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Mini Review

Antibiotic resistance in *Pseudomonas aeruginosa* and alternative therapeutic options

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ABSTRACT

Pseudomonas aeruginosa is a leading cause of nosocomial infections and is responsible for ~10% of all hospital-acquired infections worldwide. It continues to pose a therapeutic challenge because of the high rate of morbidity and mortality associated with it and the possibility of development of drug resistance during therapy. Standard antibiotic regimes against *P. aeruginosa* are increasingly becoming ineffective due to the rise in drug resistance. With the scope for developing new antibiotics being limited, alternative treatment options are gaining more and more attention. A number of recent studies reported complementary and alternative treatment options to combat *P. aeruginosa* infections. Quorum sensing inhibitors, phages, probiotics, anti-microbial peptides, vaccine antigens and antimicrobial nanoparticles have the potential to act against drug resistant strains. Unfortunately, most studies considering alternative treatment options are still confined in the pre-clinical stages, although some of these findings have tremendous potential to be turned into valuable therapeutics. This review is intended to raise awareness of several novel approaches that can be considered further for combating drug resistant *P. aeruginosa* infections.

1. Introduction

Pseudomonas aeruginosa is one of the most common pathogens in nosocomial and ventilator-associated pneumonia, cystic fibrosis (CF), meningitis, abscess, soft tissue infections, urinary tract infections, catheter associated infections, corneal infections and conjunctival erythema. In addition to acute infections, *P. aeruginosa* also causes debilitating chronic lung infections in immunocompromised patients, cystic fibrosis patients, and individuals receiving chemotherapy. It can form biofilms on indwelling medical devices such as catheters and on native airways of the CF patients; these biofilms harbor slow growing bacterial subpopulations that are extremely resistant to antibiotics (Williamson et al., 2012). *P. aeruginosa* can also colonize through disruption of the normal flora balance caused by administration of broad-spectrum antibiotics or dysfunction of the immune system (Abdelghany et al., 2012).

According to the recent Centers for Disease Control (CDC) report around 51,000 healthcare-associated *P. aeruginosa* infections occur every year in the United States, of which around 6000 (13%) are caused by multidrug-resistant (MDR) *P. aeruginosa* strains which account for roughly 400 deaths every year. MDR *P. aeruginosa* was given a threat level of serious threat in the CDC antibiotic resistance threat report. Colistin (polymyxin E), a cyclic amphipathic antibiotic, although known for its nephrotoxicity and neurotoxiciy, is the last resort of treatment option left against MDR *P. aeruginosa* strains (Sabuda et al., 2008). Recently, the emergence of colistin resistance has also been reported from several countries including Denmark, United Kingdom and Australia (Denton et al., 2002; Johansen et al., 2008). To address the difficulties in treating *P. aeruginosa* infections, several approaches were undertaken including intensifying research to develop new antibiotics, use of different antibiotic combinations, and identification of alternative treatment methods using non-antibiotic means. However, the scope for the development of new antibiotics that will be more effective than the existing antibiotics and against which the frequency of resistance development will be lower than the current antibiotics is very limited. Therefore, a lot of research was focused on developing new antibiotic combinations to treat MDR *P. aeruginosa* infections (Dubois et al., 2001; Rahal, 2006; Sobieszczyk et al., 2004; Waters and

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Smyth, 2015). In this regard, aerosolized antibiotic formulations of aztreonam, tobramycin, levofloxacin and liposomal amikacin were developed to deliver antibiotics through the pulmonary route of CF patients. Several *in vitro* experiments and clinical trials were also conducted to identify ideal antibiotic combinations (*e.g.* combinations of cefepime and amikacin; polymyxin B with carbapenem, aminoglycoside, quinolone or β-lactam) to treat *P. aeruginosa* infections. In addition to this a lot of research is being carried out to develop non-antibiotic therapeutics against this pathogen using probiotics, phages and phytomedicines. Recent investigations suggest that some of these non-antibiotic therapeutic agents alone or in combination with antibiotics are highly effective against multi-drug resistant *P. aeruginosa* strains, indicating that non-antibiotic antimicrobial agents in future may play a significant role in the management of *P. aeruginosa* infections (Veesenmeyer et al., 2009). Here, we attempt to summarize the findings that tackled *P. aeruginosa* infections by non-antibiotic means. We reviewed the role of different quorum quenchers, lectin inhibitors, iron chelators, efflux pump inhibitors, probiotic organisms, bacteriophages, antimicrobial peptides, bacteriocins and nanoparticles as antimicrobial agents against this pathogen.

