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Review Article

Antimicrobial Resistance (AMR): A Global Problem

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The diversity of the microbial world and the specific activities of antimicrobial agents virtually ensures widespread resistance among bacteria. The current global threat of antimicrobial resistance has encouraged taking action in integrating research and public health in maintaining and promoting the national and international antimicrobial resistance research community. Infectious diseases still account for 45% of deaths in low-income countries and for almost one in two premature deaths worldwide. Most of these deaths (about 90%) are due to no more than six diseases: acute respiratory infections (mainly pneumonia), diarrhoeal disease, HIV/AIDS, TB, malaria and measles. Relaunching antimicrobial drug discovery and development should be a global priority. So, this review article spectaculates the advances in current situation of antimicrobial resistance based on the basis of theoretical and experimental researches done in medical sciences and pharmaceutical sciences. Promoting research and development of novel antimicrobial drugs needs to address the issue of the challenging commercial model and approaches with therapeutic strategies to resolve public health needs with an attractive economic model for the pharmaceutical industry to embark on global threat of antimicrobial resistance.

Keywords: Antimicrobial resistance, Global, Multidrug Resistant Bacteria, Prevalence, β-lactamases

INTRODUCTION

Antibiotic resistance is the acquired ability of the pathogen to withstand an antibiotic that kills off its sensitive counterparts, such resistance usually arising from random mutations in existing genes or from intact genes. Exposure to antibiotics and other antimicrobial products, whether in the human body, in animals or the environment applies selective pressure that encourages resistance to emerge favouring both naturally resistant strains and acquired resistance strains (ASM, 2009). Resistance is neither a new phenomenon nor unexpected in an environment in which potent antimicrobial agents are used. The diversity of the microbial world and the relatively specific activities of antimicrobial agents virtually ensures widespread resistance among bacteria. Resistance as a clinical entity is essentially a relative phenomenon and exists as a gradient that reflects phenotypic and genotypic variations in natural microbial populations (Denyer et al., 2004; Forbes et al., 2007; ASM, 2009).

Resistance in many ways is a problem only related to the microbiological techniques often used to detect it and must be recognised that most problems arise from expression of resistance by bacteria that are intrinsically susceptible to the antibiotic. Several factors like inoculum effect, intrinsic susceptibility, tolerance should be taken into account before classifying organism as resistance or susceptible (Murray et al., 2003). The emergence and spread of antimicrobial resistance due to the production of β-lactamases, major defense of Gram negative bacteria against β-lactam antibiotics i.e. penicillins,

cephalosporins, carbapenems, monobactams, clavams and oxacephems. Bacteria responded with a excess of new β-lactamases including Extended Spectrum β-lactamases (ESBLs), plasmid-mediated AmpC enzymes and carbapenem hydrolyzing β-lactamases (carbapenemases) with variable success, conferred resistance to the newer β-lactam antibiotics (Jacoby et al., 2005).

In recent years, the extensive and inappropriate use of antimicrobial agents has continually resulted in the development of antibiotic resistance which has become a major public problem worldwide as infection caused by Multi-Drug Resistant (MDR) strains often leads to death (Yadav and Prakash, 2016). With increased number of infections caused by antibiotic resistant bacteria are known as "ESKAPE" pathogens i.e. *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa* and *Enterobacter* spp. (Giske *et al*., 2008; Rice, 2008). They are the most frequent human pathogen which is responsible for upper respiratory tract infection, impetigo, folliculitis, furuncle, wound infections, osteomyelitis, bacteremia with metastatic complications, food poisoning, toxic shock syndrome, scaled skin syndrome, cellulitis, etc (Sampathukumar 2007). The rapidly increasing rates of infection due to methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *E. faecium* (VRE), and fluoroquinolone-resistant *P. aeruginosa* has been frequently reported (Bradford *et al*., 2004; Falagas *et al*., 2006; Boucher *et al*., 2009). MDR among common bacterial pathogens has resulted into treatment failures and increased economic burden (ASM, 2009).

The trend of antimicrobial resistance is particularly increasing due to the severity and diversity of diseases which has been noticed as one of the supreme microbial threats of the 21st century (Smolinski et al., 2003; Yadav and Prakash, 2016). Resistance of numerous bacterial pathogens to many antibiotics continues to increase globally. Frequencies pattern and distributions of resistant bacteria varies significantly with geographical regions and often reflect the practice patterns of antibiotics (Yadav et al., 2014). Antimicrobial chemotherapy has played a vital role in the medical intervention, control and management of human infectious diseases. The sheer number and continuing development of agents available, the marked increase in frequency of the microbial resistance towards the agents and frequent reports of establishment of polyantibiotic resistance; PAN drug resistant (PDR) organism in the hospital setting make it even more difficult for the clinicians to keep up pace in the field of development of antibiotics and antibiotic resistance as well as presents significant challenges for the clinical microbiologists to decide about the inclusion of various antimicrobials in the routine and specialized susceptibility testing (Bollero et al., 2001; Yao et al., 2003; Falagas et al., 2006; Forbes et al, 2007).

Therefore, surveillance on the antimicrobial susceptibility patterns of pathogens is of chief importance in understanding new and emerging resistance trends in the management of both hospital and community-acquired infections. This is a great concern due to the high rates of resistance to antimicrobials used in the treatment of infections caused by pathogens globally.

Predisposing factors of Antimicrobial Resistance

The predisposing factors of antimicrobial resistance plays a significant role in increasing and decreasing of prevalence of resistant strains. i.e.

- Host and clone specificity
- Plasmid and clone specificity
- Virulence
- Interactions with other commensal flora
- Duration of the selection pressure, and
- Variable gene expression (WHO, 2004; ASM, 2009).

Emergence of Antimicrobial Resistance

The emergence of antimicrobial resistance is inevitably linked to the clinical use of antimicrobial agents against which the resistance is directed. The two major reasons for this association are:

- Not testing for resistance to antibiotics that are not in clinical use.
- Nature adhors vacuum and so when an effective antimicrobial eliminate susceptible members of the flora, resistant varieties soon fill the niche (Murray et al., 2003; CDC, 2002).

Spread of Antimicrobial Resistance

- Failure to adhere to appropriate infection control techniques both within and outside the hospital.
- Improper hygienic practices that cause the transmission of resistant bacteria.
- Exposure of people to various centers like Day care center, nursing homes where probably resistance harbouring microorganisms are present and may get transmitted.
- Transmission from patients to patients, presumably by transiently or persistently colonized health care workers etc.
- Non-human niches in which antibiotics are used in excess (Roberts et al., 1998; Sherertz et al., 1996; Wegener et al., 1999).

