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RESISTANCE TO β-LACTAM ANTIBIOTICS AND STRATEGIES TO COMBAT ANTIMICROBIAL RESISTANCE-A REVIEW

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ABSTRACT

Antimicrobial resistance is a problem of concern in the treatment of infectious diseases. If unnoticed, it may result in serious complications of infectious diseases. Resistance to antibiotics emerged due to the inappropriate and irrational use of antibiotics. Even though resistance can arise during natural evolution process, notable resistance has emerged from horizontal gene transfer between resistant and sensitive organisms. The Present review article describes the different types of beta lactam antibiotics, their mechanism of action and the possible mechanisms of their resistance. The mechanism of resistance is highly variable among organisms. To reduce the prevalence of antibiotic resistance, many new strategies have been developed. The article also imparts that the antibiotic surveillance studies are the prime of it which helps to record the nature of resistance in different regions. Regarding the β lactam resistance many new β lactamase inhibitors has been discovered to combat the resistance. Since the available inhibitors pose side effects, plant derived compounds also finds its way in resisting the antimicrobial resistance.

1. INTRODUCTION

History of medicine has a profound effect upon human life and society with the development of power to control infectious diseases. The publications of Pasteur and Koch family established that microorganisms are the cause of infectious disease. Chemotherapy however really began with Paul Ehrlich during the period of ten years from 1902 which governed many concepts of antimicrobial agents. Ehrlich continued his research towards the search of compounds with more curative effect and low toxicity to be safe as

chemotherapeutic agents. In 1910, the famous drug salvarsan, an organoarsenial compound was discovered for treating syphilis (Greenwood, 1995).

The term "chemotherapy" was invented by Ehrlich and he made a belief that infectious diseases could be treated by synthetic chemicals. According to Ehrlich, a chemotherapeutic substance has two functional features, the haptophore or binding group that helps to bind the drug to the specific receptor and the toxophore or toxic group which makes an adverse effect on the cell. After some 60 years of effort, the synthetic antibacterial compounds in current use include, sulphonamides, trimethoprim, imidazole, oxazolidinones etc., and semisynthetic β-lactam, aminoglycosides, macrolides etc., (Franklin and Snow, 2005a).

In 1928 Alexander Flemming discovered penicillin from the mold *Penicillium notatum*, which was found to be active against gram positive bacteria. The success of penicillin quickly diverted a great deal of scientific effort towards the search of other antibiotics. Selman Waksman discovered streptomycin and soon followed by several thousand antibiotics. Out of all 50 had some sort of clinical use and only a very few are employed in the regular therapy of infectious diseases (Franklin and Snow, 2005b). The traditional first-line available options for treatingserious infections caused by enterobacteria include penicillins, cephalosporins, monobactams, carbapenems, fluorquinolones, and in certain situations, aminoglycosides (Valverde *et al*., 2013).

2. Classes of antimicrobials

Antimicrobials are classified into many types based on their structure and mode of action. They are classified into five functional groups:

- Inhibitors of cell wall synthesis
- Inhibitors of protein synthesis
- Inhibitors of membrane function
- Antimetabolites
- Inhibitors of nucleic acid synthesis

Based on their structure they are mainly classified into two groups

1. β-lactams
2. Aminoglycosides

Among this β-lactam forms the major group. In 2002, the global market for antibiotics was estimated at 25 billion dollars, of which about 50%was β-lactam antibiotics (Coates *et al*., 2002). In addition β-lactam antibiotics are the most frequently applied in treatment of bacterial infections. β-lactam antibiotics all share the presence of the β-lactam ring, a four-membered ring in which a carbonyl and a nitrogen are joined in an amide linkage (Ganguly *et al*., 2011)

The clinical success of the first β-lactam, penicillin G (benzylpenicillin),

prompted the search for and development of additional derivatives and gave rise to the β-lactam antibiotics in clinical use today (penicillins, narrow and extended-spectrum cephalosporins, monobactams, and carbapenems). The common structural feature of these classes of antibiotics is the highly reactive four-membered β-lactam ring (Babic *et al*., 2006).

