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Enterobacteriaceae: At the verge of treatment

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ABSTRACT: Antibiotics have a history of more than 70 years, during which they have saved the lives of millions of individuals. However, the golden era of so called miracle bullets is over now-antibiotic resistance to almost every class of antibiotics particularly in Gram negative microbes has rippled the fear that we may enter to pre-antibiotic era. The situation is gruesome as eventually diarrhea would be difficult to treat because it has already developed resistance to treatment of last resort and in enterobacteriaceae, which comprises of diarrheal pathogens-the multi-drug resistance genes are present on plasmids associated with integrons and transposons that aid in dissemination of resistance genes to unrelated species. It is imperative to reverse the resistance mechanism by overwhelming those microbial enzymes that degrade the antibiotics, by increasing the uptake of antibiotics by bacterial cells, by blocking drug efflux, by discovering new natural products having antimicrobial potential and most importantly through development of nano-antibiotics.

KEYWORDS: Enterobacteraceae, Nano antibiotics, Extended Spectrum Beta Lactamases, New Delhi Metallo beta lactamases-1, Multi drug resistance, Efflux pumps.

1 INTRODUCTION

Almost a decade back, resistance in Gram positive microbes was considered to be reaching at critical level. However, with the implementation of control policies and through the development of new medicines, it is considered to be under good control now [1]. However, though the situation against Gram positive microbes has improved the same against Gram negative has exacerbated to an extent that clinical microbiologists reach agreement that multi drug resistant Gram-negative bacteria are the existent threat to public health. There are many whys and wherefores that can be attributed to this menace.

Enterobacteriaceae is a large family comprising of more than 70 genera and all microbes belonging to this family are Gram negative bacilli. It along with other microbes constitutes normal flora of GIT. Clinically significant pathogens belonging to this family are *Escherichia coli (E.coli)*, *Klebsiella* species, *Salmonella, shigella* and *Enterobacter* species [2]. As its name indicates Enterobacteriaceae family are mostly the etiological agents of gastrointestinal infection like diarrhea, though few extra abdominal infections are also caused by them [3]-[4]. Enteric infections so caused by them generally exhibit very good spontaneous recovery, where fluid and electrolyte replacement therapy is appropriate to get rid of diarrhea and subsequent dehydration so caused. Scarcely antimicrobial chemotherapy is required [2] with which the cure rate always remained very high. Enterobacteriaceae spread effortlessly between humans by various means like by hand carriage besides contaminated food and water. It has genetic plasticity that reveal tendency to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons. This combination is why emerging multidrug resistance in Enterobacteriaceae is of the utmost importance for clinical therapy [3].

It was considered to be causative agent of easily curable ailments however; past several decades have observed the spread of Enterobacteriaceae with resistance to broad-spectrum antimicrobials. Especially, the emergence of Carbapenem-resistant Enterobacteriaceae (CRE) has invalidated almost all the available therapies. It has created the havoc that once easily treatable infections like diarrhea may become untreatable or very difficult to be managed. KPC (*Klebsiella pneumoniae*

carbapenemase) and NDM (New Delhi Metallo-beta-lactamase) are the recently discovered types of CRE. KPC and NDM are the enzymes that break down carbapenems and make them ineffective [5]-[6].

It's evident that Enterobacteriaceae has developed resistance against Carbapenem, which is considered to be the drug of last resort. Second reason why resistance in Enterobacteriacece is considered to be more notorious is that the rate of spread of resistance; being very fast in Gram negative than the same in Gram-positive bacteria, the resistance genes in Enterobacteriacece are found on mobile genetic elements that can readily disseminate resistance through horizontal gene transfer to unrelated bacterial populations [7]. Moreover, unprecedented human air travel is also considered to be a cause of dissemination of resistance between countries and continents. Thirdly, there is currently no new antibiotic in the developmental pipeline that can specifically target Gram negative microbes though many against Gram positive are in the way [8].

2 DISSEMINATION OF RESISTANCE IN ENTEROBACTERIACECE

The mixing of susceptible to resistant microbes is an important cause of spread of resistance genes. Thus the most important reservoir is the gut of men and animals where all type of microbes get the opportunity to interact with each other. Particularly, in all those individuals who frequently take antibiotics courses and may have developed resistance to multiple antibiotics. In Enterobacteriaceae genetic plasticity allows acquisition and simultaneous dissemination of resistance genes to takes place very actively [7].

