P1411

Abstract (poster session)

Deciphering molecular mechanisms of resistance to daptomycin and tigecycline in Enterococcus faecium

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Objectives: Daptomycin and tigecycline are active against Gram-positive bacteria such as methicillin resistant (MRSA) and vancomycin-resistant enterococci (VRE). The mechanisms of resistance to these antibiotics are not yet known in E. faecium, an opportunistic bacteria responsible for nosocomial infections. The aim of the study was to investigate the molecular mechanisms of resistance towards these two novel antibiotics in E. faecium. Methods: Mutants strains of E. faecium HM1070 resistant to daptomycin and tigecycline (MIC of 128 mg/L and 1 mg/L, respectively) were obtained in vitro by serial passages. Whole-genome sequencing was performed for the parental strain and their mutants by using a 454 Life Sciences (Roche) GS-FLX system. When possible, observed mutations have been verified by re-sequencing of the corresponding region that were amplified by PCR. RT-qPCR experiments were performed in order to verify the transcription level of genes surrounding mutations identified into intergenic regions. Lastly, cell walls have been observed by transmission electron microscopy (TEM). Results: For the daptomycin resistant strain, 24 different mutations were identified in 16 chromosomal regions amongst which 15 were common with those observed for the tigecycline resistant mutant. Moreover, contrarily to publish data for E. faecalis, the measurement of the thickness of the cell wall of the mutant by TEM showed only a small increase compared with that of the wild-type strain. Furthermore, analysis of genes known to be involved in resistance in other Gram-positive bacteria did not reveal any mutation. We also identified 111 mutations located in 93 chromosomal regions of the genomic sequence of the strain resistant to tigecycline, of which the majority of genes were involved in regulatory functions and transport. Finally, analysis of the expression of genes flanking the mutations located in intergenic regions showed no change. Conclusion: All of these data highlights the existence of new mechanisms of resistance to daptomycin and tigecycline in E. faecium. Among these new candidates, further investigations are needed to more precisely identify genes involved in the resistance.