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Review of Efflux Pump Overexpression Resistance Mechanisms for Pseudomonas aeruginosa

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Pseudomonas aeruginosa Resistance

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Pseudomonas aeruginosa is a multi-drug resistant pathogen, fertile within the hospital setting. Known to be causal of many respiratory infections, the treatment of this gram negative microorganism is complicated by an intrinsic and acquired resistance profile. An example of intrinsic resistance would be an impermeable outer membrane common to many gram negative bacteria.

A separate method of intrinsic and acquired resistance comes through the activity of efflux pumps within mutant strains of P. aeruginosa and other gram negative bacteria[7-10]. Presently, there are four efflux pump systems that have been extensively studied: MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM. These efflux pumps operate by capturing antimicrobial compounds within the periplasm, cytoplasmic space, or plasmalemma and ejecting them outward into the extracellular matrix through a three protein conduit. Efflux pumps are energy dependent using a proton motive driving force.[7] Although these efflux pumps have broad substrate ranges, the greatest is seen with MexAB-OprM; not all compounds are susceptible to the activity of each pump. An example would be aztreonam that shows inefficacy as an antimicrobial for the treatment of P. aeruginosa when the particular strain overproduces MexAB-OprM. However with the overproduction of other Mex-Opr efflux pumps, the aztreonam Minimum Inhibitory Concentration (MIC) may remain at approximate values.

The MexAB-OprM efflux pump is produced by the mexA-mexB-oprM operon gene[7-8]. This pump is produced at low levels constitutively in all strains of P. aeruginosa. This implies that this efflux pump system is a contributor to both the intrinsic and acquired resistance profile. Overproduction occurs within nalB mutants, increasing resistance to aztreonam, the β-lactams, β-lactamase inhibitors, cephalosporins, quinolones, the penems, and tetracyclines[7-8].

MexCD-OprJ and MexEF-OprN are overproduced by nfxB and nfxC mutants, respectively. Substrates for the MexCD-OprJ efflux pump include cefepime, chloramphenicol, the quinolones, erythromycin, and tetracycline. MexEF-OprN substrates are largely similar: chloramphenicol, the quinolones, imipenem, and trimethoprim[7-8]. The fluoroquinolones tend to select for MexCD-OprJ or MexEF-OprN among other efflux types. Sub-inhibitory concentrations of the quinolones have been shown to induce increased MexCD-OprJ production.[8] However, the documentation of Efflux Pump Overexpression (EPO) with the fluoroquinolones is difficult because the MIC may not increase above the National Committee for Clinical Laboratory Standards (NCCLS) established levels[7].

Selective substrates for MexXY-OprM are the aminoglycosides[9]. Other substrates include amikacin, cefepime, cefotaxime, erythromycin, tetracycline, and the quinolones. MexXY-OprM over-expression can occur due to sub-inhibitory concentrations of respective substrates similar to the fluoroquinolones and MexCD-OprJ.[6]

Other potential causes for EPO include a greater bacterial inoculum, decreased environmental pH because of the proton motive driving force allowing efflux pump activity, and intercellular signaling[7]. Communication allows genetic activation of efflux pump expression. In P. aeruginosa, the Mex-Opr pumps regulate at least one homoserine lactone signaling molecule involved in this process[7, 11].

Previous studies have attempted to determine any causal factors that can promote the development of resistance. A six year study identified age greater than 65 years, prior exposure to antibiotics for at least fourteen days, and residence in a long term facility as independent and

significant risk factors for the harboring of multi-drug resistant gram negative organisms. [6] Other factors identified tend to rely heavily on the previous or prolonged use of certain antibiotics, in particular imipenem or the fluoroquinolones[1]. Both antibiotics tend to select for resistant forms of P. aeruginosa, in particular the presence of efflux pumps with the use of fluoroquinolones. Cancer patients, for example, showed a significant increase in multi-drug resistance (MDR) of P. aeruginosa strains if exposed to carbapenem for greater than seven days or if there was a history of chronic obstructive pulmonary disease (COPD).[2]

One study was that by B. Cao, et al.[3] This was a 44 month retrospective cohort study. Results showed, through multivariate analysis, that previous exposure to imipenem or meropenem within the previous fifteen days of isolation and previous mechanical ventilation greater than 48 hours were risk factors for MDR P. aeruginosa; this has similarly been documented with cystic fibrosis patients. While in the respiratory tract P. aeruginosa has the ability to form biofilms that serve as a form of protection and promote additional resistance.