Mechanisms of Antimicrobial Resistance

Antibiotic resistance arises by chance through mechanisms that may represent the legacy of natural competition among microorganisms. The mechanisms, genes, pathways of antibiotic production and resistance help microorganisms compete for niches in nature.

Therefore, they are fundamental components of microbial life and represent normal evolutionary phenomena. Resistance in microorganisms can be attributed to various mechanisms either acting singly or in combinations. The mechanisms of antimicrobial resistance are on the genetical bases and bichemical bases.

1. Genetic bases of Antimicrobial Resistance

The various ways in which genetic change from antibiotic sensitivity to resistant may be produced. They are:

i. Mutation of Cellular genes
Changing of a single amino acid as a result of the single base change in the gene within the protein, make the antibiotic-protein interaction unfavourable resulting in resistance to that antibiotics. Eg. Rifampin targets cellular RNA polymerase rpoB- mutation in rpoB gene confers complete resistance (Sharma and Mohan, 2006).

ii. Acquistion of Resistance genes
Bacteria acquire these resistant genes in various ways. i.e.

- **Natural transformation**

The ability of some bacteria is to absorb naked DNA molecules from the environment under appropriate circumstances. These foreign pieces of DNA are then incorporated into bacterial chromosome by recombining across region of sufficient homology (Rice, 2000).

• **Conjugation**
It is the most commonly employed mechanism for genetic exchange and occur more frequently by the transfer of cojugative plasmid. These extrachromosomal replicative DNA forms can encode a large variety of important genes. R plasmids often contain many resistance genes; they are maintained stably in the host strains of bacteria and are transferred very efficiently to neighboring drug-susceptible cells. Most drug resistance genes are effective when expressed from plasmids and remarkably, many such genes are often present on a single R plasmid, so that multidrug resistance can be transferred to a susceptible bacterium in a single conjugation event (Nikaido, 2009).

- **Transduction**

It is the process acquisition of genes via a bacterial virus called phage. Transduction can be either specialized or generalized ones (Alekshun et al., 2007).

- **Transposons**

These are the non replicative elements known to code for resistance to antibiotics. They encode their own ability to transfer between replicons and sometimes even code for their conjugation allowing them to transfer within bacterial chromosomes (Rice, 2000). The non conjugative transposons, however, integrate themselves into the transferable plasmids either transiently or permanently (Shaw et al., 1993).

iii. Mutation of Acquired genes

It is the process whereby the genes that are acquired, further mutated exhibiting even broad spectrum of antimicrobial resistance (Jacoby et al., 1991).

2. Biochemical Bases of Resistance

The biochemical mechanisms of resistance exhibited by cells to antagonise the action of antibiotics are as follows.

i. Modification of the antibiotics
Many antibiotic modifying enzymes have been known including the β-lactamases, aminoglycoside modifying enzymes and chloramphenicol acetyl transferases. These enzymes in most cases are acquired, in some cases are intrinsic though expressed at low levels. In genera like *Enterobacter* and *Pseudomonas*, these enzymes are under regulatory control with de-arrangements in these regulatory mechanisms resulting in high level of broad spectrum β-lactam resistance (Jacobs et al., 1995).

- **Modification of the target molecule**

Since minor alterations of the target molecule has a pronounced effect on antibiotic binding. For eg. Interaction between Erythromycin-Ribosomal methylase confer resistance to the macrolide-lincosamide-streptogramin B classes of antibiotics. Modification of the penicillin binding proteins (PBPs) alter the interaction of β-lactams with these proteins. Change in PBP2 or PBP2a resulted in the emergence of methicillin resistance *Staphylococcus aureus* (MRSA) (Livermore, 1992).

- **Restricted access to the target**

It is axiomatic that an antibiotic must reach its target in order to be effective. Therefore, when barriers must be crossed by the antibiotic before it can reach its target, strengthening these barriers can be a highly effective mechanism of resistance. All gram-negative bacteria have an outer membrane that must be crossed before the cytoplasmic membrane can be reached. Reduction in the quantities of presumed porins have been documented as an important contributors to resistance to imipenem in *Ps. aeruginosa*, cefepime in *Enterobacter cloacae* and cefoxitin or ceftazidime in *K. pneumoniae* (Lee *et al*., 1991; Livermore, 1992).

- **Efflux pumps**

Efflux pumps are the pumps that remove one or more antibiotics from the bacterial cell. Several classes of pumps have been described in gram-positive and gram-negative bacteria which may be quite selective and have a broad substrate specificity. The majority of these pumps are located in the cytoplasmic membrane and use

proton motive force to drive drug efflux. The major family of efflux transporters are-

- The major facilitator superfamily which includes QacA and NorA/Bmr of gram positive bacteria and EmrB of E. coli.
- The small multi-drug resistance family, including Smr of S. aureus and emrE of E. coli
- The Resistance Nodulation division family, including AcrAB-TolC of E. coli and MexAB-OprM of Ps. aeruginosa.

Multi-drug efflux pumps of the ATP-Binding Cassette Superfamily (Lee et al., 2000, Alekshun et al., 2007; Nikaido, 2009).

Multi-drug Resistance (MDR)

Multi-drug resistance (MDR) is defined as resistance to at least two antibiotics of different classes including aminoglycosides, chloramphenicol, tetracyclines and/or erythromycin (Huys et al., 2005). MDR in many bacteria is due to the action of multi-drug efflux pumps which can pump out more than one drug type and by the accumulation on resistance (R) plasmids or transposons, of genes with each coding for resistance to a specific agent (Nikaido, 2009).

