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RESEARCH ARTICLE

MULTIDRUG RESISTANCE IN PSEUDOMONAS AERUGINOSA: A GENERAL OVERVIEW.

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Abstract

Pseudomonas aeruginosa, a gram-negative bacillus, is a multidrug resistant (MDR) pathogen posing a threat to hospitalised patients and contributes to their morbidity and mortality. This organism shows a remarkable capacity to resist antibiotics, either intrinsically (because of constitutive expression of β -lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (e.g., genes for β -lactamases, or enzymes inactivating aminoglycosides or modifying their target), over-expression of efflux pumps, decreased expression of porins, or mutations in quinolone targets. There has been an accelerated increase in multidrug resistant strains of this organism while the available therapeutic options are severely limited. There is a need for new agents that can overcome the multidrug resistance of these organisms when the available therapeutic options become restricted. This review discusses about various mechanisms of multidrug resistance and possible novel therapeutics introduced from time to time.

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Introduction:-

Antibiotic resistance in bacteria is a worldwide problem. It has been recognized as a major medical and therapeutic problem as more and more cases of drug resistant strains are being reported. These bacteria can be divided as multidrug resistant (resistant to three or more classes of antimicrobials), extensively drug resistant (resistant to all but one or two classes) and pan drug resistant (resistant to all available classes) bacteria. They are causing therapeutic problems and posing infection control issues in hospitals. After the introduction of antibiotics into the medical and veterinary fields, *P. aeruginosa* has been widely recognized as a potential pathogen.

Pseudomonas aeruginosa is a Gram-negative opportunistic nosocomial pathogen responsible for a wide range of infections that may present high rates of antimicrobial resistance. The genome of this microorganism is among the largest in the bacterial world allowing for great genetic capacity and high adaptability to environmental changes. In fact, *P. aeruginosa* has 5567 genes encoded in 6.26 Mbp of DNA while *Escherichia coli* K12 for example has 4279 genes encoded in 4.46 Mbp and *Haemophilus influenzae* Rd has 1.83 Mbp encoding 1714 genes (Lambert et al, 2002). This large genetic armamentarium can be further enriched with the addition of genes acquired by transferable genetic elements via horizontal gene transfer and that is a major contributing factor to its formidable ability to develop resistance against all known antibiotics.

P. aeruginosa is of ubiquitous nature and has the ability to survive in moist environments. Due to its innate resistance to many antibiotics and antiseptics, *P. aeruginosa* is a common pathogen in hospitals and particularly in intensive care units. Resistance developed in *P. aeruginosa* is multifactorial which occurs due to mutations in genes encoding porins, efflux pumps, penicillin-binding proteins, and chromosomal β -lactamase. All these factors contribute to resistance to β -lactams, carbapenems, aminoglycosides, and fluoroquinolones (Ozer et al, 2009). Strains of *P. aeruginosa* cause several diseases in nosocomial environments namely pneumonia, bacteremia, meningitis, urinary tract infections, as well as skin and soft-tissue infections (Wróblewska et al, 2006). It is of ultimate importance to develop new antimicrobial drugs due to the emergence of MDR pathogens.

P. aeruginosa is considered as one of the most versatile microbial organisms. It has a wide span of habitats including soil, disinfectant solution and jet plane fuel (Kobayashi et al, 2009). Low permeability of its outer membrane by a complex set of efflux pump systems and secretion of alginate during biofilm formation are major factors that allow the pathogen to become highly virulent and resistant to multiple antibiotic agents. Adding to these factors, other bacterial exoproducts such as lipopolysaccharides and elastase induce harmful pathogenesis resulting in tissue destruction.

Flagellins in *P. aeruginosa* perform several functions during host infection (Verma et al, 2007). Apart from enabling motility, the flagellum of *P. aeruginosa* plays an indirect role in membrane permeabilization and surfactant protein-mediated bacterial clearance (Zhang et al, 2007). Similarly, pili are involved during inflammation due to glycosylation in the interface between pili and host cells. Flagellins are classified into two types: Type-a (polymorphic glycosylated) and type-b (non-glycosylated).

