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Is OmpK35 Specific for Ceftazadime Penetration?

In a recent article, Rasheed et al. postulated that OmpK35 in *Klebsiella pneumoniae* is specific for ceftazadime penetration (2). The conclusion is based on kinetic data from their study and an observation from a previous study by Martínez-Martínez et al. (1).

According to the kinetic data reported by Rasheed et al., the relative hydrolytic efficiency of cefotaxime was approximately 15 times greater than that of ceftazidime; cefotaxime resistance was therefore thought to be higher than that of ceftazidime due to the higher affinity. Unpredictably, the MICs of ceftazidime and cefotaxime for *K. pneumoniae* K6 were 32 and 8 μg/ml, respectively. Subsequently, the authors found ompK35 was not expressed in *K. pneumoniae* K6. Rasheed et al. thought that porin loss may help explain why the MIC of ceftazidime is higher that of cefotaxime. The study by Martínez-Martínez et al. was cited in support of this conclusion. My principal objection to this explanation is that Martínez-Martínez et al. never discussed the contribution of ceftazidime resistance due to OmpK35 loss (1). In that study, an OmpK36 gene carrier was cloned back into an OmpK35- and ompK36-deficient strain, and a reduction in the MIC of cefotaxime was observed. Although the MIC of ceftazdime had not changed, SHV-5 production in the deficient strain cannot be excluded. In addition, the MIC of cefotaxime remained at 4 μg/ml for the cloned strain, suggesting that SHV-5 plays an important role in resistance. Rasheed et al. state that SHV-5 is thought to have little effect on the hydrolysis of cefotaxime but is necessary for resistance to ceftazidime. Therefore, conclusions about OmpK35 are premature, since production of the extended-spectrum β-lactamase cannot be excluded. Secondly, for a transconjugant (HB101 TC-K6/1) of an SHV-18 carrier, the MICs of cefotaxime and ceftazidime were 1 and 8 μg/ml, respectively (2). A 16-fold reduction in the MIC of cefotaxime for TC-K6/1 was observed compared to the original K6. On the other hand, a fourfold reduction in the MIC of ceftazidime was detected. If OmpK35 is specific for ceftazidime, the MIC of ceftazidime should be significantly reduced and the MIC of cefotaxime should not be affected much. Further experimental work should be conducted.

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Authors' Reply

Our suggestion that the loss of OmpK35 by *K. pneumoniae* K6 may have specifically contributed to the elevated MIC of ceftazidime was intended to be speculative. This, indeed, remains to be proven. We do, however, appreciate Dr. Siu's alternative explanation, which may warrant further study.

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Enzyme kinetics, in particular the ratio of V_{max} to K_m, predict that cefotaxime should be a better substrate than ceftazidime for most TEM- and SHV-type extended-spectrum β-lactamases (ESBLs) (3), including SHV-18, studied by Rasheed et al. Despite these in vitro findings, MICs are typically higher for ceftazidime than for cefotaxime. This paradox can be explained by the slower rate of diffusion of ceftazidime compared to cefotaxime, measured by Yoshimura and Nikaido through porins OmpF and OmpC of *Escherichia coli* (4). To our knowledge, analogous measurements have not been performed with porins OmpK35 and OmpK36 of *K. pneumoniae*, but similar results are expected because of their high homology to OmpF and OmpC, respectively.

Using a set of isogenic strains deficient in β-lactamase and with variable levels of porin expression, it has been shown that the MICs of ceftazidime and cefotaxime against mutants deficient in either OmpK35 or OmpK36 are the same as those for a wild-type strain expressing both porins but are four (ceftazidime) or eight (cefotaxime) times lower than those for a mutant deficient in both porins (1). These findings suggest that either porin allows the diffusion of both cephalosporins. In this group of strains, MICs of ceftazidime were higher than those of cefotaxime, suggesting a higher diffusion rate for cefotaxime, although other mechanisms (for example, active efflux) could also be involved.

Most of the information on the role of *K. pneumoniae* porins in antimicrobial resistance has been gained with OmpK36. The data from our report cited by Rasheed et al. as suggesting that OmpK35 is specific for ceftazidime are inconclusive on this point. We reported that restoration of OmpK36 expression in a porin-deficient strain of *K. pneumoniae* producing SHV-5 changed the MIC of cefotaxime from 64 to 2 μg/ml while the MIC of ceftazidime remained >256 μg/ml. The value >256 μg/ml, however, could hide a considerable change in MIC. In another strain deficient in OmpK35 and OmpK36, *K. pneumoniae* C1 producing TEM-6, expression of OmpK36 caused the ceftazidime MIC to drop from >256 to 32 μg/ml (2). Thus, OmpK36 allows diffusion of ceftazidime into *K. pneumoniae*, indicating that OmpK35 is not a specific ceftazidime channel. The *ompK35* gene was recently cloned and sequenced. Preliminary data from strain CSUB10R (ESBL producer, deficient in both OmpK35 and OmpK36) indicated that expression of either OmpK35 or OmpK36 significantly decreased MICs of ceftazidime, cefotaxime, and other β-lactams and that, in absolute terms, the MICs of cephalosporins for a transconjugant expressing OmpK35 were lower than those for a transconjugant expressing OmpK36 (A. Doménech-Sánchez, S. Hernández-Allés, M. C. Conejo, L. Martínez-Martínez, and V. J.

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It should not be stated that OmpK35 is either specific or exclusive for ceftazidime, but, like the OmpF homologue in *E. coli*, it provides slower entry for ceftazidime than for cefotaxime and, for this reason, greater augmentation of β-lactamase-mediated resistance. We agree with L. K. Siu that further work is necessary to complete our understanding of the role of OmpK35 and other porins in antimicrobial resistance of *K. pneumoniae*.

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