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Review Article

MECHANISMS OF ANTIMICROBIAL RESISTANCE IN BACTERIA, GENERAL APPROACH

Olowe O Adekunle[1*]

*Corresponding Author: ***Olowe O Adekunle,*** ✉ *oaolowe@lautech.edu.ng*

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. While a spontaneous or induced genetic mutation in bacteria may confer resistance to antimicrobial drugs, genes that confer resistance can be transferred between bacterial in a horizontal fashion by conjugation, transduction or transformation. Thus, a gene for antibiotic resistance that evolves via natural selection may be shared. Evolutionary stress such as exposure to antibiotics then selects for the antibiotic resistant trait. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. If a bacterium carries several resistance genes, it is called multidrug resistant (MDR) or, informally, a superbug or super bacterium. The emerging resistance in today's world has created a major public health dilemma. The major driving force behind the emergence and spread of antibiotic-resistant pathogens is the rapid rise of antibiotic consumption. This trend reflects the growing medicalisation of societies worldwide, with its identification of microbial pathogens as the cause of infectious diseases. Antibiotics promise cure. This together with their ease of use, the usually short treatment requirements, and, for many parts of the world, availability without prescription by a doctor results in a demand that is increasingly met by a growing supply of generic drugs produced in emerging market economies. The same escalation in consumption has occurred in the animal welfare sector, prompting concerns about the transmission of antibiotic resistance through the food chain. An additional set of threats that facilitate the spread of antibiotic-resistant pathogens comes from unpredictable disasters that disrupt human livelihoods and bring about crowding, mass migration, famine and unsafe water supplies. Conflicts within and between states, environmental degradation and climate change can provide scenarios in which infectious diseases thrive and antibiotic resistance may come to the forefront.

Keywords: Antibiotics, Multidrug resistance, Resistance mechanism, Extended-spectrum beta-lactamases genes, Vaccine, Phage, cytokines.

INTRODUCTION

Increasing rates of bacterial resistance among common pathogens and serious ones are threatening the effectiveness of even the most reliable potent antibiotics. With the ever increasing spread of multidrug resistance pathogens in our

[1] Department of Medical Microbiology and Parasitology, College of Health Sciences Ladoke Akintola University, P.M.B.4400, Osogbo, 230222, Nigeria.

daily lives it becomes imperative to find a way out to suppress this menace because sooner or later the spread will eventually becomes a serious public concerns. (Fish and oblinger, 2006). In developed countries, resistance has until now been found mainly in pathogens that can be transmitted without causing disease. They can be carried for long periods and might cause an infection only when coming into contact with parts of the body that would normally be free from bacterial colonization that is, introduced by medical interventions, or in children or people with poor immune systems. Problems with resistant organisms are therefore mainly found in hospitals and nursing homes where patients are treated for acute or chronic conditions. In developing countries, on the other hand, antibiotic resistance often occurs in microorganisms transmitted in communities by person-to person contact, through contaminated food, unsafe drinking water or by insects. Resistance can mean that people infected with such organisms do not respond to conventional drugs and, if no other treatment options are available, must depend on their immune system overcoming the disease. The common resistance found easily today of importance is the extended spectrum beta-lactamase (ESBL) producing bacterial especially the gram negative in the family Entero-bacteriaceae which are constantly found both in the community and hospital environment and are becoming associated with clinical and treatment failure (Pitout *et al*., 2005). Likewise the introduction of new antibiotics has not kept pace with the increasing rate of resistance, leaving clinicians with fewer treatment options. A recent survey analysis found that of 506 new drugs in development, only 5 were antibiotics and reports shows that the pharmaceutical pipeline for new antibiotics are drying up (IDSA, 2004). The alarming nature of the super bug and the problems associated with it is fast becoming a major global health concern. According to 2007 report from the centre for disease Control and Prevention, an estimated 1.7 million health-care associated infections occur in American hospitals each year. These infections are associated with 99,000 deaths (CDC, 2007). As reported earlier, this is a huge jump from previous decades. Tertiary care centers, teaching hospitals and centers that treat critically ill patients both in rural and urban settings are particularly vulnerable to high rates of bacterial resistance. Such resistances have been reported in several classes of bacterial. Multi-drug resistant *Klebsiella* species and *Escherichia coli* have been isolated in hospitals throughout the United States and around the world even in Nigeria several reported cases of multidrug resistance to gram negatives have been reported showing resistance in different clinical samples (Olowe *et al*., 2007, 2010,). In *Klebsiela pneumponia* (Aibinu *et al*., 2005; Antoniadou *et al*., 2007; Olowe *et al*., 2010,), In *Enterobacter spp* (Aibinu *et al*., 2003b). Reports of methicillin-resistant *Staphylococcus aureus* (MRSA) a potentially dangerous type of staphyloccocci bacteria that is resistant to certain antibiotics and may cause skin and other infections in persons with no links to healthcare systems have been observed with increasing frequency in the United States and elsewhere around the globe (Taiwo *et al*., 2005; Bozdogan *et al*., 2003), In Nigeria resistance through *Salmonella typhimurium* has also been reported. (Olowe *et al*., 2007). Resistance in *Enterococus faecalis* likewise reported (David *et al*., 2010). Several reports have been seen also on *Pseudomonas aeruginosa*. This is a bacterial with clearly more resilient and dangerous pathogens

which has established themselves in hospitals as stated by (Robert, 2008). Several reports of *Pseudomonas aeruginosa* resistance have also been reported (Livermore, 2002; Arancibia *et al*., 2002 Zavascki *et al*., 2006). Likewise the usage and abuse of antibiotics in veterinary have been reported (Schneider and Garrett 2009; Ajayi et al., 2010; and 2011). Antibiotic-resistant *Streptococcus pneumoniae* infections have significantly declined, but remain a concern in some populations. Some of the risk factors associated with increased bacterial resistance among patients in the intensive care unit (ICU) include long hospital stay, advanced age, use of invasive devices, immunosuppression, lack of hospital personnel adherence to infection-control principles, and previous antibiotic use. Repeated courses of antimicrobial therapy are common in acutely ill, febrile patients, who frequently have endotracheal tubes, urinary catheters, and central venouscatheters (Fish and oblinger., 2006, Robert, 2008). In combination with host factors, in dwelling devices are routes for transmission and colonization of resistant infections (Fish and oblinger, 2006, Robert, 2008). However, two principal drivers of resistance appear to be inadequate (or inappropriate) empirical antibiotic therapy and prolonged antibiotic use (Fish and oblinger, 2006, Robert, 2008).