Pathogenesis and colonization:-

Pili, flagella, exoenzyme S, and mucoid exopolysaccharide are recognized as major adhesins in *P. aeruginosa*. Invading pathogens are recognized by Toll-like receptors (TLRs) on epithelial cells and innate immunocytes, both of which are then activated to express inflammatory mediators. Thereafter, defense systems such as mucociliary clearance, phagocytosis and humoral immunity are promoted to neutralize the danger (Kobayashi et al, 2009). Invading organisms are first trapped by the mucus layer coating the airway epithelial cells. Airway mucin, the main component of mucus, is a large heterogeneous glycoprotein with carbohydrate side chains consisting of *N*-acetylglucosamine (GlcNAc), GalNAc, D-mannose, L-fucose, and *N*-acetylneuraminic acid (NeuAc) which may promote colonization, whereas binding to the glycolipids may cause an inflammatory response (Krivan et al, 1988). These exposed oligosaccharide residues become adhesive receptors for *P. aeruginosa* and others microbes.

The diversity of oligosaccharide side chains on glycoproteins or glycolipids in mucin may determine which organisms will effectively bind to it (Lamblin et al, 1991). *P. aeruginosa* has a variety of lectin-like adhesions (Figure 1), including pili, mucoid exopolysaccharide, and non-pilus adhesins, represented by exoenzyme S, that have binding domains similar to that of the pilus. Flagella motility and pili, which mediate twitching motility in *P. aeruginosa*, are thought to be the prevailing adhesins for the initial attachment required for colonization of the airway tract (Kobayashi et al, 2009).

Alginate expression is observed afterward the initial attachment of *P. aeruginosa* to a solid surface. Alginate may be involved in cementing the primary adherence of the non-pilus adhesion found on the surface of *P. aeruginosa* so it can bind to respiratory cells or mucin in the absence of other adhesions (Kobayashi et al, 2009). When the organisms trapped in mucus multiply faster than the removal rate the production of exoproducts increases, most of which are virulent and result in decreased mucociliary transport and airway epithelial cell function. The latter results in enhanced mucus inactivity and cell surface colonization.

Following, quorum sensing and biofilm formation start. Intracellular communication is involved in *P. aeruginosa* biofilm development (Davies et al, 1998). The phenomenon of quorum sensing or cell-to-cell communication requires self-generated signal molecules, named autoinducers. As cell density increases, there is a proportional increase in autoinducer production. *P. aeruginosa* has at least two quorum-sensing systems. Each system includes a gene encoding a transcriptional activator, LasR or RhlR and a gene encoding an autoinducer *lasI* or *rhlI*. These systems contribute to the development of the biofilm. In addition, the LasR-*lasI* and RhlR-*rhlI* quorum-sensing systems regulate the expression of various virulence genes in a density-dependent fashion (Kobayashi et al, 2009). In animal models of acute and chronic infections with *P. aeruginosa* containing a mutation in quorum-sensing genes, less tissue destruction was induced and less mortality was observed compared with findings in wild-type

strains (Smith et al, 2003). Bacteria inside a mature biofilm exhibit increased resistance to antibacterials and phagocytic cells, and are less stimulatory to the mucosa; these facts are found in bacterial colonization grown particularly in inappropriate environments. It can be said that one of the ways the pathogen and host coexist is established at the site of colonization.

In a chronic colonization with *P. aeruginosa* biofilms, the lungs show chronic inflammation that is associated with the development of lymphocyte follicles around respiratory bronchioles and with the influx of PMNs into airway lumens (Kobayashi et al, 2009).