Inheritance of MDR

Bacterial antibiotic resistance can be attained through intrinsic or acquired mechanisms. Intrinsic mechanisms are those specified by naturally occurring genes found on the host's chromosome such as AmpC β-lactamase of gram-negative bacteria and many MDR efflux systems. Acquired mechanisms involve mutations in genes targeted by the antibiotic and the transfer of resistance determinants borne on plasmids, bacteriophages, transposons and other mobile genetic material. (Alekshun et al., 2007)

Table 1: Multi-drug resistance of bacterial isolates and its mechanism of resistance

Bacterial isolates	Antibiotic resistance	Mechanism of resistance	Antimicrobial agents with potential clinical use
Hospital associated MRSA	Vancomycin (both VISA and VRSA)	Thickening of cell wall; change in last amino acid of peptidoglycan precursor	Linezolid, quinpristin-dalfopristin, daptomycin, tigecycline, ceftobiprole, televancin, icaprim
	Daptomycin	Associated with changes in cell wall and cell membrane	Linezolid, quinpristin-dalfopristin, tigecycline, ceftobiprole,
	Linezolid	Mutation in 23 rRna genes, rarely acquisition of methl transferase gene *cfr*	Linezolid, quinpristin-dalfopristin, tigecycline, ceftobiprole, televancin icaprim
Vancomycin resistant Enterococcus faecium	Ampicillin	Mutation and over expression of pbp5	Linezolid, quinpristin-dalfopristin, Linezolid, quinpristin-

Table 1 Con'd

	Aminoglycosides	Aquisition of aminoglycoside modifying enzymes	No alternative for a reliable bactericidal effect alone or in combination
	Linezolid	Mutation in 23s rRNA genes	Linezolid
	Daptomycin	Unknown	Linezolid, quinpristin-dalfopristin, tigecycline
	Quinpristin-dalfopristin	Modifying enzymes and target	Quinpristin-dalfopristin
Escherichia coli, Klebsiella spp. and Enterobacter spp.	*oxyiminocephalosporins (Ceftriaxone, cefotaxime, ceftazidime, cefepime)*	ESBL (includes hyperproduction of AmpC enzyme by Enterobacteriaceae	Carbapenems and tigecycline
	Carbapenems	Production of carbapenamases and decreased permeability	Polymixins, tigecycline
Acinetobacter spp.	*Carbapenems*	Decreased permeability increased efflux and production of carbapenamases	Polymixins
Pseudomonas aeruginosa	Carbapenems	Decreased permeability increased efflux and production of carbapenamases	Polymixins

(Owens, 2009)

β-Lactams and β-lactamases

β-lactam antibiotics are the most commonly used antibiotics that kill bacteria by blocking the crucial transpeptidations that lead to mechanically strong peptidoglycan through the covalent cross-links of peptide strands. These includes Penicillins, Cephalosporins, Carbapenems, Monobactams, Clavams and Oxacephems (Walsh et al., 2003; Denyer et al., 2004). These are used in a wide variety of both systemic and localized infections including Respiratory tract infections, GI tract infections, CNS infections, Skin and soft tissue infections, Urinary tract infections etc. However, the clinical utility of β-lactam antibiotics is under threat with the rapid dissemination and emergence of β-lactamases of various types; extended spectrum β-lactamases, AmpC β-lactamases and Metallo β-lactamases (MBL) that can easily hydrolyse these antibiotics by breaking down the β-lactam ring. Carbapenems once considered the last resort antibiotics for treating infections caused by multi-drug-resistant Gram negative bacilli, has now become the substrate of the versatile β-lactamases i.e. MBL which easily hydrolyse them (Denyer et al., 2004; Lee et al., 2003; Franklin et al., 2006).

β-lactamases are a heterogenous group of proteins with structural similarities composed of α-helices, β-pleated sheets and are the members of a superfamily of active site serine proteases. β- lactamases have been designated as enzymes hydrolyzing amides, amidines and other C- N bonds separated on the basis of the substrates (Bush et al., 1995) which are the major cause of bacterial resistance to β-lactam antibiotics. They are secreted into the periplasmic space in Gram negative bacteria or into the surrounding medium by their Gram positive counterparts. (Livermore et al., 1995; Jacoby et al., 2005).

Classification of β-lactamases

Because of the diversity of the enzymatic characteristics of β-lactamases, two schemes are currently used to classify β-lactamases i.e. the Ambler classification scheme

and the Bush-Jacoby-Medeiros classification system. The Ambler classification scheme separates β-lactamases into four distinct classes based on similarities in amino acid sequence. Classes A, C and D are serine β-lactamases, whereas class B are metallo β-lactamases that require zinc for activity (Ambler, 1980). Similarly, the Bush-Jacoby-Medeiros classification system classifies β-lactamases according to functional similarities i.e. substrate-inhibitor profiles. There are four categories and multiple subgroups in this classification scheme (Group 1, 2, 3 and 2a, 2c, 3a etc) (Bush et al., 1995).

Table 2: β-lactamases Classification (Bush et al., 1995)

Bush-Jacoby-Medeiros system	**Major Subgroups**	**Ambler System**	**Main Attributes**
Group 1 Cephalosporinases		ClassC (cephalosporinases)	Usually chromosomal, resistance to all β-lactams except carbapenems, not inhibited by clavulanate
Group 2 Penicillinases (Clavulanic acid susceptible)	2a	Class A (Serine β-lactamases)	Staphylococcal penicillinases
	2b	A	Broad spectrum: TEM-1, TEM-2, SHV-1
	2be	A	Extended spectrum: TEM-3, SHV-2
	2br	A	Inhibitor resistant Tem (IRT)
	2c	A	Carbenicillin hydrolysing
	2e	A	Cephalosporinases inhibited by clavulanate
	2f	A	Carbapenemases inhibited by clavulanate
	2D	Class D (Oxacillin hydrolysing)	Cloxacillin hydrolysing (OXA)
Group 3 Metallo-β-lactamases	3a 3b 3c	Class B (Metalloenzymes) B B	Zinc dependent carbapenemases
Group 4		Not classified	Miscellaneous enzymes

Mode of Action of β-lactamases

β-lactamases catalytically disrupt the β-lactam (amide) bond to form an acyl enzyme complex. A conserved serine in the active site acts as the reactive nucleophile in the acylation reaction. A critically positioned water then acts as the attacking nucleophile in the deacylation process resulting in the release of penicilloyl and cephalosporyl moiety. Penicillin-binding proteins (PBPs) have similar mode of action, however, their structure don't allow easy acess of water such that β-lactamases have hydolysis rate for β-lactams upto 2-3000 times higher than PBPs (Ghuysen et al., 1991).