With increasing resistance to existing antibiotics, developing countries face a serious challenge in safeguarding their populations' health against killer diseases such as TB and typhoid fever and the likes other bacterial as earlier stated. According to report of (*Grundmann* 2008). Public health experts have been warning for over a decade that a 'post-antibiotic era' is rapidly approaching when the spread of antibiotic resistance means that effective antibiotic therapy will no longer be effective and the situation is deteriorating with ever-increasing speed. Despite the scale of the threat, resistance is still not taken sufficiently serious by many in the health sector. Surveillance is needed to monitor the spread of resistance, and thus understand the scale of the problem, in order to provide crucial data for the development of containment strategies

MECHANISM OF RESISTANCE

Several factors have been reported to be responsible to antibiotics resistance in bacterial. Some of the reasons includes: Reduced access to target due to slow porin channels; increased antibiotics expulsion due to multiple drug efflux pumps; inactivating enzymes due to β-lactamases, aminoglycoside-modifying enzymes; mutational resistance due to regulatory mutations that increases the expression of intrinsic genes and operons which is variable in certain circumstances (Nikkado *et al*., 2003). The antimicrobial agents in widespread clinical use were developed to inhibit targets unique to prokaryotic cells such as bacterial cell wall, the bacterial ribosome and bacterial DNA gyrase. These antibiotics have reduced the mortality resulting from infectious diseases. Use and often abuse of antibiotics has encouraged the evolution of bacterial towards resistance, resulting often in therapeutic failure. Resistance reflects the ability of a microorganism to avoid the inhibitory and lethal activity of antimicrobial agents. (Fraimow and Abrutyn, 1995).

Microorganisms Demonstrate Resistance in Several Ways

Intrinsic Resistance to an antimicrobial agents characterizes resistance that is an inherent attribute of a particular species; these organisms may lack the appropriate drug- susceptibility

targets or posses natural barriers that prevent the agents from reaching the target; examples are the natural resistance of gram- negative bacteria to vancomycin because of the drug's inability to penetrate the gram-negative outer membrane, or the intrinsic resistance of the penicillin (Bozdogan *et al*., 2003; Xie *et al*., 2011).

Circumstantial Resistance is the difference between the *in vitro* and *in vivo* effects of an antimicrobial agent. Agents that appear to be active in the laboratory may be ineffective *in vivo* because of failure to reach the site of infection, such as the inability of first generation cephalosporins to cross the blood-brain barrier. Drugs such as aminoglycosides may be inactivated; *in vivo* antagonist of trimethoprim-sulfamethoxazole can be overcome by enterococci via their inability to take up and internalize corporate environmental folate (Fraimow and Abrutyn, 1995, Hidron *et al*., 2008).

Acquired Resistance, which is the primary focus of this work, reflects a true change in the genetic composition of a bacterium so that a drug that once was effective *in vivo* no longer is effective (Fraimow and Abrutyn, 1995). The major mechanism that bacteria employ to avoid the actions of antimicrobial agents include limiting the intracellular concentration of the antimicrobial agent by decreased influx or increased efflux, neutralization of the antimicrobial agent by enzymes that reversibly or irreversibly inactivate the drug, alteration of the target so that the agents no longer will interfere with it, and elimination of the target altogether by the creation of new metabolic pathways (Neu, 1992, Jacoby and Archer, 1991 Li, X and Nikadio, 2009). Bacteria may employ or combine multiple mechanisms against a single agent or class of agents, or a single change may result in development of resistance to several different agents (Neu, 1992; Jacoby and Archer, 1991, Fraimow and Abrutyn, 1995; Li, X and Nikadio 2009)

MECHANISM OF DISSEMINATION OF RESISTANCE GENES

Bacteria avail themselves of a variety of efficient mechanisms for the transfer of resistance genes to other organisms and other species (Cohen, 1992; Courvalin, 1994). The bacterial genome consists of chromosomal DNA, which encodes for general cellular characteristics and metabolic repair pathways, and smaller circular DNA elements known as plasmids that encode for supplemental bacterial activities such as virulence factors and resistance genes. The vast majority of resistance genes are plasmid-mediated, but plasmid-mediated traits can interchange with chromosomal elements. Transfer of genetic material from a plasmid to the chromosome can occur by simple recombination events, but the process is greatly facilitated by means of transposons. Transposons are small, mobile DNA elements capable of mediating transfer of DNA by removing and inserting themselves into host chromosomal and plasmid DNA and include Insertion Sequences, Transposons and integrons. If these elements become associated with either transmissible or mobilisable plasmids, chances are increased that they will be transferred to other organisms. Many resistance genes, such as plasmid-mediated β-lactamase, tetracycline-resistance genes and aminoglycosides-modifying enzymes are organized on transposons, which can vary greatly in size and complexity. Transposons may have a broader host range than their parent plasmids and may be important in the dissemination of resistance genes among species (Ochial *et al*.,

1959; Courvalin, 1994; Berg, 1989). Resistance determinants carried on the chromosome are transmitted by clonal dissemination. Resistance determinants on plasmids also are transferred vertically, although plasmids may be lost from the bacterial population if they no longer contain particular selective advantage. In bacteria, gene transfer that can lead to recombination which may occur in any of three different easy: transformation, transduction and conjugation**. (**Ochial et al., 1959)