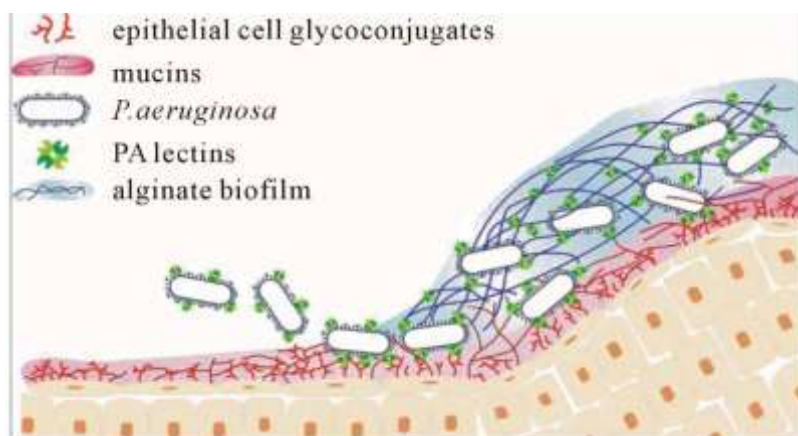


Figure 1:-Schematic interactions of the possible roles of *P. aeruginosa* lectins during host recognition and biofilm formation.

Resistance mechanisms in *P. aeruginosa*:-

Generally, antibiotic resistance mechanisms of *P. aeruginosa* can be divided in intrinsic and acquired. Intrinsic refers to resistance that is a consequence of a large selection of genetically-encoded mechanisms and acquired refers to resistance that is achieved via the acquisition of additional mechanisms or is a consequence of mutational events under selective pressure.

Multidrug Resistance:-

P. aeruginosa is naturally resistant to a significant number of antimicrobials (Table 1). Furthermore, they easily acquire resistance to new antibacterial agents by mutational changes or acquisition of genetic material. In a study, *P. aeruginosa* strains isolated presented resistance to carbenicillin and gentamicin. *P. aeruginosa* is intrinsically less susceptible to the fluoroquinolones and usually it is moderately susceptible or resistant (Araque et al, 1998).

In recent years, resistance of *P. aeruginosa* to commonly used therapeutic agents has increased. Multidrug resistance can be defined as resistance to at least four classes of antibiotics used during treatment of these infections: third-generation cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems (Karlowsky et al, 2003).

Emergence of MDR strains is often due to selective pressure of antimicrobial therapy. Genetic studies confirm the selection of resistant mutants and their subsequent spread. Outbreaks caused by MDR *P. aeruginosa* may follow an increased use of third-generation cephalosporins or carbapenems for therapy of infections caused by other resistant bacteria (Wróblewska, 2006).

All bacteria rely on a heavily cross-linked peptidoglycan layer for cell shape and morphological stability (Wilke et al, 2005). The formation of this layer depends on the catalytic activity of transpeptidase enzymes, which utilize an active site serine and perform their catalytic cycle by way of an acylation/deacylation pathway. β -lactam antibiotics inhibit the action of transpeptidases, effectively blocking the transpeptidation reaction and therefore leaving bacteria susceptible to cell lysis. β -lactamases confer significant antibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the four-membered β -lactam ring. Their mechanism depends heavily on the concentration of zinc ions, required by the four different classes of β -lactamases during hydrolysis (Estiu et al, 2006).

Bacteria exhibit two control mechanisms when in presence of metallic compounds: One based on sensing of the environment and, in proportion to what is detected, one of regulatory response (Choudhury et al, 2001).

The process occurs in the context of chemical gradients, which activate several metabolic pathways acquired by bacteria along their evolutionary history. Enthalpy plays a determinant role in this case: All non-deleterious mutations attempt to minimize metabolic costs. Toxicity of metals in the cellular medium can be either independent of concentration or regulated by it, in which case metals at appropriate concentration levels become catalytic. In the case of *P. aeruginosa* zinc is not only required for metabolic functions but fulfils a definitive role in resistance to β -lactams, also dependent on chemical gradients. A similar case occurs with iron acquisition, allowing the pathogen to degrade host iron binding proteins and act as an extracellular protein, bringing the host cell into metabolic stress (Vasil, 2007).

Table 1:-Natural resistance of *P. aeruginosa* to antibiotics (Wróblewska, 2009)

Bacteria	Natural resistance to
<i>P. aeruginosa</i>	ampicillin amoxicillin amoxicillin/clavulanate first-generation cephalosporins second-generation cefotaxime ceftriaxone nalidixic acid trimethoprim

Intrinsic resistance of *Pseudomonas aeruginosa*:-

P. aeruginosa shows inherent resistance to antimicrobial agents through a variety of mechanisms: (1) decreased permeability of the outer membrane, (2) efflux systems which actively pump antibiotics out of the cell, and (3) production of antibiotic-inactivating enzymes (Moore et al, 2011).