2. Virulence factors

The severity of the *P. aeruginosa* infections is due to its virulence factors. The virulence factors, especially the exotoxins and proteases cause extensive host tissue damage by disrupting normal cytoskeletal structure, depolymerization of actin filaments and cleavage of the immunoglobulin G (IgG) and A (IgA). *P. aeruginosa* produced exoenzymes disrupt the normal cytoskeletal structure, depolymerizes the actin filaments and cleaves IgG and IgA; thus facilitates invasion, dissemination and development of chronic infections (Sadikot et al., 2005). This bacterium possesses 5 protein secretion systems of which the type II (T2SS) and type III secretion system (T3SS) secrete the majority of known toxins (Jyot et al., 2011). The T2SS secretes exotoxin A (inhibits eukaryotic protein synthesis) (Stuart and Pollack, 1982), LasA (Zinc metalloprotease with elastase and staphylolytic activity) (Kessler et al., 1997) and LasB (elastase) (Grande et al., 2007; Toder et al., 1994), type IV protease (degrades immunoglobins, complement proteins, fibrinogen and plasminogen), alkaline protease (AprA; inhibits complement activation and neutrophil phagocytosis), protease IV (PrpL; cleaves transferrin, elastin, lactoferrin, decorin and casein) (Engel et al., 1998), and phospholipase H (hemolyse erythrocytes) (Shortridge et al., 1992), as well as lipolytic enzymes (Jyot et al., 2011). Additionally, *P. aeruginosa* uses a type III secretion system to introduce toxins into host cells (Hauser, 2009). These toxins are termed exotoxin (Exo)-S, -T, -U, and -Y. Type III secreted toxins typically mimic eukaryotic protein activities and participates in subverting the host signaling within the infected cell. ExoS and ExoT are bifunctional enzymes with GTPase activating protein (GAP) activity and ADP ribosyl transferase (ADPRT) activity, which target several proteins, including Ras and Ras-like GTPases (Jia et al., 2006). These two distinct enzymatic activities work redundantly to disrupt the actin cytoskeleton, resulting in profound effects on host cellular processes. While the ADPRT domains of ExoS and ExoT are highly homologous, their targets are very different. Expression of the ADPRT domain of ExoS is toxic to cultured cells, while expression of ExoT appears to interfere with host cell phagocytic activity (Galle et al., 2012). ExoY is an adenylate cyclase that causes cell rounding upon co-cultivation with cells and is toxic when expressed in yeast (Cowell et al., 2005). ExoU has been characterized as a member of the phospholipase family of enzymes and has at least phospholipase A2 activity (Sato and Frank, 2004). In mammalian cells, the direct injection of ExoU causes irreversible damage to cellular membranes and rapid necrotic death. Other *P. aeruginosa* secreted virulence factors such as pili, LecA and LecB, siderophores, pyoverdin, pyocyanin, alginate, hemolysins and exopolysaccharides (Suh et al., 1999) play important roles in the pathogenesis of *P. aeruginosa* by facilitating its colonization, survival and invasion within the host tissues. Pili, chitin-binding protein, LecA and LecB helps *P. aeruginosa* to adhere to host cells (Chemani et al., 2009). Siderophores (pyoverdin and pyochelin), allow this bacteria to multiply in iron limited environments. The pseudocapsule of alginate produced by the *P. aeruginosa* protects it from phagocytosis and antibiotics (Mishra et al., 2012). Additionally, alginate protects *P. aeruginosa* from immune attacks by scavenging reactive oxygen species, reducing polymorphonuclear chemotaxis and inhibiting the complement factors (Leid et al., 2005). A list of virulence proteins produced by *P. aeruginosa* and their functional role is pictorially depicted in Fig. 1.

3. Mechanisms of antibiotic resistance and need for alternative antimicrobial agents

The mechanism of antibiotic resistance in *P. aeruginosa* is multi-factorial which include the expression of multiple antibiotic modifying enzymes such as aminoglycoside modifying enzymes, β-lactamases including extended-spectrum β-lactamases and metallo-β-lactamases (Lister et al., 2009); antibiotic efflux pumps such as MexAB-OprM, MexEF-OprN, MexCD-OprJ, and MexXY-OprM (Livermore, 2002; Poole, 2001) and acquisition of chromosomally or plasmid encoded antibiotic resistance genes. Additionally, chromosomal mutations (*e.g.*, quinolone resistance due to mutation in DNA gyrase and topoisomerase IV gene) and lower membrane permeability for the antibiotics also contribute to antibiotic resistance (Lister et al., 2009).

Efflux pumps MexAB-OprM, MexEF-OprN and MexCD-OprJ confer resistance to β-lactam antibiotics. Additionally, up-regulation of MexEF-OprN and MexCD-OprJ—confer resistance to fluoroquinolones; and up-regulation of MexXY-OprM also affects aminoglycoside resistance (Livermore, 2002). Many acquired β-lactamases and aminoglycoside-modifying enzymes have been noted in *P. aeruginosa*. The most frequently acquired β-lactamases are TEM, OXA, PSE, PER, IMP and VIM. TEM, OXA and PSE enzymes confer resistance to carbapenems, oxyimino-aminothiazolyl cephalosporins (*e.g.*, ceftazidime, cefepime, or cefpirome) and aztreonam. Loss of OprD, a porin that forms narrow transmembrane channels is associated with resistance to imipenem and reduced susceptibility to meropenem. *P. aeruginosa* strains that up-regulate MexEF-OprN and exhibit reduced OprD expression, show resistance to both fluoroquinolones and imipenem, and reduced susceptibility to meropenem (Morita et al., 2015). Clearly, the incidence of infections caused by multidrug-resistant *P. aeruginosa* strains are on a raise and colistin, despite its toxicity remains the only drug of choice to treat extremely antibiotic resistant *P. aeruginosa* strains.

4. Non-antibiotic antimicrobial agents

While the incidence of infections caused by antibiotic resistant strains has increased, the discovery of novel classes of antibiotics has slowed down which made it imperative to search for alternative treatment strategies. Several non-conventional ways of treating *P. aeruginosa* infections have shown promising results in preclinical studies and few of them have succeeded in demonstrating significant clinical outcomes. The antimicrobial roles of the different non-conventional non-antibiotic agents against *P. aeruginosa* are discussed here.