Acquisition of resistance genes may takes place by consumption of contaminated crops irrigated by polluted water, livestock fed on antibiotics containing feed, consumption of fish that has been fed on unwholesome ingredients, as our oceans and rivers are polluted with industrial waste and likewise, water that we consume is also not clean and pure. There are numerous studies that indicate potable water being contaminated with highly resistant pathogens. As these microbes interact with each other they exchange their genetic material so cross resistance takes place and it is much more prominent in Enterobacteriaceae [7].

Not only the gut of humans and animals is the reservoir for exchange of genetic materials but infact, the large-scale mixing of environmental bacteria with microbes from anthropogenic sources such as farm drainage and waste processing provides the ideal selective and ecological conditions for new resistant strains to arise; thus, soil, water, and other nutrient-enriched habitats can act as hotspots for horizontal gene transfer [9]. Many antibiotics particularly, some synthetic antibiotics are not easily biodegradable and can persist in soils for many years and microbes residing there develop resistance to them [7].

In enterobacteriaceae resistance genes are usually associated with mobile genetic elements such as transposons and integrons on large plasmids therefore they easily acquire and spread resistance phenotypes [10]. Likewise they accumulate resistance genes by other species making them multidrug resistant then these multiple genes of resistance persists for years.

3 TREATMENT MODULES FOR ENTERIC INFECTIONS:

Semi-synthetic penicillins in combination with beta-lactamase inhibitors were successfully used to treat enteric infections. However, the emergence of plasmid-encoded beta-lactamases (particularly TEM) put a pressure on the use of aminoglycosides, third generation cephalosporins and quinolones. Just after few years of application, resistance against aminoglycosides invalidated its use and put the pressure on third-generation cephalosporins and quinolones. However, early in this century extended-spectrum beta-lactamases (ESBLs) has created turbulence. An ESBL confer resistance to third and fourth generation cephalosporins called oxyimino-cephalosporins [7].

The carbapenems (e.g. imipenem, meropenem) are regarded as the drug of choice against ESBL-producing organisms since they are uniformly active *in vitro* and *in vivo* against these strains. Carbapenems can easily penetrate outer membrane barrier of Gram Negatives microbes due to its zwitterionic structure and is also stable to ESBL hydrolytic activity [11].

Although it is understandable from a clinical perspective to use then carbapenems, it is equally worrisome that this practice leads to the rapid selection of carbapenem-resistant Ent. As a result, only a few antimicrobial agents (e.g., colistin, fosfomycin, tigecycline) with an uncertain in vivo efficacy and/or reported toxicity are usually left to treat infections due to multidrug-resistant Ent (MDR-Ent) [4].

4 RESISTANCE MECHANISMS IN ENTEROBACTERIACEAE

In order to understand the mechanism of resistance in Enterobacteriaceae, it is very crucial to understand their structure. As Enterobacteriaceae consists of all Gram-negative (G-ve) bacteria and these Gram-negative microbes exhibit a rather more complex outer envelope consisting of an outer (OM) and an inner (IM) membrane that demarcate a periplasmic space [12] (Fig. 1).Bacterial lipopolysaccharides (LPS) are also unique to Gram negative (G-ve) microbes. LPS gives a negative charge to the cell and makes it impermeable to certain molecules particularly, hydrophilic one and the one that bear negative charge while promote the attachment of positively charged molecules. Diverse protein channels are present in outer membranes that are involve in transportation of materials, nutrients, drugs and chemicals from both inside and outside the cell