The large number of natural, semisynthetic and synthetic β-lactam antibiotics can be subdivided into 6 different structural subtypes:

(i) penams (e.g. benzylPenicillin, Ampicillin);

(ii) cephems which include classical cephalosporins, 2nd generation cephalosporins (e.g. cefotiam, cefuroxime), and also representatives of 3rd generation cephalosporins (e.g. cefo-taxime, ceftazidime);

(iii) cephamycins as 7-β-methoxyCephalosporins (e.g. cefoxitin);

(iv) monobactams as monocyclic β- lactam molecules (e.g. aztreonam);

(v) penems with a 2,3-double bond in the fused thiazoline ring (e.g. faropenem); and

(vi) carbapenems (e.g. Imipenem) with an unsaturated fused 5-membered ring differing from penem structure by possession of a carbon atom at position 1.

3. Mechanism of action of β-lactam antibiotics

β-lactam antibiotics exhibit their bactericidal effects by inhibiting enzymes involved in cell wall synthesis. The integrity of the bacterial cell wall is essential to maintain the cell shape in a hypertonic and hostile environment. Osmotic stability is preserved by a rigid cell wall comprised of alternating N-acetyl- muramic acid (NAM) and N-acetylglucosamine (NAG) units. These glycosidic units are linked by transglycosidases. A pentapeptide is attached to each NAM unit, and the cross-linking of two D-alanine–D-alanine NAM pentapeptides is catalyzed by PBPs, which act as transpeptidases. This cross-linking of adjacent glycan strands confers the rigidity of the cell wall. The β-lactam ring is sterically similar to the D-alanine–D-alanine of the NAM pentapeptide, and PBPs "mistakenly" use the β-lactam as a "building block" during cell wall synthesis. This results in acylation of the PBP, which renders the enzyme unable to catalyze further transpeptidation reactions. As cell wall synthesis slows to a halt, constitutive peptidoglycan autolysis continues. The breakdown of the murein leads to cell wall compromise and increased permeability. Thus, the β-lactam mediated inhibition of transpeptidation causes cell lysis,, and the specific details

of penicillin's bactericidal effects are still being unravelled (Drawz1 and Bonomo 2010).

4. Antimicrobial resistance mechanisms

Antibiotics work by interacting with specific bacterial targets, inhibiting bacterial cell-wall synthesis, protein synthesis or nucleic acid replication. To accomplish this, the antibiotic must have access to and bind to its bacterial target site. Whether antibiotic resistance is intrinsic or acquired, the genetic determinants of resistance encode specific biochemical resistance mechanisms that may include

- Enzymatic inactivation of the drug,
- Alterations to the structure of the antibiotic target site, and
- Changes that prevent access of an adequate concentration of the antimicrobial agent to the active site (Neu, 1992 and Koneman *et al*., 1997).

There are four primary mechanisms by which bacteria can overcome β-lactam antibiotics (Babic *et al*., 2006):

- Production of β-lactamase enzymes is the most common and important mechanism of resistance in gram-negative bacteria.
- Changes in the active site of PBPs can lower the affinity for β-lactam antibiotics.
- Decreased expression of outer membrane proteins (OMPs).
- Efflux pumps, as part of either an acquired or intrinsic resistance phenotype, are capable of exporting a wide range of substrates from the periplasm to the surrounding environment.

4.1. Enzymatic resistance mechanism

Bacteria may produce enzymes that modify or destroy thechemical structure of an antibiotic, which renders it inactive. This mechanism of resistance is probably best exemplified by the β-lactamase family of enzymes, which act by hydrolyzing the β-lactam ring of penicillins, cephalosporins and carbapenems. There are hundreds of β-lactamase enzymes that may be distinguished by their substrate profiles and activities. Some β-lactamase genes are chromosomal, whereas others are located on plasmids or transposons. Penicillin resistance in *S.aureus* and *Neissseria gonorrheae*, ampicillin-resistance in *Haemophilus influenzae*, and resistance to extended-spectrum Cephalosporins in *E. coli* and in *Enterobacter* species are all commonly mediated by the production of β-lactamases. Resistance to extended-spectrum Cephalosporins (e.g., Cefotaxime, Ceftriaxone, and Ceftazidime) has arisen primarily by 1 of 2 mechanisms,

both of which involve the production of β-lactamases (Bradford, 2001).

4.1.1. β-lactamases

β-lactamases (β-lactamhydrolyases, EC 3.5.2.6) are enzymes that open the β-lactam ring, inactivating the antibiotics (Figure 1**).** Reports on β-lactamases have been increasing in many countries.β-lactamases are the main cause of bacterial resistance to penicillins and cephalosporins. Definitive identification of these enzymes is only possible by gene or protein sequencing aspects (Livermore and Brown, 2001).