Transformation is the simplest type of gene transfer. A recipient cell acquires genes from 'free floating' DNA molecules in the surrounding medium. In nature, the DNA may come from dead cells that lyse and release their DNA. In the laboratory, however, the DNA is extracted by chemical methods from a suspension of donor bacteria and then added to a culture of recipient bacteria. In nature or in the laboratory, a recipient bacterium can acquire one or more inheritable characteristics from a donor bacterium and become what is called transformed. Only certain species of bacteria are known to undergo transformation, and even these must be in a state of growth receptive to the incorporation of donor DNA; that is they must be competent. This condition usually occurs when the recipient bacteria are in the late logarithm phase of their growth. Competent bacteria cells produce a special protein that binds donor DNA fragments at specific sites on the cell surface. Although chromosomal DNA can be readily transferred to competent recipient bacteria, plasmid DNA is not easily transferred by ordinary transformation procedure that simply add DNA to recipient cells. However, special procedures widely used in genetic engineering can be used to accomplish transformation with plasmid DNA (Pelczar *et al*., 1992). Plasmids can also be transferred to recipient cells via phages (Pelczar *et al*., 1992).

Transduction is gene transfer in which a virus serves as the vehicle for carrying DNA from a donor bacterium to a recipient bacterium. A phage consists of a nucleic acid, usually DNA surrounded by a protein coat to form a head. A tail –like appendage serves to attach the phage to the surface of a susceptible host bacterium. After the phage injects its DNA into the host cell, the phage DNA is replicated rapidly while the bacterial DNA is degraded. The phage DNA then directs the synthesis of new phage proteins by the host cell. Within a short time the new phage, DNA molecules combine the new phage proteins to form numerous whole phages, which are released as the host cell disintegrates. During assembly of the phage progeny within the infected host cell, any fragment of the host bacterium's DNA that is approximately the same size as the phage DNA may be accidentally incorporated into a new phage head instead of the phage DNA. A phage carrying such a fragment is called a transducting phage because if it affects another bacterium, it injects the bacterial DNA fragments into the new host**.** Because the transducting phages do not contain the entire viral DNA, they do not kill the new host). The fragment can then undergo recombination with the corresponding part of the new host's chromosome and become a permanent part of that chromosome. Thus, the second bacterial host acquires one or more genes (Pelzcar *et al*., 1992).

Conjugation is a process of gene transfer that requires cell to cell contact. Plasmids also are capable of horizontal transfer via conjugation, although the efficiency of plasmid transfer both within and between species can vary tremendously**.** DNA may be transferred directly

from one bacterium to another. Bacterial conjugation differs from sexual mating in eukaryotes in that it does not involve the fusion of two gametes to form a single cell. In some types of conjugation, only a plasmid may be transferred from the donor bacterium to the recipient bacterium. In other types, large segments of the donor cell's chromosome or even the entire chromosome may be transferred to a recipient's cell. This differs from transformation and transduction, in which only relatively small chromosomal fragments may be, transferred (Pelzcar *et al*., 1992). Studies of conjugation in *E. coli* have revealed that this bacterium has two different mating types: a donor and a recipient. The 'donor' cells contain a plasmid called the F plasmid ('F' stands for fertility). Like most plasmids, this F plasmid is a small, circular piece of double-stranded DNA that is not part of the bacterial chromosome and can replicate independently. It contains about 40 genes that control the plasmid's replication and the synthesis but the host cell of a filamentous appendage called the sex pilus. Cells containing the F plasmid are referred to as 'F' cells and are donors in mating. Recipient cells lacking the 'F' plasmid are called F^- cells. When F+ and F- cells are mixed together in what is termed an F+ x F- cross, the end of the F+ sex pilus binds to a nearby F- cell and then retracts, pulling the F+ and F- cells into close contact. A channel is formed between the two cells, through which transferred one is DNA strand from the donor's F plasmid to the F- cell. Once inside the recipient's cell, the DNA strand acts as a template for the synthesis of a second, complimentary DNA strand. The end of the double stranded DNA molecule then joins to form a circular F plasmid and the recipient cell has become an F+ cell capable of donating DNA. In this way, the conjugation process can continue until all the F-cells in the culture are converted. Whilst DNA transport readily occurs at the conjugational junction, there is no general mixing of the cytoplasmic contents of conjugating bacteria. Only a single strand of DNA is transferred. The single strand is produced when the plasmid is nicked at the specific origin of transfer (oriT) site. Unwinding of the duplex by one or more DNA helicases following this, a single strand of DNA is then progressively displayed 5' to 3' and transported into the recipient. When transfer is complete, the F factor is recircularised in the recipient and a complementary strand synthesized (Lanka and Wilkins, 1993). Transfer can proceed until cell contact is interrupted or until a break in the DNA or the 3' end of oriT is reached. As transfer of the single strand proceeds 5' to 3', the F genes are transferred, the recipient will not become F+. The features of F factor transfer appear to be characteristics of other conjugative transfer systems in Gram-negative bacteria. Chromosomal genes can be transferred along with the F plasmid, but this is a rare event, occurring only 1 in 10 million matings (Pelczar *et al*., 1992).

β-LACTAMASE AND THE GRAM NEGATIVE BACTERIAL ORGANISM

The production of β-lactamase enzymes, particularly ESBLs, is an important mechanism of resistance to β-lactam antibiotics among gram-negative bacteria and most of these β-lactamase enzymes are plasmid encoded. This has strongly facilitated their spread among strains of many species of gram-negative bacteria.