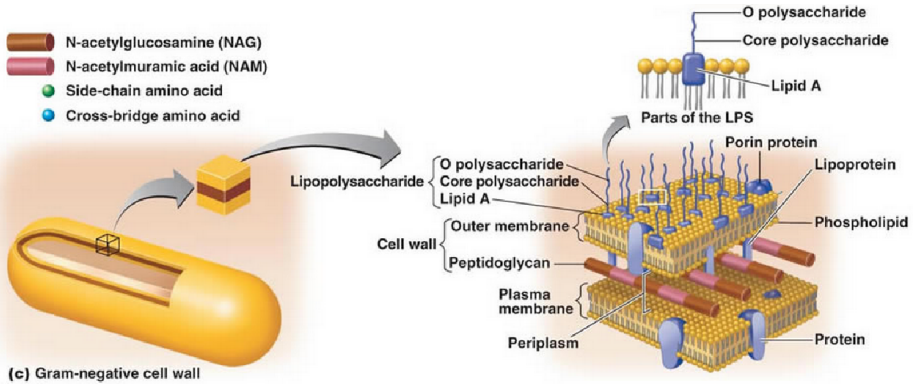

Fig. 1. Structure of Gram negative outer envelop

4.1 PORINS MODIFICATIONS

Though there are various channels but, porins and efflux transporters maintain a balance of intracellular drug accumulation and the quantity to be extruded. Hydrophobic compounds can permeate through the OM bilayer easily in a concentration dependent manner. However, hydrophilic molecules can only traverse via porins as LPS is offering a permeability barrier to them. Consequently, LPS alterations and porin modifications are a significant resistance mechanism adopted by various Gram negative microbes [12]. Mutatios in porins also reduce the ability of many beta-lactam antiobiotic (hydrophilic antibiotic) to cross the outer membrane of G-ve bacteria to bind at penicillin binding protein (PBP). Pertinent to mention here is that this resistance mechanism is specific for G-ve microbes only.

4.2 OVER-EXPRESSION OF EFFLUX PUMPS

The over-expression of efflux pumps is another effective way of resistance in enterobacteriaceae [13]-[14] (Fig. 2). In Gram negative microbes, down regulation of porins along with over-expression of efflux pump establish the first line of defense against antibiotics, biocides (disinfectants, antiseptics, preservatives), bacteriocins, detergents, surfactants, defensins, bile salts and also dyes and other chemicals.

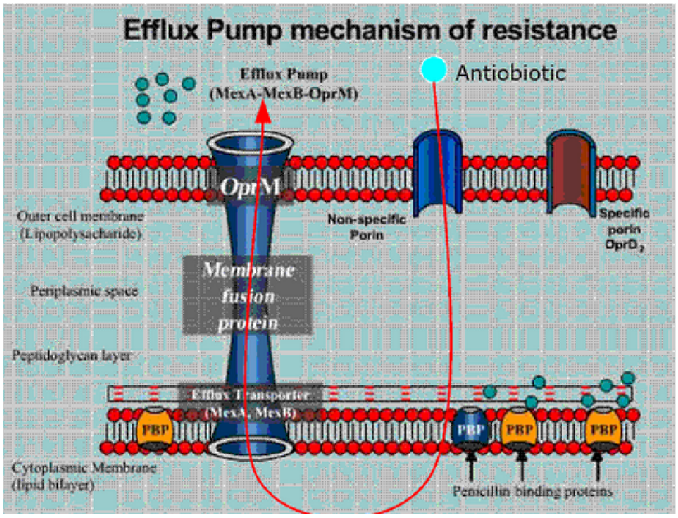

Fig. 2. Over expression of efflux pump

4.3 MODIFICATION OF ANTIBIOTIC TARGET SITES

Another mechanism of resistance in G –ve microbes are modification of target site. Penicillin-binding proteins (PBPs) forms crosslinking between peptidoglycan unit and β-lactam antibiotics have high affinity for binding with them because of structural similarity. As β-lactam antibiotics occupy PBPs, further cell wall synthesis stops because it will no longer be employed by cell wall synthesis machinery. Alteration in target site i.e., PBP will end up in losing the β-lactam to bind with PBPs.

4.4 Β-LACTAMASE PRODUCTION

In Gram-negative pathogens, most significant mechanism of resistance is the production of certain enzymes that degrade β-lactam ring of β-lactam antibiotics by hydrolysis and make them ineffective (Fig.3). These enzymes termed as β-lactamase. There are different types of β-lactamases for instance penicillinases, cephalosporinases, Extended Spectrum Beta Lactamases (ESBL), carbenicillinases, oxacillinases, carbapenemases etc., that are classified according to two schemes of classifications *viz.,* Bush-Jacoby-Medeiros classification system and Ambler classification [15] [16].