β-lactamases (ESBL) are enzymes that confer resistance to most β-lactam antibiotics, including penicillins, cephalosporins, and the monobactam-azotreonam. Community-acquired ESBL producing Enterobacteriaceae are prevalent worldwide (Rodriguez and Jones 2002). The first plasmid-mediated β-lactamase in gram-negative bacteria was discovered in Greece in the 1960s. It was named TEM after the patient from whom it was isolated (Temoniera). The first β-lactamase enzyme was identified in Bacillus (*Escherichia*) coli before the clinical use of penicillin. In a sentinel paper published nearly 75 years ago, B.coli is described as "Penicillinase" (Abraham and Chain 1940).

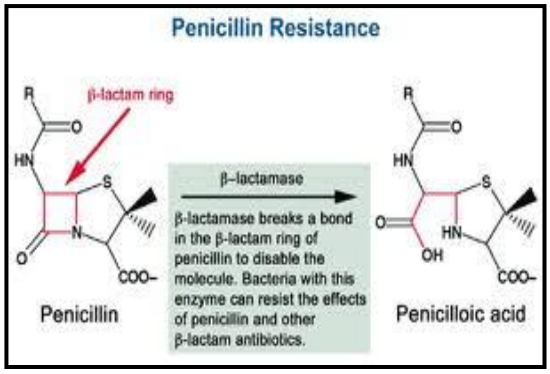

Figure 1 Hydrolysis β-lactam ring in Penicillin by β-lactamase enzymes

Extended spectrum β-lactamases are the enzymes with broad substrate specificity to β-lactam antibiotics and were first identified in the year 1983 (Knothe *et al*., 1983).They are associated with increased morbidity and mortality, especially amongst patients on intensive care and high-dependency units. Thus β-lactamases predate the antibiotic era. The widely accepted molecular classification places β-lactamases into four classes: three serine-dependent enzyme classes (classes A, C, and D) and one metal-dependent (class B).

Class A β-lactamases

This is the largest and best mechanistically characterized serine β-lactamase class. Historically, these β-lactamases were described as "penicillinases" as their ability to catalyze penicillin hydrolysis was greater than that for cephalosporins. The class A β-lactamases are closely related in sequence

to low molecular weight class C PBPs such as PBP4 of *E. coli*, *H. influenza*, and *Mycobacterium tuberculae* (Massova and Mobashery, 1998). New class A β-lactamases that are active against the more recent cephalosporins (ceftazidime and cefotaxime and the monobactamaztreonam) and others that are active against the carbapenems are known collectively (also with other class C and D enzymes) as "expanded-spectrum β-lactamases" (ESBL) (Bradford, 2001).

Class B β-lactamases

These metal-dependent (almost always divalent zinc) β-lactamases have a broad β-lactam substrate tolerance that encompasses many of the newer generation cephalosporins, carbapenems, and other β-lactamase inhibitory (clavulanate and penamsulfones) β-lactams important to the treatment of gram-negative infection (Livermore and Woodford, 2006, Nordmann and Poirel 2002).This enzyme was first observed in 1967 by Kawabata and Abraham as chromosomal enzymes of the innocuous gram positive *Bacillus cereus* – a spontaneous mutant strain producing class B β-lactamase constitutively (Walsh and Wright 2005).The structure and dynamics of metallo β-lactamases have been studied.

Class C β-lactamases

Class C β-lactamases share with the class A β-lactamases a similar mechanisms active site acylation and hydrolytic deacylations for β-lactam hydrolysis. The class C β-lactamases originally termed as cephalosporinases due to a substrate preference for cephalosporins. They are found, with few exceptions, in most Gram-negative bacteria and are chromosomally encoded in several organisms (including *Citrobacter freundii*, *Enterobacteraerogenes*, and *Enterobacter cloacae*) (Rice and Bonomo 2000, Hanson, 2003). An increased incidence of plasmid-encoded class C β-lactamases was observed 15 years after their first discovery (Hall and Barlow, 2004). Plasmid-encoded class C enzymes have been found in *E.coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *C.freundii*, *Enterobacter aerogenes*, and *Proteus mirabilis* (Bauernfeind *et al*. 1998). Most worrisome is that the rate of incidences of these enzymes is highest in *Klebsiella pneumoniae* and *E.coli*, organisms common to the hospital and community settings (Rice and Bonomo, 2000).