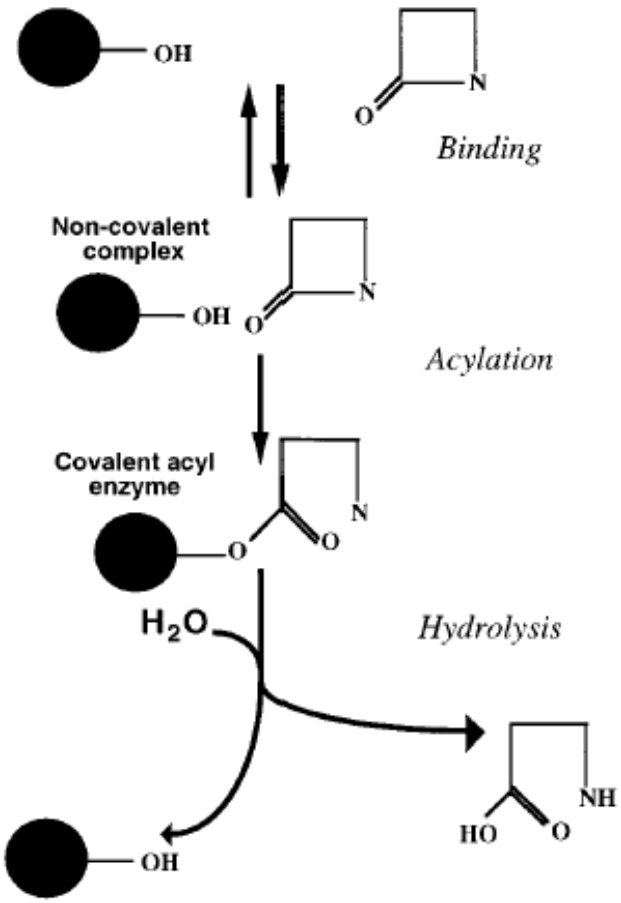

Figure 1: Action of a serine β-lactamase (Waley *et al*., 1992).

Extended Spectrum β-lactamases (ESBLs)

ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins, first, second and third generation of cephalosporins (such as cefazolin, cefuroxime, ceftazidime, cefotaxime, ceftriaxone etc) and aztreonam but not the cephamycins (eg cefoxitin and Cefotetan) and carbapenems (eg. imipenem and meropenem) by hydrolysis of these antibiotics and which are inhibited by β-lactamase inhibitors such as clavulanic acid. ESBLs are able to hydrolyze penicillins, narrow spectrum and third generation cephalosporins and monobactams with hydrolysis rate for ceftazidime, cefotaxime, or aztreonam at least 10% that for benzyl penicillin (Paterson et al., 2005; Bush, 2008; Ramphal et al., 2006; Pfaller et al., 2006).

Types of ESBLs

1. **SHV type:** It refers to sulfhydryl variable and was first reported in 1983 in Klebsiella ozaenae. Most frequently found ESBL type in clinical isolates than any other type.
2. **TEM type**: It is the derivative of TEM-1 and TEM-2. Over 100 TEM type β-lactamases have been reported.
3. **CTX-M and Toho β-lactamases:** These reflects the potent hydrolytic activity of β-lactamases toward cefotaximes than ceftazidimes.
4. **OXA types:** These β-lactamases are characterised by hydrolysis of cloxacillin and oxacillin greater than 50% that for benzylpenicillin and found predominantly in *Pseudomonas aeruginosa.*
5. **PER types:** These types of ESBLs efficiently hydrolyse penicillins and cephalosporins. However, shares only 25-27% homology with SHV and TEM types.
6. **VEB-1, BES-1, and other ESBLs** : These are either plasmid mediated or integron associated class A enzymes (Bonnet et al., 2000; Mavroidi et al., 2001).

Modes of Resistance to 2nd and 3rd generation Cephalosporins

Modes of resistance to 2nd and 3rd generation Cephalosporins in different bacterial isolates via:

- Hyper-produced chromosomal AmpC β-lactamases especially in *Enterobacter* spp.
- Plasmid-mediated AmpC β-lactamases in *Klebsiella* spp. and *E. coli*
- Hyperproduced K1 chromosomal β-lactamases in *K. oxytoca*, not *K. pneumoniae*
- Efflux-mediated resistance in *Ps. aeruginosa*

- Various ill defined mechanisms in *Acinetobacter* spp (HPA, 2005).

Logical indicator of ESBLs

The ideal indicator of cephalosporin is one to which all ESBLs confer resistance, even when their production is scanty. Choice is predicted by the following general traits -

- **TEM and SHV ESBLs** - Resistance to Ceftazidime , variable to cefotaxime.
- **CTX-M ESBLs -** Resistance to cefotaxime, variable to Ceftazidime.
- **All ESBLs -** Resistance to Cefpodoxime

Thus, the logical indicator is either cefpodoxime or both of cefotaxime and ceftazidime resistance (HPA, 2005).

AmpC β-lactamases (ABLs)

AmpC beta lactamases are clinically important cephalosporinases encoded on the chromosome of many Enterobacteriaceae and a few other organisms where they mediate resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and beta lactamase inhibitor/beta lactam combinations (Jacoby et al., 2009; Beceiro et al., 2004). The first bacterial enzyme reported to hydrolyze penicillin was the AmpC beta lactamase of *Escherichia coli* (Abraham et al., 1940). β-lactams with greater β-lactamase stability including cephalosporins, carbapenems and monobactams were resistance toward these antibiotics in *Enterobacter cloacae*, *Citrobacter freundii, Serratia marcescens* and *Pseudomonas aeruginosa* due to overproducing their chromosomal AmpC beta lactamase (Ruppe et al., 2006; Philippon et al., 2002). However, the most common cause of AmpC overexpression in clinical isolate is the mutation in ampD leading to AmpC hyperinducibility or hyperinducible phenotypes. ampR are less common but can result in high constitutive or hyperinducible phenotypes (Kaneko et al., 2005).

AmpC β-lactamases are produced to a greater or lesser degree by almost all gram negative bacteria including clinically important isolates of *Citrobacter freundii, Enterobacter aerogenes, E. cloccae, Morganella morganii, Pseudomonas aeruginosa* and *Serratia marcescens* with *Salmonella* and *Klebsiella* being the exceptions (Tenover et al., 2009). In many bacteria AmpC enzymes are inducible and can be expressed at high levels by mutations. Transmissible plasmids have acquired genes for AmpC enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal blaampc gene, such as *E. coli, K. pneumoniae* and *Proteus mirabilis* . Resistance due to plasmid mediated AmpC enzyme is less common than ESBL production in most parts of the world but may be both harder to detect and broader in spectrum (Jacoby et al., 2009).