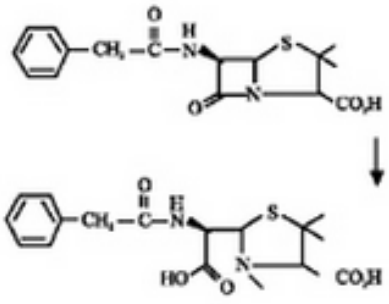

Fig. 3. Hydrolysis of β-lactam ring

4.4.1 BETA LACTAM ANTIBIOTICS:

All antibiotics included in this category contains beta lactam ring and antibiotics included in this category named as Penicillins (Ampicillin, Amoxicillin, Piperacillin), Cephalosporins (1st gen: cephalothin, 2nd gen (cephamycins): cefoxitin, cefotetan, 3rd gen: ceftazidime, cefotaxime, ceftriaxone and 4th gen: cefepime), Monobactam: aztreonam and Carbapenems:Imipenem, Meropenem and Ertapenem.

4.4.2 CLASSIFICATION SCHEMES FOR B-LACTAMASES

MOLECULAR OR AMBLER CLASSIFICATION (AND NUMBERING) SCHEME

In this scheme of classification, β-lactamases enzymes are classified based upon amino acid sequence similarity. Ambler et al., distributed β-lactamases into four classes (A, B, C and D), based on conserved and distinguishing amino acid motifs. Enzymes included in Class A, C, and D hydrolyze their substrates through an active site serine, whereas class B β-lactamases are metalloenzymes that utilize at least one active-site zinc ion to facilitate β-lactam hydrolysis [16].

FUNCTIONAL OR BUSH'S CLASSIFICATION SCHEME

Another way to classify β-lactamases is proposed by Karen Bush. According to this scheme of classification β-lactamases are divided into various classes according to the substrate profile, inhibitor profiles, and physical characteristics such as molecular weight and isoelectric point. For the most part, b-lactamases of a particular molecular class correlate with a specific group in the Bush classification system [11] Table 1.

Table.1: Classification scheme of β-lactamases

Bush's Group	Ambler Group	Enzymes	Inhibited by		Substrate	examples
			CA/TZB[1]	EDTA[2]		
1	Class C	cephalosporinases	No	No	cephalosporin	AmpC
2a	A	penicillinases	yes	No	Penicillin, cephalosporin,	
2b	A	Penicillinases+ cephalosporinases	yes	No	Penicillin, early cephalosporin	TEM-1, TEM-2, SHV-1
2be (ESBL)	A		yes	No	Inactivating 3rd-generation cephalosporins and monobactams (aztreonam)	TEM-3, SHV-2, CTX-M-15.
2br (inhibitor resistant)	A		No	No	Penicillin	TEM-30, SHV-10,
2ber	A		no	No	3rd and 4th generation cephalosporins, monobactams	TEM-50
2c	A	Carbenicillinase	Yes	No	Carbenicillins	
2ce	A		Yes	No	Carbenicillins and cefipime	
2d	D	Oxacillinase	variable	No	Cloxacillin or oxacillin	OXA-1, OXA-10
2de	D		do	No	3rd and 4th generation cephalosporins+cloxacillin or oxacillin	OXA-11, OXA-15
2df	D		do	No	Cloxacillin or oxacillin+carbapenems	OXA-23, OXA-48,
2e	A	Cephalosporinase	Yes	No	Cephalosporins(extended spectrum)+manobactams	CepA
2f	A	Carbapenemase	variable	No	Carbapenems, 3rd and 4th generation cephalosporins and cephamycins	KPC-2, SME-1
3a	B	MBL	No	Yes	Carbapenem but not monobactams+penicillns+cephalosporins.	IMP-1, VIM-1
3b	B	MBL	No	Yes	Carbapenems	

1: Clavulonic Acid/ Tazobactam
2: Ethylene diammine tetra acetic acid

4.4.3 EXTENDED SPECTRUM BETA-LACTAMASES (ESBLS)

Most imperative type of β-lactamases in current practice is extended-spectrum β-lactamases (ESBLs). These extended spectrum beta-lactamases (ESBLs) are emerging as a foremost threat for patients in the patient care centers and in community.

All those enzymes that have the capacity to hydrolyze penicillins (ampicillin and piperacillin), third and fourth generation cephalosporins and monobactams (aztreonam), are termed ESBL. Cephamycins (i.e. cefoxitin and cefotetan) and carbapenems (i.e. imipenem, meropenem, doripenem and ertapenem) are susceptible to their action. ESBL enzymes are readily inhibited by lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam.

As ESBLs are inhibited by beta lactamase inhibitors so this unique property serves as an important phenotypic test that is conveniently exploited to identify ESBLs in bacteria [17].