Class D β-lactamases

The class D β-lactamases are increasingly encountered among the defensive β-lactamase ensemble of certain gram-negative pathogens (Nordmann and Poirel, 2002). These β-lactamases were first termed as oxacillinases for their ability to hydrolyze the 5-methyl- 3-

phenylisoxazole-4-carboxy side chain penicillin class, exemplified by oxacillin and cloxacillin. Over 50 class D OXA variants are now known (Heritier *et al*., 2004). The first studies on class D β-lactamases showed that the domain folding was similar to serine β-lactamases (Paetzel *et al*., 2000). But later it was demonstrated that lysine lies in the active site of this class of enzyme (Maveyraud *et al*., 2002). Class D gene in gram positive bacteria possesses structural and functional relationship with Penicillin binding protein.

4.1.2. β-lactamase genes

β-lactamase genes are generally located on large transferable plasmids that often carry other resistance determinants such as those for aminoglycosides, tetracycline, sulphonamides and chloramphenicol (Jacoby and Mederios 1991). All enterobacteriaceae can harbor plasmid-mediated ESBL genes (Bouchillon *et al*., 2002 and Lautenbach *et al*., 2001). β-lactamase TEM is found to be predominant and very lesser amount of *blaSHV* and *blaOXA*-1 were reported in Enterobacteriaceae. *blaTEM* and bla*SHV* genes were also found to be in combination form (Colom *et al*., 2003). β-lactamase genes families of *blaTEM*, *bla*SHV, *blaVEB* and *blaCTX-M*, were reported to be highly prevalent in many countries (Bradford, 2001; Chanawong *et al.*, 2007; Empel *et al*., 2007). Plasmid analysis of β-lactamase genes in *Klebsiella pneumoniae* showed that Kp4940 and Kp1 resistance gene was carried by one of two small plasmids with estimated sizes of 6 and 14 kb, respectively. The small size of the Kp1 plasmids suggested that they were not self-transferable, but probably mobilized by the 60 kb plasmid (Laksania *et al*., 2000). Mobilisation of ESBL genes in environment led to the rise of *blaCTX* family enzyme in Enterobacteriaceae (Bonnet, 2004).

β-lactamase*TEM* gene

TEM and *SHV*-derived extended-spectrum β-lactamase genes producing enterobacteriaceae had been reported from throughout the world, but there has been limited data for the molecular characterization of these enzymes (Tash and Bahar, 2005). Zeba *et al*. (2004) had reported that β-lactamases bla*SHV* gene may be common among *Klebsiella*spp and *E.coli* species. *Klebsiella pneumoniae* strain BDK0419 contained a transferable plasmid with a molecular size 21 kbp that carries both *blaSHV-2a* and *blaCTX-M*-54 β-lactamase genes, along with two other plasmids. (Bae *et al*., 2006).

β-lactamase*SHV* gene

SHV type β-lactamases can be easily detected using PCR and melting curve analysis in which enzymes of nearly

32 clinical isolates can be detected within one hour (Randegger and Hachiler, 2001). LCR typing permitted for the definition the *SHV* families with simplicity and reliability and can be applied to the detailed characterization and molecular epidemiology of *SHV* type β-lactamases (Kim and Lee, 2000).

4.1.3. Mutations in β-lactamase genes

Mutations in genes that encode resistance to β-lactam antibiotics were described in different studies, which commonly include mutations in *blaTEM* at position 21,164 and 265. These mutations may be associated with either increase or decrease in β- lactam resistance (Stobberingh, *et al*., 1999).

A substantial number of *blaTEM* was associated with mutations with absence of a fragment of 136 base pairs located upstream the promoter region including the -35 region and not the -10 region of the promoter. This finding was associated with increased resistance to cefaclor when compared to the normal isolates. In addition 3.9% of isolates carried the bla Rob gene (Molina *et al*., 2003). Single nucleotide specific PCR was used to discriminate the polymorphic nucleotides at positions 32 and 317 of the *blaTEM* genes from a collection of TEM-positive strains (Tristram *et al*., 2005).According to the amino acid sequence, SHV β-lactamases in Taiwan were basically derived through stepwise mutation from *SHV*-1 or *SHV*-11 and further subdivided by four routes. (Chang *et al*., 2001).