Univariate analysis cited MDR strains were largely greater in elderly, ICU, COPD / bronchiectasis, and higher APACHE II scoring patients. This also included patients with polymicrobial infections and those that received fluoroquinolones or imipenem / meropenem within the previous fifteen days before strain isolation. Of further interest is that of all the antibiotics, imipenem had the highest ratio for the emergence of antibiotic resistance in P. aeruginosa. This response also induced resistance to other agents including the fluoroquinolones, cephalosporins, and anti-pseudomonal penicillins.[3]

Considering imipenem resistance, a three year case control study by Harris et al. compared patients that either had imipenem resistant or susceptible P. aeruginosa strains.[4] The purpose of this study was to find risk factors for the development of imipenem resistance.

Bivariate and multivariate analysis showed that imipenem resistant strains were significantly greater in the diabetic population and in those patients with an increased length of stay, ICU transfer, or previous admission. Patients were also significantly more apt to have imipenem resistant P. aeruginosa if previously treated with imipenem, piperacillin-tazobactam, vancomycin, first or third generation cephalosporins, aminoglycosides, or quinolones.[4]

As shown, even though imipenem is largely known to be a consistently repeating cause of MDR, other antibiotics and even comorbidities may also be responsible or influential. This even includes antibiotics that have no anti-pseudomonal activity, such as the glycopeptides.

Certain disease states tend to be associated more with resistance which includes circulatory, cardiovascular, and genitourinary disorders. The fact that diseases of the genitourinary system prefer the development of resistant isolates is not surprising. This is because most patients with urinary tract infections are treated with trimethoprim, fluoroquinolones, or the β-lactams. Considering the fluoroquinolones that are hepatically metabolized, subtherapeutic concentrations most likely accumulate within the urinary tract promoting resistance. This is especially applicable to EPO, since again sub – therapeutic concentrations of fluoroquinolones have been shown to induce the expression of MexCD-OprJ. Furthermore, considering that older patients tend to have renal diseases and insufficiencies, this can provide additional reasons for the easy accumulation of bacterium within this setting.

Considering chronic alcoholism, one hypothesis is that the lack of proper dietary habits and malnutrition upset the balance of normal gastrointestinal flora. Thereafter a virulent bacterium, such as P. aeruginosa may thrive, evolve into a resistant organism, and translocate. Chronic alcoholics additionally exhibit an endocrine profile similar to a diabetic, and as such this

would agree with the results of the Harris et al. study that found a greater amount of MDR P. aeruginosa isolates in the diabetic population.[4]

One study by C. Defez et al. studied patients if they presented with a MDR P. aeruginosa nosocomial infection for the first time. Univariate analysis showed MDR to be significantly greater in older patients. Bivariate analysis stated that MDR was greater in patients that were exposed to previous antimicrobial medications, in particular the β-lactams, macrolides, glycopeptides, fluoroquinolones, and imidazoles. This was further analyzed using multivariate analysis that showed previous exposure to β-lactams or fluoroquinolones were significantly associated with MDR P. aeruginosa.[5]

In relation to chronic alcoholism and diabetes though this study also noted that urinary catheterization was also associated with MDR within these patient subtypes. An additional interesting discovery was that patients fed through a nasogastric tube were also more likely to have MDR. This was possibly due to the weakening of the digestive mucosa causing easier translocation of P. aeruginosa post an imbalance of the normal gut flora.[5] This could have possible application to chronic alcoholism or diabetes, making these comorbidities possible risk factors.

Although much research has investigated the genetics of Mex-Opr efflux pumps, knowledge of the clinical prevalence and significance of EPO is novel. These topics tend to not be included with most epidemiologic studies. Further complications include the lack of literature addressing differences in duration of treatment, outcome, and therapy prescribed between EPO positive and negative strains. In the clinical setting current guidelines for susceptibility testing is to label an antibiotic as either susceptible or resistant based on MIC breakpoints established by the NCCLS. However the data presented does not routinely show progressive changes in the MIC over the duration of antibiotic treatment. EPO is both an innate and acquired resistance mechanism that does not necessarily increase the MIC above the breakpoint levels but can still significantly impact clinical progress of a patient and eventually be a cause of treatment failure. Considering EPO and other resistance mechanisms it is important to design appropriate therapy based not only if a strain is susceptible or resistant but also upon the strain's degree of susceptibility. This knowledge can help to ensure microbiological and clinical treatment success, reduce costs, and abstain from the promotion of resistance.

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