The majority of ESBLs belongs to the class A (TEM, SHV, CTX-M) and D (OXA type ESBLs) in Ambler classification and from groups 2be (TEM, SHV, CTX-M) and 2d (OXA type ESBLs) in Bush's classification [18]. Pertinent to mention is that OXA type ESBLs have been found mainly in Pseudomonas aeruginosa and only rarely in Enterobacteriaceae [3].

ESBL genes (blaESBL) are mostly encoded by large plasmids (up to 100 kb and even more) that are transferable from strain to strain and between different bacterial species through conjugation; these are not chromosomally mediated [11]. Thus, ESBL genes can be transmitted between different gram-negative bacteria. In addition, their association with mobile genetic elements (e.g, integrons and transposons) facilitate dissemination of resistance pattern many folds. This is not the only resistance gene present on plasmid rather the situation is more gruesome because of the very fact that ESBL -producing bacteria show cross resistance to other classes of antibiotics such as aminoglycosides, fluoroquinolones, cotrimoxazole, and tetracycline [18]. There are more than 700 types of ESBLs that have been detected all over the world [19].

TYPES OF ESBLS

TEM, SHV, CTX-M and OXA β-lactamases are the types of ESBLs. More than 140 variants of TEM β-lactamases have been discovered so far, while greater than 60 for SHV β-lactamases. *E. coli* producing CTX-M- β-lactamases are the most frequently reported etiological agent for urinary tract infections (UTIs). Currently, these enzymes are the most prevalent type of ESBL found in most areas of the world [20]. Particularly, CTX-M-15 is the most widely distributed and most commonly recorded type in the world, having reached endemic prevalence in much of Asia, southern Europe, and South America. The widespread and increasing prevalence of CTX-M is causing a shift in prescribing away from third generation cephalosporins to carbapenem. Of grave concern at present is the rise in carbapenemase genes. This rise was because of the high incidence of CTX-M ESBLs, which resulted in heavy use of carbapenem antibiotics to treat patients infected with these bacteria. The resistance genes emerging in India include NDM-1 [7].

ESBLs together with the chromosomally encoded class C cephalosporinases (AmpC) constitute today's predominant resistance mechanism of gram-negative bacteria.

4.4.4 RESISTANCE TO CARBAPENEMS:

Carbapenemases are β-lactamases able to hydrolize β-lactam antibiotics, including carbapenems and cephamycins which are not hydrolyzed by ESBL. However, they cannot catalyze the hydrolysis of Aztreonam. These are termed metallo β-lactamases because metal ion is necessary for their activity. They are inhibited by metal chelators for instance, EDTA but not by beta lactamase inhibitors.

Pertinent to mention here is that carbapenems (imipenem, ertapenem, meropenem, and doripenem) are the latest developed molecules that possess the broadest spectrum of activity and are considered to be as last resort therapy. CRE has become notorious and deadly with a death toll up to 50%.

TYPES OF CARBAPENEMASES

Class A carbapenemases (Ambler Group and 2f from Bush's grouping) can be chromosomally encoded (SME) or plasmid-encoded (KPC). The KPC-types are the most clinically common carbapenemases found in Ent and are responsible for hospital outbreaks. Class B carbapenemases (Ambler Group and 3a from Bush's grouping) are called as metallo-β-lactamases (MBLs). They are usually of VIM and IMP types but the recently emerged NDM-types [21] are becoming the most threatening carbapenemases and have spread rapidly among Ent in all continents [22]. In Ent, class D carbapenemases (Ambler Group 2df from Bush's grouping) are mainly represented by the OXA-48-like enzymes (e.g., OXA-48, -162, and -181). These genes are extensively reported among *E. coli* and *K. pneumoniae* isolates in the European and African Mediterranean countries. However, very recently, OXA-48 producers have been reported in North America [10].

Carbapenem Resistant Enterobacteriaceae (CRE) develops a form of infection that is hard-to-treat and it is displaying an escalating trend amongst patients in medical facilities. CRE have become resistant to nearly all the antibiotics. Particularly, CRE with NDM-1 carbapenemase are a particular intimidation, as the blaNDM-1 gene is highly promiscuous and is readily transmitted between species and genera [23], with concomitant transfer of up to 14 antibiotic resistance genes. There is much evidence that CPE with NDM-1 carbapenemase are widespread in the population of the Indian subcontinent [23]-[24].