4.1.4. Amino acid substitutions in β-lactamase genes

Zeba *et al*. (2004) had highlighted a typical *blaSHV*11 (clone 5) gene of 861 bp isolated from *K. pneumoniae* N°39 which was not identical with *blaSHV*-11 genes available in gene Bank (NCBI-DB). The nucleotide, alignment of *blaSHV*-11 gene and that of clone 5 revealed substitutions at position 324 (cytosine replace thymine), 357 (thymine replace cytosine), 762 (cytosine replace thymine) and 795 (cytosine replace thymine). This report shows that the amino acid change always occurs between cytosine and thymine. Therefore, on the basis of nucleotide sequences, the two genes (classical *blaSHV*-11 and clone 5) are slightly different, but the translation of the two genes yields the same variant *SHV*-11 β-lactamase.

5. Strategies to combat antimicrobial resistance

5.1. β-lactamase inhibitors

β-lactamase inhibitors generally inhibit ESBL producing strains.β-lactamase inhibitors, such as clavulanic acid, sulbactam, or tazobactam are largely

prescribed in association with amino and ureidopenicillins for treating gram-negative infections. Clavulanic acid, the first β-lactamase inhibitor introduced into clinical medicine, was isolated from *Streptomyces clavuligerus* in the 1970s, (Reading and Cole, 1977). Clavulanate (the salt form of the acid in solution) showed little antimicrobial activity alone, but when combined with amoxicillin, clavulanate significantly lowered the amoxicillin MICs against *S. aureus, K. pneumoniae*, *Proteus mirabilis* and *E. coli* (Brown, 1986). However with the increased use of amoxyclav resulted with resistance to them (Guibout *et al*., 2000).

5.1.2. Class A β-lactamase inhibitors

There are suicide inactivators of Ambler class A β-lactamases (CTX-M and the ESBL derivatives of TEM-1, TEM-2, and SHV-1). Several mechanisms, however, allow enterobacteriaceae to overcome the efficacy of these molecules, such as overproduction of a cephalosporinase or of narrow-spectrum class A enzymes, limited uptake of the antibiotics, production of OXA-type enzymes, and production of β-lactamase inhibitor-resistant TEM or SHV derivatives (Therrien and Levesque, 2000).

5.1.2.Class C β-lactamase inhibitors

Boronic acid is used as an effective inhibitor for class C β-lactamases whereas clavulinic acid acts as an effective inhibitor for ESBL (Liebana *et al*., 2004). β-lactamase enzyme activity was also inhibited by inhibitors such as EDTA, iodine, Cu^{2+}, and Hg^{2+}, sulbctam. Mercaptoacetic acid thiol esters (Yang and Crowder, 1999) and thiomandelic acid (Mollard *et al*., 2001) had been identified as metallo-β-lactamase inhibitors. Boronic acid is used as an effective inhibitor for class C β-lactamases whereas clavulinic acid acts as an effective inhibitor for ESBL (Liebana *et al*., 2004). β-lactamase enzyme activity was also inhibited by inhibitors such as EDTA, iodine, Cu^{2+}, and Hg^{2+}, sulbctam. Mercaptoacetic acid thiol esters (Yang and Crowder, 1999) and thiomandelic acid (Mollard *et al*., 2001) had been identified as metallo-β-lactamase inhibitors.

5.2. Antimicrobial surveillance studies

The surveillance of antimicrobial resistance has as its goal in the gathering of information for several purposes at every level where health care is provided. No country has a reliable, longitudinal, full-service antimicrobial resistance surveillance program with comprehensive focus, nor is there a comprehensive database for monitoring trends in antimicrobial usage. Thousands of clinical and basic research laboratories throughout the world generate resistance data. But

very few labs submit these data to appropriate databases that could allow local analysis or linking with a surveillance network (Obrien *et al*., 2001).

The effectiveness of surveillance data can be enhanced by integrating with other types of information. For instance, molecular studies of resistance can help to observe resistance phenotypes.. Surveillance of resistance can and should build on existing resources. Clinical laboratories in more than eighty countries have begun to build databases and link them into international networks using free software (WHONET) downloadable from a World Health Organization Web site (Obrien *et al*., 2001).

Surveillance data come essentially from three sources:

(1) Active surveillance,

(2) Passive surveillance involving reference laboratories, and

(3) Outbreak investigations.