Plasmid-encoded AmpC genes have been known since 1989. Various plasmid mediated AmpC β-lactamases include CMY-1, CMY-2, MIR-1, MOX-1, LAT-1, FOX-1, DHA-1, ACT-1, ACC-1, CFE-1 etc. Like the chromosomally mediated AmpC β-lactamases, plasmid mediated AmpC β-lactamases also confer resistance to a broad spectrum of β-lactams including penicillins, oxyimino-β-cephalosporins, cephamycins and variably aztreonam. Plasmid carrying the gene for AmpC β-lactamases often carry multiple other resistances including genes for resistance to aminoglycosides, chloramphenicol, quinolones, sulphonamides, tetracycline, and trimethoprim as well as genes for other beta lactamases such as TEM-1, PSE-1, CTX-M-3, SHV varieties and VIM-1 thus raising a significant issue about the use of above mentioned antibiotics in pathgens harbouring AmpC β-lactamases (Shahid et al., 2009).

Metallo β-lactamases (MBLs)

MBL was first discovered in Pseudomonas aeruginosa in Japan and has been reported to spread to other species (Lee et al., 2000). Some bacteria of environmental habitat ubiquitously carry chromosomal MBLs which are opportunistic pathogens with the arguable exception of *Stenotrophomonas maltophila* and *Bacillus anthracis* , seldom cause serious infections. Some bacteria harbouring chromosomal MBLs are B. cereus, B. *anthracis, Aeromonas hydrophila, Legionella gormanii , Pseudomonas aeruginosa* and *Acinetobacter* spp. etc (Walsh et al., 2005) However, concerns have been raised that acquired MBL genes are located on integron structure that reside on mobile genetic elements such as plasmids or transposons thus enabling widespread dissemination (Franklin et al., 2006).

The MBL producers that are most clinically significant are primarily those where the gene encoding the enzyme is transferable and include *Ps. aeruginosa, Acinetobacter* spp. and to a lesser extent enterobacterial species (Espedido et al., 2007). The fact that in many cases the MBL genes may be located on plasmids along with genes encoding other antibiotic resistant determinants i.e. aminoglycoside resistance gene (Projan, 2003). The presence of bacteria possesing transferable MBLs have been reported in more than 30 countries and 5 continents.

Types of MBLs

1. **IMP-type:** It is the dominant MBL type. Most commonly found in *Ps. aeruginosa, Acinetobacter baumanii, Klebsiella pneumoniae, E. coli, Citrobacter* spp. etc.
2. **VIM-type:** It is the second most predominant type of acquired MBLs. It was first described in *Ps. aeruginosa* and also found in *Klebsiella pneumoniae, E. coli* etc.
3. **Others:** SPM-1 and GIM-1 are the other two most important acquired MBL types. These both have similarities with the IMP type MBLs.

Global problem of AMR

Problems occur in both Developed and Developing Countries when antimicrobials are:

- Not equitably available
- Used by too many people
- To treat the wrong disease
- In the wrong dosage
- For the wrong period of time
- Not in the correct formulation or strength (WHO, 2000)

Emerging problems of AMR

- Fluoroquinolones-resistant Salmonella
- 3rd generation Cephalosporin-resistant Salmonella (ESBL)
- Fluoroquinolone and macrolide resistant Campylobacter
- Vancomycin-resistant enterococci (VRE)
- Multi-drug resistant E. coli
- MRSA in humans and animals (report of high prevalence of MRSA in pigs in the Netherlands - now also found in Danish animals) (ASM, 2009).

Prevalence of AMR in National and International Scenario

International Scenario

Johnson *et al* ., 2012 reported 90% of *S. auerus* resistant to penicillin in the UK while in some communities more than 50% of strains are resistant to methicillin (Klevens *et al.,* 2007). Resistance among Gram positive organisms is familiar territory. In contrast, the new landscape of resistant Gram negative bacteria is unfamiliar and even more alarming. Organisms producing extended spectrum beta lactamases (ESBL) have increased their prevalence in Europe and elsewhere (Brolund *et al.,* 2013, Colpan *et al*., 2013), and in some areas are crossing the border from hospital settings to the community. Reuland *et al.,* 2013 in the Netherlands carried out a prospective surveillance study of 1,713 asymptomatic urban dwelling volunteers revealed the presence of ESBL producing enterobacteriaceae was 8% of stool samples; prevalence is only slightly lower than that found in symptomatic patients of the same region (10.6%).

In a study of urinary isolates in India, m ost isolates were resistant to 4 or more number of antibiotics with 42% of isolates producing ESBL (Akram *et al*., 2007). Similarly in a study of susceptibility pattern of *Pseudomonas aeruginosa* isolated from various clinical specimen in turkey, 36% percent of isolates were resistant to more than one group of antibiotics (Gencer *et al*., 2002). In contrast, in a study of 11,865 *E. coli* urinary isolates obtained from community and hospitalised patients in East London, h igh rates of resistance to ampicillin (55%) and trimethoprim (40%), often in combination were observed in both sets of isolates. Although isolates exhibiting resistance to multiple drug classes were rare, resistance to cefpodoxime, indicative of extended spectrum β-lactamase production, was observed in 5.7% of community and 21.6% of nosocomial isolates (Bean *et al*., 2008). Cotrimoxazole as a prophylactic therapy are used to prevent pulmonary infection with *Pneumocystis jirovecii* in AIDS patients has led to emergence of around 80% of *S. pneumoniae* resistant to this drug (ASM, 2009).

The emergence and rapid spread of carbapenemase producing Gram negatives such as extensively drug resistant *Acinetobacter* spp. and enterobacteriacae producing New Delhi metalloprotease1 (NDM1), *Klebsiella pneumoniae* carbapenemase (KPC) or oxacillinase 48 (OXA48) are disturbing, as these multi-drug resistant infections leave patients with few or no antimicrobial options. Invasive CRE infections carry mortality rates of 40 – 50% (Patel *et al.,* 2008). A recent environmental point prevalence study conducted by Walsh and colleagues in New Delhi, India, revealed the presence of the NDM1 gene in two of fifty community drinking water samples and twelve of 172 seepage samples (Walsh *et al.,* 2011).

Organisms that are almost entirely community acquired such as *Neisseria gonorrhoeae* also continue to increase their resistance to ceftriaxone (CDC, 2012). The H041 *N. gonorrhoeae* strain, which carries high level ceftriaxone and cefixime resistance was detected in Japan in 2011 (Ohnishi *et al.,* 2011) and its phenotypic homologue F89, was isolated in France in 2012 (Unemo *et al.,* 2012). In a study conducted in France in 241 clinical strains of IPM-nonsusceptible *P. aeruginosa* isolated from 2002 to 2004, 110/241 (46%) were MBL positive using phenotypic methods while 107/241 (45%) were PCR positive for MBL genes: 103/241 (43%) for bla_{VIM} and 4/241 (2%) for bla_{IMP} (Pitout *et al*., 2005).