HISTORY OF β–LACTAMASE

In 1940, Abraham and Chain made the first report of β-lactamase activity. The enzyme extracted from a strain of *Escherichia coli* was shown to inactivate a solution of benzyl penicillin, and was named 'penicillinase". The term 'penicillinase' was formerly used to describe β-lactamase enzymes. Penicillinase was born on December 28th, 1940. The term made its first appearance in the Quarterly Cumulated Index Medicus (QCIM) in 1944 (volume 36) and was originally a purely functional word meaning in classical biochemical language, an enzyme, the substrate of which is a penicillin. The age of penicillin saw the rapid emergence of resistance in *Staphylococcus aureus* due to a plasmid-encoded penicillinase. A few years later, penicillinase activity in *Staphylococcus aureus* was shown to be responsible for clinical resistance, and thus for therapeutic failure. This enzyme quickly spread to most clinical isolates of *S.aureus* as well as other species of *staphylococci*. The bacterial enzyme (penicillinase) responsible was shown to open the β-lactam ring, and penicillinase-producing *Staphylococcus aureus* became of great importance in outbreaks of hospital infections around the 1950s. Similar activity was subsequently discovered in a wide variety of microorganisms (Hamilton-Miller, 1979). The year 1959 was a date that marked the introduction of the first semi synthetic penicillin, phenethicillin ('Broxil'). It was soon followed by methicillin, which had been designed specifically to neutralize the effect of staphylococcal, penicillinase, and ampicillin.

Likewise results of studies on the resistance to the latter compound, both natural (as in *Klebsiella aerogenes*) and acquired (as transmitted by (R-TEM) was what started β-lactamase on the road to becoming such a popular enzyme. It had been realized for some time that the name 'penicillinase' was not very satisfactory, as three entirely different enzymes could be fairly be given this name. First, the acylase (or amidase) which is used for the production of 6-amino-penicillinate from benzyl penicillin; secondly, the classic enzymes which break the β-lactam bond ('penicillinase' and 'cephalosporinase'); thirdly the enzyme which liberates a penicillinate from a penicillin-3-amide. Pollock (1960) rationalized the situation by suggested the name 'β-lactamase' for the enzymes 'penicillinase' and 'cephalosporinase'. (Hamilton-Miller, 1979). As far as the hospital Microbiologist was concerned, β-lactamase was an enzyme, which caused hospital Staphylococci to be resistant to penicillin. Although β-lactamase was known to occur in many bacterial species, the importance of the enzyme does not seem to be recognized in terms of the determination of penicillin resistance in species other than the staphylococcus. It should be remembered that some pathogens species such as, *Bacillus anthracis* are known to produce β-lactamase (Barnes, 1947) and yet are extremely sensitive to benzyl-penicillin. β-lactamase enjoyed a brief spell of popularity as a resistance mechanism to treatment with penicillin. Even the introduction of the first semi-synthetic penicillin phenethicillin (Broxil) in 1959 did not kindle much renewed interest in β-lactamase. When methicillin became available in 1960, it appeared that the clinical relevance of β-lactamase had completely vanished. It was not until after ampicillin came into wide use following its release in 1961 that it was appreciated that bacterial β-lactamase might be of crucial importance in determining resistance to β-lactam antibiotics in gram-negative bacteria as well as in Staphylococci.

The realization that a new situation existed was soon reinforced by two events. First, semi-synthetic cephalosporins appeared for clinical use (cephaloridine in 1964 and was first used in great Britain) and it was soon discovered that although they were virtually unaffected by the staphylococcal enzymes, these compounds were rapidly destroyed by β-lactamases from many gram-negative bacteria (Hamilton-Miller, 1979). Secondly, Datta and Kontomichalou (1965) reported that a β-lactamase from an ampicillin-resistant strain of *E. coli* (designated TEM) was carried on an R-factor. This finding was to have far reaching consequences, as it predicted that an ampicillin resistance would spread not only among strains of *E. coli* but also to other genera (Hamilton-Miller, 1979; Datta and Kontomichalou 1965). This soon proved true, resistance appearing not only in enteric bacteria but also in *Haemophilus influenzae* and *Neisseria gonorrhoeae.* By 1973, distinct biochemical patterns were beginning to emerge among many β-lactamase, which had been reported. The first comprehensive classification was put forward by (Richmond and Sykes, 1973). This proved extremely useful, because for the first time all workers in this rapidly expanding field could describe the enzyme with which they were working in a unified way that was intelligible to other workers; and in 1974, it was discovered that the gene specifying TEM β-lactamase was carried on a transposon. This explains why the enzyme is so widely distributed among different bacterial genera. Many genera of gram-negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase. These enzymes are thought to have evolved from penicillin-binding proteins (PBP), with which they show some sequence homology (Datta and Kontomichalou., 1965). The first plasmid-mediated β-lactamase in gram-negatives, TEM-1, was described in the early 1960s (Datta and Kontomichalou., 1965). The TEM-1 enzyme was originally found in a single strain of *E.coli* isolated from a blood culture of a patient named Temoniera in Greece hence the designation TEM (Medeiros, 1984). Being plasmid and transposon mediated has facilitated the spread of TEM-1 to other species of bacteria. Within a few years after its first isolation, the TEM-1 β-wide and is now found in many different species of members of the family Entero-bacteriaceae and other generas such as *Pseudomonas aeruginosa, Haemophilus influenza,* and *Neisseria gonorrhoea.* Another common plasmid-mediated β-lactamase found in *Klebsiella pneumoniae* and *E.coli* is SHV-1 (for sulphydryl variable). The SHV-1 lactamase *is chromosomally encoded* in the majority of isolates of *K.pneumoniae* but is usually *plasmid-mediated* in *E. coli* (Bradford, 2001). Over the last 20 years, many new β-lactamase resistant antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new β-lactamases emerged that caused resistance to that class of drug. Presumably, the selective pressure of the use and overuse of new antibiotics in the treatment of patients has selected for new variants of β-lactamase. One of these new classes was the *oxyimino-cephalosporins*, which became widely used for the treatment of serious infections due to gram-negative bacteria in the 1980s. Not surprisingly, resistance to these expanded-spectrum β-lactam antibiotics due to β-lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer β-lactams, SHV-2, was found in a single strain of

Klebsiella ozaenae isolated in Germany (Kliebe *et al*., 1985). Due to their increased spectrum of activity, especially against the oxymino-cephalosporins, these enzymes were called extended-spectrum β-lactamases (ESBLs). Today over 150 different ESBLs have been described and found worldwide in many different genera of Enterobacteriaceae and *P. aeruginosa.*

β-lactamases of Gram-Positive Bacteria

Gram-positive bacteria such as *S. aureus*, lacking the protection of an outer membrane, hyper-produce their mediated class A β-lactamases. These β-lactamases are induced by transmembrane spanning penicillin sensitive receptors upon detection of extracellular antibiotics (Phillipon *et al*., 1989). Large quantities of β-lactamases are secreted, conferring resistance to the host and surrounding bacterial flora (Medeiros, 1997). The genes that determine staphylococcal β-lactamases are usually carried on small plasmids or transposons. Larger plasmids encoding β-lactamase and encoding β-lactamase and other resistance also exist and can transfer by conjugation, not only between strains of *S. aureus* but also between *S aureus* and *S. epidermidis* (Phillipon *et al*., 1989).