5.2.1. Types of AMR surveillance

Three types of surveillance can be done for AMR

- Comprehensive surveillance - gives actual estimate of AMR burden, includes the study of the whole population at risk / under study and needs the involvement of a large number of laboratories which is not practical, especially in our country.
- Point prevalence studies - useful for validation of the representativeness of the surveillance data.
- Sentinel surveillance studies - suitable mode of surveillance when prolonged and detailed data are needed. This seems to be the best approach for our country.

5.2.3. Local surveillance

Local level surveillance forms the foundation for the national and international comprehension of antimicrobial resistance. These data highlight the importance of understanding 'microtrends' within larger patterns. Susceptibility testing should be performed in all local laboratories and hospitals that help to draw a conclusion for a particular antibiotic (Osterholm, 1998).

5.2.4. National systems

Surveillance should not be limited to the health-care sector. Mechanisms of resistance to any new antibiotic may already exist in nature, so any resistance encountered in nonpathogenic organisms in the environment or antibiotic producers should also be entered into the database. Clinicians and developers of diagnostics would then be aware of resistance mechanisms that may be encountered in the clinic. Though there are definite policies / standard treatment guidelines for appropriate use of antimicrobials in

specific national health programmes e. g. RNTCP (Revised National Tuberculosis Control Programme), NACP (National AIDS Control Programme), NVBDCP (National Vector Borne Disease Control Programme), the same are not available for other diseases of public health importance like enteric fever, diarrhoea / dysentery, pneumonia, etc.

In this regard a task force has been constituted with the following terms of reference (Srivastava, 2011):

- To review the current situation regarding manufacture, use and misuse of antibiotics in the country.
- To recommend the design for creation of a national Surveillance System for antibiotic resistance.
- To initiate studies documenting prescriptions patterns and establish a monitoring system for the same.
- To enforce and enhance regulatory provisions for use of antibiotics in human & veterinary and industrial use.
- To recommend specific intervention measures such as rational use of antibiotics and antibiotic policies in hospitals.

Diagnostic methods pertaining to monitor antimicrobial resistance:

The Central Drugs Standard Control Organization (CDSCO), headed by the Drugs Controller General (India) in the Directorate General of Health Services, is concerned with the regulatory control over the quality of drugs, cosmetics and certain notified medical devices under the Drugs and Cosmetics Act, 1940 and rules made thereunder.

5.2.4. International systems

The surveillance network should not only be nationwide, but linked to international efforts to integrate worldwide data seamlessly. Multiple surveillance activities around the globe are attempting in different ways and at different speeds to move toward the ideal depicted in this report, but these systems, as a group, are uncoordinated and unstandardized. Thus, the magnitude of the resistance problem and its impact are really unknown and may be considerably understated.

SENTRY is the first collaborative, worldwide, longitudinal antimicrobial surveillance program to provide timely data on both community and hospital acquired infections with standard methodology. This project was launched in February 1997 in four regions; currently, there are 38 program sites in North America, 27 sites in 13 European countries, 10 sites in 7 South American countries, and 3 sites in Turkey. Japan, Australia, and countries in Asia and Africa are slated to join in 1998.CEM/NET (Centre for Epidemiologic molecules /

Network for epidemiologic tracking of antibiotic resistance pathogens) is an independent international alliance between clinical microbiologists and molecular biologists (Kristonsson, 1998).

5.3. Approach to new antimicrobials

There are several indications that new approaches are required to combat emerging infections and the global spread of drug-resistant bacterial pathogens.

One is the pattern in rates of death from infectious disease in the 20th century: from 1900 to 1980, the rate dropped from 797 per 100,000 people to 36 per 100,000 people, a reduction by a factor of more than 20 and a testament in part to the efficacy of antibiotics (Armstrong *et al*., 1999).

A second indication of the need for novel antibacterial therapeutics is the almost 40-year innovation gap between introductions of new molecular classes of antibiotics: fluoroquinolones in 1962 and the oxazolidinone linezolid in 2000 (Walsh, 2003). A third indication is the recent trend by several large pharmaceutical companies to leave the antibacterial and anti-fungal therapeutic arenas, suggesting a future decrease in scientific expertise in antibacterial-drug discovery and development skills (Projan, 2003, Shlaes 2003). It is very hard to displace the establishing resistance and newer resistance continues to emerge and proliferate at new sites. Consequently there remains strong need for new antibiotics particularly towards Multidrug resistance in gram negative isolates (Vashishtha, 2010).