This poses a significant challenge to effective antimicrobial therapy of patients in this region and there is a clear danger of global dissemination of NDM-1 via international travel [25].

NDM-1 has been disseminated in different countries and is being reported globally. Because of its association with Indian sub-continent, a number of studies have included samples from Pakistan and India to evaluate the prevalence and spread of this enzyme. A study conducted on stool samples from patients at Military hospitals in Pakistan revealed an overall prevalence of 18.5% NDM-1 positive Enterobacteriaceae with 27.1% from inpatients while 13.8% were from out patients [26]. Out of 356 clinical isolates, 160 showed carbapenem resistance. Of these 160 isolates, 131 displayed MBLs production as accessed by combined disk method. In MBLs producing organisms, PCR amplification confirmed 31 (23.6%) isolates harboring blaNDM-1 gene, 33 (25.1%) isolates having blaVIM gene and 2 (1.5%) isolates displaying blaIMP gene. Plasmid profile analysis of NDM-1 positive organisms showed variable number of plasmids which were stable during serial passages in antibiotic free media. The prevalence of ESBL producing organisms was recorded to be 87.5% [27].

TREATMENT OF CRE

Treatment of carbapenemase resistant Enterobacteriaceae (CRE) now includes combination regimens of carbapenems, colistin, aminoglycosides, aztreonam, and tigecycline, as well as prolonged infusions of carbapenems [24].

5 COUNTER STRATEGIES TO COMBAT RESISTANCE

5.1 OVERCOMING BETA-LACTAMASES

β-lactamases constitute the main mechanism of resistance in enterobacteriaceae and it can be overcome by adopting two strategies, firstly by finding it's inhibitor or by discovering new antibiotics that are not target for β-lactamases. Right now, three beta-lactamase inhibitors are available: clavulanic acid, sulbactam, and tazobactam, these inhibitors because of their structural similarity with that of beta-lactam are targeted by β-lactamases and they spare actual drug moiety [15].

5.2 INCREASING THE ANTIBIOTIC INFLUX

Second most significant resistance mechanism in Gram negative microbes is the inability of hydrophilic drugs to cross the outer membrane barrier due to alteration in porins. On the other hand hydrophobic drugs cannot trespass barrier due to modifications in LPS.

To combat resistance in Gram negative microbes because of limited entry there is a need to facilitate the diffusion of antibiotics through the bacterial envelope in order to increase their intracellular concentration. Certain chemical facilitators and chemosensitizers, such as detergents, surfactants, chaotropic agents, polymyxines, and antimicrobial peptides can be employed for this purpose.

5.3 DESTABILIZATION OF LPS BARRIER

Another way to circumvent the membrane barrier is to destabilize the LPS layer by using chaotropic agents or detergents that consequently facilitate the diffusion of hydrophilic compounds through the membrane lipid bilayer. Treatment by Tris/EDTA leads to massive release of LPS in the medium, and it is believed that the reduced amount of LPS in the OM leaflet is compensated by glycerophospholipids, essentially creating patches of phospholipid bilayer, which are much more permeable to lipophilic compounds

5.4 BLOCKING THE EFFLUX

Many of the antibiotics if traverse the outer membrane barrier extruded out by efflux pumps. Efflux pumps inhibitors in combination with conventional antibiotic therapy are now considered as an attractive target for the development of a combinational therapy [13]. At the moment, efflux pump activity blockers are the main group that are described and tested on Gram-negative bacteria from both, natural and synthetic sources [28]-[29].

5.5 NATURAL ANTIBIOTICS

Natural products and their analogs continue to play a prominent role in medicine, accounting for two-thirds of new antibacterial therapies approved from 1980 to 2010 as well as several antibacterials currently in clinical trials. In the past 80

years numerous classes of natural product antibacterials have been discovered. The efficacy of natural products as antibacterial agents likely stems from the fact that they have been honed by evolutionary processes to be bioactive, thereby giving the producing organisms a selective advantage in the environment. Discovery and development of new antibacterial agents is undergoing a renaissance brought on by the confluence of diverse fields and expertise; and once again natural products are coming to the forefront [5].

5.6 NANOANTIBIOTICS

One of the recent struggles in addressing the challenge of resistance lies in exploring antimicrobial nanomaterials, to which microbial pathogens may not be able to develop resistance, and novel nanosized platforms for efficient antibiotics delivery [30].