β-Lactamase of Anaerobic Bacteria

The resistance of anaerobic bacteria to β-lactam antibiotic also involves the production of β-lactamase (Appelbaum, 1992). The β-lactamase of *fusobacteria* and *clostridia* are principally penicillinase. Those produced *by Bacteriodes fragilis* are predominantly cephalosporinase, some of which have been found to hydrolyze cefocitine and imipenem and may be transferable (Hedberg *et al*., 1992). Most of the cephalo-sporinases are inhibited by clavulanate, sulbactam or tazobactam. The carbapenemase however are metalo-enzymes inhibited by EDTA, but not clavulanate or sulbactam.

β-lactamases of Gram Negative Bacteria: Gram negative bacteria produce a much greater varieties than do gram positive bacteria. They produced both inducible and constitutive β-lactamase enzymes (Richmond and Sykes, 1973). The enzymes are almost always cell bound. The chromosomal β-lactamases of gram negative bacteria are induced by an increase in peptidoglycan degradation fragment resulting from β-lactam activity. The enzymes are synthesized at a lower rate than in gram positive bacteria and are confined in the periplasm (Ambler, 1980). Here they act synergistically with outer membrane porins' to effectively protect against susceptible antibiotics (Medeiros, 1997). Almost all the enzymes are produced constitutively and can be grouped into six broad classes.

- Those that hydrolyses benzyl penicillin and cephaloridine at similar rates (broad spectrum enzymes).
- Those that hydrolyses oxacillin and related penicillin rapidly (Oxacillinases).
- Those that breakdown carbenicillin readily (Carbenicillinases).
- Enzymes that inactivate Oxymino-β-lactams such as cefotaxime, ceftazidime, aztreonam (extended beta-lactamases).
- Enzymes that breakdown Oxymino β-lactams and are resistant to clavulanate (Cephalosporinases; the genes that encode these enzymes are similar in nucleotide sequence to chromosomal β-lactamase gene of *Enterobacter, Citrobacter or Klebsiella oxytoca* and have similar biochemical

characteristics (Payne *et al*., 1992; Baerthelemy *et al*., 1992; Papanicolau *et al*.,1990).

- An unusual β-lactamase found in pseudomonas aeruginosa that hydrolyses imipenem (Carbapenemases). (Watanabe *et al*., 1991).

Classification of β-lactamase: Although arbitrary, the classification of â-lactamase allows for a level of uniformity in reference material and aids in the identification of new enzymes. There are two schemes generally accepted today, the Ameber and Buch-Jacob-Mederios classifications. Currently numbering around 340, the β-lactamases were initially classified according to amino acid sequence into classes A or in a system developed by Ambler (1980). Class C was introduced after the enzymes were found to have no sequence homology with classes A or B (Jaurin and Grundstrom, 1981). Huovinen *et al*., (1988) identified class D. The Bush-Jacoby-medeiros (1995) scheme sought to update the classification of the â-lactamases based upon properties such as substrate and inhibitor profiles. However, molecular point mutations can result in changes in properties resulting in the potential for variations in defining characteristics (Bush, 1989). For instance, a point mutation in a class A TEM-1 enzyme resulted in a substrate profile similar to a class C enzyme.

Class A (Group 2) Serine Penicillinases

The β-lactamases designated as class A by Ambler (1980) are classified as the group 2 family under the Bush-Jacoby-Medeiros system (1995) and are present in gram-negative and gram-positive bacteria. Class A β-lactamases contain 260 to 270 amino acid residues and have molecular weight around 29 kD (Ambler, 1980). Their sequence homology is great enough to suggest class A enzyme evolved from a single ancestral gene (Ambler, 1980). All class A enzymes have a serine residue in the active site at position 70. They hydrolyze ampicillin and Penicillin G preferentially. They are generally inhibited by clavulanic acid as they have an arginine at position 244 that facilitates inhibitor attack (Bush *et al*., 1995; Medeiros, 1997). Many are carried along with other resistance genes plasmids or transposons (Medeiros, 1997). The predominant families are the TEM and SHV plasmid mediated enzymes. TEM â-lactamases are the most widespread â-lactam resistance mechanism amongst Enterobacteriaceae with TEM-1 being the world's most common (Blazquez *et al*., 2000). The success of TEM-1 may be attributed to its efficiency in hydrolyzing clinically used antibiotic and its location on a highly class 2 transposon (Amyes, 1997). Increased spectrum of activity against â-lactams occurs in class enzymes via mutations that increase the entrance to the active site (Medeiros, 1997).

Class B (Group 3) Metallo-β-lactamases

Since the β-lactamase of *Bacillus cereus* studied by Ambler (1980) differed from the original class A enzyme in so many ways, he established class B to accommodate it. The Bush-Jacoby-Medeiros scheme (1995) classified the class B enzyme as group 3. Their molecular weight is generally 23 kD. They have no active site serine residue but require a metal cofactor, usually zinc, and are able to hydrolyse most â-lactam, including imipenem (Ambler, 1980). Class B â-lactamases are poorly inhibited by clavulanic acid, but inhibition by EDTA and restoration of activity upon addition of Zn^{2+} easily identifies a class B β-lactamase (Bush, 1989). Most class B enzymes occur in bacteria that produce at least one other class of

β-lactamase, resulting in extended-spectrum resistance phenotype.