In a study of prevalence of plasmid mediated AmpC β-lactamases (PABLs) in China, a total of 1,935 consecutive non-repeat clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were tested by PCR for the presence of bla_{AmpC} genes and sequenced. Fifty-four (2.79%) isolates harbored PABLs, as demonstrated by PCR and isoelectric focusing (Li *et al*., 2008) . In Taiwan, a total of 291 *E. coli* and 282 *K. pneumoniae* isolates tested for the production of AmpC enzymes, the results showed a confirmed presence of ABL in 43.6% (127 isolates) of the 291 *E. coli* isolates, and in 14.5% (41 isolates) of the 282 *K. pneumoniae* isolates (Yan *et al*., 2006).

Patients infected with nalidixic acid-resistant serovar Typhi showed 36% and prolonged fecal carriage when treated with an older-generation fluoroquinolone such as ofloxacin **(**Chinh *et al.,* 2000**;** Parry *et al.,* 2007**).** The antimicrobial resistance data from southern Vietnam are complemented by the results of a cross-sectional study from eight Asian countries: Bangladesh, China, India, Indonesia, Laos, Nepal, Pakistan and Vietnam which have approximately 80% of the world's typhoid fever cases (Crump *et al.,* 2004). Roumagnac et al. suggested that fluoroquinolone use has driven the clonal expansion

of a nalidixic acid-resistant serovar Typhi haplotype H58 in Southeast Asia (Roumagnac et al., 2006). The emergence of resistance of serovar Typhi to ciprofloxacin (6/149 isolates; 4%) in Nepal, together with reports of high-level ciprofloxacin resistance in India and Bangladesh (Gaind et al., 2006; Renuka et al., 2005; Saha et al., 2006) might be the prelude to a worsening drug resistance problem in Asia. The European arm of the SENTRY surveillance program identified 2.7% of polymyxin B-resistant A. baumannii isolates collected between 2001-2004 (Gales et al., 2006).

Souli et al., 2006 conducted a surveillance study in Greece, reported among 100 A. baumannii strains derived from ICU patients, 3% were colistin-resistant whereas the minimum inhibitory concentration (MIC) levels of tigecycline ranged from 0.12 µg/ml to 4µg/ml. Sporadic cases of infections caused by colistin-resistant isolates have been increasingly frequently reported (Falagas et al., 2008; Giamarellou, 2007; Matthaiou et al., 2008). A surveillance study performed in 34 centres across UK during 2000 reported a 2% resistance rate to colistin among 443 A. baumannii tested while tigecycline MICs ranged from <0.032 µg/ml to 16 µg/ ml (Henwood et al., 2002). Sporadic strains exhibiting colistin resistance have also been reported in Slovakia (Beno et al., 2006). In vitro activity of tigecycline against MDR strains of A. baumannii showed promising results (Souli et al., 2006; Rodloff et al., 2008). In a recent surveillance study from Germany, tigecycline resistance among 215 A. baumannii was 6% whereas colistin resistance was 2.8% (Seifert et al., 2006). Alarmingly high resistance rates to tigecycline (25%) have recently been reported from Turkey.

In the MYSTIC 2006 results, Turner reported that among 1,012 P. aeruginosa isolates collected from 40 European centres, resistance to piperacillin/tazobactam was the lowest (15%), followed by meropenem (22%), amikacin (23%), ceftazidime (25%), gentamicin (29%), imipenem (32%), ciprofloxacin (33%) and tobramycin (35%) (Turner, 2008). Compared to imipenem, meropenem was more potent and was active against up to one third of imipenem-resistant strains, which indicates that a considerable percentage of these strains have lost the OprD porin, which is influential mainly against imipenem (Giamarellou and Kanellakopoulou, 2008., Turner, 2006).

Most authors have found that mortality among patients infected by XDR Enterobacteriaceae, mostly carbapenem-resistant isolates was high (Tato et al., 2007; Cagnacci et al., 2008; Souli et al., 2008; Schwaber et al., 2008). Infections by PDR Enterobacteriaceae, although still rare, have been associated with a high mortality was 33.3% from January 2006 to May 2007 (Falagas et al., 2008). The isolation of PDR (MBL-positive and colistin-resistant) K. pneumoniae was associated with a crude mortality of 100% but with an attributable mortality of 25% in a cohort of patients from Greece (Carrer et al., 2008).

Table 3: Antibiotic Resistant Bacterial Infections causing Deaths in US

Antibiotic resistant organism	Deaths	Year	References
Methicillin resistant *staphylococcus aureus*	11, 285	Per year	Gross, 2013
Vancomycin resistant Enterococci	1, 300	Per year	CDC, 2013
Drug resistant Streptococcus pneumoniae	7, 000	Per year	CDC, 2013
Drug resistant Mycobacterium tuberculosis	1, 70, 000	2012	Sengupta *et al.,* 2013
Carbapenem resistant Enterobacteriaceae	600	Per year	Gross, 2013
Muti drug resistant Pseudomonas aeruginosa	400	Per year	CDC, 2013
Multi drug resistant Acinetobacter	500	Per year	CDC, 2013
ESBL producing Enterobacteriaceae	1, 700	Per year	CDC, 2013

National Scenario

A study conducted by Yadav and Prakash (2015) at Janaki Medical College Teaching Hospital (JMCTH), Tribhuvan University, Nepal reported S.mutans was highly resistant to penicillin (66.15%) followed by tetracycline (60.76%) and less resistant to cotrimoxazole (20%). S. aureus was found to be very highly resistant towards penicillin (91.48%) followed by tetracycline (86.17%) and ampicillin (61.70%). S. mitis was resistant to tetracycline (78.12%) followed by ciprofloxacin (65.62%). Pseudomonas spp showed highly resistant towards tetracycline followed by cotrimoxazole (90.90%) (Yadav and Prakash, 2015). A total of 71 isolates of S. aureus isolated from upper respiratory tract infection, 28% isolates were defined as MRSA (Khushbu and Prakash, 2016).

In a study conducted at Tribhuvan University Teaching Hospital (TUTH), 47.57% of the isolates from the sputum and 60.40% of urinary isolates were MDR strains among which 24.27% and 16% of the isolates from sputum and urine respectively were ESBL producers (Pokhrel et al., 2004). In a study of 541 blood isolates of Salmonella enterica in TUTH, 5% isolates were found to be MDR strains with 3 isolates of Salmonella Paratyphi A demonstrating ESBL activity (Pokhrel et al., 2006).