Outer membrane permeability:-

The outer membrane of Gram-negative bacteria is a barrier which prevents large hydrophilic molecules to pass through it. Aminoglycosides and colistin interact with lipopolysaccharides changing the permeability of the membrane in order to pass whereas β -lactams and quinolones need to diffuse through certain porin channels. Bacteria produce two major classes of porins: general; which allow almost any hydrophilic molecule to pass (Hancock et al, 2002) and specific; which have binding sites for certain molecules, allowing them to be oriented and pass in the most energy-efficient way (Tamber et al, 2006). Most bacteria possess lots of general porins and relatively few specific ones. However, the exact opposite occurs for *P. aeruginosa* that expresses mainly specific porins (Hancock et al, 2002).

Efflux systems:-

P. aeruginosa expresses several efflux pumps that expel drugs together with other substances out of the bacterial cell. These pumps consist of three proteins: (1) a protein transporter of the cytoplasmic membrane that uses energy in the form of proton motive force, (2) a periplasmic connective protein, and (3) an outer membrane porin (Lambert, 2002). Most antibiotics, except polymyxins, are pumped out by these efflux systems (Table 2) therefore their first two components are named multidrug efflux (Mex) along with a letter (e.g. MexA and MexB) (Lister et al, 2009; Strateva et al, 2009). The outer membrane porin is called Opr along with a letter (e.g. OprM) (Schweizer et al, 2003).

Table 2:-Efflux pump systems associated to antibiotics resistance in *P. aeruginosa* (Poole k, 2005).

Efflux system	Efflux pump family	Substrates
MexAB-OprM	Resistance Nodulation Division (RND)	Fluoroquinolones Aminoglycosides β -Lactams (preferably Meropenem, Ticarcillin) Tetracycline Tigecycline Chloramphenicol

MexCD-OprJ	Resistance Nodulation Division (RND)	Fluoroquinolones β-Lactams (preferably Meropenem, Ticarcillin) Tetracycline Tigecycline Chloramphenicol Erythromycin Roxythromycin
MexEF-OprN	Resistance Nodulation Division (RND)	Fluoroquinolones β-Lactams (preferably Meropenem, Ticarcillin) Tetracycline Tigecycline Chloramphenicol
MexXY-OprM	Resistance Nodulation Division (RND)	Fluoroquinolones Aminoglycosides β-Lactams (preferably Meropenem, Ticarcillin, Cefepime) Tetracycline Tigecycline Chloramphenicol
AmrAB-OprA	Resistance Nodulation Division (RND)	Aminoglycosides
PmpM	Multidrug And Toxic compound Extrusion (MATE)	Fluoroquinolones
Mef(A)	Major Facilitator Superfamily (MFS)	Macrolides
ErmEPAF	Small Multidrug Resistance (SMR)	Aminoglycosides

Antibiotic-inactivating enzymes:-

P. aeruginosa belongs to the SPICE group of bacteria (*Serratia* spp., *P. aeruginosa*, Indole positive *Proteus*, *Citrobacter* spp., *Enterobacter* spp.). These microorganisms share a common characteristic: the ability to produce chromosomal-encoded and inducible AmpC β-lactamases. These are cephalosporinases that hydrolyze most β-lactams and are not inhibited by the β lactamase inhibitors. Another endogenous β-lactamase produced by *P. aeruginosa* is the class D oxacillinase PoxB (Girlich et al, 2004; Kong et al, 2005). This enzyme however has only been found in laboratory mutants and is not clinically significant.

Acquired resistance of *Pseudomonas aeruginosa*:-

Apart from being resistant to a variety of antimicrobial agents, *P. aeruginosa* develops resistance to anti-pseudomonal drugs as well. This acquired resistance is a consequence of mutational changes or the acquisition of resistance mechanisms via horizontal gene transfer and can occur during chemotherapy (Poole K, 2011). Mutational events may lead to over-expression of endogenous β-lactamases or efflux pumps, diminished expression of specific porins and target site modifications while acquisition of resistance genes mainly refers to transferable β-lactamases and aminoglycoside modifying enzymes (Table 3).