All those nanomaterials, which possess their intrinsic antimicrobial activity or augment the overall efficacy and safety of enclosed or adsorbed antibiotics are termed “nano-antibiotics. This definition includes nano-carriers as well. Nano-carriers are drug vectors which retain and transport drug; deliver it within or in the vicinity of target. In general, nano-carriers may protect a drug from degradation, enhance drug absorption, modify pharmacokinetic and pharmacodynamics and improve intracellular penetration [31].

Bio-based nano-carrier systems (including liposomes, chitosan etc.,) [32] as drug delivery vehicle are replacing metallic NPs because of various advantages rendered by them being biodegradable, biocompatible, economical, easy to manufacture with minimal or no side effects [33].

Nanoparticles (NPs) can aid in overcoming existing drug resistance mechanisms, including decreased uptake and increased efflux of drug from the microbial cell, biofilm formation, and intracellular bacteria. In addition, NPs can target antimicrobial agents to the site of infection, so that higher doses of drug are given at the infected site, thereby overcoming resistance (Fig. 4).

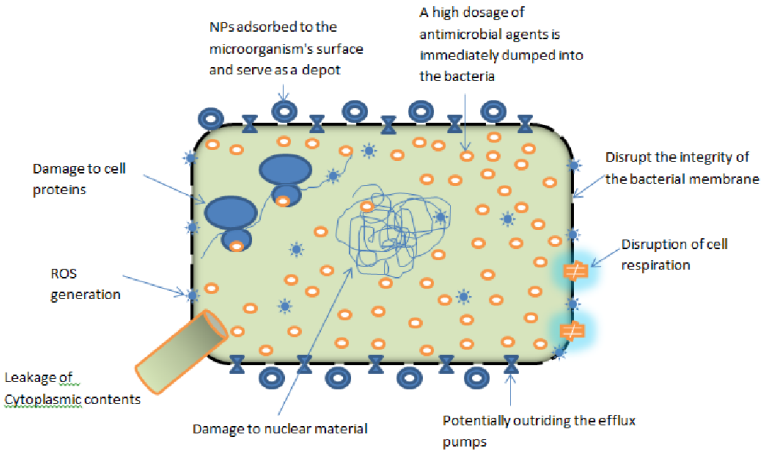

Fig. 4. Possible mechanism of action of nano antibiotics

Nanoparticles use multiple mechanisms of action simultaneously to combat microbes resulting in decreasing the likelihood of developing resistance to these NPs [34]-[35].

6 CONCLUSION

The recent emergence and spread of a novel carbapenemase, New Delhi Metallo β-lactamase (NDM) producing organisms are an example of that situation where available antibiotics are ineffective. This novel enzyme along with other antibiotic resistance factors is carried by mobile genetic elements such as plasmids or transposons. Horizontal gene transfer (HGT) is one of the most common mechanisms by which antibiotic resistance traits are transferred from one organism to another. It is imperative to find out quick method for the diagnosis of these MDR microbes. In particular, blaCTX-M, blaTEM, blaSHV, blaCMY, blaKPC, blaNDM, blaOXA-48, the 16S rRNA methylases genes and mutations in gyrA and parC are the most important targets that should be always tested for Ent [4].

In enterobacteriaceae, resistance is mainly caused by impaired intake of antibiotics by the microbial cells or by active efflux of these drugs by efflux pumps and by the activity of beta lactamases. Nonetheless, it can be overcome by reversing these resistance mechanisms. Consequently, in order to bypass the membrane barrier, we need to develop various strategies to increase the diffusion of antibiotics through bacterial membranes (target the influx), and we have to circumvent the pump mechanism to preserve a high intracellular concentration of antibiotics (target the efflux). Nano technology is a promising field that has lot of potential in many areas including medical field. Nano antibiotics could be an attractive alternative to conventional antibiotics in combating many resistance mechanisms. Likewise, the potential of natural products could be further explored to bulwark resistance menace.

One good thing for CRE infections is that normal healthy people cannot get it. It is usually associated with hospitals and other healthcare settings, where many type of resistance mechanisms prevail under one roof and CRE has the propensity to acquire these resistance mechanisms actively. Strict measurement should be taken to contain CRE and to avoid its spread to community.

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