Class C (Group 1) Serine Cephalosporinases

Jaurin and Grundstrom (1981), studying enzymes with only limited sequence to classes A and B, introduced class C to the Ambler classification, which the Bush-Jacoby-Medeiros (1995) scheme designates as group 1. Class C β-lactamases have a high substrate affinity for cephalosporins and are not inhibited by clavulanic acid (Bush, 1989). They have molecular weights greater than 30kD, containing between 360 and 370 amino acid, and basic isoelectric points. Class C enzymes have active site serines at position 80, indicating an evolutionary origin distinct from the other serine β-lactamases (Juarin and Grundstrom, 1981). They occur only in Gram-negative bacilli and amongst the *Enterobacteriaceae;* the majority of species-specific β-lactamases are chromosomal class C genes (*amp*C). Class C enzymes have larger active sites than the class A enzymes, allowing them to hydrolyse cephalosporins. Mutations in the genes regulating the quantity of class C enzymes synthesized are the main mechanism for enhanced β-lactam resistance by class C enzymes (Medeiros, 1997). Class C β-lactamases also occur on high-copy number plasmids, which are readily transmitted among *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae*.

Class D (Group 2d)

Expanding on the Ambler scheme, Huovinen *et al*. (1988) introduced class D to incorporate those enzymes with little structural similarity to classes A or C. There is no sequence homology with class B. however; there is a region of homology with classes A and C at the active site, suggesting a convergent evolution. Class D makes up group 2d of the Bush-Jacoby-Medeiros scheme. Class D enzymes are generally inhibited by clavulanic acid, but are not as susceptible as Class A enzymes. The isoelectric point of class D enzymes range from 6.1 to 7.7 (Bush, 1989). Comprising the plasmid-mediated OXA-1, OXA-2 and PSE-2 enzymes, class D β-lactamases are similar in size to class A enzymes and preferentially hydrolyse oxacillin and cloxacillin.

β–LACTAMASE

Current Situation and Clinical Importance β-lactamase may be classified into four categories:

1. The well known traditional plasmid-mediated enzymes,
2. The Carbapenemases
3. Chromosomally mediated β-lactamases and
4. The more recently encountered extended-spectrum beta-lactamase (ESBLs).

Traditional, Well Known, Plasmid-Mediated β-Lactamases

Over 50 plasmid-mediated β-lactamases have been described among Gram-negative bacteria. Most plasmid-encoded β-lactamases are constitutively produced and many are encoded by genes on transposons. For example, TEM-1 enzymes were initially confined to *enterobacteriaceae* and have now spread to other genera and species, including *Haemophilus influenza*e and *Neisseria gonorrhoea*. Currently plasmid-mediated β-lactamases are found in 30-80% of the isolates of many enterobacteria particularly in developing countries (Kesah and Odugbemi, 2002). A high percentage of isolates tested for β-lactamases production were found to be producing β-lactamases. In Spain, 55-60% of *E. coli* strains are resistant to ampicillin, and

resistance are primarily due to these plasmid-mediated β-lactamases (Garau, 1994). Surveys of *Neisseria* spp and *E. coli* provide excellent examples of the clinical importance of plasmid-mediated β-lactamases production as a mechanism of bacterial resistance. A survey in Spain, as well as data from the National Reference center in Madrid demonstrated that of 2010 different isolates of *N. gonorrhoea*, 23% were producing TEM-1 β-lactamases and as a result were resistant to ampicilllin. The prevalence of *Pseudomonas aeruginosa*-lactamases-producing gonococci was found to vary widely, however in Northern Spain; only 3% of isolates were producing β-lactamases; whereas in Catalonia, the figure was close to 28%. *Neisseria meningitidis* has also been shown to produce β-lactamases. During the past few years in Barcelona area, two strains of *N.meningitidis* that produce β-lactamase were found. Although these organisms are rare, their existence illustrates the growing problem of β-lactamase resistance (Garau, 1994).

Carbapenemase: A Problematic β-Lactamase

The carbapenemase that was first characterized in a strain of *Pseudomonas aeruginosa* in Japan 1991 is similar to metalloenzymes of broad-spectrum chromosomal β-lactamases. This enzyme, which has been described only in a single strain of *Pseudomonas aeruginosa*, is capable of hydrolyzing a very broad range of different β-lactams including the newer cephalosporins, cephamycins. (Watanabe *et al*., 1991). The MICs against these bacteria are 50 μg/ml for imipenem and 100 μg/ml for meropenem. Currently available â-lactam inhibitors do not inhibit Carbapenemase. The importance of this enzyme is two-fold: the broad range of resistance it confers and the eventual possibility of dissemination, given its plasmid origin (Garau, 1994).

CHROMOSOMALLY MEDIATED β-LACTAMASES

Chromosomally mediated β-lactamases are enzymes encoded by genes located in the bacterial chromosome. Chromosomally encoded β-lactamases are common in Gram-negative bacterial and have been described in enterobacteria. *Pseudomonas, Moraxella, Bacteroides, Campylobacter, Acinebacter, Legionella*, and *Pasteurella spp.;* they have not been described in *Nesseria* or in *Haemophilus*. Clinically significant production of class 1 chromosomally mediated β-lactamases normally occurs only in the presence of an inducer. In genera such as *Enterobacter, Citrobacter, Serratia* and *Pseudomonas*, which represent a major source of nosocomial infection, clinically significant production of β-lactamase occurs on exposure to β-lactam inducer. If the β-lactam is removed or hydrolysed, the induction is normally stopped, so that β-lactamase production returns to basal limits. There can be, however, a spontaneous mutation within the bacterial genome that results in a stably depressed state in which β-lactamase production is permanently hyper-produced even in the absence of an inducer (Garau, 1994). It is important to distinguish between the more potent β-lactamase inducers, such as the cefamycins, imipenem, and the first generation cephalosporins, and weaker inducers, such as ureido penicillins, mono-bactams, and the third generation cephalosporins. The antibacterial activity of weak inducers is strongly dependent on their weak inducer activity. That is, these

