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Treating bacterial virulence systems: we are not there yet

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In the manuscript by van Delden and colleagues [1], the claim is made that azithromycin was successful in inhibiting quorum sensing (QS) and decreased the incidence of *Pseudomonas aeruginosa*-ventilator-associated pneumonia (VAP). This conclusion belies the complexity of what happens in patients colonized and ultimately infected by *P. aeruginosa*.

In an earlier publication by van Delden and colleagues in Thorax [2], 320 *P. aeruginosa* isolates were obtained from a subset of the study patients (see below); they collected isolates from 29 patients over 20 days. Interestingly, only 7/29 (24 %) patients had “QS-proficient isolates”, and only four of these patients developed VAP. In fact, the majority of patients were colonized by QS-deficient *P. aeruginosa* isolates. VAP occurred more frequently in patients colonized during the entire

observation period by *P. aeruginosa* isolates that produced high levels of rhamnolipids; other virulence products associated with quorum sensing (elastase) did not have an association with VAP [2]. One could conclude that the QS virulence system is involved in about 1/4 of the patients who are colonized with *P. aeruginosa* isolates in ventilated patients and clearly involved in some of the patients who developed VAP.

This same group then collected more specimens during this double-blind, placebo-controlled randomized study to assess the efficacy of azithromycin as a quorum-sensing inhibitor in preventing VAP development in *P. aeruginosa*-colonized patients. This study took 3 years and included 21 medical centers in Europe. The patients were randomized when they were found to have proven colonization and were given placebo or 300 mg/day iv azithromycin for 20 days. Patients were allowed to receive antibiotics that were inactive against *P. aeruginosa*, and some patients also received antibiotics with activity against *P. aeruginosa* when considered mandatory.

Notably the trial was stopped prematurely because its funding was discontinued with the dissolution of the sponsoring corporation. Forty-two placebo and 43 azithromycin patients were analyzed; 21 azithromycin and 25 placebo patients received antibiotics devoid of activity for *P. aeruginosa*. However, seven azithromycin and eight placebo patients received antibiotics that were active against *P. aeruginosa*. There was no difference between the two groups in terms of VAP; 2/43 azithromycin-treated patients and 6/42 placebo patients developed VAP. Therefore, for this primary endpoint, the trial documented a failure of azithromycin to protect against VAP.

The authors noted that many of the patients in the trial had QS-deficient isolates, which meant azithromycin might not be effective treatment for these patients. Secondary analysis documented that five patients in the azithromycin group had strains that produced high levels

of rhamnolipids and so would have potentially been affected by the azithromycin treatment. The authors suggest that in this secondary subgroup analysis, azithromycin was effective because only 1/5 of the azithromycin patients that had *P. aeruginosa* strains that produced high levels of rhamnolipids developed VAP compared to 5/5 patients in the placebo group.

The secondary subgroup analysis suggests a difference in VAP rates in patients with high levels of rhamnolipids; however, the subgroup sizes are very small (5 per group). The *p* value is “borderline,” and no adjustment was made for the multiple post hoc comparisons. Additionally it seems surprising that (5/5) 100 % of the patients with high levels of rhamnolipids in the placebo group developed VAP when only (5/8) 63 % with high levels of rhamnolipids developed VAP in the earlier [2] analysis. The small number of patients in the secondary analysis makes this analysis extremely sensitive to minor misclassification [3]. For example, if only 4/5 patients with high levels of rhamnolipids in the placebo group developed VAP (instead of 5/5), a proportion more consistent with the earlier analysis [2], the *p* value would be 0.206, which is not suggestive of a difference between groups.

Notwithstanding the small number of patients in the subgroup analysis, the authors appropriately allude to the fact that potential effects of azithromycin on the resident lung microbial flora cannot be excluded. Indeed, the diversity of microbiota that can reside in the respiratory tract, particularly in compromised or diseased airways, brings another layer of complexity to interpreting the results. More than 50 % of patients in both intervention groups received systemic antibiotics of any type, which

clearly would impact the overall diversity or burden of the microbial community present. It is also conceivable that azithromycin could reduce bacterial diversity or burden through direct antimicrobial effects, or perhaps inhibition of QS expressed by other organisms. QS-proficiency was determined only for *P. aeruginosa* isolates, the main organism of interest. However, upregulation of quorum-sensing genes with enhanced virulence of *P. aeruginosa* has been shown to occur upon co-infection with certain oropharyngeal-derived bacterial strains, compared to *P. aeruginosa* infection alone [4]. Thus, it is important to keep in mind potential contributions made by other members of the microbiota, as well as the potential effects of therapeutics on organisms other than the primary pathogen(s).

What can we conclude from this study? According to the author’s own work, only 10 patients out of the 85 studied could have possibly benefited from a treatment targeting QS. A rapid test to identify these patients would have spared 75 patients a lot of excess treatment. The numbers of patients who developed *P. aeruginosa*-VAP were extremely small; despite multiple centers and 3 years of work, only eight patients developed VAP. Again, this suggests this particular therapy needs to be used sparingly and for specific patients with *P. aeruginosa* strains that produce large quantities of QS. The ultimate conclusion is that we need to develop a molecular paradigm, similar to that utilized in cancer patients, where we treat specific virulence systems when we show they are active in our bacterial strains.

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