In a study of fluoroquinolone susceptibility pattern of the Salmonella isolates in NPHL, of the 41 Salmonella isolates obtained during a seven month period, 2 (4.88%) isolates of Salmonella Typhi were multi-drug resistant (Acharya, 2008). In a study of Salmonella serovars isolated from urban drinking water supply of Nepal, 35 Salmonella isolates were MDR and all the isolates of S. enteritidis and four isolates of S. Typhimurium were resistant to ceftriaxone and indicated presence of one of the ESBL genes blaSHV on PCR amplification (Bhatta et al., 2007).

In a study of nosocomial isolates in Kathmandu Medical College (KMC), Citrobacter spp. was accounted as the most frequently isolated nosocomial pathogen with high prevalence of MDR strain followed by K. pneumoniae and E. coli (Thapa et al., 2009). In a study of antibiotic resistance pattern of S. aureus, carried out in Manipal Teaching Hospital, of the 117 S. aureus isolates tested 15.4% were found to be MRSA with fourteen (77.8%) of the methicillin-resistant isolates resistant to all agents tested (Subedi et al., 2005).

In a study of prevalence of multi-drug resistance clinical isolates in Kathmandu Model Hospital, 41.07% of the clinical isolates were found to be MDR with E. coli (46.12%) being the most predominant MDR strain. Of the MDR E. coli 100%, 81.03% and 75.75% strains respectively demonstrated ESBL, ABL and MBL activity (Baral, 2008). In a similar study conducted at TUTH, 68.33% of the urinary and 71.43% of the sputum isolates were MDR with 12 urinary isolates and 3 isolates from sputum demonstrating ESBL activity (Bomjan, 2005).

Laboratory Diagnosis of AMR

Antibiotic sensitivity test for the isolated organism is done by using Kirby Bauer Disc Diffusion Method following the definition of the National Committee of Clinical Laboratory Standards. Interpretation as 'Sensitive' or 'Resistant' is done on the basis of the diameters of zones of inhibition of bacterial growth as recommended by CLSI.

Detection of ESBL Producers

1. Screening test for ESBL Producers

Clinical and Laboratory Standards Institute (CLSI) has developed disk diffusion and broth microdilution screening tests for the possible ESBL production. The CLSI has proposed disk diffusion methods for screening for ESBL production by Klebsiella spp., Escherichia coli and proteus mirabilis which can be detected by noting specific zone diameters whereas in dilution methods for screening ESBL production by Klebsiella spp. and E. coli noting minimum inhibition concentration as ≥2 µg/ml (NCCLS, 2005).

Table 4: Organisms should be reported as potential ESBL producers by disk diffusion and Micro-dilution method

Antibiotics	Disk diffusion	Minimum inhibition concentration (MIC)
Cefpodoxime	≤ 17 mm	≥2 µg/ml
Ceftazidime	≤ 22 mm	≥2 µg/ml
Cefotaxime	≤ 27 mm	≥2 µg/ml
Ceftriaxone	≤ 25 mm	≥2 µg/ml
Aztreonam	≤ 27 mm	≥2 µg/ml

2. Phenotypic confirmatory test for ESBL production

The CLSI has recommended phenotypic confirmatory test for the suspected ESBL producers. Several of the phenotypic confirmatory tests include:-

- **Cephalosporins/Clavulanate Combination Disks**

The CLSI advocates the use of Cefotaxime (30µg), Ceftazidime (30µg) disks with or without clavulanate (10µg) or Cefpodoxime (10µg) plus clavulanate (1µg). A difference of ≥5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBL production.

- **Broth Microdilution**

It utilizes ceftazidime (0.25 to128µg/ml), ceftazidime plus clavulanic acid (0.25 to 128/4 µg/ml), cefotaxime (0.25 to 64µg/ml), and cefotaxime plus clavulanic acid (0.25 to 64/4 µg/ml). Phenotypic confirmation is considered as ≥3 fold serial dilution decrease in MIC of either of cephalosporin in the presence of clavulanic acid compared to its MIC when tested alone.

- **Double Disk Synergy Test**

This test incorporates the use of cefotaxime (30 µg) and ceftazidime (30 µg) disks which are placed on either side co-amoxiclav (20+10 µg) on a already inoculated Mueller Hinton Agar plate. ESBL production is inferred when the zone of either cephalosporin is expanded by the clavulanate (Livermore, 2004).

- **E-test for ESBLs**

These have a cephalosporin gradient at one end and a cephalosporin + clavulanate gradient at the other. ESBL production is inferred if ≥ 8-fold reduction is seen in cephalosporin MICs in the presence of clavulanate.

Others

- Vitek ESBL Cards
- MicroScan Panels
- BD Phoenix automated Microbiology System
- Agar supplemented with clavulanate

Detection of ABLs

Some of the currently available methods for the detection of the AmpC beta lactamases are:

- Three Dimensional Test
- Cefoxitin agar test
- Use of beta lactam inhibitors and non-β-lactam inhibitors
- PCR (Gold Standard) (Jacoby *et al*., 2009).

Detection of MBLs

Table 5: MBL Detection Techniques

Techniques	Test	Substrate-inhibitor combination	Advantages	Disadvantages
Clinical microbiology	Disk approximation	Ceftazidime and 2-mercapto-propionic acid	Easy to use	Distance of disk placement not standardized, difficult to interpret
	Disk diffusion	Imipenem EDTA	Easy to use and relatively easy to interpret	Disk not standardized and MBL producing bacteria can be imipenem sensitive
	Microdilution test	Imipenem and EDTA and 1,10-phenanthroline	Based on reduction in MIC, easy to interpret	Borderline cases can be missed, Imipenem sensitive cases
	E-test	Imipenem and EDTA	Easy use and interpretation	Borderline cases can be missed imipenem sensitive cases

Table 5 Con't

	Carbapenem hydrolysis	Meropenem and EDTA	Very sensitive and deemed to be the gold standard	Highly specialized, labour intensive and interpretation not straight forward
Molecular detection	PCR for genes of IMP, VIM etc		Easy to perform specific for gene family	Requires tailor made DNA, unable to differentiate between variants
	DNA probes		Specialized, labour intensive	Probe required for each gene family, cannot differentiate between variants
	Cloning and sequencing		Molecular gold standard	Labour intensive and interpretation requires experience

(Walsh *et al*., 2005)

Treatment of AMR

Resistance can be effectively treated by ideal drug usage involves:

- The correct drug
- Administered by the best route
- In the right amount
- At optimum intervals
- For the appropriate period
- After an accurate diagnosis (WHO, 2000)

Prevention and Control of AMR

Following measures can be taken to prevent and control the emergence and spread of antibiotic resistance worldwide:

- Rational use of antibiotics in all settings
- Implementation of infection control measures in healthcare settings
- Development of strategies to mitigate the risks of environmental exposure
- Development of rapid diagnostic tests
- Promotion of research on antibacterial resistance prevention and surveillance
- Promotion of research and development of novel antimicrobial strategies and antibacterial agents
- Improved general awareness of antibiotic use and risk of increasing resistance.