agents remain active against β-lactamase-inducible species of Gram negative bacilli because they fail to induce β-lactamase synthesis, not because they are stable to the enzyme. However, selection of stably depressed mutants has been reported with most third generation cephalosporins, with aztreonam and occasionally, with ureidopenicillins (Garau, 1994). Organisms that produce inducible β-lactamases include *Enterobacter spp., Serrantia marcescens, Citrobacter freundii, Morganella morganii, Provindencia spp*., indole positive Proteus *spp., and pseudomonas aeruginosa.* Any population of these species of gram negative bacteria normally contains stably derepressed mutants. These occur most frequently in *E. cloacae*. The clinical importance of selection of depressed mutants is further illustrated in the review of the rates of emergence of resistance in patients infected with organisms possessing β-lactamases and treated with the newer cephalosporins (Sanders and Sanders., 1988). Emergence of resistance during cephalosporin therapy range from 14% to56% with a mean of about 30%; among patients in whom resistance was detected, the rate of relapse was 25% to 75% of cases. The drugs, which may be hydrolysed by class 1 β-lactamases, including almost all of the cephalosporins, cephamycins, monobactams and expended spectrum penicillins. Use of these agents has been associated with the emergence of multiple β-lactam resistances due to selection of stably derepressed mutants. Inducible class 1 β-lactamases are not inhibited by clavulanic acid and few are moderately inhibited by tazobactam. This type of resistance is most likely to appear and be of clinical significant in patients with respiratory tract infections, in granulocytopenic patients admitted to intensive care units, in patients with major burns and in patients with cystic fibrosis (Bauernfeind et al., 1996). The wide spread occurrence of antibiotic resistance associated with these enzymes and the clinical implications should be carefully considered during the establishment of an antibiotic policy in a hospital setting.

Extended Spectrum Plasmid–Mediated β-lactamases (ESBLs)

The ESBLs are a relatively new group of plasmid –mediated enzymes. The first ESBLs, an oxyimino β-lactamase were described in 1983 in Frankfurt, Germany (Knothe *et al*., 1983). Since that time nearly 40 different ESBLs have been described. Over the past years bacterial have acquired genetic information that permits inactivation of a large group or number of β-lactam antibiotics. Extended-spectrum plasmid-mediated β-lactamases have been identified in enterobacteriaceae particularly on *Escherichia coli* and *Klebsiella pneumoniae*. The majority of ESBLs are derived via mutation of TEM-1, TEM-2 and SHV-1. In general ESBLs are variably capable of hydrolysis second and third generation cephalosporins as well as older β-lactamase inhibitors such as tazobactam, clavulanic acid and sulbactam. The term ESBLs generally refers to the oxyimino β-lactamases (Garau, 1994).

TEM β-LACTAMASE ENZYME

TEM-1 is the most commonly encountered β-lactamase gram-negative bacteria. Up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1 (Livermore 1995). This enzyme is also responsible for the penicillin and ampicillin resistance that is seen in *Haemophilus influenza* and *N. gonorrhoea* in increasing

numbers. TEM-1 is able to hydrolyse penicillins and cephalosporins such as cephalothin and cephaloridine. TEM-2, the first derivative of TEM-1 had a single amino acid substitution from the original β-lactamase. This caused a shift in the isoelectric point from a pl of 5.4 to 5.6, but it did not change the substrate profile. TEM-3 originally reported in 1989, was the first TEM- type β-lactamase that displayed the ESBL phenotype. In the years since the first report, over 90 additional TEM derivatives have been described. The amino substitution that occurs within the TEM enzyme occurs at a limited number of positions. The combinations of these amino acid changes result in various subtle alterations in the phenotypes such as the ability to hydrolyze specific oxyimino-cephalosporins such as ceftazidime and cefotaxime, or a change in their isoelectric points. A number of amino acid residues are especially important for producing the various phenotypes when substitution occurs at that position. In addition to β-lactamases TEM-1 through TEM-92, there has been a report of a naturally TEM-like enzyme, TEM-AQ that contained a number of amino acid substitution and one amino acid delection that have not been noted in other TEM enzymes. Although TEM type β-lactamases are mostly found in *E .coli* and other enteropathogens e.g. *Klebsiella spp*., they are also found in other species of gram-negative bacterial with increasing frequency. TEM-type ESBL has been reported in general of Enterobactriaceae (Marchandin *et al*., 1999; Bonnet *et al*., 1999).

Inhibitor-Resistant β-Lactamase enzyme:

Of the over 90 additional TEM derivatives that have been described, some of these are inhibited resistant enzymes, but the majority of the new derivatives are ESBLs. Although the inhibitor resistant β-lactamases are not ESBLs, they are often discussed with ESBL s because they are also derivative of the classical TEM- or SHV- type enzymes. In the early 1990s β-lactamases that were resistant to inhibition by clavulanic acid were discovered. Nucleotide sequencing revealed that these enzymes were variants of the TEM-1 or TEM-2 β-lactamases. These enzymes were at first given the designation IRT for inhibition – resistant TEM β-lactamase; however, all have subsequently been renamed with numerical TEM designations. There are least 19 distinct inhibitor resistant TEM β-lactamases (Bradford, 2001). Inhibitor-resistant β-lactamases have been found mainly in clinical isolates of *E. coli* but also some strains of *K. pneumoniae, K. oxytoca, P. mirabilis and Citrobacter freundii* (Bret *et al*., 1996; Lemozy *et al*., 1995).

SHV β-lactamase Enzyme

Unlike the TEM-type β-lactamases, there are relatively few derivatives of SHV-1. The SHV-1 β-lactamases is most commonly found in *E. coli* and *K. pneumonia* and is responsible for up to 20 % of the plasmid mediated ampicillin resistance in these species (Tzouvelekis and Bonomo, 1999). In many strains of *E. coli* bla shv -1 or a related gene is integrated into the bacterial chromosome (Livermore, 1995). The changes that have been observed in bla shv to give rise to the SHV variants occur in fewer positions within the structural gene. To date, the majority of SHV-type derivatives possess the ESBL phenotype, one variant; SHV-10 is reported to have an inhibitor-resistant phenotype.

