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MACROLIDE RESISTANCE IN PSEUDOMONAS AERUGINOSA DUE TO UL4 AND UL22 RIBOSOMAL MUTATIONS

Abstract. Antibiotics called macrolides are frequently used to treat bacterial airway infections. Testing for antibiotic sensitivity to macrolides is not done since Pseudomonas aeruginosa is regarded as innately resistant due to its high minimum inhibitory concentration in standardized culture medium. However, macrolides are utilized to treat persistent P. aeruginosa infections because of their immunomodulatory action and reduction of virulence factors. Here, we use an AirLiquid Interface (ALI) infection model system that mimics the human airways to show that macrolides are, in fact, effective antibiotics against P. aeruginosa airway infections. Crucially, the use of macrolides in patients with primary ciliary dyskinesia and cystic fibrosis induces P. aeruginosa to accumulate uL4 and uL22 ribosomal protein mutations, which results in antibiotic resistance.

Key words: Antibiotics, macrolides, effects, mutations.

Macrolides are widely used antibiotics for the treatment of several bacterial airway infections. They inhibit protein synthesis by binding the antibiotic binding site within the nascent polypeptide exit tunnel (NPET) of the bacterial ribosome. This modifies the translational capacity of the cell and blocks its growth [1–3]. Recent implementations of in vivo-like model of airway epithelium in an Air-Liquid Interphase (ALI) have disclosed mechanisms of antibiotic resistance that could not be identified by classical Minimum Inhibitory Concentration (MIC) evaluations[1]. Indeed, the localization of the bacteria within the epithelium, the ability of the antibiotics to cross the cell layer and the availability of nutrients can modify the bacterial susceptibility. This is not evident from standardized MIC assays, but it requires taking into account the complexity of the host environment including the relevant interactions between the host and the pathogen. Previously, we identified and characterized clinical P. aeruginosa isolates from pwCF which were significantly less susceptible to erythromycin and azithromycin than their most closely related ancestors [4]. In addition, macrolide-induced effects on redox sensitivity and motility were also reduced. This was caused by mutations in the uL4 ribosomal protein which together with the uL22 ribosomal protein and other ribosomal proteins, form the NPET of the ribosome close to the antibiotic binding site [4]. Similar mutations have been identified in other bacterial species susceptible to macrolide antibiotics such as Escherichia coli, Streptococcus pneumoniae, Legionella pneumophila and Neisseria gonorrhoeae[5]. Moreover, mutations in the 23S rRNA have also been identified as drivers of resistance to macrolides in several clinical strains [6]. However, thorough investigations of mechanisms of resistance have not been carried out in P. aeruginosa because of its perceived intrinsic macrolide resistance. Moreover, it is often challenging to evaluate the effect of ribosomal mutations on antibiotic susceptibility in clinical isolates. This is because clinical isolates harbour a high genomic complexity due to a large number of mutations with epistatic interactions, which drastically modify the bacterial phenotype. Therefore, whether P. aeruginosa is intrinsically susceptible to macrolides, whether mutations in the uL4 and uL22 ribosomal proteins provide increased antibiotic resistance and if such mutations lead to other secondary effects remains unclear. Ribosomal mutations can have unforeseen consequences due to the key function of the translation machinery . For example, they can change the translational landscape and induce or

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abolish ribosomal pausing at certain sequence motifs providing changes in gene expression[6-7]. Moreover, whether macrolide-dependent virulence modulation is dependent on the specific historical contingency of the clinical isolates or if such effect is generalizable, is largely unclear. By transferring a number of previously discovered uL4 and uL22 mutations into the identical genetic background of a reference strain, we employ a reverse engineering technique in this instance. From the tens or hundreds of other mutations present in the clinical isolates, this separates the effects of the ribosomal alterations. Additionally, it makes it possible to directly compare the mutants' virulence, growth rate, proteome allocation, and susceptibility. We conducted infection experiments in an ALI model system of human airway bronchial epithelium to evaluate the biological effects of uL4 and uL22 ribosomal mutations during an infection. Together with its bacteriostatic effect, macrolides exert an antivirulence effect suppressing quorum sensing dependent virulence[8]. Therefore, we quantified various parameters such as swimming diameter, protease secretion, redox sensitivity, pyoverdine and pyocyanin production in the absence or presence of sub-MIC concentrations of azithromycin for all strains . We analyzed the effect of sub-inhibitory concentrations of azithromycin since uL4 and uL22 mutant strains showed a high difference in MIC relative to the PAO1 wild-type and since azithromycin is widely used for the treatment of airway infections in both pwCF and PCD patients. These results confirm that each of the selected mutations did in fact confer increased macrolide resistance. Furthermore, changes in antibiotic susceptibility only apply to ribosome-targeting compounds, ruling out collateral sensitivity or cross-resistance toward other drug classes. Lastly, azithromycin exerts reduced suppression of virulence traits as a consequence of the reduced susceptibility of uL4 and uL22 mutant strains. In addition to altering the growth physiology of the cell, ribosomal mutations may also alter other phenotypic traits independent of antibiotic susceptibility. Motility (swimming and twitching), biofilm formation, secondary metabolites secretion (pyoverdine and pyocyanin), protease secretion and redox sensitivity were, therefore, investigated for all uL4 and uL22 mutants in the absence of antibiotics. No substantial differences between the mutant strains and the PAO1 wildtype strain were observed for any of the tested phenotypes, health. In the case of macrolides and P. aeruginosa infections, this is exacerbated by the lack of specific knowledge on the mode of action in vivo and on the unrecognized ability of infecting bacteria to develop antibiotic resistance. The assumption that P. aeruginosa is intrinsically resistant to macrolides underlines the limitations in mimicking antibiotic efficacy within the unique infectious microenvironment and modelling susceptibility accurately [8]. This misconception - based on standardized AST - has inadvertently allowed the emergence of macrolide-resistant bacteria, which evade detection since routine AST for macrolides on P. aeruginosa is not commonly performed in clinical laboratories. Given their continuous use for the treatment of pwCF, COPD and PCD, it is imperative to recognize macrolide antibiotics' effectiveness against P. aeruginosa and implement improved protocols for the early detection of resistant strains. In conclusion, our review findings highlight the importance of systematic investigations of bacterial pathogens under in vivo-like conditions to comprehensively understand antibiotic efficacy, identify mechanisms of antibiotic resistance, and update antibiotic use guidelines which take into consideration secondary effects of antibiotic resistance mutations. These efforts will contribute to a new understanding of treatment failure and provide new therapies, which are essential for combatting the growing threat of increasing antibiotic resistance.

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