1826. Molecular characterization of the mmpT5 transcriptional repressor in Mycobacterium smegmatis and Mycobacterium intracellulare

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Background. Bacterial colonies associated with mutant transcriptional repressor MmpT5 were also examined. We then compared the use of active transcriptional repression therapy between patients whose patients with CRE BSI from January 2016 to June 2018 at 8 New York and New Jersey medical centers. Patients" "were compared between patients who died within 30 days of BSI onset and survivors. Differences in the diagnosis, indication for and cost of mortality were identified using single PCR tests.

Results. Of 178 patients with CRE BSI (K. pneumoniae: n=14, 84%; Enterobacter cloacae: n=26, 15%; Escherichia coli: n=26, 15%), the 30-day mortality rate was 38%. An increasing Acute Physiology and Chronic Health Evaluation II score (adjusted OR 0.86, 95% CI 0.72-1.04, P = 0.09) and source control (aOR 0.25, 95% CI 0.11-0.57, P = 0.001), and source control (aOR 0.25, 95% CI 0.11-0.57, P = 0.001) were independently associated with survival. Initial targeted therapy with CAZ-AVI was associated with a 28% 30-day mortality rate, compared with a 49% 30-day mortality rate among patients who received a polymyxin combination with drug efflux. The use of PCR to rapidly identify blood cultures with blaKPC and their clinical impact is unclear.

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1828. Bedaquiline Resistance in Mycobacterium intracellulare Infiltrated by the Transcriptional Repressor MmpT5

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Background. Bedaquiline (BDQ) is an FDA approved antibiotic with antimycobacterial activity. BDQ resistance has been observed in several Mycobacterium species. High-level resistance is due to mutations in ATP synthase. Low-level resistance is attributed to drug efflux. Previously, we suggested that the MmpS5L5 efflux system mediates BDQ resistance in M. intracellulare. Here, we examine the role of MmpT5 in transcriptional regulation of mmpS5L5 and BDQ resistance.

Methods. In this study, mmpS5L5-mmpT5 genes were cloned from 2 pre-treatment (wild-type mmpT5) and 2 relapse (mutant mmpT5) isolates of M. intracellulare and transformed into M. smegmatis. BDQ MICs were determined as well as changes observed after 24 hours of exposure to BDQ instead of 72 hours. Transcriptomes of BDQ. Transcription of the M. intracellulare mmpS5L5 and mmpT5 promoters was monitored with luciferase reporter gene fusions in the presence of wild-type and mutant alleles of mmpT5. Single and multigene constructs were created using the MoClo system, and transformed into E. coli DH5α. Constructs containing the M. tuberculosis rv0678 gene, which mediates low-level BDQ resistance in M. tuberculosis, were also examined.

Results. The BDQ MIC for the M. smegmatis control strain, and all strains containing the wild-type mmpT5 control, was 0.007 μg/ml. However, the mutant containing mutant mmpT5 alleles showed enhanced survival after 24 hours exposure to 0.007 μg/ml BDQ. BDQ-resistant colonies associated with mutant mmpT5 alleles exhibited altered morphology relative to wild-type strains. Transcriptome of mmpS5L5 was repressed by wild-type mmpT5, but neither mutant mmpT5 nor rv0678 repressed transcription. The mmpT5 luciferase reporter was not active.

Conclusion. MmpT5 represses transcription of mmpS5L5 whereas the operon is dysregulated by mmpT5 mutations. Although rv0678 regulates mmpS5L5 expression in M. tuberculosis, it cannot repress the M. intracellulare mmpS5L5 genes. The mmpS5L5 alleles have no impact on the BDQ MIC for M. smegmatis, but constructs containing mutant mmpT5 alleles do enhance bacterial survival. The altered morphology of these colonies suggests that BDQ resistance is mediated by cell wall changes in combination with drug efflux.

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1829. The Paradox of KPC Bearing Strains of Klebsiella pneumoniae with high-level resistance to Vancomycin (VAN)

Vancomycin (VAN) is a first-line therapeutic option for severe infections, especially in Latin America where other options are limited. However, reduced susceptibility to VAN may lead to therapeutic failures. The molecular mechanisms leading the development of VAN-intermediate S. aureus (VISA) and heterogeneous-VISA (hVISA) phenotypes are still unclear. We explored genetic signatures associated with hVISA phenotype in MRSA isolates recovered from bacteremic patients in 9 Latin American countries (2011-2014) in order to develop a genomic platform to identify these isolates.

Methods. From 538 VAN-susceptible MRSA (MIC < 1 μg/ml) we identified 30 hVISA isolates using GRD and macromethod E-tests; from these, 3 were confirmed by PAP-AUC. Whole-genome sequencing was performed in all 30 isolates using Illumina platform. Based on previous studies, we selected 46 genes involved in hVISA development. Multiple Blast alignments were performed using genomes of ATCC29213 and N315 (VAN-susceptible), Mu3 (hVISA) and Mu50 (VISA) as references.

Results. A total of 130 changes in 46 predicted proteins belonging to 8 functional categories were determined: 48 changes related to cell wall biogenesis, 22 to DNA/RNA processing, 17 to regulatory systems, 12 to cofactors and enzymes, 11 to membrane biosynthesis, 9 to virulence, 6 to amino acid metabolism, and 5 to transport of nitrogen and putrescine/spermidine. The most common changes identified in all the hVISA were: Y318H in Adj, N165 in PBP4, S166G in RpoB, L141 in WaaK and E156G in YopQ; compared with VISA strains. The proteins with the highest number of changes detected in the isolates confirmed by PAP-AUC were: CapD, DfrA, Php4, TcaA, LyrM (Cell wall biogenesis); MurL, RpoB (DNA/RNA processing); Gra5 (Regulatory systems).

Conclusion. Changes in genes associated with cell wall biogenesis, DNA/RNA processing, regulatory systems, and membrane biosynthesis were the most prevalent in Latin American hVISA strains. Genetic signatures in genes encoding GraR (N197S), RpoB (H481Y, H481N), Vca (T5N), WaaK (L141F, R127K2) and MsrR (E146K) are potentially associated with this phenotype. These changes could be used to develop a platform for possible identification of hVISA isolates.

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