Table 3:-Resistance mechanisms of *P. aeruginosa* to anti-pseudomonal drugs

Resistance to	Resistance mechanism
β-lactams	Endogenous β-lactamases
	Acquired β-lactamases
	Efflux
	Diminished permeability
Fluoroquinolones	Target site mutations
	Efflux

Aminoglycosides	Aminoglycoside-modifying enzymes
	Efflux
	16S rRNA methylases
polymyxins	LPS modification

Resistance to β -lactams:-

Resistance to β -lactam antibiotics is multi-factorial but is mediated mainly by inactivating enzymes called β -lactamases. These enzymes cleave the amide bond of the β -lactam ring causing antibiotic inactivation.

Presence of AmpC β -lactamases is the main cause of resistance to β -lactams in clinical isolates (Upadhyay et al, 2010). Furthermore, the production of AmpC β -lactamases in *P. aeruginosa* can be induced by a number of β -lactam antibiotics such as benzyl penicillins, narrow spectrum cephalosporins and imipenem (Dunne et al, 2005). In fact, this mutational derepression is one of the most common mechanisms of resistance to β -lactams in *P. aeruginosa*. AmpC enzymes are not carbapenemases, they possess however a low potential of carbapenem hydrolysis and their overproduction combined with efflux pumps over-expression and/or diminished outer membrane permeability has been proven to lead also to carbapenem resistance in *P. aeruginosa* (Quale et al, 2006).

Acquired β -lactamases are typically encoded by genes which are located in transferable genetic elements such as plasmids or transposons (Giedraitiene et al, 2011) often on integrons (Poirel et al, 2002). Integrons are genetic elements that capture and mobilize genes (Cambray et al, 2010).

Resistance to fluoroquinolones:-

High-level resistance to fluoroquinolones is mediated by target site modifications. Efflux plays a contributing role as well (Jacoby, 2005) and the two mechanisms often coexist (Tam et al, 2007).

Resistance to aminoglycosides:-

Acquired resistance to aminoglycosides is mediated by transferable aminoglycosidemodifying enzymes (AMEs), rRNA methylases and derepression of endogenous efflux systems (Poole, 2011).

Other Resistance Mechanisms:-

Another mechanism present in *P. aeruginosa* is the formation of permeability barriers (OM-outer membrane) (Kadry et al, 2003). Impaired penetration of different substances through the membrane (e.g. imipenem) is due to diminished expression of specific OM protein. It has been shown that OM permeabilizers such as EDTA increase susceptibility to antibiotics, indicating that the lack of OprD protein leads to a reduction of active antibiotic molecules capable of reaching the target penicillin-binding-proteins.

Two-component systems (2CS) are common molecular mechanisms that allow diverse bacteria to have adaptive regulation in response to complex environments, often composed by a sensor histidine kinase and a response regulator (Chen et al, 2004). The sensor kinase is composed of at least one signal recognition domain coupled to an autokinase domain in an input-transmitter arrangement. Two hypotheses exist regarding the evolution of 2CS. The co-evolution model proposes that 2CS genes have appeared as a result of duplication and further differentiation of these in bacterial genomes. The recruitment model on the other hand proposes that some of the 2CS operons have appeared as a result of an assembly of a sensor gene and a regulator gene from heterologous 2CS genes. Both are supported by evidence from phylogenetic analysis and gene regulatory network modeling in the *P. aeruginosa* PA01 strain.

Finally, it has been shown that cytotoxicity is an important mechanism that contributes to high morbidity and mortality in *P. aeruginosa* infections, particularly in cystic fibrosis (Guespin-Michel et al, 2004). Along with mucoidy resultant from the release of alginate, *P. aeruginosa* synthesizes a secretory apparatus (Type III) that allows it to inject toxins from their cytoplasm into the target cell. The latter mechanism allows mucoid bacteria to lyse the host's macrophages and overcome various defence such as in the case of cystic fibrosis lung infection.