(https://www.combacte.com/Portals/1/Documents/The%20global%20threat.pdf)

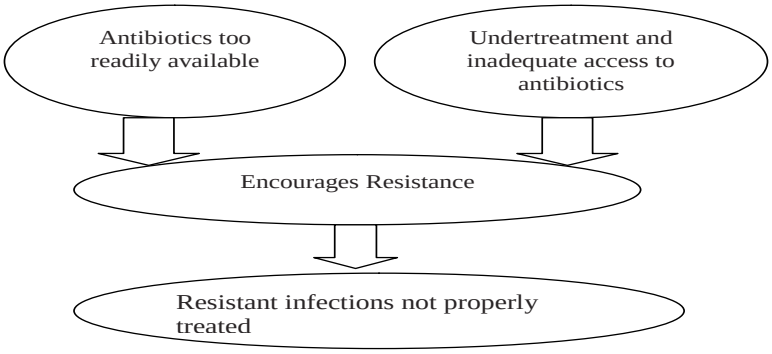

Figure 2: Paradox of controlling drug resistance (WHO, 2000)

Table 6: Common interventions to avoid the transmission of antimicrobial-resistant Microorganisms

Parameters	Common interventions
Early detection	• Enhanced provision of testing (lab capacity, point of care testing, eg: TB rapid molecular assay) • Enhanced infrastructure and new devices • Screening of patients at risk
Reducing infectivity	• Isolation of patients (Predominantly in hospital setting, specialist staff, training) • Decolonisation
Increasing hygiene	• Improved cleaning practices (specialist staff, training, peer quality control) • Alcohol-based hand hygiene • Contact precautions (gowns, gloves, masks) • Promote sanitation in schools
Reduce Susceptibility	• Probiotics • Early removal of catheters • Health promotion campaigns

(WHO, 2014)

Prioritising Interventions to contain Antimicrobial Resistance

The WHO Global Strategy to contain resistance identifies 64 interventions in total. Of these, 44 interventions or below are aimed at improving the use of antimicrobial drugs in humans at the national level (i.e. excluding interventions concerning animal use, new vaccine and drug development and international measures).

Table 7: Agreed list of Interventions to contain Antimicrobial Resistance

Target group	Recommended interventions
Group A **Patients and the public**	• Education on appropriate use • Education on hygiene • Discourage self-medication
Group B **Prescribers and dispensers**	• Training • Guidelines and formularies • Monitoring and supervision • Regulation of professionals • Educate prescribers about promotion
Group C **Health systems**	• Therapeutic committees • Infection control committees • Guidelines for antimicrobial use • Antimicrobial use surveillance • Laboratory network and epidemiological resistance surveillance

Table 7 Cont'd

Group D **Government policies, strategies and regulations**	• National AMR task force with budget • Drug policies e.g. essential drugs list, standard treatment guidelines • Registration of all drug outlets • Antimicrobials by prescription-only • Dispensing of antimiocrobials by licensed staff only • Quality assurance system • Drug licensing to include resistance data • Undergraduate and postgraduate training on AMR • Access to evidence-based drug information • Cut perverse rational drug use economic incentives • Monitor and supervise drug promotion • Monitor and link AMR and drug use data
Group E **Pharmaceutical industry**	• Incentives for industry to do research and development • Monitor and supervise drug promotion • Production according to Good Manufacturing Practice standards
Group F **Non-human antimicrobial use**	• Surveillance of resistance and use • Phase-out growth promoters • Educate farmers and vets

(http://www.who.int/emc/amr)

Strategies in the Development of Novel Antibacterial Drugs

There are six strategies in the development of novel antibacterial drugs which are as given below:

Table 8: Strategies for discovery and development of novel antibacterial drugs

Strategy	Description
Drug derivatives	Modification of the basic structure of known antimicrobial agents or development of inhibitors of a specific mechanism of resistance (i.e. new β-lactamases or efflux pump inhibitors).
Discovery of new antimicrobial agents	Classical or whole-cell antibacterial assay to find antibiotics produced by microorganism of different sources. Genomic or target-base antibiotic discovery with the use of new tools such as combinatorial chemistry and genomics
Antivirulence drugs	Antibodies or compounds blocking or inhibiting virulence factors.
Nanoparticle	Development of antibacterial peptides or peptidomimetics.
Bacteriophages or enzybiotics	Delivery of bacteriophages or phage-lytic enzymes.
Ecology/evolutionary biology approaches	Aimed at targeting the ecology and evolution of antibiotic resistance, including inhibitors of plasmid transfer of resistance, and gene-silencing antisense oligomers.

(Pelgrift and Friedman, 2013; Goemaere *et al.,* 2012; Singh *et al.,* 2011; Pastagia *et al.,* 2013; Bragg *et al.,* 2014; Mosqueda *et al.,* 2014).

CONCLUSION

The recent trends in globalisation, trade liberalisation, the rising number of travellers and growing interdependence all contribute to the increasing risk of the spread of existing infections which is a global threat that spans in all countries. The present review documents inappropriate prescription and use of Antimicrobial Therapies (AMTs), poor adherence to the prescribed therapy, insufficient hygiene practices are the factors that play a crucial role in helping in increasing antimicrobial resistant microorganisms (ARMs) rapidly. Multi-drug resistance among bacterial pathogens is a major health problem all over the world that threats the management of several infectious diseases and compromises therapy. Early detection may help avoid spread of the MDR isolates and maintain first and second line therapies.

Since β-lactamases confer a high level of resistance to β-lactam antibiotics and these traits usually being carried in transferable genes and capable of being acquiesced in normally non pathogenic bacteria as well as their high prevalence among bacterial isolates. Thus, controlling antibotic resistant bacteria to β-lactam antibiotics, to prevent the needless use of antibiotics, to improve the rapid prescription of appropriate antibiotics, immediate infection control and coupled with antibiotic stewardship programs in order to limit the spread of β-lactamase producing organisms to a patient so as to prevent the spread of infection with more resistance character.

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