

Overexpression of Efflux Pump in Multiresistant *Pseudomonas aeruginosa*: How You Will Discover and Treat It?

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The Emergence and spread of multiresistant strains of *Pseudomonas aeruginosa* is increasing problem, because it is associated with high morbidity and mortality. *P. aeruginosa* has intrinsic resistance to a wide variety of antibiotics, including most β -lactams, chloramphenicol, tetracyclines and fluoroquinolones through its impermeable outer membrane. Recently, *P. aeruginosa* strains, which has acquired resistance to antipseudomonal antibiotics such as ciprofloxacin, ceftazidime, imipenem, and piperacillin, has been frequently reported [1]. In addition to impermeability of membrane, efflux, target alteration, enzyme inactivation, salvage pathway, and target protein protection are known mechanisms of antibiotic resistance. Among these mechanisms, active efflux of drug through efflux pump system is thought to be associated with acquired resistance in *P. aeruginosa*.

There are various efflux pump systems in bacteria. The resistance-nodular-cell division (RND) family type of secondary multidrug transporters, which use the transmembrane electrochemical gradient of protons or sodium ions to drive the extrusion of drugs from the cell, has been found in *P. aerugi-*

nosa [2]. Especially, 4 out of 12 RND efflux pump system is thought to play a role in the exportation of antimicrobial agents in *P. aeruginosa* [3]. MexAB-OprM is expressed constitutively and related to fluoroquinolones, β -lactams, tetracycline, macrolides, novobiocin, chloramphenicol, trimethoprim, sulfonamides, meropenem resistance, but not imipenem. MexXY-OprM is also constitutively expressed and known to cause resistant to aminoglycosides, ciprofloxacin, levofloxacin, cefepime, but not ceftazidime. Unlike MexAB-OprM and MexXY-OprM, MexCD-OprJ and MexEF-OprN are induced by their substrates. MexCD-OprJ is not detectable in wild type cell and it cause resistant to tetracycline, macrolides, chloramphenicol, novobiocin, trimethoprim and some β -lactams. MexEF-OprN is quiescent in wild type cell and related to fluoroquinolones, chloramphenicol, trimethoprim and imipenem resistance [4].

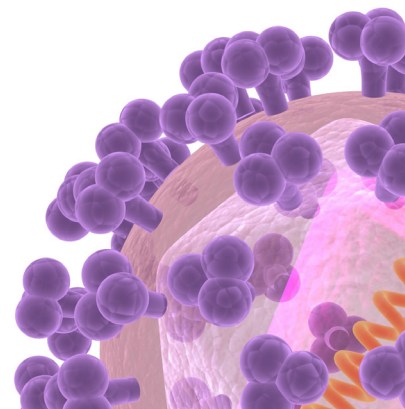
JDumas, et al. [5] reported that gene expression changes of efflux pump in *P. aeruginosa* cause resistant to antimicrobial agents. Using quantitative realtime-PCR, they observed several fold increasing of efflux pump genes, *mexA*, *mexB* and

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oprM, in antibiotic-resistant clinical strains. Shigemura, et al. [6] also reported that overexpression of *mexC* leads to levofloxacin resistance, and increased expression of *mexB* or *mexC* is related to complicated urinary tract infection (UTI) in *P. aeruginosa* strains clinically isolated from UTI patients. Other than in *P. aeruginosa*, association of efflux pump gene overexpression and antimicrobial resistance is also reported in other microorganisms [7, 8].

Adabi, et al. [9] demonstrated that efflux pump activity is related to ciprofloxacin resistance in *P. aeruginosa* strains clinically isolated from burned patients, using efflux pump inhibitor (EPI), carbonyl cyanide-*m*-chlorophenylhydrazine (CCCP). Compared to the absence of EPI, they detected ciprofloxacin susceptibility is increased in the presence of EPI. However, they only checked the presence of efflux pump genes by PCR, which cannot assess expression level of efflux pump. Although detection method using EPI for efflux pump overexpression was used in some previous researches [10], it is indirect and non-quantitative method. Instead, recent researches use quantitative real-time PCR to discover overexpression of efflux pump [5-7]. Adabi, et al. [9] also showed susceptibility to a variety of antibiotics, beside ciprofloxacin, increase in presence of EPI, and suggested use of EPI in combination with antibiotics. Multiresistant *P. aeruginosa* infection is associated with higher mortality rate and difficult to treat. A point that most of multiresistant strains show overexpressed efflux pump, it is reasonable treatment strategy to use EPI together with antibiotics [11]. However, toxic property of EPI in clinical application is not to be known clearly. Furthermore, safety and effectiveness of combination therapy are key point to disclose through further studies.

Spread of multiresistant *P. aeruginosa* is very serious problem in terms of immunocompromised patients and hospital infection control system. To control multiresistant *P. aeruginosa*, which has overexpressed efflux pump, choice of detection method and treatment strategy is very important thing. Further studies should consider not only expression level of efflux pump via quantitative real-time PCR, but also effectiveness and safety of EPI and antibiotic combination therapy in various multiresistant *P. aeruginosa* strains.

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