Current therapeutic options:-

Antimicrobial therapy:-

Polymyxin B agents were used in the therapy of infections in the 1970s, but due to reported toxicity and the subsequent development of less toxic drugs such as nephrotoxicity, ototoxicity and neuromuscular blockade, their use has been discontinued. Polymyxin B is a polypeptide antibiotic produced by a strain of *Bacillus polymyxa* and is

primarily used for resistant Gram-negative infections. Now, with the emergence of MDR strains, their clinical use is being reconsidered (Wróblewska, 2006). Several reports in the past five years showed that colistin toxicity is not as frequent as previously reported (Falagas et al, 2005). Renal failure was rare and usually reversible, while neurotoxicity was not reported.

Furthermore, colistin (polymyxin E) has been used in several cases as a salvage agent during therapy of infections caused by strains resistant to all available antimicrobials (Falagas et al, 2006). However, clinical strains with reduced susceptibility to polymyxin B have been reported (Landman et al, 2005). Colistin, in combination with antibiotics from other classes, may be a useful agent for the treatment of infections caused by pandrug-resistant *P. aeruginosa* (Huh et al, 2011). Aztreonam may be used in the therapy of infections caused by *P. aeruginosa*. Combination therapy of aztreonam with other antimicrobials may be effective. A two-drug (aztreonam and amikacin) and a three-drug combination (aztreonam, ceftazidime, and amikacin) were very active against MDR strains of *P. aeruginosa* in an in vitro study (Oie et al, 2003).

Imipenem and meropenem are carbapenems commonly used in hospital practice. Many reports confirm their usefulness in the therapy of nosocomial infections caused by MDR Gram-negative bacilli. Apart from imipenem and meropenem, new carbapenems are being evaluated for their efficacy against MDR pathogens (Wróblewska et al, 2006). Carbapenems may be administered as monotherapy, but with the emergence of MDR *P. aeruginosa*, combination therapies are being evaluated.

Combination therapy:-

The application of combination therapy instead of mono-therapy in cases of non-MDR *P. aeruginosa* remains to date a controversial issue (Moore, 2011). Combination treatment against MDR strains instead seems to be some times necessary (for example in cases of pan-resistance or resistance to all except a single agent). In such cases better results are expected by the additive or sub additive activity of a combination or by the enhancement of a single active agent by an otherwise inactive drug. Several old and newer studies have showed the increased activity *in vitro* of various antibiotic combinations against MDR *P. aeruginosa* (Table 4) even though, the mechanisms of positive interaction between the various agents are rarely known (Rahal et al, 2006).

Table 4:-Enhanced activity of antibiotic combinations against MDR *P. aeruginosa*

Antibiotic combinations
Ticarcillin, Tobramycin, Rifampin
Cephalosporins, Quinolones
Ceftazidime, Colistin
Macrolides, Tobramycin, Trimethoprim, Rifampin
Polymyxin B, Rifampin
Polymyxin B, Imipenem
Colistin, Meropenem

Immunisation and genetic therapy:-

A new avenue for preventing chronic pulmonary colonisation in cystic fibrosis patients, while limiting antibiotic use, could involve immunotherapy. Many efforts have been made in this direction (Holder et al, 2004), but clinical efficacy has, to date, been disappointing, especially for heterologous strains (Sedlak et al, 2005). However, potential candidate immunotherapies are currently being assessed in a phase III clinical trial (Malfroot et al, 2005). Cystic fibrosis patients also benefit from other vaccinations (viruses, *Strep. pneumoniae*), which contribute to a reduction in both the number of infective episodes and the number of antibiotics used (Malfroot et al, 2005)

Novel Antimicrobials:-

Efflux pump inhibitors are under development for use in therapy of infections with resistant strains. In *P. aeruginosa*, two enzymes are involved: the enoyl-acyl carrier protein (ACP) reductase FabI and the alternative enoyl-ACP reductase FabK. Triclosan and other novel FabI- and FabK-directed inhibitors could prove to be broad spectrum antibacterial agents, particularly for the therapy of infections caused by MDR pathogens (Hoang et al, 1999).