CTX-M Enzymes

A new family of plasmid-mediated enzymes called CTX-M that preferentially hydrolyzes cefotaxime was found mainly in *Salmonella*

enterica serovar Typhimurium and *E. coli* but have also been described in other species of Enterobacteriaceacea. They include the CTX-M type enzymes (CTX-M-1 formerly called MEN-1), CTX-M-2 through CTX-M-10 (Bonnet *et al*., 2000; Bradford *et al*., 1998; Bauernfeind *et al*., 1996; Barthelemy *et al*., 1992; Bauernfeind *et al*., 1990; Olowe et al., 2010). These enzymes are not very closely related to TEM or SHV β-lactamases in that they show only approximately 40% identity with these two commonly isolated β-lactamase (Tzouvelekis *et al*., 1999).

OXA Enzymes

The OXA enzymes are another growing family of ESBLs. These β-lactamases differ from the TEM and SHV–enzymes in that they belong to the molecular class D and functional group 2d (Bush *et al*., 1995). The OXA–type ampicillin also confers resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid (Bush et al., 1995). While most of the ESBLs have been found mainly in *E. coli*, *K. pneumoniae* and other Enterobacteriaceae, the OXA-type ESBLs have been found mainly in Pseudomonas aeruginosa. Several of the OX- types ESBLs have been derived from OXA-10.

CONCLUSION

Antimicrobial drug resistance occurs everywhere in the world and is not limited to industrialized nations. Hospitals and other healthcare settings are battling drug-resistant organisms that spread inside these institutions. Drug-resistant infections also spread in the community at large. Examples include drug-resistant pneumonias, sexually transmitted diseases (STDs), and skin and soft tissue infections. Until the discovery and approval of new compounds, strategies can be employed to slow the development of resistance. For example, we must avoid under-dosing, which is a common yet often unrecognized factor associated with treatment failure and bacterial resistance. An understanding of pharmacokinetic and pharmacodynamic principles can optimize antibiotic use, such as by increasing the time above the minimum inhibitory concentration with β-lactams, and by maximizing the peak level or area under the concentration curve with fluoroquinolones and aminoglycosides. (Craig 1998). Resistance containment depends on very early empirical and aggressive treatment for potentially resistant pathogens, followed by de-escalation and narrowing of the antimicrobial spectrum after identifying the pathogen. Empirical therapy should be discontinued altogether if a diagnosis of infection seems unlikely. De-escalation is a crucial infection-management technique and an effective strategy that balances the need to provide early adequate antibiotic therapy to high-risk patients and the objective of avoiding antibiotic overuse (Kollef, 2001).

The WHO estimates that the effect of communicable diseases on global health will fall steadily over the next 25 years. But these projections are based largely on estimates of economic, social and demographic developments, and their historical association with mortality rates. These predictions are extrapolations of improvements in the last 50 years, largely through pharmaceutical interventions. But the forecasts do not take into account one of the most striking trends in recent years the reversal of antibiotic effectiveness. Estimates of global health are one of the most important instruments for decision-makers on national and global health issues. But current predictions underestimate the

potential role of antibiotic resistance in the emergence and resurgence of infectious diseases in the coming decades. Underestimates are usually due to lack of data, making it difficult to generalise about the impact of antibiotic resistance on treatment outcomes, and on global health and economic burdens. It would thus be justifiable and timely to encourage the implementation of international surveillance systems on antibiotic resistance. This could be achieved by connecting already existing national and international initiatives and by agreements on data collection and exchange. The Pan American Health Organization, which successfully supports national surveillance in all Latin American countries by providing quality control and diagnostic standards, has shown that this can be achieved in low-income and middle-income countries. An international exchange of surveillance data like the one currently funded by the European Centre for Disease Prevention and Control would be the ultimate aim and indeed a formidable task for the WHO.

Vaccines do not have the problem of resistance because a vaccine enhances the body's natural defenses, while an antibiotic operates separately from the body's normal defenses. Nevertheless, new strains may evolve that escape immunity induced by vaccines; for example an updated influenza vaccine is needed each year. While theoretically promising, antistaphylococcal vaccines have shown limited efficacy, because of immunological variation between *Staphylococcus* species, and the limited duration of effectiveness of the antibodies produced. Development and testing of more effective vaccines is under way. The Australian Commonwealth Scientific and Industrial Research Organization (CSIRO), realizing the need for the reduction of antibiotic use, has been working on two alternatives. One alternative is to prevent diseases by adding cytokines instead of antibiotics to animal feed These proteins are made in the animal body "naturally" after a disease and are not antibiotics, so they do not contribute to the antibiotic resistance problem. Furthermore, studies on using cytokines have shown they also enhance the growth of animals like the antibiotics now used, but without the drawbacks of nontherapeutic antibiotic use.

Phage therapy an approach that has been extensively researched and used as a therapeutic agent for over 60 years, especially in the Soviet Union, represents a potentially significant but currently underdeveloped approach to the treatment of bacterial disease (Keen, 2012). Phage therapy was widely used in the United States until the discovery of antibiotics, in the early 1940s. Bacteriophages or "phages" are viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. (Chanishvili *et al*., 2001; Jikia *et al*., 2005; Weber-Dabrowska *et al*., 2003).

One of the major causes of antibiotic resistance is the decrease of effective drug concentrations at their target place, due to the increased action of ABC transporters. Since ABC transporter blockers can be used in combination with current drugs to increase their effective intracellular concentration, the possible impact of ABC transporter inhibitors is of great clinical interest. ABC transporter blockers that may be useful to increase the efficacy of current drugs have entered clinical trials and are available for therapeutic regimens (PSA, 2009). Great effort,

rigorous research, enlightens and a continued commitment to these challenges with proper planning can help us to overcome the problems associated with drug resistance in bacteria.

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