruginosa* is an example of bacteria that exhibits such mechanism (Adegboye *et al*., 2012).

**2.4 Mechanism of Resistance to B-lactam Antibiotics**

Resistance to B-lactam antibiotics is in accordance with the general mechanism of antibiotic resistance. These mechanisms are as follows:

1. Production of B-lactamases, which is the major resistant mechanism. These enzymes break the B-lactam ring of such antibiotics.
2. Alteration of Penicillin Binding Proteins (PBPs), which hinders the antibiotic from binding effectively with the PBPs.
3. Porin mediated resistance will halt antibiotic from reaching its target.
4. Effective efflux pumps system (Lakshmi *et al*., 2014).

**2.4.1 B-lactamases**

The B-lactamases are the major cause of resistance to B-lactam antibiotics. The B-lactamases are enzymes that inactivate B-lactam antibiotics, many of which are susceptible to hydrolysis. The hydrolysis of these target sites will render the antibiotic inactive even before it reaches its target in the bacterial cell (Dzidic *et al*., 2008).

B-lactamase was described as Penicillinase and its activity was first discovered in 1940. B-lactamase was first extracted from *E. coli* and it was observed that its activity rendered benzyl-penicillin ineffective. B-lactamase activity in gram positive bacteria was first observed in *Staphylococcus aureus* and soon after it was extended to other species of *Staphylococcus.* The variation of B-lactamases produced in gram negative bacteria is much more than those produced by gram positive bacteria (Adekunle, 2012).

There are several types of B-lactamases but the typical ones include: Extended B-lactamases (ESBLs), AmpC, Carbapenemases and OXA (Oxallinases).

**2.4.2 Extended Spectrum, Beta Lactamases (ESBLs)**

The ESBLs are mutant B-lactamases that have wide spectrum of activity against B-lactam antibiotics such as penicillins, monobactams, third generation (extended spectrum) cephalosporins such as cefotaxime, ceftriaxone, ceftazidime, but not carbapenems (e.g. imipenem, meropenem, and ertapenem) and cephamycins (e.g. cefoxitin and cefotetan) (Adekunle, 2012; Pitout and Laupland, 2008).

The mutation that leads to emergence of ESBLs are caused by alterations of amino acids in the B-lactamases which will result into different phenotypes with wider spectrum of activity, such that can hydrolyze third generation cephalosporins (Ghafourian *et al*., 2014). Substitutions of amino acids in

B-lactam genes lead to the formation of ESBL. These substitutions create more tendencies to the enzyme’s active site, and hence confer resistance to extended spectrum B-lactam antibiotics such as the third generation cephalosporins (Thenmozhi *et al*., 2014).

Genes responsible for ESBLs are always located on the plasmids (Adekunle, 2012; Pitout and Laupland, 2008).

ESBLs have become a global issue in hospitals and health care settings (Ghafourian *et al*., 2014).

Most of these ESBLs are gotten from enzymes that have come from class A b-lactamases which are typically expressed in gram negative bacteria. These enzymes are TEM-1, TEM-2, and SHV-1 (Paterson, 2006).



Figure Amino acid substitution of TEM-1 and make new TEM-52. Ghafourian, S., Sadeghifard, N., Soheili, S., and Sekawi, Z. (2014). Extended spectrum beta-lactamases: definition, classification and epidemiology. Extended Spectrum Beta-lactamases.

ESBLs have a wide spread around the globe but were first reported in Europe, particularly in Germany and England. In 1986, a total of 54 patients from three intensive care units in France tested ESBL positive and in the 19190s statistics showed that about 25-35% of all *Klesiella pneumoniae* nosocomial infections that occurred in France were ESBLs positive. In another study conducted by European Antimicrobial Resistance Surveillance-Network (EARS-Net) showed that ESBL producing *E. coli* were 3% in Sweden and 36% in Cyprus, indicating a higher percentage of ESBL producing *E. coli* strains in Southern Europe (Ghafourian *et al*., 2014).

In the Middle East precisely Iran, a study was conducted on several *E. coli* isolated from patients with urinary tract infections. The result showed that 25% of the total numbers of E. coli isolated were ESBL producers (Pakzad *et al*., 2014).

In a recent study conducted from 2010-2014 in Northern Cyprus, *E. coli* isolates were obtained from both hospitalized patients and from the community. 53% of the E. coli from hospitalized patients tested ESBL positive and 44% of the E. coli isolated from the community also tested ESBL positive (Cantas *et al*., 2016).

And in the year 2012, 300 claocal samples were collected from healthy broilers from several poultries in Turkey. 101 of the samples had E. coli isolated from them and 33 of the *E. coli* isolates tested ESBL positive. This shows a zoonotic risk for humans (Unal *et al*., 2017).

**Types of Extended Spectrum Beta lactamases (ESBLs)**

ESBLs are grouped into the classical and non classical ESBLs.

1. **Classical ESBLs**

The classical ESBLs are able to hydrolyze extended spectrum (third generation) cephalosporins and they are gotten from the mutation of classical B-lactamases TEM-1, TEM-2 and SHV-1. In the 1960s, the TEM-1 was discovered in Greece from blood culture of a person named ‘’Temoniera’’ The TEM type B-lactamases are found in numerous species of Enterobacteriaceae and *Pseudomonas aeruginosa* and few cases have also been reported for *Hemophilus influenza* and *Neisseria gonorrhoeae.* The SHV-1 (Sulphydryl Variable type-1) is commonly found in *Klebsiella* and *E. coli.* The TEM-3 and SHV-2 were the first set of B-lactamases to be described as extended spectrum B-lactamases (ESBLs). The classical ESBLs are the most common type of ESBLs, previous studies shows that ESBLs are derived majorly from TEM-1, TEM-2 and SHV-1 type B-lactamases either by one or several point mutations (Deepti and Deepti, 2010).

1. **Non classical ESBLs**

Non classical ESBLs are less common than the classical ESBLs and are derived from enzymes other than TEM and SHV. The non classical ESBLs include the CTX-M and OXA types (Lakshmi *et al*., 2014).

**CTX-M type**

The CTX-M type ESBL is a large group, having about 40 subgroups. The origin of CTX-M has been traced to the escape of chromosomal B-lactamase genes from *Kluvera* spp. which is a genus of bacteria with little or no clinical significance. Several members of the Enterobacteriaceae producing the CTX-M enzymes, especially *E. coli* have been identified as the predominant cause of community acquired urinary tract infections (Lakshmi *et al*., 2014).

**OXA (Oxacillinas)**

As the name implies, they have ability to hydrolyze oxicillin, cloxacillin and Methicillin. These enzymes are mostly found in Pseudomona aeruginosa, but have also been detected in the Enterobacteriaceae.

Other type of ESBLs include the PER, VEB, GES, BES, TLA, SFO, IBC groups. They are not products of point mutations from known B-lactamases and are also plasmid mediated (Deepti and Deepti, 2010).