Bacteriophage therapy of bacterial infections has also been investigated for many years. It has now received renewed attention as a result of the emergence of MDR strains of pathogenic bacteria. Several studies have shown the efficacy of bacteriophages in the treatment of experimental infections caused by *P. aeruginosa* in animals (Soothill et al, 1994). These studies indicate bacteriophages might also be useful in the therapy of infections caused by MDR bacterial strains in humans. Bacteriophages may be administered alone or in combination with antibiotics, and can be given prophylactically or as a therapy of infection. They offer several advantages, as they are very specific, replicate at the site of infection, and no serious adverse effects of their administration have been described. However further studies are needed in order to assess their therapeutic use in humans.

Nanomedicine:-

Use of nanotechnology in the treatment of infections consist in designing, delivering antimicrobial drugs, and diagnosis and control of infections, in particular in overcoming multidrug-resistant microorganisms, has been explored as a good alternative to the current antibiotics.

The recent development of nanotechnology has allowed the study of the effect of nanostructures in the biomedical area, and has promoted studies around the use of nanomaterials and nanoparticles as antimicrobial agents. Nanomaterials can be useful for *in vivo* and *in vitro* biomedical research and applications. The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, molecular sensors and drug delivery vehicles. From all nanomaterials with antibacterial properties, metallic nanoparticles provide the best results.

The importance of studying and developing bactericidal nanomaterials is given by the increase of new bacteria strains resistant against most potent antibiotics available and antimicrobial nanoparticles board multiple biological pathways (Figure 2), found in broad species of microbes and many concurrent mutations would have to occur in order to develop resistance against nanoparticles antimicrobial activities (Huh et al, 2011).