Among fifth generation of cephalosporin antibiotics, two compounds were introduced: ceftobiprolemedocaril (EMA - 2008) and ceftarolinefosamil (FDA - 2010, EMA 2012). Two new compounds *CXA*-101 and S-649266 are at the stage of clinical trials. In April 2010, Calixa Therapeutics, Inc. completed phase II of clinical trials on safety and efficacy of the *CXA*-101 (ceftolozane, FR264205) in comparison to ceftazidime, among patients with complicated urinary tract infections (cUTI).The advantage of this compound is low tendency to induce resistance and increased stability to β-lactamase type AmpC (Karpiuk and Tyski, 2013). The combination of *CXA*-101 with β-lactamase inhibitor – tazobactam in a concentration of 8μg/mL was found to be active against Enterobacteriaceae producing ESBL. This combination called *CXA*-201 shows efficacy against more than 90% of the Enterobacteriaceae producing extended spectrum β-lactamase type *CTX-M* (Bush, 2012).

5.4. Need for new molecules

The development of new antimicrobial agents is urgent. However, only a few new agents have entered full clinical development; these include newer aminoglycosides, β-lactams, β-lactam inhibitors, and tetracycline derivatives with activity against enterobacteria (Bush, 2012). New antibiotics can help stave off the catastrophe. But since 1987, no major antibiotic has been discovered. The science of antibiotics is complex and research on antibiotics is expensive and time consuming. It's not profitable for global big pharma as antibiotics are for short-term use. As the root of the crisis is a "patent cliff", a term coined for the sheer number of major drugs coming off patent between 2010- 2014. Patents protect the rights of original makers of a branded drug for 20 years to sell it exclusively. Once it expires, others can make and sell cheaper versions. Loss of avenues is the biggest challenge to R&D innovation (Datta, 2013).

5.5. Status for new antibiotics

Oxazolidinones was the only new antibiotic class joined in 1990s. All other introductions have been variants of existing classes. Only a few new antibacterial agents have received approval by the US Food and Drug Administration in the last 10 years, including linezolid in 2001, cefditorenpivoxil and ertapenem in 2002, gemifloxacin and daptomycin in 2003,telithromycin in 2004, and tigecycline in 2005 (Raghunath, 2008). Only a single new antibacterial doripenem has been approved in the USA since 2006. Many of these agents are improved derivatives from established classes of antibiotics, and several are directed primarily at resistant gram-positive bacteria (e.g., linezolid and daptomycin). These modified agents suffer from the disadvantages of the parent molecules. In view of the crossover of resistance across related compounds, the future may see sharply depleting antibiotic resources (Vashishtha, 2010).

5.6. New Therapeutic Approaches in Combating Antimicrobial Resistance

Understanding the substrate evolution, properties and modes of spread of these clinically important β-lactamases can help in formulating effective antibiotic policies and developing new antimicrobial agents. One strategy employed to overcome these resistance mechanisms is the use of combination of drugs, such as β-lactams together with β-lactamase inhibitors. Several plant extracts have exhibited synergistic activity against microorganisms. Synergy and mechanism of action between natural products including flavonoids and essential oils with synthetic drugs can be used in

effectively combating bacterial infections. (Hemaishwarya *et al*., 2008).

Using plants as the inspiration for new drugs provides an infusion of novel compounds or substances for healing disease (Iwu *et al*., 1999). Braga *et al*. (2005) have reported pomegranate extract can enhance the *in vitro* activity of certain antibiotics against strains of MDR *Staphylococcus aureus* and other pathogens. With an increasing demand to new drugs, plants are being utilized (Nasim *et al*., 2010). *Allium sativum* (Garlic) is a perennial, erect, bulbous plant and belongs to family Liliaceae (Pathmanathan *et al*., 2010). *Allium sativum* can be used as an adjunct with antibiotics to combat the effect of antibiotic resistance. (Abouelfetouch and Moussa 2012). Oxygenated sulfur compound in *Allium sativum* is reported as an important antimicrobial compound (Shobana *et al*., 2009).

6. CONCLUSION

The Problem of antibiotic resistance is major problem of concern in the treatment of infectious diseases. Many new surveillance studies should be carried out at regional and National level to monitor the existing resistance pattern of microbes against certain antibiotics. Surveillance studies should also be carried out at periodic intervals to record the changing pattern of resistance. Physicians should prescribe the antibiotics only after analyzing the susceptibility pattern. Public should be made aware of antibiotic resistance through many campaigns and certain guidelines should be followed nationwide for the usage of antibiotics.

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