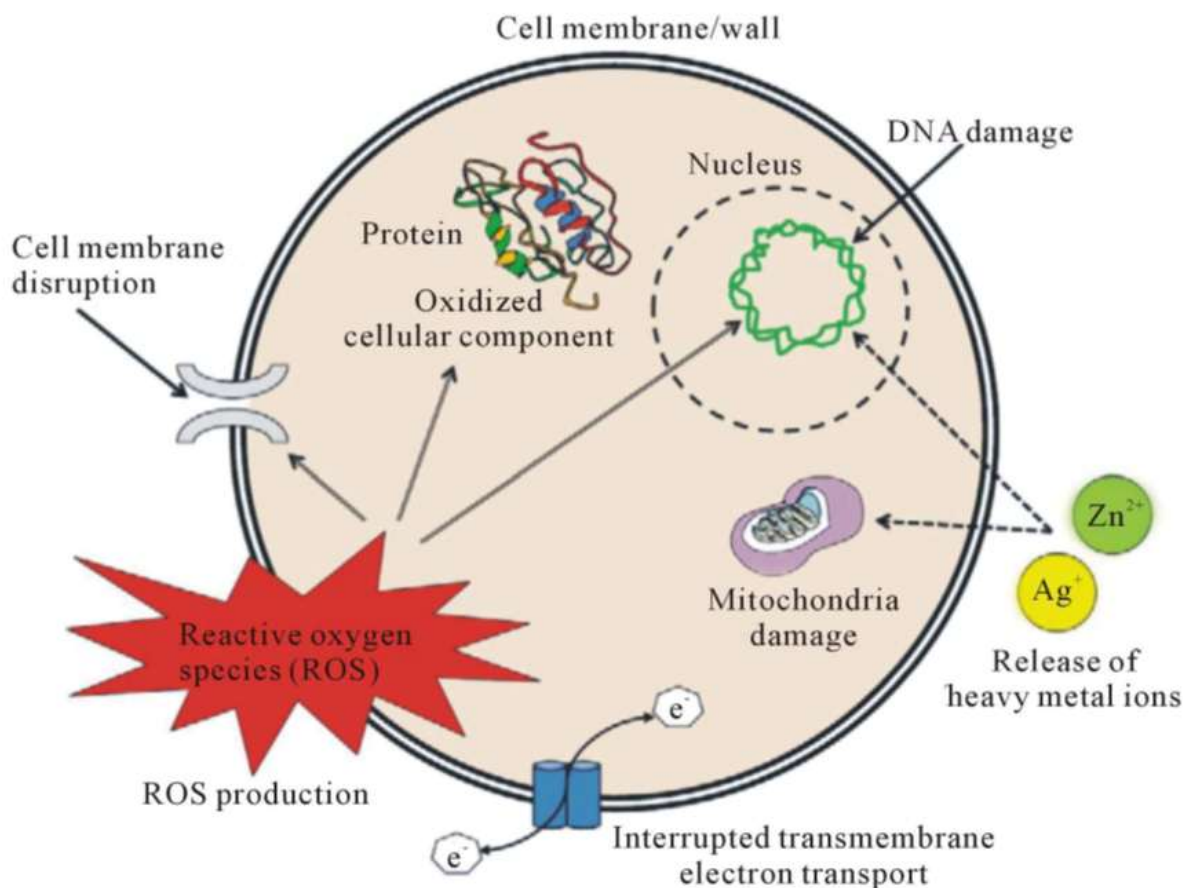


Figure 2:-Various antimicrobial mechanisms of nanomaterials (Huh et al, 2011)

The latter has promoted research in the well-known activity of silver ions and silver-based compounds, including silver nanoparticles. Their effect was shown to be size and dose dependent, and was more pronounced against gram-negative bacteria than gram-positive organisms (Singh et al, 2008).

Silver nanoparticles (AgNP) are intrinsically anti-bacterial, whereas gold nanoparticles (AuNP) have antimicrobial effect only when ampicillin was bound to their surface. Both AuNP and AgNP functionalized with ampicillin are bactericides against Gram-negative and Gram-positive bacteria. Most importantly, when AuNP and AgNP are functionalized with ampicillin, they became potent bactericidal agents with unique properties that subverted antibiotic resistance mechanisms of multiple-drug-resistant bacteria as *P. aeruginosa* (Brown et al, 2012).

Currently nanoparticles such as chitosan nanoparticles, quantum dots, dendrimers and liposomes are under study as antimicrobial agents. Polymyxin B-loaded liposomes represent a successful example of liposomal antimicrobial drug delivery (Zhang et al, 2010). As mentioned before, polymyxin B has been recognized as a viable treatment for *P. aeruginosa* related infections. However, its systemic use has been limited due to toxic side effects. It has been reported that liposomal encapsulation of polymyxin B dramatically diminishes side effects and improves its antimicrobial activity against resistant strains of *P. aeruginosa* (Alipour et al, 2008). The action mechanism of liposomal polymyxin B against bacteria has been identified as membrane fusion. Membrane fusion between liposomes and bacteria is a rapid and spontaneous process driven by non-covalent forces such as van der Waals force and hydrophobic interactions that minimize the free energy within the system. Antibiotic efflux is a widely accepted mechanism of microbial drug resistance, in which proteinaceous transports located in bacterial membranes preferentially pump antimicrobial drugs out of the cells. When liposomes fuse with cell membranes, a high dosage of drug contents is immediately delivered to the bacteria, potentially suppressing the antimicrobial resistance of the bacteria by overwhelming the efflux pumps, thereby improving drug's antimicrobial activity (Zhang et al, 2010).

Concluding remarks:-

P. aeruginosa is a nosocomial pathogen of particular clinical concern not only because of its extraordinary resistance mechanisms armamentarium but also for its formidable ability to adapt very well to the hospital environment. There are important challenges in the treatment of MDR *P. aeruginosa* strains and their isolation in healthcare settings poses serious infection control issues. For these reasons, the prudent use of antibiotics, mainly those used as last resort treatment like carbapenems is of utmost importance in order to prevent evolutionary pressure that may lead to the emergence of highly resistant clones.

Due to their promising antimicrobial properties, nanomaterials and nanoparticles are currently being studied as potential, highly potent antimicrobial agents for a variety of medical applications. Different kinds of nanoparticles have been investigated for carrying and delivering antibiotics. Moreover, nanoparticles enable combining multiple approaches in order to enhance antimicrobial activity and overcome the various resistance mechanisms in *P. aeruginosa*.

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